

LISTA DE SECUENCIAS

<110> Syngenta Participations AG
Chae, Hyunsook Sarah

<120> Proteínas pesticidas modificadas y métodos para controlar plagas de plantas

<130> 81090-WO-REG-ORG-P-1

<150> US 62/432,909

<151> 2016-12-12

<160> 35

<170> PatentIn versión 3.5

<210> 1

<211> 715

<212> PRT

<213> Secuencia artificial

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<223> Secuencia de BT-0022 ensamblada a partir de la prospección genómica de Bacillus.

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			20					25					30		

Glu	Leu	Gln	Asn	Phe	Asn	His	Glu	Gly	Ile	Glu	Pro	Phe	Val	Ser	Val
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Ser	Thr	Ile	Gln	Thr	Gly	Ile	Gly	Ile	Ala	Gly	Lys	Ile	Leu	Gly	Asn
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Leu	Gly	Val	Pro	Phe	Ala	Gly	Gln	Val	Ala	Ser	Leu	Tyr	Ser	Phe	Ile
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Leu	Gly	Glu	Leu	Trp	Pro	Lys	Gly	Lys	Ser	Gln	Trp	Glu	Ile	Phe	Met
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Glu His Val Glu Glu Leu Ile Asn Gln Lys Ile Ser Thr Tyr Ala Arg
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Asn Lys Ala Leu Ala Asp Leu Lys Gly Leu Gly Asp Ala Leu Ala Val
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Tyr His Glu Ser Leu Glu Ser Trp Ile Lys Asn Arg Asn Asn Thr Arg
130 135 140

Thr Arg Ser Val Val Lys Ser Gln Tyr Ile Thr Leu Glu Leu Met Phe
145 150 155 160

Val Gln Ser Leu Pro Ser Phe Ala Val Ser Gly Glu Glu Val Pro Leu
165 170 175

Leu Pro Ile Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Leu Arg
180 185 190

Asp Ala Ser Ile Phe Gly Lys Glu Trp Gly Leu Ser Asp Ser Glu Ile
195 200 205

Ser Thr Phe Tyr Asn Arg Gln Val Glu Arg Thr Ser Asp Tyr Ser Asp
210 215 220

His Cys Thr Lys Trp Phe Asp Thr Gly Leu Asn Arg Leu Lys Gly Ser
225 230 235 240

Asn Ala Glu Ile Trp Val Lys Tyr Asn Gln Phe Arg Arg Asp Met Thr
245 250 255

Leu Met Val Leu Asp Leu Val Ala Leu Phe Gln Ser Tyr Asp Thr His
260 265 270

Met Tyr Pro Ile Lys Thr Thr Ala Gln Leu Thr Arg Glu Val Tyr Thr
275 280 285

Asn Ala Ile Gly Thr Val His Pro His Pro Ser Phe Thr Ser Thr Thr
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Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu Ala Ala Val
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Ile Arg Ser Pro His Leu Leu Asp Phe Leu Glu Gln Val Thr Ile Tyr
325 330 335

Ser Leu Leu Ser Arg Trp Ser Asn Thr Gln Tyr Met Asn Met Trp Gly
340 345 350

Gly His Lys Leu Glu Phe Arg Thr Ile Gly Gly Thr Leu Asn Thr Ser
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Thr Gln Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Pro Phe
370 375 380

Thr Ser Arg Asp Ile Tyr Arg Thr Glu Ser Leu Ala Gly Leu Asn Leu
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Phe Leu Thr Gln Pro Val Asn Gly Val Pro Arg Val Asp Phe His Trp
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Lys Phe Val Thr His Pro Ile Ala Ser Asp Asn Phe Tyr Tyr Pro Gly
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Tyr Ala Gly Ile Gly Thr Gln Leu Gln Asp Ser Glu Asn Glu Leu Pro
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Pro Glu Thr Thr Gly Gln Pro Asn Tyr Glu Ser Tyr Ser His Arg Leu
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Ser His Ile Gly Leu Ile Ser Ala Ser His Val Lys Ala Leu Val Tyr
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Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile His Ser Asp
485 490 495

Ser Ile Thr Gln Ile Pro Leu Val Lys Ala His Thr Leu Gln Ser Gly
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Thr Thr Val Val Lys Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg
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Arg Thr Ser Gly Gly Pro Phe Ala Phe Ser Asn Val Asn Leu Asp Trp
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Asn Leu Ser Gln Arg Tyr Arg Ala Arg Ile Arg Tyr Ala Ser Thr Thr
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Asn Leu Arg Met Tyr Val Thr Ile Ala Gly Glu Arg Ile Phe Ala Gly
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Gln Phe Asn Lys Thr Met Asn Thr Gly Asp Pro Leu Thr Phe Gln Ser
580 585 590

Phe Ser Tyr Ala Thr Ile Asp Thr Ala Phe Thr Phe Pro Thr Lys Ala
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Ser Ser Leu Thr Val Gly Ala Asp Thr Phe Ser Ser Gly Asn Glu Val
610 615 620

Tyr Val Asp Arg Phe Glu Leu Ile Pro Val Thr Ala Thr Leu Glu Ala
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Val Thr Asp Leu Glu Arg Ala Gln Lys Ala Val His Glu Leu Phe Thr
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Ser Thr Asn Pro Gly Gly Leu Lys Thr Asp Val Ala Lys Asp His Tyr
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Thr Asn Thr Ile Ser Lys Ser Val Gln Ser Val Phe Arg Cys Arg Cys
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<212> PRT
<213> Secuencia artificial

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<223> Secuencia de BT-0029 ensamblada a partir de la prospección genómica de Bacillus.

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Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
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Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
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Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
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Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
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Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
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Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
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Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
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Val Lys Ala Phe Asn Leu His Ser Gly Ala Thr Ile Val Lys Gly Pro
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Gly Asp Met Arg Val Asn Ile Thr Ala Pro Leu Ser Gln Arg Tyr Arg
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Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu
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Lys Thr Asp Val Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val
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Glu Lys Val Lys His Ala Lys Arg Leu Cys Asp Glu Arg Asn Leu Leu
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740 745 750

Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
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Glu Pro Asn Arg Cys Ala Thr His Leu Glu Trp Asn Pro Asp Leu Asp
785 790 795 800

Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
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Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu
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Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu Ala Leu
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Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys
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Leu Glu Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Lys Ser Val
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Asn Ile Ala Ile Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg
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Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
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Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Tyr Ser Leu Tyr
945 950 955 960

Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Ser
965 970 975

Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His
980 985 990

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Tyr	Asp	Gly	Ala	Tyr	Glu	Ser	Asn	Ser	Ser	Val	Pro	Ala	Asp	Tyr
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Pro	Ala	Gly	Tyr	Val	Thr	Lys	Glu	Leu	Glu	Tyr	Phe	Pro	Glu	Thr
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Asp	Lys	Val	Trp	Ile	Glu	Ile	Gly	Glu	Thr	Glu	Gly	Thr	Phe	Ile
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<223> proteína quimérica Bt29-Bt22.

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Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
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Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
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Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
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Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
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Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
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Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
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Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
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Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
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Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
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Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
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Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys Ala
595 600 605

Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu
610 615 620

Lys Thr Asp Val Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val
625 630 635 640

Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser
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Glu Lys Val Lys His Ala Lys Arg Leu Cys Asp Glu Arg Asn Leu Leu
660 665 670

Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg Gly Trp
675 680 685

Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys
690 695 700

Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr
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Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg
725 730 735

Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr
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Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
755 760 765

Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly
770 775 780

Glu Pro Asn Arg Cys Ala Thr His Leu Glu Trp Asn Pro Asp Leu Asp
785 790 795 800

Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
805 810 815

Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly
820 825 830

Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu
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Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu Ala Leu
850 855 860

Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys
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Leu Glu Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Lys Ser Val
885 890 895

Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr
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Asn Ile Ala Ile Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg
915 920 925

Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
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Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Tyr Ser Leu Tyr
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Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Ser
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Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His
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Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu
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Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala
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Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile
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Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu
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Glu Ile Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Ala
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Ala Ser Ala Tyr Glu Glu Lys Ala Tyr Thr Asp Gly Arg Arg Asp
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Asn Thr Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu
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Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr
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quimérica Bt29-Bt22.

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Arg Pro Phe Tyr Arg Thr Leu Ser Asp Pro Val Phe Val Arg Gly Gly
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Gln Thr Gly Thr Asn His Thr Arg Thr Phe Arg Asn Ser Gly Thr Ile
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Asp Ser Leu Asp Glu Ile Pro Pro Gln Asp Asn Ser Gly Ala Pro Trp
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Asn Asp Tyr Ser His Val Leu Asn His Val Thr Phe Val Arg Trp Pro
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Gly Glu Ile Ser Gly Ser Asp Ser Trp Arg Ala Pro Met Phe Ser Trp
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Thr His Arg Ser Ala Thr Pro Thr Asn Thr Ile Asp Pro Glu Arg Ile
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Thr Gln Ile Pro Leu Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr
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Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr
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Ser Gly Gly Pro Phe Ala Tyr Thr Ile Val Asn Ile Asn Gly Gln Leu
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Pro Gln Arg Tyr Arg Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu
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Asn Lys Thr Met Asp Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser
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Tyr Ala Thr Ile Asn Thr Ala Phe Thr Phe Pro Met Ser Gln Ser Ser
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Asn Val Leu Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Gln Ser Pro
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His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu
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Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu
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Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
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Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
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Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu

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Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His				
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Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile				
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Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu				
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Glu Ile Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Ala				
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Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr				

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Asn Ser Trp Phe Arg Asn Gly Ala Ser Phe Ser Ala Ile Glu Asn Ala
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Ile Ile Arg Gln Pro His Leu Tyr Asp Phe Leu Thr Asn Leu Thr Ile
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Tyr Thr Arg Arg Ser Gln Val Gly Thr Thr Ile Met Asn Leu Trp Ala
340 345 350

Gly His Arg Ile Thr Phe Asn Arg Ile Gln Gly Gly Ser Thr Ser Glu
355 360 365

Met Val Tyr Gly Ala Ile Thr Asn Pro Val Ser Val Ser Asp Ile Pro
370 375 380

Phe Val Asn Arg Asp Val Tyr Arg Thr Val Ser Leu Ala Gly Gly Leu
385 390 395 400

Gly Ser Leu Ser Gly Ile Arg Tyr Gly Leu Thr Arg Val Asp Phe Asp
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Met Ile Phe Arg Asn His Pro Asp Ile Val Thr Gly Leu Phe Tyr His
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Pro Gly His Ala Gly Ile Ala Thr Gln Val Lys Asp Ser Asp Thr Glu
435 440 445

Leu Pro Pro Glu Thr Thr Glu Gln Pro Asn Tyr Arg Ala Phe Ser His
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Leu Leu Ser His Ile Ser Met Gly Pro Thr Thr Gln Asp Val Pro Pro
465 470 475 480

Val Tyr Ser Trp Thr His Gln Ser Ala Asp Arg Thr Asn Thr Ile Asn
485 490 495

Ser Asp Arg Ile Thr Gln Ile Pro Leu Val Lys Ala His Thr Leu Gln
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Ser Gly Thr Thr Val Val Lys Gly Pro Gly Phe Thr Gly Gly Asp Ile
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Leu Arg Arg Thr Ser Gly Gly Pro Phe Ala Phe Ser Asn Val Asn Leu
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Asp Phe Asn Leu Ser Gln Arg Tyr Arg Ala Arg Ile Arg Tyr Ala Ser
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Thr Thr Asn Leu Arg Ile Tyr Val Thr Val Ala Gly Glu Arg Ile Phe
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Ala Gly Gln Phe Asp Lys Thr Met Asp Ala Gly Ala Pro Leu Thr Phe
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Gln Ser Phe Ser Tyr Ala Thr Ile Asn Thr Ala Phe Thr Phe Pro Glu
595 600 605

Arg Ser Ser Ser Leu Thr Ile Gly Ala Asp Thr Phe Ser Ser Gly Asn
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Glu Val Tyr Val Asp Arg Phe Glu Leu Ile Gln Val Thr Ala Thr Phe
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Glu Ala Glu Ser Asp Leu Glu Arg Ala Arg Lys Ala Val Asn Ala Leu
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Phe Thr Ser Thr Asn Pro Arg Gly Leu Lys Thr Asp Val Thr Asp Tyr
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His Ile Asp Gln Val Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe
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Cys Leu Asp Lys Lys Arg Glu Leu Leu Glu Glu Val Lys Tyr Ala Lys
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Arg Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Thr Phe Thr Ser
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Ile Ser Gly Gln Thr Asp Arg Gly Trp Ile Gly Ser Thr Gly Ile Ser
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Ile Gln Gly Gly Asp Asp Ile Phe Lys Glu Asn Tyr Val Arg Leu Pro
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Gly Thr Val Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp
755 760 765

Glu Ser Gln Leu Lys Ser Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile
770 775 780

Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys
785 790 795 800

His Glu Thr Leu Ser Val Pro Gly Thr Glu Ser Pro Trp Pro Ser Ser
805 810 815

Gly Val Tyr Pro Ser Gly Arg Cys Gly Glu Pro Asn Arg Cys Ala Pro
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Arg Ile Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg Tyr Gly Glu
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Lys Cys Val His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly
850 855 860

Cys Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile
865 870 875 880

Lys Thr Gln Asp Gly His Ala Lys Leu Gly Asn Leu Glu Phe Ile Glu
885 890 895

Glu Lys Pro Leu Leu Gly Lys Ala Leu Ser Arg Val Lys Arg Ala Glu
900 905 910

Lys Lys Trp Arg Asp Lys Tyr Glu Lys Leu Gln Leu Glu Thr Lys Arg
915 920 925

Val Tyr Thr Glu Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asp Ser
930 935 940

Gln Tyr Asp Lys Leu Gln Ala Asn Thr Asn Ile Gly Ile Ile His Gly
945 950 955 960

Ala Asp Lys Gln Val His Arg Ile Arg Glu Pro Tyr Leu Ser Glu Leu
965 970 975

Pro Val Ile Pro Ser Ile Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly
980 985 990

His Ile Phe Lys Ala Tyr Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys
995 1000 1005

Asn Gly Asp Phe Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly
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His Val Asp Val Gln Gln Asn His His Arg Ser Val Leu Val Leu
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Asp Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr
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Gly Glu Gly Cys Val Thr Ile His Glu Phe Glu Asp Asn Thr Asp
1070 1075 1080

Val Leu Lys Phe Arg Asn Phe Val Glu Glu Glu Val Tyr Pro Asn
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Asn Thr Val Thr Cys Asn Asp Tyr Thr Thr Asn Gln Ser Ala Glu
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Tyr Asn His Cys Val Ser Asp Arg Gly Tyr Arg Asn His Thr Pro
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
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Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
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Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
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Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
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Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
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Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
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Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
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Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
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Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
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Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
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Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
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His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
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Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
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Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

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Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

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420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
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Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
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Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
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Lys Thr Asp Val Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val
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Glu Lys Val Lys His Ala Lys Arg Leu Cys Asp Glu Arg Asn Leu Leu
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Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg Gly Trp
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Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys
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Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr
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Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg
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Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr
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Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
755 760 765

Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly
770 775 780

Glu Pro Asn Arg Cys Ala Thr His Leu Glu Trp Asn Pro Asp Leu Asp
785 790 795 800

Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
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Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly
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Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu
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Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys
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Leu Glu Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Lys Ser Val
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Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr
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Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
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Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Tyr Ser Leu Tyr
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Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Ser
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Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His
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Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu
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Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala
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Ala Ser Ala Tyr Glu Glu Lys Ala Tyr Thr Asp Gly Arg Arg Asp
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Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr
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tgggaagcag aagtgtcaca agaggttcgt gtctgtccag gtcgtggcta tatccttcgt
3060

gttacagcgt acaaagaggg atatggagag ggctgtgtaa cgattcatga gatcgaagac
3120

aatacagacg aactgaaatt cagcaactgt gtagaagagg aaatatatcc aaacaacacg
3180

gtaacgtgta atgattatac tgcgactcaa gaagaatatg agggtagcga cacttctcgt
3240

aatcgaggat atgacggagc ctatgaaagc aattcttctg taccagctga ttatgcatca
3300

gcctatgaag aaaaagcgtg tacagatgga agaagagaca atacttgtga atctaacaga
3360

ggatatgggg attacacacc actaccagct ggctatgtga caaagaatt agagtacttc
3420

ccagaaaccg ataaggtatg gattgagatt ggagaaacgg aaggaacatt tatcgtggac
3480

agcgtggaat tactccttat ggaggaatag
3510

<210> 15

<211> 1169

<212> PRT

<213> Secuencia artificial

<220>

<223> Proteína quimérica Bt29-1Kav2

<400> 15

Met	Glu	Ile	Asn	Asn	Gln	Asn	Gln	Cys	Val	Pro	Tyr	Asn	Cys	Leu	Asn
1				5					10					15	

Asn	Pro	Glu	Ser	Glu	Ile	Leu	Asn	Val	Ala	Ile	Phe	Ser	Ser	Glu	Gln
			20					25					30		

Val	Ala	Glu	Ile	His	Leu	Lys	Ile	Thr	Arg	Leu	Ile	Leu	Glu	Asn	Phe
		35					40					45			

Leu	Pro	Gly	Gly	Ser	Phe	Ala	Phe	Gly	Leu	Phe	Asp	Leu	Ile	Trp	Gly
	50					55					60				

Ile	Phe	Asn	Glu	Asp	Gln	Trp	Ser	Ala	Phe	Leu	Arg	Gln	Val	Glu	Glu
65					70					75					80

Leu	Ile	Asn	Gln	Arg	Ile	Thr	Glu	Phe	Ala	Arg	Gly	Gln	Ala	Ile	Gln
				85					90					95	

Arg	Leu	Val	Gly	Phe	Gly	Arg	Ser	Tyr	Asp	Glu	Tyr	Ile	Leu	Ala	Leu
			100					105					110		

Lys	Glu	Trp	Glu	Asn	Asp	Pro	Asp	Asn	Pro	Ala	Ser	Lys	Glu	Arg	Val
		115					120					125			

Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro
	130					135					140				

Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala
145					150					155					160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Phe Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Ile Tyr Val Thr
515 520 525

Val Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asp Lys Thr Met Asp
530 535 540

Ala Gly Ala Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asn
545 550 555 560

Thr Ala Phe Thr Phe Pro Glu Arg Ser Ser Ser Leu Thr Ile Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Gln Val Thr Ala Thr Phe Glu Ala Glu Ser Asp Leu Glu Arg Ala
595 600 605

Arg Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu
610 615 620

Lys Thr Asp Val Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val
625 630 635 640

Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser
645 650 655

Glu Lys Val Lys His Ala Lys Arg Leu Cys Asp Glu Arg Asn Leu Leu
660 665 670

Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg Gly Trp
675 680 685

Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys
690 695 700

Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr
705 710 715 720

Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg
725 730 735

Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr
740 745 750

Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
755 760 765

Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly
770 775 780

Glu Pro Asn Arg Cys Ala Thr His Leu Glu Trp Asn Pro Asp Leu Asp
785 790 795 800

Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
805 810 815

Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly
820 825 830

Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu
835 840 845

Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu Ala Leu
850 855 860

Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys
865 870 875 880

Leu Glu Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Lys Ser Val
885 890 895

Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr
900 905 910

Asn Ile Ala Ile Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg
915 920 925

Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
930 935 940

Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Tyr Ser Leu Tyr
945 950 955 960

Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Ser
965 970 975

Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His
980 985 990

Arg	Ser	Val	Leu	Val	Val	Pro	Glu	Trp	Glu	Ala	Glu	Val	Ser	Gln	Glu
		995					1000					1005			

Val	Arg	Val	Cys	Pro	Gly	Arg	Gly	Tyr	Ile	Leu	Arg	Val	Thr	Ala
	1010					1015					1020			

Tyr	Lys	Glu	Gly	Tyr	Gly	Glu	Gly	Cys	Val	Thr	Ile	His	Glu	Ile
	1025					1030					1035			

Glu	Asp	Asn	Thr	Asp	Glu	Leu	Lys	Phe	Ser	Asn	Cys	Val	Glu	Glu
	1040					1045					1050			

Glu	Ile	Tyr	Pro	Asn	Asn	Thr	Val	Thr	Cys	Asn	Asp	Tyr	Thr	Ala
	1055					1060					1065			

Thr	Gln	Glu	Glu	Tyr	Glu	Gly	Thr	Tyr	Thr	Ser	Arg	Asn	Arg	Gly
	1070					1075					1080			

Tyr	Asp	Gly	Ala	Tyr	Glu	Ser	Asn	Ser	Ser	Val	Pro	Ala	Asp	Tyr
	1085					1090					1095			

Ala	Ser	Ala	Tyr	Glu	Glu	Lys	Ala	Tyr	Thr	Asp	Gly	Arg	Arg	Asp
	1100					1105					1110			

Asn	Thr	Cys	Glu	Ser	Asn	Arg	Gly	Tyr	Gly	Asp	Tyr	Thr	Pro	Leu
	1115					1120					1125			

Pro	Ala	Gly	Tyr	Val	Thr	Lys	Glu	Leu	Glu	Tyr	Phe	Pro	Glu	Thr
	1130					1135					1140			

Asp	Lys	Val	Trp	Ile	Glu	Ile	Gly	Glu	Thr	Glu	Gly	Thr	Phe	Ile
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Val	Asp	Ser	Val	Glu	Leu	Leu	Leu	Met	Glu	Glu
	1160					1165				

<210> 16
<211> 3510

<212> ADN

<213> Secuencia artificial

<220>

<223> Quimera Bt29-1Ka

<400> 16

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120

acgcgcttaa ttttagagaa ttttttacca ggtgggagtt ttgcattcgg cttatttgat
180

ttaatatggg ggatttttaa tgaagatcaa tggagcgcac ttcttcggca ggtagaagaa
240

ttaattaatc aaaggataac ggaattcgcg agagggaag caattcagag actagtaggg
300

tttgaagga gttatgatga atatatttta gcactaaaag aatgggaaaa cgatcctgat
360

aaccagctt caaaggaaag agtgcgcact cgatttcgga caactgatga tgccttgcta
420

accggtgttc ctcttatggc aattccaggt tttgaattag ctactttatc tgtttatgct
480

caatcagcca atctacattt agccctatta agagatgctg tatttttttg ggagagatgg
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ggattgacac aaacaaatat aaatgattta tatagtagat taaaaaactc cattcgtgat
600

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660

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720

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780

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840

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cgcggaattc gtcactgggc aggacatgaa gtagaatcta gtagaacagg tatgatgact
960

aatataagat ttcctttgta tggaacagcc gcaacagcag aaccaacacg atttataact
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gatcaattac cacttgaggg tgagagtagt ctaactgaat atagtcatcg attatgccat
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1380

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1800

gcagaatcag atttagagag agcacgaaaa gcggtgaatg cgctgtttac ttcttccaat
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1920

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1980

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2040

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2100

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2160

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2220

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2280

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2340

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2400

tgttcgtgta gggatggaga aaagtgtgcc catcattcgc atcatttctc cttagacatt
2460

gatgtaggat gtacagacct aaatgaggac ctaggtgtat gggatgatctt taagattaag
2520

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2580

ggagaagcgc tagctcgtgt gaagagagcg gagaaaaaat ggagagacaa acgcgaaaaa
2640

ttggaattgg aaacaaatat tgtttataaa gaggcaaaaa aatctgtaga tgctttattt
2700

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2760

gataaacgcg ttcatagcat tcgagaagca tatcttccag agttgtctgt aattccgggt
2820

gtaaatgcag ctatTTTTga agaattagag ggacgtatTT tcacagccta ctctctatat
2880

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3000

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3180

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aatcgaggat atgacggagc ctatgaaagc aattcttctg taccagctga ttatgcatca
3300

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3360

ggatatggggg attacacacc actaccagct ggctatgtga caaaagaatt agagtacttc
3420

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3480

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3510

<210> 17
<211> 1189
<212> PRT
<213> Bacillus thuringiensis

<400> 17

Met	Glu	Glu	Asn	Asn	Gln	Asn	Gln	Cys	Ile	Pro	Tyr	Asn	Cys	Leu	Ser
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Asn	Pro	Glu	Glu	Val	Leu	Leu	Asp	Gly	Glu	Arg	Ile	Ser	Thr	Gly	Asn
			20					25					30		

Ser	Ser	Ile	Asp	Ile	Ser	Leu	Ser	Leu	Val	Gln	Phe	Leu	Val	Ser	Asn
		35					40					45			

Phe	Val	Pro	Gly	Gly	Gly	Phe	Leu	Val	Gly	Leu	Ile	Asp	Phe	Val	Trp
	50					55					60				

Gly Ile Val Gly Pro Ser Gln Trp Asp Ala Phe Leu Val Gln Ile Glu
65 70 75 80

Gln Leu Ile Asn Glu Arg Ile Ala Glu Phe Ala Arg Asn Ala Ala Ile
85 90 95

Ala Asn Leu Glu Gly Leu Gly Asn Asn Phe Asn Ile Tyr Val Glu Ala
100 105 110

Phe Lys Glu Trp Glu Glu Asp Pro Asn Asn Pro Ala Thr Arg Thr Arg
115 120 125

Val Ile Asp Arg Phe Arg Ile Leu Asp Gly Leu Leu Glu Arg Asp Ile
130 135 140

Pro Ser Phe Arg Ile Ser Gly Phe Glu Val Pro Leu Leu Ser Val Tyr
145 150 155 160

Ala Gln Ala Ala Asn Leu His Leu Ala Ile Leu Arg Asp Ser Val Ile
165 170 175

Phe Gly Glu Arg Trp Gly Leu Thr Thr Ile Asn Val Asn Glu Asn Tyr
180 185 190

Asn Arg Leu Ile Arg His Ile Asp Glu Tyr Ala Asp His Cys Ala Asn
195 200 205

Thr Tyr Asn Arg Gly Leu Asn Asn Leu Pro Lys Ser Thr Tyr Gln Asp
210 215 220

Trp Ile Thr Tyr Asn Arg Leu Arg Arg Asp Leu Thr Leu Thr Val Leu
225 230 235 240

Asp Ile Ala Ala Phe Phe Pro Asn Tyr Asp Asn Arg Arg Tyr Pro Ile
245 250 255

Gln Pro Val Gly Gln Leu Thr Arg Glu Val Tyr Thr Asp Pro Leu Ile
260 265 270

Asn Phe Asn Pro Gln Leu Gln Ser Val Ala Gln Leu Pro Thr Phe Asn
275 280 285

Val Met Glu Ser Ser Ala Ile Arg Asn Pro His Leu Phe Asp Ile Leu
290 295 300

Asn Asn Leu Thr Ile Phe Thr Asp Trp Phe Ser Val Gly Arg Asn Phe
305 310 315 320

Tyr Trp Gly Gly His Arg Val Ile Ser Ser Leu Ile Gly Gly Gly Asn
325 330 335

Ile Thr Ser Pro Ile Tyr Gly Arg Glu Ala Asn Gln Glu Pro Pro Arg
340 345 350

Ser Phe Thr Phe Asn Gly Pro Val Phe Arg Thr Leu Ser Asn Pro Thr
355 360 365

Leu Arg Leu Leu Gln Gln Pro Trp Pro Ala Pro Pro Phe Asn Leu Arg
370 375 380

Gly Val Glu Gly Val Glu Phe Ser Thr Pro Thr Asn Ser Phe Thr Tyr
385 390 395 400

Arg Gly Arg Gly Thr Val Asp Ser Leu Thr Glu Leu Pro Pro Glu Asp
405 410 415

Asn Ser Val Pro Pro Arg Glu Gly Tyr Ser His Arg Leu Cys His Ala
420 425 430

Thr Phe Val Gln Arg Ser Gly Thr Pro Phe Leu Thr Thr Gly Val Val
435 440 445

Phe Ser Trp Thr His Arg Ser Ala Thr Leu Thr Asn Thr Ile Asp Pro
450 455 460

Glu Arg Ile Asn Gln Ile Pro Leu Val Lys Gly Phe Arg Val Trp Gly
465 470 475 480

Gly Thr Ser Val Ile Thr Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu
485 490 495

Arg Arg Asn Thr Phe Gly Asp Phe Val Ser Leu Gln Val Asn Ile Asn
500 505 510

Ser Pro Ile Thr Gln Arg Tyr Arg Leu Arg Phe Arg Tyr Ala Ser Ser
515 520 525

Arg Asp Ala Arg Val Ile Val Leu Thr Gly Ala Ala Ser Thr Gly Val
530 535 540

Gly Gly Gln Val Ser Val Asn Met Pro Leu Gln Lys Thr Met Glu Ile
545 550 555 560

Gly Glu Asn Leu Thr Ser Arg Thr Phe Arg Tyr Thr Asp Phe Ser Asn
565 570 575

Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Gly Ile Ser Glu Gln
580 585 590

Pro Leu Phe Gly Ala Gly Ser Ile Ser Ser Gly Glu Leu Tyr Ile Asp
595 600 605

Lys Ile Glu Ile Ile Leu Ala Asp Ala Thr Phe Glu Ala Glu Ser Asp
610 615 620

Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn
625 630 635 640

Gln Ile Gly Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val
645 650 655

Ser Asn Leu Val Asp Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys
660 665 670

Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu
675 680 685

Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro
690 695 700

Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp
705 710 715 720

Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr Val Asp Glu
725 730 735

Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys
740 745 750

Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
755 760 765

Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Ile Val Asn
770 775 780

Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile
785 790 795 800

Gly Lys Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn
805 810 815

Pro Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His
820 825 830

Ser His His Phe Thr Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn
835 840 845

Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly
850 855 860

His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Leu
865 870 875 880

Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp
885 890 895

Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala
900 905 910

Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu
915 920 925

Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val
930 935 940

His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly
945 950 955 960

Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala
965 970 975

Tyr Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn
980 985 990

Asn Gly Leu Leu Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu
995 1000 1005

Gln Asn Asn His Arg Ser Val Leu Val Ile Pro Glu Trp Glu Ala
1010 1015 1020

Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile
1025 1030 1035

Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val
1040 1045 1050

Thr Ile His Glu Ile Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser
1055 1060 1065

Asn Cys Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys
1070 1075 1080

Asn Asn Tyr Thr Gly Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr
1085 1090 1095

Ser Arg Asn Gln Gly Tyr Asp Glu Ala Tyr Gly Asn Asn Pro Ser
 1100 1105 1110

Val Pro Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ser Tyr Thr
 1115 1120 1125

Asp Gly Arg Arg Glu Asn Pro Cys Glu Ser Asn Arg Gly Tyr Gly
 1130 1135 1140

Asp Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Asp Leu Glu
 1145 1150 1155

Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr
 1160 1165 1170

Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu
 1175 1180 1185

Glu

<210> 18
 <211> 1181
 <212> PRT
 <213> Secuencia artificial

<220>
 <223> Proteína quimérica Bt29-1Ca modificada.

<400> 18

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
 20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
 35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Gly Phe Arg Val Trp Gly Gly Thr Ser Val Ile Thr Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Asn Thr Phe Gly Asp Phe
485 490 495

Val Ser Leu Gln Val Asn Ile Asn Ser Pro Ile Thr Gln Arg Tyr Arg
500 505 510

Leu Arg Phe Arg Tyr Ala Ser Ser Arg Asp Ala Arg Val Ile Val Leu
515 520 525

Thr Gly Ala Ala Ser Thr Gly Val Gly Gly Gln Val Ser Val Asn Met
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Pro Leu Gln Lys Thr Met Glu Ile Gly Glu Asn Leu Thr Ser Arg Thr
545 550 555 560

Phe Arg Tyr Thr Asp Phe Ser Asn Pro Phe Ser Phe Arg Ala Asn Pro
565 570 575

Asp Ile Ile Gly Ile Ser Glu Gln Pro Leu Phe Gly Ala Gly Ser Ile
580 585 590

Ser Ser Gly Glu Leu Tyr Ile Asp Lys Ile Glu Ile Ile Leu Ala Asp
595 600 605

Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys Ala Gln Lys Ala Val
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Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu Lys Thr Asp Val
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Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val Glu Cys Leu Ser
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Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser Glu Lys Val Lys
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His Ala Lys Arg Leu Cys Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn
675 680 685

Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg Gly Trp Arg Gly Ser Thr
690 695 700

Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val
705 710 715 720

Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln
725 730 735

Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Glu Leu Arg
740 745 750

Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg Tyr
755 760 765

Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr Gly Ser Leu Trp
770 775 780

Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly Glu Pro Asn Arg
785 790 795 800

Cys Ala Thr His Leu Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg
805 810 815

Asp Gly Glu Lys Cys Ala His His Ser His His Phe Ser Leu Asp Ile
820 825 830

Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile
835 840 845

Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu
850 855 860

Phe Leu Glu Glu Lys Pro Leu Val Gly Glu Ala Leu Ala Arg Val Lys
865 870 875 880

Arg	Ala	Glu	Lys	Lys	Trp	Arg	Asp	Lys	Arg	Glu	Lys	Leu	Glu	Leu	Glu			
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Thr	Asn	Ile	Val	Tyr	Lys	Glu	Ala	Lys	Lys	Ser	Val	Asp	Ala	Leu	Phe			
			900					905					910					
Val	Asn	Ser	Gln	Tyr	Asp	Arg	Leu	Gln	Ala	Asp	Thr	Asn	Ile	Ala	Ile			
			915				920					925						
Ile	His	Ala	Ala	Asp	Lys	Arg	Val	His	Ser	Ile	Arg	Glu	Ala	Tyr	Leu			
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Pro	Glu	Leu	Ser	Val	Ile	Pro	Gly	Val	Asn	Ala	Ala	Ile	Phe	Glu	Glu			
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				965					970					975				
Val	Ile	Lys	Asn	Gly	Asp	Phe	Asn	Asn	Gly	Leu	Ser	Cys	Trp	Asn	Val			
			980					985					990					
Lys	Gly	His	Val	Asp	Val	Glu	Glu	Gln	Asn	Asn	His	Arg	Ser	Val	Leu			
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Val	Val	Pro	Glu	Trp	Glu	Ala	Glu	Val	Ser	Gln	Glu	Val	Arg	Val				
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Cys	Pro	Gly	Arg	Gly	Tyr	Ile	Leu	Arg	Val	Thr	Ala	Tyr	Lys	Glu				
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Gly	Tyr	Gly	Glu	Gly	Cys	Val	Thr	Ile	His	Glu	Ile	Glu	Asp	Asn				
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Thr	Asp	Glu	Leu	Lys	Phe	Ser	Asn	Cys	Val	Glu	Glu	Glu	Ile	Tyr				
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Pro	Asn	Asn	Thr	Val	Thr	Cys	Asn	Asp	Tyr	Thr	Ala	Thr	Gln	Glu				
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Glu Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Arg Gly Tyr Asp Gly
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Ala Tyr Glu Ser Asn Ser Ser Val Pro Ala Asp Tyr Ala Ser Ala
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Tyr Glu Glu Lys Ala Tyr Thr Asp Gly Arg Arg Asp Asn Thr Cys
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Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro Ala Gly
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Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val
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Val Glu Leu Leu Leu Met Glu Glu
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acgcgcttaa ttttagagaa ttttttacca ggtgggagtt ttgcattcgg cttatttgat
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240

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Val	Ala	Glu	Ile	His	Leu	Lys	Ile	Thr	Arg	Leu	Ile	Leu	Glu	Asn	Phe
		35					40					45			

Leu	Pro	Gly	Gly	Ser	Phe	Ala	Phe	Gly	Leu	Phe	Asp	Leu	Ile	Trp	Gly
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Ile	Phe	Asn	Glu	Asp	Gln	Trp	Ser	Ala	Phe	Leu	Arg	Gln	Val	Glu	Glu
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Leu	Ile	Asn	Gln	Arg	Ile	Thr	Glu	Phe	Ala	Arg	Gly	Gln	Ala	Ile	Gln
				85					90					95	

Arg	Leu	Val	Gly	Phe	Gly	Arg	Ser	Tyr	Asp	Glu	Tyr	Ile	Leu	Ala	Leu
			100					105					110		

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val

115							120								125
Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro
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Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala
145					150					155					160
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe
			165						170					175	
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser
			180					185					190		
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe
		195					200					205			
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr
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Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu
225					230					235					240
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln
				245					250					255	
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln
			260					265					270		
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp
		275					280					285			
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg
	290					295					300				
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr
305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr

				325					330					335			
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg		
			340					345					350				
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile		
		355					360					365					
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro		
	370					375					380						
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu		
385					390					395					400		
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His		
				405					410					415			
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro		
			420					425					430				
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala		
		435					440					445					
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu		
	450					455					460						
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Lys	Gly	Pro		
465					470					475					480		
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe		
				485					490					495			
Ala	Phe	Ser	Asn	Val	Asn	Leu	Asp	Trp	Asn	Leu	Ser	Gln	Arg	Tyr	Arg		
			500					505					510				
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Met	Tyr	Val	Thr		
		515					520					525					
Ile	Ala	Gly	Glu	Arg	Ile	Phe	Ala	Gly	Gln	Phe	Asn	Lys	Thr	Met	Asn		

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Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp				
545		550		555
Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala				
		565		570
				575
Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu				
		580		585
				590
Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys Ala				
		595		600
				605
Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu				
		610		615
				620
Lys Thr Asp Val Thr Asp Tyr His Ile Asp Lys Val Ser Asn Leu Val				
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Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys				
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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln				
		20		25
				30
Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe				
		35		40
				45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys Ala
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Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu
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Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
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Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe

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Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln
				245					250					255	
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln
			260					265					270		
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp
		275					280					285			
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg
290						295					300				
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr
305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr
				325					330					335	
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg
			340					345					350		
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile
		355					360					365			
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro
370						375					380				
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu
385					390					395					400
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His

				405					410					415					
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro				
			420					425					430						
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala				
		435					440					445							
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu				
	450					455						460							
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Lys	Gly	Pro				
465					470					475					480				
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe				
				485					490					495					
Ala	Phe	Ser	Asn	Val	Asn	Leu	Asp	Trp	Asn	Leu	Ser	Gln	Arg	Tyr	Arg				
			500					505					510						
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Met	Tyr	Val	Thr				
		515					520					525							
Ile	Ala	Gly	Glu	Arg	Ile	Phe	Ala	Gly	Gln	Phe	Asn	Lys	Thr	Met	Asn				
	530					535					540								
Thr	Gly	Asp	Pro	Leu	Thr	Phe	Gln	Ser	Phe	Ser	Tyr	Ala	Thr	Ile	Asp				
545					550					555					560				
Thr	Ala	Phe	Thr	Phe	Pro	Thr	Lys	Ala	Ser	Ser	Leu	Thr	Val	Gly	Ala				
				565					570					575					
Asp	Thr	Phe	Ser	Ser	Gly	Asn	Glu	Val	Tyr	Val	Asp	Arg	Phe	Glu	Leu				
			580					585					590						
Ile	Pro	Val	Thr	Ala	Thr	Phe	Glu	Ala	Glu	Tyr	Asp	Leu	Glu	Lys	Ala				
		595					600					605							
Gln	Lys	Ala	Val	Asn	Ala	Leu	Phe	Thr	Ser	Ser	Asn	Gln	Ile						

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615

620

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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
 20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
 35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
 50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
 65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
 85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
 100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
 115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
 130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
 145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys Ala
595 600 605

Gln Lys
610

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Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val

115							120								125
Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro
130						135					140				
Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala
145					150					155					160
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe
			165						170					175	
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser
			180					185					190		
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe
		195					200					205			
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr
	210					215					220				
Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu
225					230					235					240
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln
				245					250					255	
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln
			260					265					270		
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp
		275					280					285			
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg
	290					295					300				
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr
305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr

				325					330					335					
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg				
			340					345					350						
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile				
		355					360					365							
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro				
	370					375					380								
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu				
385					390					395					400				
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His				
				405					410					415					
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro				
			420					425					430						
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala				
		435					440					445							
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu				
	450					455					460								
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Lys	Gly	Pro				
465					470					475					480				
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe				
				485					490					495					
Ala	Phe	Ser	Asn	Val	Asn	Leu	Asp	Trp	Asn	Leu	Ser	Gln	Arg	Tyr	Arg				
			500					505					510						
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Met	Tyr	Val	Thr				
		515					520					525							
Ile	Ala	Gly	Glu	Arg	Ile	Phe	Ala	Gly	Gln	Phe	Asn	Lys	Thr	Met	Asn				

530 535 540
 Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
 545 550 555 560

 Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
 565 570 575

 Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
 580 585 590

 Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys
 595 600 605

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 Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
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 Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
 20 25 30

 Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
 35 40 45

 Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
 50 55 60

 Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
 65 70 75 80

 Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
 85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr
595 600

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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu

65					70					75				80		
Leu	Ile	Asn	Gln	Arg	Ile	Thr	Glu	Phe	Ala	Arg	Gly	Gln	Ala	Ile	Gln	
				85					90					95		
Arg	Leu	Val	Gly	Phe	Gly	Arg	Ser	Tyr	Asp	Glu	Tyr	Ile	Leu	Ala	Leu	
			100					105					110			
Lys	Glu	Trp	Glu	Asn	Asp	Pro	Asp	Asn	Pro	Ala	Ser	Lys	Glu	Arg	Val	
		115					120					125				
Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro	
	130					135						140				
Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala	
145					150					155					160	
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe	
				165					170					175		
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser	
			180					185					190			
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe	
		195					200					205				
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr	
	210					215					220					
Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu	
225					230					235					240	
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln	
				245					250					255		
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln	
			260					265					270			
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp	

275								280							285
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg
290						295					300				
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr
305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr
				325					330					335	
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg
			340					345					350		
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile
		355					360					365			
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro
370						375					380				
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu
385					390					395					400
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His
				405					410					415	
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro
			420					425					430		
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala
		435					440					445			
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu
	450					455					460				
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Lys	Gly	Pro
465					470					475					480
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe

				485						490						495			
Ala	Phe	Ser	Asn	Val	Asn	Leu	Asp	Trp	Asn	Leu	Ser	Gln	Arg	Tyr	Arg				
			500					505					510						
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Met	Tyr	Val	Thr				
		515					520					525							
Ile	Ala	Gly	Glu	Arg	Ile	Phe	Ala	Gly	Gln	Phe	Asn	Lys	Thr	Met	Asn				
	530					535					540								
Thr	Gly	Asp	Pro	Leu	Thr	Phe	Gln	Ser	Phe	Ser	Tyr	Ala	Thr	Ile	Asp				
545					550					555					560				
Thr	Ala	Phe	Thr	Phe	Pro	Thr	Lys	Ala	Ser	Ser	Leu	Thr	Val	Gly	Ala				
				565					570					575					
Asp	Thr	Phe	Ser	Ser	Gly	Asn	Glu	Val	Tyr	Val	Asp	Arg	Phe	Glu	Leu				
			580					585					590						
Ile	Pro	Val	Thr	Ala	Thr	Leu	Glu	Ala	Val	Thr	Asp	Leu	Glu	Arg	Ala				
		595					600					605							
Gln	Lys	Ala	Val	His	Glu	Leu	Phe	Thr	Ser	Thr	Asn	Pro	Gly	Gly	Leu				
	610					615					620								
Lys	Thr	Asp	Val	Ala	Lys	Asp	His	Tyr	Thr	Asn	Thr	Ile	Ser	Lys	Ser				
625					630					635					640				
Val	Gln	Ser	Val	Phe	Arg	Cys	Arg	Cys	Ser	Glu	Arg	Thr	Arg	Ile	Tyr				
				645					650					655					
Arg	Trp	Gly	Tyr	Pro	Ser	Lys	Lys	Glu	Tyr	Trp	Tyr	Ile	Trp	Gly	Tyr				
			660					665					670						
Thr	Ser	Lys	Tyr																
		675																	

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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Leu Glu Ala Val Thr Asp Leu Glu Arg Ala
595 600 605

Gln Lys Ala Val His Glu Leu Phe Thr Ser Thr Asn Pro Gly Gly Leu
610 615 620

Lys Thr Asp Val Ala Lys Asp His Tyr Thr Asn Thr Ile Ser Lys Ser
625 630 635 640

Val Gln Ser Val

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<223> BT29BT22-TL22v3

<400> 28

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu

				100					105						110				
Lys	Glu	Trp	Glu	Asn	Asp	Pro	Asp	Asn	Pro	Ala	Ser	Lys	Glu	Arg	Val				
		115					120					125							
Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro				
	130					135						140							
Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala				
145					150					155					160				
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe				
				165					170					175					
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser				
			180					185					190						
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe				
		195					200					205							
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr				
	210					215					220								
Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu				
225					230					235					240				
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln				
				245					250					255					
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln				
			260					265					270						
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp				
		275					280					285							
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg				
	290					295					300								
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr				

305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr
				325					330					335	
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg
			340					345					350		
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile
		355					360					365			
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro
	370					375					380				
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu
385					390					395					400
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His
				405					410					415	
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro
			420					425					430		
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala
		435					440					445			
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu
	450					455					460				
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Lys	Gly	Pro
465					470					475					480
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe
				485					490					495	
Ala	Phe	Ser	Asn	Val	Asn	Leu	Asp	Trp	Asn	Leu	Ser	Gln	Arg	Tyr	Arg
			500					505					510		
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Met	Tyr	Val	Thr

515

520

525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
 530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
 545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
 565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
 580 585 590

Ile Pro Val Thr Ala Thr Leu Glu Ala Val Thr Asp Leu Glu Arg Ala
 595 600 605

Gln Lys Ala Val His Glu Leu Phe Thr Ser Thr Asn Pro Gly Gly Leu
 610 615 620

Lys Thr Asp Val Ala Lys Asp His Tyr Thr
 625 630

<210> 29

<211> 622

<212> PRT

<213> Secuencia artificial

<220>

<223> BT29BT22-TL22v4

<400> 29

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
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Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
 20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
 35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Leu Glu Ala Val Thr Asp Leu Glu Arg Ala
595 600 605

Gln Lys Ala Val His Glu Leu Phe Thr Ser Thr Asn Pro Gly
610 615 620

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<211> 610
<212> PRT
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<220>
<223> BT29BT22-TL22v5

<400> 30

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn

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Asn	Pro	Glu	Ser	Glu	Ile	Leu	Asn	Val	Ala	Ile	Phe	Ser	Ser	Glu	Gln	
		20					25					30				
Val	Ala	Glu	Ile	His	Leu	Lys	Ile	Thr	Arg	Leu	Ile	Leu	Glu	Asn	Phe	
		35				40						45				
Leu	Pro	Gly	Gly	Ser	Phe	Ala	Phe	Gly	Leu	Phe	Asp	Leu	Ile	Trp	Gly	
	50					55					60					
Ile	Phe	Asn	Glu	Asp	Gln	Trp	Ser	Ala	Phe	Leu	Arg	Gln	Val	Glu	Glu	
65					70					75					80	
Leu	Ile	Asn	Gln	Arg	Ile	Thr	Glu	Phe	Ala	Arg	Gly	Gln	Ala	Ile	Gln	
				85					90					95		
Arg	Leu	Val	Gly	Phe	Gly	Arg	Ser	Tyr	Asp	Glu	Tyr	Ile	Leu	Ala	Leu	
			100					105					110			
Lys	Glu	Trp	Glu	Asn	Asp	Pro	Asp	Asn	Pro	Ala	Ser	Lys	Glu	Arg	Val	
		115					120					125				
Arg	Thr	Arg	Phe	Arg	Thr	Thr	Asp	Asp	Ala	Leu	Leu	Thr	Gly	Val	Pro	
	130					135						140				
Leu	Met	Ala	Ile	Pro	Gly	Phe	Glu	Leu	Ala	Thr	Leu	Ser	Val	Tyr	Ala	
145					150					155					160	
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe	
				165					170					175		
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser	
			180					185					190			
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe	
		195					200					205				
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr	

210						215										220
Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu	
225					230					235					240	
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln	
				245					250					255		
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln	
			260					265					270			
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp	
		275					280					285				
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg	
	290					295					300					
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr	
305					310					315					320	
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr	
				325					330					335		
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg	
			340					345					350			
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile	
		355					360					365				
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro	
	370					375					380					
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu	
385					390					395					400	
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His	
				405					410					415		
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro	

420	425	430
Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala		
435	440	445
Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu		
450	455	460
Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro		
465	470	475
Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe		
	485	490
Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg		
	500	505
Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr		
	515	520
Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn		
530	535	540
Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp		
545	550	555
Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala		
	565	570
Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu		
	580	585
Ile Pro Val Thr Ala Thr Leu Glu Ala Val Thr Asp Leu Glu Arg Ala		
	595	600
Gln Lys		
610		

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<220>
<223> BT29BT22-TL22v6

<400> 31

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
1 5 10 15

Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala
145 150 155 160

Gln Ser Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ala Val Phe Phe
165 170 175

Gly Glu Arg Trp Gly Leu Thr Gln Thr Asn Ile Asn Asp Leu Tyr Ser
180 185 190

Arg Leu Lys Asn Ser Ile Arg Asp Tyr Thr Asn His Cys Val Arg Phe
195 200 205

Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
210 215 220

Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
225 230 235 240

Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
245 250 255

Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
260 265 270

Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
275 280 285

Phe Leu Asn Arg Leu Ile Ile Tyr Thr Gly Glu Tyr Arg Gly Ile Arg
290 295 300

His Trp Ala Gly His Glu Val Glu Ser Ser Arg Thr Gly Met Met Thr
305 310 315 320

Asn Ile Arg Phe Pro Leu Tyr Gly Thr Ala Ala Thr Ala Glu Pro Thr
325 330 335

Arg Phe Ile Thr Pro Ser Thr Phe Pro Gly Leu Asn Leu Phe Tyr Arg
340 345 350

Thr Leu Ser Ala Pro Ile Phe Arg Asp Glu Pro Gly Ala Asn Ile Ile
355 360 365

Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
370 375 380

Asn Asn Gly Glu Gln Leu Tyr Arg Val Arg Gly Thr Leu Asp Ser Leu
385 390 395 400

Asp Gln Leu Pro Leu Glu Gly Glu Ser Ser Leu Thr Glu Tyr Ser His
405 410 415

Arg Leu Cys His Val Arg Phe Ala Gln Ser Leu Arg Asn Ala Glu Pro
420 425 430

Leu Asp Tyr Ala Arg Val Pro Met Phe Ser Trp Thr His Arg Ser Ala
435 440 445

Thr Pro Thr Asn Thr Ile Asp Pro Asp Val Ile Thr Gln Ile Pro Leu
450 455 460

Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Lys Gly Pro
465 470 475 480

Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe
485 490 495

Ala Phe Ser Asn Val Asn Leu Asp Trp Asn Leu Ser Gln Arg Tyr Arg
500 505 510

Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Met Tyr Val Thr
515 520 525

Ile Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asn
530 535 540

Thr Gly Asp Pro Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asp
545 550 555 560

Thr Ala Phe Thr Phe Pro Thr Lys Ala Ser Ser Leu Thr Val Gly Ala
565 570 575

Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu
580 585 590

Ile Pro Val Thr Ala Thr Leu Glu
595 600

<210> 32
<211> 610
<212> PRT
<213> Secuencia artificial

<220>
<223> Bt29-1FaTr1

<400> 32

Met Glu Ile Asn Asn Gln Asn Gln Cys Val Pro Tyr Asn Cys Leu Asn
1 5 10 15

Asn Pro Glu Ser Glu Ile Leu Asn Val Ala Ile Phe Ser Ser Glu Gln
20 25 30

Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
35 40 45

Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
50 55 60

Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
65 70 75 80

Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln
85 90 95

Arg Leu Val Gly Phe Gly Arg Ser Tyr Asp Glu Tyr Ile Leu Ala Leu
100 105 110

Lys Glu Trp Glu Asn Asp Pro Asp Asn Pro Ala Ser Lys Glu Arg Val
115 120 125

Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Leu Thr Gly Val Pro
130 135 140

Leu Met Ala Ile Pro Gly Phe Glu Leu Ala Thr Leu Ser Val Tyr Ala

145					150					155				160	
Gln	Ser	Ala	Asn	Leu	His	Leu	Ala	Leu	Leu	Arg	Asp	Ala	Val	Phe	Phe
			165						170					175	
Gly	Glu	Arg	Trp	Gly	Leu	Thr	Gln	Thr	Asn	Ile	Asn	Asp	Leu	Tyr	Ser
			180					185					190		
Arg	Leu	Lys	Asn	Ser	Ile	Arg	Asp	Tyr	Thr	Asn	His	Cys	Val	Arg	Phe
		195					200					205			
Tyr	Asn	Ile	Gly	Leu	Gly	Asn	Leu	Asn	Val	Ile	Arg	Pro	Glu	Tyr	Tyr
	210					215					220				
Arg	Phe	Gln	Arg	Glu	Leu	Thr	Ile	Ser	Val	Leu	Asp	Leu	Val	Ala	Leu
225					230					235					240
Phe	Pro	Asn	Tyr	Asp	Ile	Arg	Thr	Tyr	Pro	Ile	Pro	Thr	Lys	Ser	Gln
				245					250					255	
Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asp	Pro	Ile	Ile	Ser	Pro	Gly	Ala	Gln
			260					265					270		
Ala	Gly	Tyr	Thr	Leu	Gln	Asp	Val	Leu	Arg	Glu	Pro	His	Leu	Met	Asp
		275					280					285			
Phe	Leu	Asn	Arg	Leu	Ile	Ile	Tyr	Thr	Gly	Glu	Tyr	Arg	Gly	Ile	Arg
	290					295					300				
His	Trp	Ala	Gly	His	Glu	Val	Glu	Ser	Ser	Arg	Thr	Gly	Met	Met	Thr
305					310					315					320
Asn	Ile	Arg	Phe	Pro	Leu	Tyr	Gly	Thr	Ala	Ala	Thr	Ala	Glu	Pro	Thr
				325					330					335	
Arg	Phe	Ile	Thr	Pro	Ser	Thr	Phe	Pro	Gly	Leu	Asn	Leu	Phe	Tyr	Arg
			340					345					350		
Thr	Leu	Ser	Ala	Pro	Ile	Phe	Arg	Asp	Glu	Pro	Gly	Ala	Asn	Ile	Ile

355							360						365			
Ile	Arg	Tyr	Arg	Thr	Ser	Leu	Val	Glu	Gly	Val	Gly	Phe	Ile	Gln	Pro	
	370					375					380					
Asn	Asn	Gly	Glu	Gln	Leu	Tyr	Arg	Val	Arg	Gly	Thr	Leu	Asp	Ser	Leu	
385					390					395					400	
Asp	Gln	Leu	Pro	Leu	Glu	Gly	Glu	Ser	Ser	Leu	Thr	Glu	Tyr	Ser	His	
				405					410					415		
Arg	Leu	Cys	His	Val	Arg	Phe	Ala	Gln	Ser	Leu	Arg	Asn	Ala	Glu	Pro	
			420					425					430			
Leu	Asp	Tyr	Ala	Arg	Val	Pro	Met	Phe	Ser	Trp	Thr	His	Arg	Ser	Ala	
		435					440					445				
Thr	Pro	Thr	Asn	Thr	Ile	Asp	Pro	Asp	Val	Ile	Thr	Gln	Ile	Pro	Leu	
	450					455					460					
Val	Lys	Ala	His	Thr	Leu	Gln	Ser	Gly	Thr	Thr	Val	Val	Arg	Gly	Pro	
465					470					475					480	
Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe	
				485					490					495		
Ala	Tyr	Thr	Ile	Val	Asn	Ile	Asn	Gly	Gln	Leu	Pro	Gln	Arg	Tyr	Arg	
			500					505					510			
Ala	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Ile	Tyr	Val	Thr	
		515					520					525				
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Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr Val Val Arg Gly Pro
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Ala Tyr Thr Ile Val Asn Ile Asn Gly Gln Leu Pro Gln Arg Tyr Arg
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Val Ala Gly Glu Arg Ile Phe Ala Gly Gln Phe Asn Lys Thr Met Asp
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Val Ala Glu Ile His Leu Lys Ile Thr Arg Leu Ile Leu Glu Asn Phe
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Leu Pro Gly Gly Ser Phe Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Ile Phe Asn Glu Asp Gln Trp Ser Ala Phe Leu Arg Gln Val Glu Glu
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Leu Ile Asn Gln Arg Ile Thr Glu Phe Ala Arg Gly Gln Ala Ile Gln

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Lys	Glu	Trp 115	Glu	Asn	Asp	Pro	Asp 120	Asn	Pro	Ala	Ser	Lys 125	Glu	Arg	Val		
Arg	Thr 130	Arg	Phe	Arg	Thr	Thr 135	Asp	Asp	Ala	Leu	Leu 140	Thr	Gly	Val	Pro		
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Tyr Asn Ile Gly Leu Gly Asn Leu Asn Val Ile Arg Pro Glu Tyr Tyr
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Arg Phe Gln Arg Glu Leu Thr Ile Ser Val Leu Asp Leu Val Ala Leu
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Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Pro Ile Pro Thr Lys Ser Gln
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Leu Thr Arg Glu Ile Tyr Thr Asp Pro Ile Ile Ser Pro Gly Ala Gln
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Ala Gly Tyr Thr Leu Gln Asp Val Leu Arg Glu Pro His Leu Met Asp
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Ile Arg Tyr Arg Thr Ser Leu Val Glu Gly Val Gly Phe Ile Gln Pro
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Asp Thr Phe Ser Ser Gly Asn Glu Val Tyr Ile Asp Arg Phe Glu Leu
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Ile Pro Val Thr Ala Thr Phe Glu
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 81090-WO-REG	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2017/063722	International filing date (day/month/year) 29 November 2017	(Earliest) Priority Date (day/month/year) 12 December 2016
Applicant SYNGENTA PARTICIPATIONS AG		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ Certain claims were found unsearchable (see Box No. II).

3. ☐ Unity of invention is lacking (see Box No. III).

4. With regard to the title,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract:

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. 1A
☐ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☒ as selected by this Authority, because this figure better characterizes the invention.
- b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-3, 8, and 9 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01H 5/00; A01H 5/10; A01N 63/02; C07K 14/325; C12N 5/10; C12N 15/32 (2018.01)

CPC - A01N 37/46; A01N 65/22; A01N 65/44; C07K 14/325 (2018.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 514/4.5; 530/300; 530/350 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 8,735,560 B1 (ENGLISH et al) 27 May 2014 (27.05.2014) entire document	1-4
Y	US 2015/0148288 A1 (VESTARON CORPORATION) 28 May 2015 (28.05.2015) entire document	1-4
A	WO 2011/084631 A1 (DOW AGROSCIENCES LLC et al) 14 July 2011 (14.07.2011) entire document	1-4
A	WO 2016/061377 A2 (MONSANTO TECHNOLOGY LLC) 21 April 2016 (21.04.2016) entire document	1-4
A	WO 2016/061197 A1 (PIONEER HI-BRED INTERNATIONAL, INC.) 21 April 2016 (21.04.2016) entire document	1-4
P, A	WO 2017/003811 A1 (SYNGENTA PARTICIPATIONS AG et al) 05 January 2017 (05.01.2017) entire document	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 January 2018

Date of mailing of the international search report

16 FEB 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774



(51) International Patent Classification:

A01H 5/00 (2018.01) C07K 14/325 (2006.01)
A01H 5/10 (2018.01) C12N 5/10 (2006.01)
A01N 63/02 (2006.01) C12N 15/32 (2006.01)

(21) International Application Number:

PCT/US2017/063722

(22) International Filing Date:

29 November 2017 (29.11.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/432,909 12 December 2016 (12.12.2016) US

(71) Applicant: SYNGENTA PARTICIPATIONS AG
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(71) Applicant (for US only): SYNGENTA CROP PROTECTION, LLC [US/US]; 9 Davis Drive, Research Triangle Park, North Carolina 27709 (US).

(72) Inventor: CHAE, Hyunsook S.; 9 Davis Drive, Research Triangle Park, North Carolina 27709 (US).

(74) Agent: WARREN, Gregory W.; 9 Davis Drive, Research Triangle Park, North Carolina 27709 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: ENGINEERED PESTICIDAL PROTEINS AND METHODS OF CONTROLLING PLANT PESTS

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CrylKa 101 MEHVEQIVRQQQITDSVRDITARLEGLGRGYRSYQQALETWLDNRNDARSRIINERYIALELDITTAIFLSIRNEEVPLLMVYAQANHLALLLRDA

BT-0029 174 VFFGERMGLTQININDLYSRLKNSIRDYINHCVRFYNIIGLNLNVRP---EYFRQRELTISVLDLVALFPNYDIRTYPIPTKSQLTREIYTDPIISP
BT-0022 195 SIFGKWLSDSEISIFYNRQVETSDYSDHCTKFDFTGLNRLKGSNAEIVWKYNQFRDMLVLDLVALFQSYDTHMYPKTTAQLTREYVTHAICTV
CrylFa 174 VSPFGQWGLDIATVNNHYNRLINLIHRYTKHCLDTYNQGLENLRGNTNRQWARFNQFRDMLVLDLVALFQSYDTHMYPKTTAQLTREYVTHAICTV
CrylKa 201 MEHVEQIVRQQQITDSVRDITARLEGLGRGYRSYQQALETWLDNRNDARSRIINERYIALELDITTAIFLSIRNEEVPLLMVYAQANHLALLLRDA

BT-0029 270 GAQAG-----YTLDQVLRPHLMDFLNLRIIYTGEGYRIRHWAGHEVSSRTGMMINIRFPLYCTAATAEPTFRITPSTPGLNLFYRTL
BT-0022 295 HPHSPFTSTWYNNNAFSESAIEAAVIRSPHLLDFLEQVITYSLLSRWSNTQYMNWGG-HKLEFRTGG-TUNSTQSTNITSINPVTPPTSRDLYRT
CrylFa 274 SPVSA-----IFNGFNRAEFGRPPHLMDFMNSLFVIAETVRSQTVWGGHLVSS-RNTAGNRINFPSTYGVNFGGAIWAIEDPRP----FYRTL
CrylKa 301 AGP-----NNSWFRNGASFSAIENAIIRQPHLYDFLTNLITYTRRS-QVGTITMNLWAG-HRITFNRIQGGSTSEMWYGAITNPVSVSDIPFNVDVYRT

BT-0029 355 SAPIFRDEPGANIIIRYRSLVEGVGFIQPNNGEQLYRVGRTDLSLDQPLEGES--SLTEYSHRLCHVRFQALRNAEPLDYARVPMFSWTHRSATPTN
BT-0022 393 ESLAGLNLFLIQPVNGVPRVDHWFVTHP-IASDNFYYPGYAGITGLQDSENELPPETTGQPNYESYSHRLSHIGLISASHVKALVSWTHRSADPTN
CrylFa 360 SDPVFVRGGFGNPHYVLGRGVAFQQTG--TNHTRTRFNSGTIDSLDIEPPQDNGAPWNDYSHVLNHFVTRWPGGEISGSDSWRPPVSWTHRSATPTN
CrylKa 394 VSLAGLGLSGIRYGLTRVDFMIFRNHDPDITVGLFYHYPGHAGIATQPKDSDELTPPETTEQPNYRAFSHLLSHISMGTITQDQPPVSWTHRSADPTN

BT-0029 453 TIDPDVITQIPLVKAFLNLSGATIVKGGPFTGGDILRRTNVGSFGDMRVNITAPI.SQRYRVRIYASTITDQFYTNINGTTINIGFSSMTSDSGDDLQYG
BT-0022 492 ITHSDSITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTNVGSFGDMRVNITAPI.SQRYRVRIYASTITDQFYTNINGTTINIGFSSMTSDSGDDLQYG
CrylFa 458 TIDPERITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTNVGSFGDMRVNITAPI.SQRYRVRIYASTITDQFYTNINGTTINIGFSSMTSDSGDDLQYG
CrylKa 494 TINSDRITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTNVGSFGDMRVNITAPI.SQRYRVRIYASTITDQFYTNINGTTINIGFSSMTSDSGDDLQYG

BT-0029 553 RFRVAGFTTPTFTSDANSTFTIGAFSFSNNNEVYIDRIEFVPAEIVFEAEYOLEKAQKAVNALFTSSNQIGLKTDTV-DYHIDQVSNLVECLSDDEFCLDE
BT-0022 558 SFSYATIDTAFTFTKASSLTVGADTFSSGNEVYIDRIEFVPAEIVFEAEYOLEKAQKAVNALFTSSNQIGLKTDTV-DYHIDQVSNLVECLSDDEFCLDE
CrylFa 558 SFSYATINTAFTFPMSSQSTVGADTFSSGNEVYIDRIEFVPAEIVFEAEYOLEKAQKAVNALFTSSNQIGLKTDTV-DYHIDQVSNLVECLSDDEFCLDE
CrylKa 594 SFSYATINTAFTFPERSSSLTIGADTFSSGNEVYIDRIEFVPAEIVFEAEYOLEKAQKAVNALFTSSNQIGLKTDTV-DYHIDQVSNLVECLSDDEFCLDE

Fig. 1A

(57) Abstract: The invention provides nucleic acids, polypeptides, transgenic plants, compositions and methods for conferring pesticidal activity (e.g., insecticidal activity) to bacteria, plants, plant cells, tissues and seeds. Nucleic acids encoding the insecticidal proteins can be used to transform prokaryotic and eukaryotic organisms to express the insecticidal proteins. The recombinant organisms or compositions containing the recombinant organisms or insecticidal proteins or in combination with an appropriate agricultural carrier can be used to control an insect pest in various environments.

[Continued on next page]



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ENGINEERED PESTICIDAL PROTEINS AND METHODS OF CONTROLLING PLANT PESTS

FIELD OF THE INVENTION

[0001] This invention relates to engineered pesticidal proteins and the nucleic acid molecules that encode them, as well as compositions and methods for controlling plant pests.

BACKGROUND

[0002] *Bacillus thuringiensis* (*Bt*) is a gram-positive spore forming soil bacterium characterized by its ability to produce crystalline inclusions that are specifically toxic to certain orders and species of plant pests, including insects, but are harmless to plants and other non-target organisms. For this reason, compositions comprising *Bacillus thuringiensis* strains or their insecticidal proteins can be used as environmentally-acceptable insecticides to control agricultural insect pests or insect vectors of a variety of human or animal diseases.

[0003] Crystal (Cry) proteins from *Bacillus thuringiensis* have potent insecticidal activity against predominantly lepidopteran, dipteran, and coleopteran pest insects. These proteins also have shown activity against pests in the Orders Hymenoptera, Homoptera, Phthiraptera, Mallophaga, and Acari pest orders, as well as other invertebrate orders such as Nematelminthes, Platyhelminthes, and Sarcomastigophora (Feitelson, J. 1993. The *Bacillus Thuringiensis* family tree. In *Advanced Engineered Pesticides*. Marcel Dekker, Inc., New York, N.Y.). These proteins were originally classified as CryI to CryVI based primarily on their insecticidal activity. The major classes were Lepidoptera-specific (I), Lepidoptera- and Diptera-specific (II), Coleoptera-specific (III), Diptera-specific (IV), and nematode-specific (V) and (VI). The proteins were further classified into subfamilies;

more highly related proteins within each family were assigned divisional letters such as CryIA, CryIB, CryIC, *etc.* Even more closely related proteins within each division were given names such as CryIC(a), CryIC(b), *etc.*

[0004] The terms “Cry toxin” and “delta-endotoxin” have been used interchangeably with the term “Cry protein.” Current nomenclature for Cry proteins and genes is based upon amino acid sequence homology rather than insect target specificity (Crickmore *et al.* (1998) Microbiol. Mol. Biol. Rev. 62:807-813). In this more accepted classification, each toxin is assigned a unique name incorporating a primary rank (an Arabic number), a secondary rank (an uppercase letter), a tertiary rank (a lowercase letter), and a quaternary rank (another Arabic number). In the current classification, Roman numerals have been exchanged for Arabic numerals in the primary rank. For example, “CryIA(a)” under the older nomenclature is now “Cry1Aa” under the current nomenclature. According to Ibrahim et al. (2010, Bioeng. Bugs, 1:31-50), the Cry toxins can still be separated into six major classes according to their insect host specificities and include: Group 1—lepidopteran (*e.g.*, Cry1, Cry9 and Cry15); group 2—lepidopteran and dipteran (*e.g.*, Cry2); group 3—coleopteran (Cry3, Cry7 and Cry8); group 4—dipteran (Cry4, Cry10, Cry11, Cry16, Cry17, Cry19 and Cry20); group 5—lepidopteran and coleopteran (Cry11); and group 6—nematodes (Cry6). The Cry11, Cry2, Cry3, Cry10 and Cry11 toxins (73–82 kDa) are unique because they appear to be natural truncations of the larger Cry1 and Cry4 proteins (130–140 kDa).

[0005] Cry proteins are globular protein molecules which accumulate as protoxins in crystalline form during the sporulation stage of *Bt*. After ingestion by a pest, the crystals are typically solubilized to release protoxins, which can range in size, for example, from 130-140 kDa for many of the lepidopteran-active Cry proteins, such as Cry1 and Cry9, and 60-80 kDa for the coleopteran-active Cry3 proteins and the lepidopteran/dipteran-active Cry2 proteins. After the crystals are solubilized by a susceptible insect the released protoxins are processed by proteases in the insect gut, for example trypsin and chymotrypsin, to produce a protease-resistant core Cry protein toxin. This proteolytic processing involves the removal of amino acids from

different regions of the various Cry protoxins. For example, Cry protoxins that are 130-140 kDa are typically activated through the proteolytic removal of an N-terminal peptide of 25-30 amino acids and approximately half of the remaining protein from the C-terminus resulting in an approximately 60-70 kDa mature Cry toxin. The protoxins that are 60-80 kDa, *e.g.* Cry2 and Cry3, are also processed but not to the same extent as the larger protoxins. The smaller protoxins typically have equal or more amino acids removed from the N-terminus than the larger protoxins but less amino acids removed from the C-terminus. For example, proteolytic activation of Cry2 family members typically involves the removal of approximately 40-50 N-terminal amino acids. Many of the Cry proteins are quite toxic to specific target insects, but many have narrow spectrums of activity.

[0006] The toxin portions of Cry proteins generally have five conserved sequence blocks, and three conserved structural domains (*see, for example, de Maagd et al. (2001) Trends Genetics 17:193-199*). The first conserved structural domain, called Domain I, typically consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II typically consists of three beta-sheets arranged in a Greek key configuration, and domain III typically consists of two antiparallel beta-sheets in 'jelly-roll' formation (*de Maagd et al., 2001, supra*). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

[0007] The five conserved sequence blocks are numbered CB1 to CB5 from N-terminus to C-terminus of the Cry protein (*Hofte & Whitely, 1989, Microbiol. Rev. 53:242-255*). Conserved block 1 (CB1) comprises approximately 29 amino acids, conserved block 2 (CB2) comprises approximately 67 amino acids, conserved block 3 (CB3) comprises approximately 48 amino acids, conserved block 4 (CB4) comprises approximately 10 amino acids and conserved block 5 (CB5) comprises approximately 12 amino acids. The sequences before and after these five conserved blocks are highly variable and thus are designated the "variable regions," V1-V6. Domain I of a *Bt* delta-endotoxin typically comprises variable region 1, conserved block 1, variable region 2, and the N-terminal 52 amino acids of conserved block 2.

Domain II typically comprises approximately the C-terminal 15 amino acids of conserved block 2, variable region 3, and approximately the N-terminal 10 amino acids of conserved block 3. Domain III typically comprises approximately the C-terminal 38 amino acids of conserved block 3, variable region 4, conserved block 4, variable region 5, and conserved block 5. The Cry1 lepidopteran active toxins, among other delta-endotoxins, have a variable region 6 with approximately 1-3 amino acids lying within domain III.

[0008] Numerous commercially valuable plants, including common agricultural crops, are susceptible to attack by plant pests including insect and nematode pests, causing substantial reductions in crop yield and quality. For example, plant pests are a major factor in the loss of the world's important agricultural crops. About \$8 billion are lost every year in the United States alone due to infestations of invertebrate pests including insects. Insect pests are also a burden to vegetable and fruit growers, to producers of ornamental flowers, and to home gardeners.

[0009] Insect pests are mainly controlled by intensive applications of chemical pesticides, which are active through inhibition of insect growth, prevention of insect feeding or reproduction, or cause death. Biological pest control agents, such as *Bacillus thuringiensis* strains expressing pesticidal toxins such as Cry proteins, have also been applied to crop plants with satisfactory results, offering an alternative or complement to chemical pesticides. The genes coding for some of these Cry proteins have been isolated and their expression in heterologous hosts such as transgenic plants have been shown to provide another tool for the control of economically important insect pests.

[0010] Good insect control can thus be reached, but the continued use of certain chemical and biological control methods heightens the chance for insect pests to develop resistance to such control measures. This has been partially alleviated by various resistance management practices, but there remains a need to develop new and effective pest control agents. Particularly needed are control agents that can target to a wider spectrum of economically important insect pests and/or that

efficiently control insect strains that are or could become resistant to existing insect control agents.

SUMMARY

- [0011]** The invention provides nucleic acids, polypeptides, compositions and methods for conferring pesticidal activity (*e.g.*, insecticidal activity) to bacteria, plants, plant cells, tissues and seeds. In particular, the invention provides novel chimeric pesticidal proteins (*e.g.*, chimeric insecticidal proteins), optionally with altered or enhanced activity as compared with the parent molecule.
- [0012]** In embodiments, the chimeric proteins of the invention are toxic to economically important insect pests (*e.g.*, by inhibiting the ability of the insect pest to survive, grow and/or reproduce), particularly insect pests that infest plants. For example, in embodiments, the chimeric insecticidal proteins of the invention can be used to control one or more economically important lepidopteran pests including without limitation black cutworm (*Agrotis ipsilon*), European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), sugarcane borer (*Diatraea saccharalis*), velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Chrysodeixis includes*), southwest corn borer (*Diatraea grandiosella*), western bean cutworm (*Richia albicosta*), tobacco budworm (*Heliothis virescens*), Asian corn borer (*Ostrinia furnacalis*), cotton bollworm (*Helicoverpa armigera*), striped stem borer (*Chilo suppressalis*), pink stem borer (*Sesamia calamistis*), rice leaffolder (*Cnaphalocrocis medinalis*), and the like. In embodiments, the chimeric insecticidal protein has activity against a fall armyworm insect pest that is resistant to a Vip3A protein and/or a Cry1F protein.
- [0013]** Accordingly, as one aspect, the invention provides a chimeric insecticidal protein that is toxic to a lepidopteran insect pest comprising in an N-terminal to C-terminal direction: (a) an N-terminal region of a first Cry1 protein, which is optionally an N-terminal region of a BT-0029 protein of SEQ ID NO:2 or an amino acid sequence that is substantially identical thereto, fused to (b) a C-terminal region of a different Cry1 protein; wherein a crossover position between the first Cry1 protein

and the different Cry1 protein is located in conserved block 3. In representative embodiments, the different Cry1 protein is a Cry1F (*e.g.*, a Cry1Fa), a Cry1G, a Cry1I (*e.g.*, a Cry1Ia or a Cry1If, such as BT-0022), a Cry1C (*e.g.* a Cry1Ca) or a Cry1K protein.

[0014] In embodiments, the chimeric insecticidal protein according to the invention has insecticidal activity against a *Spodoptera frugiperda* insect pest or an insect pest population with resistance to a Vip3A protein and/or a Cry1F protein.

[0015] As a further aspect, the invention provides a nucleotide sequence encoding the chimeric insecticidal proteins of the invention, and expression cassettes and vectors comprising the same. In embodiments, the polynucleotide is codon optimized for expression in a plant (*e.g.*, a monocot plant such as maize or a dicot plant such as soybean).

[0016] As a further aspect, the invention provides a transgenic cell (*e.g.*, a transgenic plant cell such as a dicot cell or monocot cell, or a transgenic bacterial cell), transgenic plant part, transgenic plant culture, and transgenic plant seed that comprises a nucleotide sequence, expression cassette, vector and/or chimeric insecticidal protein of the invention.

[0017] As still a further aspect, the invention encompasses transgenic plants comprising a plant cell, plant part, nucleotide sequence, expression cassette, vector and/or chimeric insecticidal protein of the invention.

[0018] As a further aspect are seeds that produce the transgenic plants of the invention and seeds produced by the transgenic plants of the invention.

[0019] Also provided are harvested products derived from the transgenic plants of the invention, wherein the harvested product optionally comprises a nucleotide sequence, expression cassette, vector and/or chimeric insecticidal protein of the invention. Further provided are processed products derived from the harvested products of the invention, wherein the harvested product optionally comprises a nucleotide sequence, expression cassette, vector and/or chimeric insecticidal protein of the invention. In embodiments, the harvested product or processed product

comprises an chimeric insecticidal protein of the invention and has increased resistance to an insect pest (*e.g.*, a lepidopteran insect pest).

[0020] As still a further aspect, the invention provides an insecticidal composition comprising a chimeric insecticidal protein of the invention and an agriculturally acceptable carrier.

[0021] Still further, the invention provides as an additional aspect a method of producing a transgenic plant with increased resistance to an insect pest (*e.g.*, a lepidopteran insect pest). In embodiments, the method comprises introducing into a plant a polynucleotide, expression cassette, or vector of the invention, wherein the chimeric insecticidal protein is expressed in the plant, thereby producing a transgenic plant with increased resistance to an insect pest. Optionally, the introducing step comprises: (i) transforming a plant cell with the polynucleotide, expression cassette or vector and regenerating a transgenic plant; or (ii) crossing a first plant comprising the polynucleotide, expression cassette or vector with a second plant. In embodiments, the method further comprises producing a seed from the transgenic plant. In embodiments, the method further comprises obtaining a progeny plant from the transgenic plant, wherein the progeny plant comprises the polynucleotide, the expression cassette or the vector, expresses the chimeric insecticidal protein and has increased resistance to an insect pest.

[0022] As yet another aspect, the invention provides a method of producing a transgenic plant with increased resistance to an insect pest (*e.g.*, a lepidopteran insect pest), the method comprising: (a) planting a seed comprising a polynucleotide, expression cassette or vector of the invention; and (b) growing a transgenic plant from the seed, wherein the transgenic plant comprises the polynucleotide, expression cassette or vector and produces the chimeric insecticidal protein and has increased resistance to an insect pest. In embodiments, the method further comprises: (c) harvesting a seed from the transgenic plant of (b), wherein the harvested seed comprises the polynucleotide, expression cassette, vector and/or the chimeric insecticidal protein. Optionally, the seed has increased resistance against an insect pest (*e.g.*, a lepidopteran insect pest).

[0023] Still further, as another aspect, the invention provides a method of producing a seed. In embodiments, the method comprises: (a) providing a transgenic plant that comprises a polynucleotide, expression cassette or vector of the invention; and (b) harvesting a seed from the transgenic plant of (a), wherein the harvested seed comprises the polynucleotide, expression cassette or vector and/or a chimeric insecticidal protein of the invention. Optionally, the seed has increased resistance against an insect pest (*e.g.*, a lepidopteran insect pest).

[0024] The invention further contemplates a method of producing a hybrid plant seed. In representative embodiments, the method comprises: (a) crossing a first inbred plant, which is a transgenic plant comprising a polynucleotide, expression cassette or vector of the invention with a different inbred plant, which may or may not comprise a polynucleotide, expression cassette or vector of the invention; and (b) allowing a hybrid seed to form. In embodiments, the hybrid seed comprises a polynucleotide, expression cassette or vector and/or a chimeric insecticidal protein of the invention. Optionally, the seed has increased resistance against an insect pest (*e.g.*, a lepidopteran insect pest).

[0025] As another aspect, the invention provides a method of controlling an insect pest (*e.g.*, a lepidopteran insect pest, such as fall armyworm), the method comprising delivering to the insect pest or an environment thereof a composition comprising an effective amount of a chimeric insecticidal protein or insecticidal composition of the invention. In embodiments, the method is a method of controlling a lepidopteran insect pest (*e.g.*, fall armyworm) that is resistant to a Vip3A protein and/or a Cry1F protein.

[0026] Accordingly, as another aspect, the invention provides methods of reducing the development of resistance to a Vip3A protein and/or a Cry1F protein in a population of a target lepidopteran insect pest (*e.g.*, fall armyworm). In embodiments, the method comprises delivering to the target population or an environment thereof a transgenic plant comprising: (i) a polynucleotide, expression cassette, or vector according to the invention; and (ii) a polynucleotide comprising a nucleotide sequence encoding a Vip3A protein and/or a nucleotide sequence

encoding a Cry1F protein; wherein the chimeric insecticidal protein and the Vip3A protein and/or the Cry1F protein are produced in the transgenic plant.

[0027] The invention is also drawn to methods of using the polynucleotides of the invention, for example, in DNA constructs or expression cassettes or vectors for transformation and expression in organisms, including plants and microorganisms, such as bacteria. The nucleotide or amino acid sequences may be native or synthetic sequences that have been designed for expression in an organism such as a plant or bacteria. The invention is further drawn to methods of making the insecticidal proteins of the invention and to methods of using the polynucleotide sequences and insecticidal proteins, for example in microorganisms to control insects or in transgenic plants to confer protection from insect damage.

[0028] Another aspect of the invention includes insecticidal compositions and formulations comprising the chimeric insecticidal proteins of the invention, and methods of using the compositions or formulations to control insect populations, for example by applying the compositions or formulations to insect-infested areas, or to prophylactically treat insect-susceptible areas or plants to confer protection against the insect pests. Optionally, the compositions or formulations of the invention may, in addition to the chimeric insecticidal protein of the invention, comprise other pesticidal agents such as chemical pesticides, other pesticidal proteins, or dsRNA, *e.g.*, in order to augment or enhance the insect-controlling capability of the composition or formulation and/or for insect resistance management.

[0029] The compositions and methods of the invention are useful for controlling insect pests that attack plants, particularly crop plants. The compositions of the invention are also useful for detecting the presence of a chimeric insecticidal protein or a nucleic acid encoding the same in commercial products or transgenic organisms.

[0030] The invention also provides for uses of the chimeric insecticidal proteins, nucleic acids, transgenic plants, plant parts, seed and insecticidal compositions of the invention, for example, to control an insect pest, such as a lepidopteran pest.

[0031] In embodiments, the invention provides a method of using a polynucleotide, expression cassette, vector or host cell of the invention to produce an insecticidal composition for controlling an insect pest (*e.g.*, a lepidopteran insect pest).

[0032] In embodiments, the invention provides a method of using a polynucleotide, expression cassette or vector of the invention to produce a transgenic seed, where the transgenic seed grows a transgenic plant with increased resistance to an insect pest.

[0033] As another aspect, the invention also contemplates the use of a transgenic plant of the invention to produce a transgenic seed, which is optionally a hybrid seed.

[0034] In embodiments, the invention provides a method of using a chimeric insecticidal protein, polynucleotide, expression cassette, vector, transgenic plant or insecticidal composition of the invention to prevent the development of resistance in a population of a target lepidopteran insect pest to a Vip3A protein and/or Cry1F protein.

[0035] These and other features, aspects, and advantages of the invention will become better understood with reference to the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] Figures 1A and 1B show an alignment of the amino acid sequences of full-length BT-0029 (SEQ ID NO: 2), BT-0022 (SEQ ID NO: 1), Cry1Fa (SEQ ID NO: 8) and Cry1Ka (SEQ ID NO: 12). The Domain III core and Conserved Block 3 (CB3) are indicated. Identical amino acids in the aligned sequences are shaded.

[0037] Figures 2A and 2B show an alignment of the amino acid sequences of full-length BT-0029 (SEQ ID NO: 2) with exemplary BT-0029 chimeras: Bt29-Bt22 (SEQ ID NO: 3), Bt29-1Fa (SEQ ID NO: 9), and Bt29-1Ka (SEQ ID NO: 13). The Domain III core and Conserved Block 3 (CB3) are indicated. The Domain III core indicated in the figure is derived from the second Cry protein. Identical amino acids in the aligned sequences are shaded.

[0038] Figures 3A, 3B, 3C and 3D show a full-length Bt29-Bt22 chimera (SEQ ID NO:3) aligned with a series of Bt29-Bt22 chimeras, Bt29-Bt22Tr1 (SEQ ID NO:20), Bt29-Bt22Tr2 (SEQ ID NO:21), Bt29-Bt22Tr3 (SEQ ID NO:22), Bt29-Bt22Tr4 (SEQ ID NO:23), Bt29-Bt22Tr5 (SEQ ID NO:24) and Bt29-Bt22Tr6 (SEQ ID NO:25), with C-terminal truncations within the BT-0029 protoxin tail.

BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

- [0039]** SEQ ID NO: 1 is the amino acid sequence of a BT-0022 protein.
- [0040]** SEQ ID NO: 2 is the amino acid sequence of a BT-0029 protein.
- [0041]** SEQ ID NO: 3 is the amino acid sequence of a Bt29-Bt22 chimera. The BT-0022 sequence is amino acid 459-597.
- [0042]** SEQ ID NO: 4 is a nucleotide sequence encoding the Bt29-Bt22 chimera of SEQ ID NO: 3.
- [0043]** SEQ ID NO: 5 is an exemplary maize-optimized sequence encoding the Bt29-Bt22 chimera of SEQ ID NO: 3.
- [0044]** SEQ ID NO: 6 is an exemplary maize-optimized sequence encoding the Bt29-Bt22 chimera of SEQ ID NO: 3.
- [0045]** SEQ ID NO: 7 is an exemplary maize-optimized sequence encoding the Bt29-Bt22 chimera of SEQ ID NO: 3.
- [0046]** SEQ ID NO: 8 is the amino acid sequence of a full-length Cry1Fa.
- [0047]** SEQ ID NO: 9 is the amino acid sequence of a Bt29-1Fa chimera. The Cry1Fa sequence is amino acids 459-597.
- [0048]** SEQ ID NO: 10 is a nucleotide sequence encoding the Bt29-1Fa chimera of SEQ ID NO: 9.
- [0049]** SEQ ID NO: 11 is an exemplary maize-optimized sequence encoding the Bt29-1Fa chimera of SEQ ID NO: 9.
- [0050]** SEQ ID NO: 12 is the amino acid sequence of a full-length Cry1Ka.
- [0051]** SEQ ID NO: 13 is the amino acid sequence of a Bt29-1Ka chimera. The Cry1Ka sequence is amino acids 459-597.

- [0052] SEQ ID NO: 14 is a nucleotide sequence encoding the Bt29-1Ka chimera of SEQ ID NO: 13.
- [0053] SEQ ID NO: 15 is the amino acid sequence of a Bt29-1Kav2 chimera.
- [0054] SEQ ID NO: 16 is the nucleotide sequence encoding the Bt29-1Ka chimera of SEQ ID NO: 15.
- [0055] SEQ ID NO: 17 is the amino acid sequence of a full-length Cry1Ca.
- [0056] SEQ ID NO: 18 is the amino acid sequence of a Bt29-1Ca chimera. The Cry1Ca sequence is amino acids 459-597.
- [0057] SEQ ID NO: 19 is a nucleotide sequence encoding the Bt29-1Ca chimera of SEQ ID NO: 18.
- [0058] SEQ ID NO: 20 is the amino acid sequence of Bt29-Bt22Tr1 protein.
- [0059] SEQ ID NO: 21 is the amino acid sequence of Bt29-Bt22Tr2 protein.
- [0060] SEQ ID NO: 22 is the amino acid sequence of Bt29-Bt22Tr3 protein.
- [0061] SEQ ID NO: 23 is the amino acid sequence of Bt29-Bt22Tr4 protein.
- [0062] SEQ ID NO: 24 is the amino acid sequence of Bt29-Bt22Tr5 protein.
- [0063] SEQ ID NO: 25 is the amino acid sequence of Bt29-Bt22Tr6 protein.
- [0064] SEQ ID NO: 26 is the amino acid sequence of BT29BT22-TL22v1 protein.
- [0065] SEQ ID NO: 27 is the amino acid sequence of BT29BT22-TL22v2 protein.
- [0066] SEQ ID NO: 28 is the amino acid sequence of BT29BT22-TL22v3 protein.
- [0067] SEQ ID NO: 29 is the amino acid sequence of BT29BT22-TL22v4 protein.
- [0068] SEQ ID NO: 30 is the amino acid sequence of BT29BT22-TL22v5 protein.
- [0069] SEQ ID NO: 31 is the amino acid sequence of BT29BT22-TL22v6 protein.
- [0070] SEQ ID NO: 32 is the amino acid sequence of BT291FaTr1 protein.
- [0071] SEQ ID NO: 33 is the amino acid sequence of BT291FaTr2 protein.
- [0072] SEQ ID NO: 34 is the amino acid sequence of BT291FaTr3 protein.
- [0073] SEQ ID NO: 35 is the amino acid sequence of BT291FaTr4 protein.

DETAILED DESCRIPTION

- [0074] This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be

added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Thus, the invention contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

[0075] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0076] All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

[0077] Nucleotide sequences provided herein are presented in the 5' to 3' direction, from left to right and are presented using the standard code for representing nucleotide bases as set forth in 37 CFR §§1.821 - 1.825 and the World Intellectual Property Organization (WIPO) Standard ST.25, for example: adenine (A), cytosine (C), thymine (T), and guanine (G).

[0078] Amino acids are likewise indicated using the WIPO Standard ST.25, for example: alanine (Ala; A), arginine (Arg; R), asparagine (Asn; N), aspartic acid (Asp; D), cysteine (Cys; C), glutamine (Gln; Q), glutamic acid (Glu; E), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

[0079] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

Definitions.

[0080] As used in the description of the invention and the appended claims, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0081] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0082] The term “about,” as used herein when referring to a measurable value such as a dosage or time period and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount. As used herein, phrases such as “between about X and Y” mean “between about X and about Y” and phrases such as “from about X to Y” mean “from about X to about Y.”

[0083] As used herein, phrases such as “from X to Y” should be interpreted to include X and Y, unless the context indicates otherwise.

[0084] By “activity” of an insecticidal protein of the invention is meant that the insecticidal protein functions as an orally active insect control agent, has a toxic effect, for example, by inhibiting the ability of the insect pest to survive, grow, and/or reproduce (*e.g.*, causing morbidity and/or mortality) and/or is able to disrupt and/or deter insect feeding, which may or may not cause death of the insect. Thus, when an insecticidal protein of the invention is delivered to the insect, the result is typically morbidity and/or mortality of the insect and/or the insect reduces or stops feeding upon the source that makes the insecticidal protein available to the insect.

[0085] In the context of the present invention, a "chimeric" protein is a protein created by fusing all or a portion of at least two different proteins. In embodiments of the present invention, the chimeric protein is a chimeric Cry protein comprising all or a portion of two different Cry proteins fused together into a single polypeptide. A "chimeric insecticidal protein" is a chimeric protein that has insecticidal activity (as described herein).

[0086] A "coding sequence" is a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. In embodiments, the RNA is then translated to produce a protein.

[0087] As used herein, a "codon optimized" nucleotide sequence means a nucleotide sequence of a recombinant, transgenic, or synthetic polynucleotide wherein the codons are chosen to reflect the particular codon bias that a host cell or organism may have. This is typically done in such a way so as to preserve the amino acid sequence of the polypeptide encoded by the codon optimized nucleotide sequence. In certain embodiments, a nucleotide sequence is codon optimized for the cell (*e.g.*, an animal, plant, fungal or bacterial cell) in which the construct is to be expressed. For example, a construct to be expressed in a plant cell can have all or parts of its sequence codon optimized for expression in a plant. See, for example, U.S. Pat. No. 6,121,014. In embodiments, the polynucleotides of the invention are codon-optimized for expression in a plant cell (*e.g.*, a dicot cell or a monocot cell) or bacterial cell.

[0088] To "control" an insect pest means to inhibit, through a toxic effect, the ability of the insect pest to survive, grow, feed and/or reproduce and/or to limit insect-related damage or loss in a crop plant caused by the insect pest and/or to protect the yield potential of a crop caused by the pest when grown in the presence of an insect pest. To "control" an insect pest may or may not mean killing the insect, although in embodiments of the invention, "control" of the insect means killing the insect.

[0089] The term "comprise", "comprises" or "comprising," when used in this specification, indicates the presence of the stated features, integers, steps,

operations, elements, or components, but does not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0090] As used herein, the transitional phrase “consisting essentially of” (and grammatical variants) means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim “and those that do not materially alter the basic and novel characteristic(s)” of the claimed invention. Thus, the term “consisting essentially of” when used in a claim of this invention is not intended to be interpreted to be equivalent to “comprising.”

[0091] In the context of the invention, “corresponding to” or “corresponds to” means that when the amino acid sequences of modified or homolog proteins are aligned with each other, the amino acids that “correspond to” certain enumerated positions in the modified or homolog protein are those that align with these positions in a reference protein, but are not necessarily in the same exact numerical positions relative to the particular reference amino acid sequence of the invention. For example, if SEQ ID NO: 2 (BT-0029) is the reference sequence and is aligned with SEQ ID NO: 1 (BT-0022) as in Figure 1, the sequence TLEAVT immediately following Domain III in SEQ ID NO: 1 (BT-0022) “corresponds” to the sequence TFEAEY immediately following Domain III of SEQ ID NO: 2 (BT-0029).

[0092] As used herein, the term “Cry protein” means an insecticidal protein of a *Bacillus thuringiensis* crystal delta-endotoxin type. The term “Cry protein” can refer to the protoxin form or any insecticidally active fragment or toxin thereof including partially processed and the mature toxin form (*e.g.*, without the N-terminal peptidyl fragment and/or the C-terminal protoxin tail).

[0093] As used herein, to “deliver” or “delivering” (and grammatical variations) a composition or insecticidal protein means that the composition or insecticidal protein comes in contact with an insect, which facilitates the oral ingestion of the composition or insecticidal protein, resulting in a toxic effect and control of the insect. The composition or insecticidal protein can be delivered in many recognized ways, including but not limited to, by transgenic plant expression, a formulated protein

composition(s), a sprayable protein composition(s), a bait matrix, or any other art-recognized protein delivery system.

[0094] The term "domain" refers to a set of amino acids conserved at specific positions along an alignment of sequences of evolutionarily related proteins. While amino acids at other positions can vary between homologues, amino acids that are highly conserved at specific positions indicate amino acids that are likely essential in the structure, stability or function of a protein. Identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers to determine if any polypeptide in question belongs to a previously identified polypeptide group.

[0095] "Effective insect-controlling amount" means that concentration of an insecticidal protein that inhibits, through a toxic effect, the ability of an insect to survive, grow, feed and/or reproduce and/or that limits insect-related damage or loss in a crop plant. An "effective insect-controlling amount" may or may not mean killing the insect, although in embodiments it indicates killing the insect.

[0096] "Expression cassette" as used herein means a nucleic acid molecule capable of directing expression of at least one polynucleotide of interest, such as a polynucleotide that encodes an insecticidal protein of the invention, in an appropriate host cell, comprising a promoter operably linked to the polynucleotide of interest which is operably linked to a termination signal. An "expression cassette" also typically comprises additional polynucleotides to facilitate proper translation of the polynucleotide of interest. The expression cassette may also comprise other polynucleotides not related to the expression of a polynucleotide of interest but which are present due to convenient restriction sites for removal of the cassette from an expression vector. In embodiments, at least one of the components in the expression cassette may be heterologous (*i.e.*, foreign) with respect to at least one of the other components (*e.g.*, a heterologous promoter operatively associated with a polynucleotide of interest). The expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is

heterologous with respect to the host, *i.e.*, the expression cassette (or even the polynucleotide of interest) does not occur naturally in the host cell and has been introduced into the host cell or an ancestor cell thereof by a transformation process or a breeding process. The expression of the polynucleotide(s) of interest in the expression cassette is generally under the control of a promoter. In the case of a multicellular organism, such as a plant, the promoter can also be specific or preferential to a particular tissue, or organ, or stage of development (as described in more detail herein). An expression cassette, or fragment thereof, can also be referred to as "inserted polynucleotide" or "insertion polynucleotide" when transformed into a plant.

[0097] A "gene" is defined herein as a hereditary unit comprising one or more polynucleotides that occupies a specific location on a chromosome or plasmid and that contains the genetic instruction for a particular characteristic or trait in an organism.

[0098] As used herein, a "gut protease" refers to a protease naturally found in the digestive tract of an insect. This protease is usually involved in the digestion of ingested proteins. Examples of gut proteases include trypsin, which typically cleaves peptides on the C-terminal side of lysine (K) or arginine (R) residues, and chymotrypsin, which typically cleaves peptides on the C-terminal side of phenylalanine (F), tryptophan (W) or tyrosine (Y).

[0099] As used herein, the term "heterologous" means foreign, exogenous, non-native and/or non-naturally occurring. In embodiments, a "heterologous" polynucleotide or polypeptide is a polynucleotide or polypeptide that is not naturally associated with a host cell into which it is introduced, including non-naturally occurring multiple copies of a naturally occurring nucleotide sequence or polypeptide. In embodiments, a nucleotide sequence is heterologous to another sequence with which it is operatively associated, *e.g.*, a promoter may be heterologous (*i.e.*, foreign) to an operatively associated coding sequence.

[00100] As used here, "homologous" means native. For example, a homologous nucleotide sequence or amino acid sequence is a nucleotide sequence or amino

acid sequence naturally associated with a host cell into which it is introduced, a homologous promoter sequence is the promoter sequence that is naturally associated with a coding sequence, and the like.

[00101] The terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof) and similar terms, as used herein, describe an elevation in the control of a plant pest, *e.g.*, by contacting a plant with a polypeptide of the invention (such as, for example, by transgenic expression or by topical application methods). This increase in control can be in reference to the level of control of the plant pest in the absence of the polypeptide of the invention (*e.g.*, a plant that is not transgenically expressing the polypeptide or is not topically treated with the polypeptide). Thus, in embodiments, the terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof), and similar terms can indicate an elevation of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 300%, 400%, 500% or more as compared to a suitable control (*e.g.*, a plant, plant part, plant cell that is not contacted with a polypeptide of the invention).

[00102] “Insecticidal” as used herein is defined as a toxic biological activity capable of controlling an insect pest, optionally but preferably by killing them.

[00103] A nucleic acid sequence is “isocoding” with a reference nucleic acid sequence when the nucleic acid sequence encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the reference nucleic acid sequence.

[00104] In representative embodiments, the nucleic acids molecules, polynucleotides or proteins of the invention are “isolated.” An “isolated” nucleic acid molecule, polynucleotide or protein, and the like, is a nucleic acid molecule, polynucleotide or protein, and the like that no longer exists in its natural environment. An isolated nucleic acid molecule, polynucleotide or protein of the invention may exist in a purified form or may exist in a recombinant host such as in a transgenic bacteria or a transgenic plant. In embodiments, an isolated nucleic acid molecule, nucleotide

sequence or polypeptide exists in a purified form that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In other embodiments, an “isolated” nucleic acid molecule, nucleotide sequence or polypeptide may exist in a non-native environment such as, for example, a recombinant host cell. Thus, for example, with respect to nucleotide sequences, the term “isolated” can mean that the nucleotide sequence is separated from the chromosome and/or cell in which it naturally occurs. A polynucleotide is also isolated if it is separated from the chromosome and/or cell in which it naturally occurs in and is then inserted into a genetic context, a chromosome and/or a cell in which it does not naturally occur (*e.g.*, a different host cell, different regulatory sequences, and/or different position in the genome than as found in nature). Accordingly, recombinant nucleic acid molecules, nucleotide sequences and their encoded polypeptides are “isolated” in that, by the hand of man, they exist apart from their native environment and therefore are not products of nature, however, in some embodiments, they can be introduced into and exist in a recombinant host cell. In representative embodiments, the isolated nucleic acid molecule, the isolated nucleotide sequence and/or the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more pure.

[00105] The term “motif” or “consensus sequence” or “signature” refers to a short conserved region in the sequence of evolutionarily related proteins. Motifs are frequently highly conserved parts of domains, but may also include only part of the domain, or be located outside of conserved domain (if all of the amino acids of the motif fall outside of a defined domain).

[00106] A “native” or “wild type” nucleic acid, nucleotide sequence, polypeptide or amino acid sequence refers to a naturally occurring or endogenous nucleic acid, nucleotide sequence, polypeptide or amino acid sequence. Thus, for example, a “wild type mRNA” is an mRNA that is naturally occurring in or endogenous to the organism.

[00107] The terms “nucleic acid,” “nucleic acid molecule,” “nucleotide sequence,” “oligonucleotide” and “polynucleotide” are used interchangeably herein, unless the context indicates otherwise, and refer to a heteropolymer of nucleotides. These terms include without limitation DNA and RNA molecules, including cDNA, genomic DNA, synthetic (*e.g.*, chemically synthesized) DNA and RNA, plasmid DNA, mRNA, anti-sense RNA, and RNA/DNA hybrids, any of which can be linear or branched, single stranded or double stranded, or a combination thereof. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2'-hydroxy in the ribose sugar group of the RNA can also be made. In embodiments, the “nucleic acid,” “nucleic acid molecule,” “nucleotide sequence,” “oligonucleotide” or “polynucleotide” refer to DNA.

[00108] By “operably linked” or “operably associated” as used herein, it is meant that the indicated elements are functionally related to each other, and are also generally physically related. Thus, the term “operably linked” or “operably associated” as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Thus, a first nucleotide sequence that is operably linked to a second nucleotide sequence, means a situation when the first nucleotide sequence is placed in a functional relationship with the second nucleotide sequence. For instance, a promoter is operably associated with a nucleotide sequence if the promoter effects the transcription or expression of said nucleotide sequence. Those skilled in the art will appreciate that the control sequences (*e.g.*, promoter) need not be contiguous with the nucleotide sequence to which it is operably associated, as long as the control sequences function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a nucleotide sequence, and the promoter can still be

considered “operably linked” to or “operatively associated” with the nucleotide sequence.

[00109] A “plant” as used herein, refers to any plant at any stage of development.

[00110] Any plant (or groupings of plants, for example, into a genus or higher order classification) can be employed in practicing the present invention including angiosperms or gymnosperms, monocots or dicots.

[00111] Exemplary plants include, but are not limited to corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), alfalfa (*Medicago saliva*), rice (*Oryza sativa*, including without limitation Indica and/or Japonica varieties), rape (*Brassica napus*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tobacum*), potato (*Solanum tuberosum*), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Cofea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), apple (*Malus pumila*), blackberry (*Rubus*), strawberry (*Fragaria*), walnut (*Juglans regia*), grape (*Vitis vinifera*), apricot (*Prunus armeniaca*), cherry (*Prunus*), peach (*Prunus persica*), plum (*Prunus domestica*), pear (*Pyrus communis*), watermelon (*Citrullus vulgaris*), duckweed (*Lemna* spp.), oats (*Avena sativa*), barley (*Hordium vulgare*), vegetables, ornamentals, conifers, and turfgrasses (e.g., for ornamental, recreational or forage purposes), and biomass grasses (e.g., switchgrass and miscanthus).

[00112] Vegetables include without limitation Solanaceous species (e.g., tomatoes; *Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), carrots (*Caucus carota*), cauliflower (*Brassica oleracea*), celery (*Apium graveolens*), eggplant (*Solanum melongena*), asparagus (*Asparagus officinalis*), ochra (*Abelmoschus esculentus*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus*

spp.), members of the genus *Cucurbita* such as hubbard squash (*C. hubbard*), butternut squash (*C. moschata*), zucchini (*C. pepo*), crookneck squash (*C. crookneck*), *C. argyrosperma*, *C. argyrosperma ssp sororia*, *C. digitata*, *C. ecuadorensis*, *C. foetidissima*, *C. lundelliana*, and *C. martinezii*, and members of the genus *Cucumis* such as cucumber (*Cucumis sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*).

[00113] Ornamentals include without limitation azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherima*), and chrysanthemum.

[00114] Conifers, which may be employed in practicing the present invention, include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*).

[00115] Turfgrass include but are not limited to zoysiagrasses, bentgrasses, fescue grasses, bluegrasses, St. Augustinegrasses, bermudagrasses, bufallograsses, ryegrasses, and orchardgrasses.

[00116] Also included are plants that serve primarily as laboratory models, *e.g.*, *Arabidopsis*.

[00117] A "plant cell" is a structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in the form of an isolated single cell or a cultured cell, or as a part of a higher organized unit such as, for example, plant tissue, a plant organ, or a whole plant.

- [00118]** A "plant cell culture" means a culture of plant units such as, for example, protoplasts, cell culture cells, cells in plant tissues, pollen, pollen tubes, ovules, embryo sacs, zygotes and embryos at various stages of development.
- [00119]** "Plant material" refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant.
- [00120]** A "plant organ" is a distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower bud, or embryo.
- [00121]** As used herein, the term "plant part" includes but is not limited to embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, stalks, roots, root tips, anthers, and/ or plant cells including plant cells that are intact in plants and/or parts of plants, plant protoplasts, plant tissues, plant cell tissue cultures, plant calli, plant clumps, and the like.
- [00122]** "Plant tissue" as used herein means a group of plant cells organized into a structural and functional unit. Any tissue of a plant in planta or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture and any groups of plant cells organized into structural or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant tissue.
- [00123]** A "polynucleotide of interest" refers to any polynucleotide which, when transferred to an organism, *e.g.*, a plant, confers upon the organism a desired characteristic such as insect resistance, disease resistance, herbicide tolerance, antibiotic resistance, improved nutritional value, improved performance in an industrial process, production of a commercially valuable enzyme or metabolite, an altered reproductive capability, and the like.
- [00124]** A "portion" or "fragment" of a polypeptide of the invention will be understood to mean an amino acid sequence of reduced length relative to a reference amino acid sequence of a polypeptide of the invention. Such a portion or fragment according to the invention may be, where appropriate, included in a larger

polypeptide of which it is a constituent (*e.g.*, a tagged or fusion protein). In embodiments, the “portion” or “fragment” substantially retains insecticidal activity (*e.g.*, at least 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or even 100% of the activity of the full-length protein, or has even greater insecticidal activity than the full-length protein).

[00125] The terms “protein,” “peptide” and “polypeptide” are used interchangeably herein.

[00126] The term “promoter” as used herein refers to a polynucleotide, typically upstream (5') of a coding polynucleotide, which controls the expression of the coding polynucleotide by providing the recognition for RNA polymerase and other transcriptional machinery.

[00127] A “protoplast” as used herein, refers to an isolated plant cell without a cell wall or with only parts of the cell wall.

[00128] As used herein, the term “recombinant” refers to a form of nucleic acid (*e.g.*, DNA or RNA) or protein or an organism that would not normally be found in nature and as such was created by human intervention. As used herein, a “recombinant nucleic acid molecule” (and similar terms) is a nucleic acid molecule comprising a combination of polynucleotides that would not naturally occur together and is the result of human intervention, *e.g.*, a nucleic acid molecule that is comprised of a combination of at least two polynucleotides heterologous to each other, or a nucleic acid molecule that is artificially synthesized and comprises a polynucleotide that deviates from the polynucleotide that would normally exist in nature, or a nucleic acid molecule that comprises a transgene artificially incorporated into a host cell's genomic DNA and the associated flanking DNA of the host cell's genome. An example of a recombinant nucleic acid molecule is a DNA molecule resulting from the insertion of a transgene into a plant's genomic DNA, which may ultimately result in the expression of a recombinant RNA or protein molecule in that organism. In embodiments, a “recombinant” protein is a protein that does not normally exist in nature or is present in a non-naturally occurring context, and is expressed from a recombinant nucleic acid molecule. As used herein, a “recombinant plant” is a plant

that would not normally exist in nature, is the result of human intervention, and contains a recombinant polynucleotide (*e.g.*, a transgene or heterologous nucleic acid molecule incorporated into its genome). As a result of such genomic alteration, the recombinant plant is distinctly different from the related wild-type plant.

[00129] The terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” and “suppress” (and grammatical variations thereof) and similar terms, as used herein, refer to a decrease in the survival, growth and/or reproduction of a plant pest, *e.g.*, by contacting a plant with a polypeptide of the invention (such as, for example, by transgenic expression or by topical application methods). This decrease in survival, growth and/or reproduction can be in reference to the level observed in the absence of the polypeptide of the invention (*e.g.*, a plant that is not transgenically expressing the polypeptide or is not topically treated with the polypeptide). Thus, in embodiments, the terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” and “suppress” (and grammatical variations thereof) and similar terms mean a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more as compared with a plant that is not contacted with a polypeptide of the invention (*e.g.*, a plant that is not transgenically expressing the polypeptide or is not topically treated with the polypeptide). In representative embodiments, the reduction results in no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10%, less than about 5% or even less than about 1%) detectable survival, growth and/or reproduction of the plant pest.

[00130] A “regulatory element” refers to a nucleotide sequence involved in controlling the expression of a polynucleotide. Examples of regulatory elements include promoters, termination signals, and nucleotide sequences that facilitate proper translation of a polynucleotide.

[00131] As used herein, “selectable marker” means a nucleotide sequence that when expressed imparts a distinct phenotype to the plant, plant part and/or plant cell expressing the marker and thus allows such transformed plants, plant parts and/or plant cells to be distinguished from those that do not have the marker. Such a nucleotide sequence may encode either a selectable or screenable marker,

depending on whether the marker confers a trait that can be selected for by chemical means, such as by using a selective agent (*e.g.*, an antibiotic, herbicide, or the like), or on whether the marker is simply a trait that one can identify through observation or testing, such as by screening (*e.g.*, the R-locus trait).

[00132] As used herein, “specific activity” refers to the amount of protein required to have an insecticidal effect. Therefore, when a first protein has a higher specific activity than a second protein means that it takes a lesser amount of the first protein compared the second protein to have an insecticidal effect on the same percentage of insects.

[00133] The phrase “substantially identical,” in the context of two nucleic acids or two amino acid sequences, refers to two or more sequences or subsequences that have at least about 50% nucleotide or amino acid residue identity when compared and aligned for maximum correspondence as measured using one of the following sequence comparison algorithms or by visual inspection. In certain embodiments, substantially identical sequences have at least about 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more nucleotide or amino acid residue identity. In certain embodiments, substantial identity exists over a region of the sequences that is at least about 50 residues, 100 residues, 150 residues, 200 residues, 250 residues, 300 residues, 350 residues, 400 residues or more in length. In further embodiments, the sequences are substantially identical when they are identical over the entire length of the coding regions.

[00134] “Identity” or “percent identity” refers to the degree of identity between two nucleic acid or amino acid sequences. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

- [00135]** For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.
- [00136]** Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally, Ausubel et al., infra*).
- [00137]** One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, 1990). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a

scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W , T , and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, $M=5$, $N=-4$, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89: 10915 (1989)).

[00138] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90: 5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[00139] Another widely used and accepted computer program for performing sequence alignments is CLUSTALW v1.6 (Thompson, et al. *Nuc. Acids Res.*, 22: 4673-4680, 1994). The number of matching bases or amino acids is divided by the total number of bases or amino acids, and multiplied by 100 to obtain a percent identity. For example, if two 580 base pair sequences had 145 matched bases, they would be 25 percent identical. If the two compared sequences are of different lengths, the number of matches is divided by the shorter of the two lengths. For example, if there were 100 matched amino acids between a 200 and a 400 amino acid proteins, they are 50 percent identical with respect to the shorter sequence. If

the shorter sequence is less than 150 bases or 50 amino acids in length, the number of matches are divided by 150 (for nucleic acid bases) or 50 (for amino acids), and multiplied by 100 to obtain a percent identity.

[00140] Two nucleotide sequences can also be considered to be substantially identical when the two sequences hybridize to each other under stringent conditions. In representative embodiments, two nucleotide sequences considered to be substantially identical hybridize to each other under highly stringent conditions.

[00141] The terms "stringent conditions" or "stringent hybridization conditions" include reference to conditions under which a nucleic acid will selectively hybridize to a target sequence to a detectably greater degree than other sequences (*e.g.*, at least 2-fold over a non-target sequence), and optionally may substantially exclude binding to non-target sequences. Stringent conditions are sequence-dependent and will vary under different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified that can be up to 100% complementary to the reference nucleotide sequence. Alternatively, conditions of moderate or even low stringency can be used to allow some mismatching in sequences so that lower degrees of sequence similarity are detected. For example, those skilled in the art will appreciate that to function as a primer or probe, a nucleic acid sequence only needs to be sufficiently complementary to the target sequence to substantially bind thereto so as to form a stable double-stranded structure under the conditions employed. Thus, primers or probes can be used under conditions of high, moderate or even low stringency. Likewise, conditions of low or moderate stringency can be advantageous to detect homolog, ortholog and/or paralog sequences having lower degrees of sequence identity than would be identified under highly stringent conditions.

[00142] The terms "complementary" or "complementarity" (and similar terms), as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be partial, in which only some of the nucleotides bind, or it

may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between the molecules. As used herein, the term “substantially complementary” (and similar terms) means that two nucleic acid sequences are at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more complementary. Alternatively, the term “substantially complementary” (and similar terms) can mean that two nucleic acid sequences can hybridize together under high stringency conditions (as described herein).

[00143] As used herein, “specifically” or “selectively” hybridizing (and similar terms) refers to the binding, duplexing, or hybridizing of a molecule to a particular nucleic acid target sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular DNA or RNA) to the substantial exclusion of non-target nucleic acids, or even with no detectable binding, duplexing or hybridizing to non-target sequences. Specifically or selectively hybridizing sequences typically are at least about 40% complementary and are optionally substantially complementary or even completely complementary (*i.e.*, 100% identical).

[00144] For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267-84 (1984): $T_m = 81.5^{\circ}\text{C} + 16.6 (\log M) + 0.41 (\% \text{ GC}) - 0.61 (\% \text{ formamide}) - 500/L$; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % formamide is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired degree of identity. For example, if sequences with >90% identity are sought, the T_m can be decreased 10°C . Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However,

highly stringent conditions can utilize a hybridization and/or wash at the thermal melting point (T_m) or 1, 2, 3 or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20°C lower than the thermal melting point (T_m). If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), optionally the SSC concentration can be increased so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, New York (1993); Current Protocols in Molecular Biology, chapter 2, Ausubel, et al., eds, Greene Publishing and Wiley-Interscience, New York (1995); and Green & Sambrook, In: Molecular Cloning, A Laboratory Manual, 4th Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2012).

[00145] Typically, stringent conditions are those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at about pH 7.0 to pH 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for longer probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt's (5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g bovine serum albumin in 500 ml of water). Exemplary low stringency conditions include hybridization with a buffer solution of 30% to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulfate) at 37°C and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50°C to 55°C. Exemplary moderate stringency conditions include hybridization in 40% to 45% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 0.5X to 1X SSC at 55°C to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 0.1X SSC at 60°C to 65°C. A further non-

limiting example of high stringency conditions include hybridization in 4X SSC, 5X Denhardt's, 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65°C and a wash in 0.1X SSC, 0.1% SDS at 65°C. Another illustration of high stringency hybridization conditions includes hybridization in 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, alternatively with washing in 1X SSC, 0.1% SDS at 50°C, alternatively with washing in 0.5X SSC, 0.1% SDS at 50°C, or alternatively with washing in 0.1X SSC, 0.1% SDS at 50°C, or even with washing in 0.1X SSC, 0.1% SDS at 65°C. Those skilled in the art will appreciate that specificity is typically a function of post-hybridization washes, the relevant factors being the ionic strength and temperature of the final wash solution.

[00146] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical (*e.g.*, due to the degeneracy of the genetic code).

[00147] A further indication that two nucleic acids or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with the protein encoded by the second nucleic acid. Thus, a protein is typically substantially identical to a second protein, for example, where the two proteins differ only by conservative substitutions.

[00148] As used herein, if a modified polypeptide or fragment (and the like) “substantially retains” insecticidal activity, it is meant that the modified polypeptide or fragment retains at least about 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or even 100% of the insecticidal activity of the reference protein, or has even greater insecticidal activity.

[00149] “Synthetic” refers to a nucleotide sequence comprising bases or a structural feature(s) that is not present in the natural sequence. For example, an artificial sequence encoding a protein of the invention that resembles more closely the G+C content and the normal codon distribution of dicot or monocot plant genes is said to be synthetic.

[00150] As used herein, a protein that is “toxic” to an insect pest is an orally-active insect control agent that kills the insect pest, causes a reduction in growth and/or

reproduction of the insect pest and/or is able to disrupt or deter insect feeding, the latter two of which may or may not cause death of the insect. When a protein of the invention is delivered to an insect or an insect comes into contact with the protein, the result is typically death of the insect, the insect's growth and/or reproduction is slowed and/or the insect reduces or stops feeding upon the source that makes the toxic protein available to the insect.

[00151] The terms “toxin fragment” and “toxin portion” are used interchangeably herein to refer to a fragment or portion of a longer (*e.g.*, full-length) chimeric insecticidal protein of the invention, where the “toxin fragment” or “toxin portion” retains insecticidal activity. For example, it is known in the art that native Cry proteins are expressed as protoxins that are processed at the N-terminal and C-terminal ends to produce a mature toxin. In embodiments, the “toxin fragment” or “toxin portion” of a chimeric insecticidal protein of the invention is truncated at the N-terminus and/or C-terminus. In embodiments, the “toxin fragment” or “toxin portion” is truncated at the N-terminus to remove part or all of the N-terminal peptidyl fragment, and optionally comprises at least about 400, 425, 450, 475, 500, 510, 520, 530, 540, 550, 560, 570, 580 or 590 contiguous amino acids of chimeric insecticidal protein specifically described herein or an amino acid sequence that is substantially identical thereto. Thus, in embodiments, a “toxin fragment” or “toxin portion” of a chimeric insecticidal protein is truncated at the N-terminus (*e.g.*, to omit part or all of the peptidyl fragment), for example, an N-terminal truncation of one amino acid or more than one amino acid, *e.g.*, up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more amino acids. In embodiments, a “toxin fragment” or “toxin portion” of a chimeric insecticidal protein is truncated at the C-terminus (*e.g.*, to omit part or all of the protoxin tail), for example, a C-terminal truncation of one amino acid or more than one amino acid, *e.g.*, up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 70,

80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 560 or more amino acids. In embodiments, the “toxin fragment” or “toxin portion” comprises domains 1 and 2, and the core domain III (*e.g.*, as shown in Figure 2). In embodiments, the “toxin fragment” or “toxin portion” is the mature (*i.e.*, processed) toxin (*e.g.*, Cry toxin).

[00152] “Transformation” is a process for introducing a heterologous nucleic acid into a host cell or organism. In particular embodiments, “transformation” means the stable integration of a DNA molecule into the genome of an organism of interest (*e.g.*, a plant cell).

[00153] The terms “transformed” and “transgenic” as used herein refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. “Transformed” or “transgenic” cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also progeny thereof comprising the heterologous nucleic acid molecule. A “non-transformed” or “non-transgenic” host refers to a wild-type organism, *e.g.*, a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

[00154] The term “vector” refers to a composition for transferring, delivering or introducing a nucleic acid (or nucleic acids) into a cell. A vector comprises a nucleic acid molecule comprising the nucleotide sequence(s) to be transferred, delivered or introduced.

Chimeric Insecticidal Proteins.

[00155] The present invention provides novel chimeric insecticidal proteins comprising at least one region from a first Cry protein (*e.g.*, a BT-0029 protein [SEQ ID NO: 2] and substantially identical variants thereof). In embodiments, the invention provides a chimeric insecticidal protein comprising a region from two or more different Cry proteins. In embodiments, the resulting chimeric insecticidal protein has increased

activity against one or more insect pests (*e.g.*, an enhanced activity or activity against a new target pest) and/or a different mode of action against one or more insect pests as compared with one or more (or even all) of the parent proteins. In representative embodiments, the chimeric insecticidal protein is a chimera comprising regions from two different Cry (*e.g.*, Cry1) proteins, and the chimera has increased insecticidal activity against one or more insect pests as compared with both parent proteins.

[00156] In embodiments, the chimeric insecticidal proteins of the invention can provide new modes of action against one or more target insect pests. For example, the chimeric insecticidal protein can have insecticidal activity against an insect pest or colony that is generally resistant to the insecticidal activity of another agent (*e.g.*, an insecticidal protein, including without limitation a *Bt* protein, such as a Cry protein or a Vip protein). In embodiments, the parent proteins themselves are not insecticidally active, or only weakly active, against the resistant insect pest or colony, which may suggest that the chimera has a novel mode of action as compared with the parent proteins. For example, if the parent proteins are active against a target insect pest ("susceptible"), but not against a resistant counterpart, and the chimeric insecticidal protein is active against the resistant insect pest, that is an indication that the chimeric insecticidal protein is toxic to the resistant pest via a novel mode of action.

[00157] Accordingly, in embodiments, the invention provides a chimeric insecticidal protein that is toxic to an insect pest (*e.g.*, a lepidopteran insect pest), the chimeric insecticidal protein comprising a region from a BT-0029 protein (SEQ ID NO: 2) or a polypeptide that is substantially identical to the region from a BT-0029 protein. In embodiments, the chimeric insecticidal protein comprises an N-terminal region of a first Cry1 protein, which is optionally a BT-0029 protein (SEQ ID NO: 2) or a polypeptide comprising an amino acid sequence that is substantially identical to the N-terminal region of the BT-0029 protein. In embodiments, the N-terminal region of the first Cry protein is fused to a C-terminal region from a different Cry protein (*e.g.*,

a different Cry1 protein) to form a chimeric insecticidal protein (*e.g.*, a chimeric insecticidal Cry protein).

[00158] In representative embodiments, the C-terminal region from a different Cry protein can be a C-terminal region from a different Cry1 protein or a polypeptide comprising an amino acid sequence that is substantially identical to the C-terminal region from the different Cry1 protein.

[00159] Thus, in a representative embodiment, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of, in an N-terminal to a C-terminal direction: (a) an N-terminal region of a first Cry protein (*e.g.*, a Cry1 protein), which is optionally an N-terminal region of a BT-0029 protein or an amino acid sequence that is substantially identical to the N-terminal region of the BT-0029 protein, fused to (b) a C-terminal region of a different Cry protein (*e.g.*, a different Cry1 protein).

[00160] In representative embodiments, the different Cry protein (*e.g.*, Cry1 protein) is selected based on an alignment of the C-terminal region from the different Cry protein and the corresponding region from the first Cry protein (*e.g.*, a Cry1 protein), for example, see the exemplary alignments in Figures 1A and 1B. In embodiments, the different Cry protein is selected such that the amino acid sequence of the C-terminal region of the different Cry protein is substantially identical to the corresponding region of the first Cry protein. In exemplary embodiments, the amino acid sequence of the C-terminal region of the different Cry protein (*e.g.*, Cry1 protein) is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical with an amino acid sequence of the corresponding C-terminal region from the first Cry protein. Methods of aligning and determining amino acid sequence identity across an aligned region are well known by those skilled in the art.

[00161] In embodiments, the different Cry1 protein includes without limitation a Cry1F protein (*e.g.*, a Cry1Fa or a Cry1Fb protein), a Cry1G protein (*e.g.*, a Cry1Ga, a Cry1Gb, or a Cry1Gc protein), a Cry1I protein (*e.g.*, a Cry1Ia protein, a Cry1Ib protein, a Cry1Ic protein, a Cry1Id protein, a Cry1Ie protein, a Cry1If protein, or a

Cry1Ig protein), a Cry1K (e.g., a Cry1Ka protein), or a Cry1C (e.g. a Cry1Ca protein),. In embodiments, the different Cry1 protein is a Cry1If protein, optionally a BT-0022 protein (SEQ ID NO: 1). In embodiments, a different Cry1 protein is a Cry1Fa protein (SEQ ID NO: 8). In embodiments, a different Cry1 protein is a Cry1K protein (SEQ ID NO: 12). In embodiments, a different Cry1 protein is a Cry1Ca protein (SEQ ID NO: 17).

[00162] The terms “N-terminal region” and a “C-terminal region” do not necessarily indicate that the most N-terminal or C-terminal amino acids (e.g., the N-terminus or C-terminus), respectively, of the full-length protein are included within the region. For example, it is well-known by those skilled in the art that Cry protoxins are processed at both the N-terminus and C-terminus to produce a mature (*i.e.*, processed) toxin. Thus, in embodiments, the “N-terminal region” and/or the “C-terminal region” omit some or all of the processed out portions of the protoxin such that the chimeric insecticidal protein comprises the mature toxin protein (e.g., Cry protein Domains I, II and III), without some or all of the N-terminal peptidyl fragment and/or the C-terminal protoxin tail, or a polypeptide that is substantially identical to the mature toxin protein. In embodiments, the chimeric insecticidal protein comprises the peptidyl fragment and/or protoxin tail. In embodiments, the chimeric insecticidal protein does not comprise the peptidyl fragment or protoxin tail, *i.e.*, corresponds to the mature processed toxin.

[00163] In embodiments, the N-terminal region of the first Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 to about position 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of SEQ ID NO: 2 (full-length

BT-0029), and any combination of lower and upper positions as if each such combination were specifically set forth herein.

[00164] In embodiments, the N-terminal region of the first Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 1 to about position 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490 or 491 of SEQ ID NO: 2.

[00165] In a representative embodiment, the N-terminal region of the first Cry1 protein comprises, consists essentially of, or consists of an amino acid sequence corresponding to an amino acid sequence from about position 1 to about amino acid position 458 of SEQ ID NO: 2.

[00166] In representative embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 to about position 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 587, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619 or 620 of SEQ ID NO: 8 (full-length Cry1Fa), and any combination of lower and upper positions as if each such combination were specifically set forth herein.

[00167] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 464 to about position 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 587, 598, 599, 600, 601,

602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619 or 620 of SEQ ID NO: 8.

[00168] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 to about position 602 of SEQ ID NO: 8.

[00169] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 464 to about position 602 of SEQ ID NO: 8.

[00170] In representative embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, or 535 to about position 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654 or 655 of SEQ ID NO: 1 (full-length BT-0022), and any combination of lower and upper positions as if each such combination were specifically set forth herein.

[00171] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 498 to about position 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636,

637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654 or 655 of SEQ ID NO: 1.

[00172] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, or 535 to about position 636 of SEQ ID NO: 1.

[00173] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 498 to about position 636 of SEQ ID NO: 1.

[00174] In representative embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536 or 537 to about position 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656 or 657 of SEQ ID NO: 12 (full-length Cry1Ka), and any combination of lower and upper positions as if each such combination were specifically set forth herein.

[00175] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 500 to about position 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627,

628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656 or 657 of SEQ ID NO: 12.

[00176] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537 to about position 597 of SEQ ID NO: 12.

[00177] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537 to about position 610 of SEQ ID NO: 12.

[00178] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 500 to about position 597 of SEQ ID NO: 12.

[00179] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 500 to about position 610 of SEQ ID NO: 12.

[00180] In representative embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466 or 467 to about position 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604,

605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616 or 617 of SEQ ID NO: 17 (full-length Cry1Ca), and any combination of lower and upper positions as if each such combination were specifically set forth herein.

[00181] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 467 to about position 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616 or 617 of SEQ ID NO: 17.

[00182] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466 or 467 to about position 617 of SEQ ID NO: 17.

[00183] In embodiments, the C-terminal region of the second Cry1 protein comprises, consists essentially of, or consists of a polypeptide corresponding to an amino acid sequence from about position 467 to about position 617 of SEQ ID NO: 17.

[00184] As is well known in the art, Cry proteins from *Bt* have 5 conserved sequence domains (Conserved Block [CB] 1 to 5) separated by more variable regions (Hofte & Whitely, 1989, Microbiol. Rev. 53:242-255), and three conserved structural domains (Domains I, II and III) (de Maagd et al., 2001, Trends Genetics 17:193-199). Figures 1A and 1B show an alignment of the Cry proteins BT-0029, BT-0022, Cry1Fa and Cry1Ka, with CB3 and Domain III indicated. Those skilled in the art can use the well-known sequence and structural information available for Cry proteins to create a chimeric insecticidal Cry protein according to the invention, for example, to select a suitable crossover region(s) between two Cry proteins. In embodiments, a crossover region is located within a conserved block, for example, within CB3. The term “within a conserved block” includes the positions at each end of the conserved block. To illustrate, the term “within CB3” includes the position immediately before

(*e.g.*, between the methionine and phenylalanine residues within the sequence VPMFSW in the BT-0029 sequence of Figure 2A) and immediately after (*e.g.*, between the threonine and asparagine residues within the sequence RRTNVG in the BT-0029 sequence of Figure 2A) conserved block 3. Figures 2A and 2B show exemplary chimeric insecticidal proteins according to the invention with a crossover region within CB3.

[00185] With reference to Figure 2A, in embodiments, a crossover is located in a position corresponding to a position immediately before amino acid residue 1 or immediately following amino acid residue 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or 51, or any combination thereof, in CB3. In embodiments, a crossover is located within a region (including the residues at each end of the region) corresponding to amino acid residue 2 to amino acid residue 7, 8, 9 or 10 of CB3, within a region corresponding to amino acid residue 11 to amino acid residue 14, 15, 16, 17 or 18 of CB3, within a region corresponding to amino acid residue 19 to amino acid residue 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 of CB3 and/or within a region corresponding to amino acid residue 37, 38 or 39 to amino acid residue 51, and any combination of such regions. In representative embodiments, a crossover is located in a position within a region corresponding to amino acid residue 19 to amino acid residue 51, amino acid residue 19 to amino acid residue 33, amino acid residue 19 to amino acid residue 28, amino acid residue 19 to amino acid residue 27, or amino acid residue 19 to amino acid residue 26 in CB3. In embodiments, a crossover position is located at the specific site in between amino acid residue 18 and amino acid residue 19 in CB3 (*e.g.*, specifically in between the valine and isoleucine in the sequence DPDVITQ in BT-0029, SEQ ID NO: 2).

[00186] The chimeric insecticidal proteins can also be defined with respect to the structural domains derived from each of the parent molecules. For example, in embodiments, the N-terminal region of the first Cry protein comprises domain I of the first Cry protein and all or essentially all of domain II. In embodiments, the C-

terminal region of the different Cry protein comprises all or essentially all of domain III of the different Cry protein. Those skilled in the art understand that there is some variability in delineating the precise location of the ends of the Cry protein structural domains and the linker regions between the domains, for example, the location of the end of the linker region between domains II and III and the beginning of domain III, although the core of domain III is readily identifiable to those skilled in the art (*e.g.*, corresponding to the location of the core domain III as shown in Figure 1A and Figure 2A). In embodiments, the crossover between the N-terminal region of the first Cry protein and the C-terminal region derived from the different Cry protein is located in CB3.

[00187] In representative embodiments, the C-terminal region of the different Cry protein (*e.g.*, Cry1 protein) is selected based on an alignment of domain III (*e.g.*, a core domain III as shown in Figure 1A) from the different Cry protein and the corresponding domain III sequence from the first Cry protein (*e.g.*, a Cry1 protein, such as BT-0029), for example, see the exemplary alignments in Figures 1A and 1B, with domain III specifically indicated. In embodiments, the different Cry protein is selected such that the amino acid sequence of domain III of the different Cry protein is substantially identical to the corresponding domain III region of the first Cry protein (*e.g.*, BT-0029). In exemplary embodiments, the amino acid sequence of the domain III region of the different Cry protein (*e.g.*, Cry1 protein) is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical with an amino acid sequence of the corresponding domain III region from the first Cry protein. Methods of aligning and determining amino acid sequence identity across an aligned region are well known by those skilled in the art.

[00188] In particular embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of (a) the amino acid sequence of any one of amino acids 1 to 597 of SEQ ID NO: 3, SEQ ID NO: 9, or SEQ ID NO: 13 or amino acids 1 to 610 of SEQ ID NO: 15, or amino acids 1 to 617 SEQ ID NO: 18, or a toxin fragment of any of SEQ ID Nos: 3, 9, 13, 15, or 18; or (b) an amino acid sequence that is substantially identical to the amino acid sequence of (a).

[00189] In particular embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of (a) the amino acid sequence of any one of amino acids 1 to 603 of SEQ ID NO: 3, SEQ ID NO: 9 or SEQ ID NO: 13, or amino acids 1 to 615 of SEQ ID NO:15 or SEQ ID NO:18, or a toxin fragment thereof; or (b) an amino acid sequence that is substantially identical to the amino acid sequence of (a).

[00190] In particular embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of (a) the amino acid sequence of any one of amino acids 1 to 607 of SEQ ID NO: 3, SEQ ID NO: 9 or SEQ ID NO: 13, or amino acids 1 to 620 of SEQ ID NO: 15 or SEQ ID NO: 18, or a toxin fragment thereof; or (b) an amino acid sequence that is substantially identical to the amino acid sequence of (a).

[00191] In particular embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of (a) the amino acid sequence of any one of amino acids 1 to 610 of SEQ ID NO: 3, SEQ ID NO: 9 or SEQ ID NO: 13, or amino acids 1 to 620 of SEQ ID NO: 15 or SEQ ID NO: 18, or a toxin fragment thereof; or (b) an amino acid sequence that is substantially identical to the amino acid sequence of (a).

[00192] As is understood by those skilled in the art, native Cry toxins are expressed as protoxins that are processed to produce a mature processed toxin by cleaving an N-terminal peptidyl fragment and a C-terminal protoxin tail. The N-terminal peptidyl fragment and/or the C-terminal protoxin tail may function to enhance the stability and/or the insecticidal activity of the Cry toxin. In embodiments of the invention, the chimeric insecticidal protein comprises all or a portion of an N-terminal peptidyl fragment and/or a protoxin tail. In embodiments, the chimeric insecticidal protein does not comprise a full N-terminal peptidyl fragment and/or a full protoxin tail. In embodiments, the chimeric insecticidal protein does not comprise an N-terminal peptidyl fragment and/or a protoxin tail, *i.e.*, corresponds to the mature processed toxin.

[00193] In embodiments, the N-terminal peptidyl fragment is derived from a Cry protein (*e.g.*, from the first Cry protein, which is optionally a Cry1 protein). In other embodiments, the N-terminal peptidyl fragment is heterologous to the first Cry protein, for example, is not derived from a Cry protein and/or is partially or completely synthetic. In embodiments, the peptidyl fragment comprises at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more amino acids, optionally from the first Cry protein, including without limitation a first Cry1 protein (*e.g.*, BT-0029, SEQ ID NO: 2). In embodiments, the peptidyl fragment comprises amino acids from about amino acid 1 to about amino acid 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 of the first Cry protein, *e.g.*, a Cry1 protein such as BT-0029.

[00194] In embodiments, the chimeric insecticidal protein of the invention comprises at the C-terminus a protoxin tail region of a *Bt* Cry protein, including modifications of native Cry protein protoxin tails that are substantially identical to a native Cry protein protoxin tail. In embodiments, the protoxin tail is from a lepidopteran-active Cry protein. In embodiments, the protoxin tail is not derived from a Cry protein and/or is partially or completely synthetic. In embodiments, the Cry protein is heterologous to the first Cry protein and/or the different Cry protein. In embodiments, the protoxin tail is from a Cry1 protein, *e.g.*, a BT-0029, a BT-0022, a Cry1F (*e.g.*, Cry1Fa), a Cry1I (*e.g.*, a Cry1Ia or a Cry1If), a Cry1K (*e.g.*, a Cry1Ka), or a Cry1C (*e.g.* Cry1Ca), or is a polypeptide that is substantially identical to a protoxin tail (or fragment thereof) from any of the foregoing. The protoxin tail region may comprise an entire protoxin tail of a Cry protein or any portion thereof. In embodiments, the protoxin tail region comprises at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 38, 40, 45, 50 or more contiguous amino acids of a Cry protein protoxin tail (*e.g.*, a Cry1 protoxin tail, such as a BT-0029), for example, as shown for the various Cry proteins in Figure 1A. In embodiments, the protoxin tail

comprises at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 38, 40, 45, 50 or more contiguous amino acids starting with amino acid position 598 of SEQ ID NO: 2 (BT-0029) or the corresponding region from another Cry protein, such as a Cry1 protein (*e.g.*, BT-0022, a Cry1I, a Cry1F, a Cry1K or a Cry1C protein). In embodiments, the protoxin tail comprises amino acids 598 to 1169, or 598 to 652, or 598 to 636, or 598 to 622, or 598 to 610, or 598 to 607, or 598 to 603, or 598 to 600 of SEQ ID NO: 2 (BT-0029) or the corresponding region from another Cry protein (*see, e.g.*, the alignment of BT-0029 with BT-0022, Cry1Fa and Cry1Ka in Figures 1A and 1B). In some embodiments, the chimeric insecticidal protein comprises any of SEQ ID Nos:20-25 or SEQ ID Nos:32-35. In other embodiments, the protoxin tail comprises amino acids 637 to 715, or 637 to 691, or 637 to 675, or 637 to 661, or 637 to 649, or 637 to 646, or 637 to 642 of SEQ ID NO: 1 (BT-0022). In still other embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of any of SEQ ID Nos: 26-31.

[00195] Accordingly, in embodiments, a chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of (a) the amino acid sequence of any one of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID Nos:20-35, or a toxin fragment thereof; or (b) an amino acid sequence that is substantially identical to the amino acid sequence of (a). In optional embodiments, the chimeric insecticidal protein of the invention comprises, consists essentially of, or consists of the amino acid sequence of any one of amino acids SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 18 or SEQ ID Nos: 20-35.

[00196] Those skilled in the art will appreciate that the chimeric insecticidal proteins of the invention can further comprise other functional domains and/or peptide tags, for example a peptide tag on the N-terminus and/or C-terminus. For example, it may be useful to express the chimeric insecticidal protein with a peptide tag that can be recognized by a commercially available antibody (*e.g.*, a FLAG motif) or with a peptide tag that facilitates purification (*e.g.*, by addition of a poly-His tag) and/or detection. Alternatively, an epitope can be introduced into the chimeric protein to

facilitate the generation of antibodies that specifically recognize the modified chimeric protein to distinguish the modified chimeric protein from the unmodified chimera and/or a parent protein(s). For example, one or more amino acids can be substituted into an antigenic loop of the native sequence to create a new epitope. In one embodiment, the antigenic loop is located in a non-conserved region outside of domain I of the native Cry protein. In embodiments, the antigenic loop is not a loop involved in insect gut receptor recognition by the Cry protein and/or is not involved in the protease activation of the Cry protein. In other embodiments, the chimeric protein can be modified to enhance its stability, for example, by fusing a maltose binding protein (MBP) or glutathione-S-transferase to the polypeptide. As another alternative, the fusion protein can comprise a reporter molecule.

[00197] Chimeric insecticidal proteins that are modified by introduction or elimination of a protease processing site at an appropriate position(s) to provide, or eliminate, proteolytic cleavage by an insect, plant and/or microorganism protease are also within the scope of the invention. In embodiments, the modified chimeric insecticidal protein substantially retains insecticidal activity. In embodiments, the stability and/or insecticidal activity of such modified chimeric proteins is increased as compared with the chimeric insecticidal protein that does not contain such modification to introduce/eliminate a protease cleavage site.

[00198] Thus, the invention encompasses polypeptides having amino acid sequences that are substantially identical to those specifically disclosed herein, and toxin fragments thereof. It will be understood that the chimeric insecticidal proteins specifically disclosed herein will typically tolerate modifications in the amino acid sequence and substantially retain biological activity (*e.g.*, insecticidal activity). Such modifications include insertions, deletions (including truncations at either terminus), and substitutions of one or more amino acids, including up to about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 100, about 105, about 110,

about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, or more amino acid substitutions, deletions and/or insertions.

[00199] To identify substantially identical polypeptides to the chimeric insecticidal proteins specifically disclosed herein, amino acid substitutions may be based on any characteristic known in the art, including the relative similarity or differences of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like.

[00200] For example, in identifying amino acid sequences encoding insecticidal polypeptides other than those specifically disclosed herein, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (see, Kyte and Doolittle, (1982) J. Mol. Biol. 157:105; incorporated herein by reference in its entirety). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

[00201] Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, Id.), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[00202] Accordingly, the hydropathic index of the amino acid (or amino acid sequence) may be considered when modifying the chimeric polypeptides specifically disclosed herein.

[00203] It is also understood in the art that the substitution of amino acids can be made on the basis of hydrophilicity. U.S. Pat. No. 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

[00204] As detailed in U.S. Pat. No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (.+-3.0); aspartate (+3.0.+-1); glutamate (+3.0.+-1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5+1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

[00205] Thus, the hydrophilicity of the amino acid (or amino acid sequence) may be considered when identifying additional insecticidal polypeptides beyond those specifically disclosed herein.

[00206] The chimeric insecticidal proteins of the invention, including modifications and toxin fragments of the chimeric polypeptide specifically disclosed herein, can be made by any suitable method known in the art, generally by modifying the coding nucleic acid sequences. Methods of manipulating and modifying nucleic acids to achieve a desired modification are well-known in the art. In addition, gene editing techniques can also be used produce a chimeric insecticidal protein of the invention or to make further modifications thereto.

[00207] As another approach, the polypeptide to be modified can be expressed in a host cell that exhibits a high rate of base mis-incorporation during DNA replication, such as XL-1 Red (Stratagene, La Jolla, CA). After propagation in such strains, one can isolate the DNA (for example, by preparing plasmid DNA or by PCR amplification and cloning of the resulting PCR fragment into a vector), culture the protein mutations in a non-mutagenic strain, and identify mutated genes with insecticidal activity, for example, by performing an assay to test for insecticidal activity. In exemplary methods, the protein is mixed and used in feeding assays. See, for example, Marrone et al. (1985) J. of Economic Entomology 78:290-293. Such assays can include contacting plants with one or more pests and determining the plant's ability to survive or cause the death of the pests. Examples of mutations that result in increased toxicity are found in Schnepf et al. (1998) Microbiol. Mol. Biol. Rev. 62:775-806.

[00208] In embodiments, the chimeric insecticidal protein (including substantially similar polypeptides and toxin fragments) of the invention is isolated. In embodiments, the chimeric insecticidal protein (including substantially similar polypeptides and toxin fragments) of the invention is a recombinant protein.

[00209] The chimeric insecticidal proteins of the invention have insecticidal activity against a lepidopteran pest. In embodiments, the chimeric insecticidal protein has activity against one or more of the following non-limiting examples of a lepidopteran pest: *Ostrinia* spp. such as *O. nubilalis* (European corn borer) and/or *O. furnacalis* (Asian corn borer); *Plutella* spp. such as *P. xylostella* (diamondback moth); *Spodoptera* spp. such as *S. frugiperda* (fall armyworm), *S. littoralis* (Egyptian cotton leafworm), *S. ornithogalli* (yellowstriped armyworm), *S. praefica* (western yellowstriped armyworm), *S. eridania* (southern armyworm) and/or *S. exigua* (beet armyworm); *Agrotis* spp. such as *A. ipsilon* (black cutworm), *A. segetum* (common cutworm), *A. gladiaria* (claybacked cutworm), and/or *A. orthogonia* (pale western cutworm); *Striacosta* spp. such as *S. albicosta* (western bean cutworm); *Helicoverpa* spp. such as *H. zea* (corn earworm), *H. punctigera* (native budworm), and/or *H. armigera* (cotton bollworm); *Heliothis* spp. such as *H. virescens* (tobacco budworm); *Diatraea* spp. such as *D. grandiosella* (southwestern corn borer) and/or *D. saccharalis* (sugarcane borer); *Trichoplusia* spp. such as *T. ni* (cabbage looper); *Sesamia* spp. such as *S. nonagroides* (Mediterranean corn borer) and/or *S. calamistis* (pink stem borer); *Pectinophora* spp. such as *P. gossypiella* (pink bollworm); *Cochylis* spp. such as *C. hospes* (banded sunflower moth); *Manduca* spp. such as *M. sexta* (tobacco hornworm) and/or *M. quinquemaculata* (tomato hornworm); *Elasmopalpus* spp. such as *E. lignosellus* (lesser cornstalk borer); *Pseudoplusia* spp. such as *P. includens* (soybean looper); *Anticarsia* spp. such as *A. gemmatilis* (velvetbean caterpillar); *Plathypena* spp. such as *P. scabra* (green cloverworm); *Pieris* spp. such as *P. brassicae* (cabbage butterfly), *Papaipema* spp. such as *P. nebris* (stalk borer); *Pseudaletia* spp. such as *P. unipuncta* (common armyworm); *Peridroma* spp. such as *P. saucia* (variegated cutworm); *Keiferia* spp. such as *K. lycopersicella* (tomato pinworm); *Artogeia* spp. such as *A. rapae*

(imported cabbageworm); *Phthorimaea* spp. such as *P. operculella* (potato tuberworm); *Chrysodeixis* spp. Such as *C. includes* (soybean looper); *Feltia* spp. such as *F. ducens* (dingy cutworm); *Chilo* spp. such as *C. suppressalis* (striped stem borer), *Cnaphalocrocis* spp. such as *C. medinalis* (rice leaffolder), or any combination of the foregoing.

[00210] Optionally, the chimeric insecticidal protein has increased activity against one or more lepidopteran pests as compared with one or more of the parent molecules (*e.g.*, the first Cry protein and the different Cry protein). In embodiments, the chimeric insecticidal protein has increased activity against one or more lepidopteran pests as compared with BT-0029. In embodiments, the chimeric insecticidal protein has increased activity against one or more lepidopteran pests as compared with BT-0022, a Cry1Fa, a Cry1Ia, a Cry1If, a Cry1Ka, or a Cry1C.

[00211] In embodiments, the chimeric insecticidal protein has enhanced insecticidal activity against fall armyworm (*Spodoptera frugiperda*) as compared with one or more of the parent molecules (*e.g.*, the first Cry protein and the different Cry protein). In embodiments, the chimeric insecticidal protein has increased activity against fall armyworm as compared with BT-0029. In embodiments, the chimeric insecticidal protein has increased activity against fall armyworm as compared with BT-0022, a Cry1Fa, a Cry1Ia, a Cry1If, a Cry1Ka and/or a Cry1Ca. According to the foregoing embodiments, the chimeric insecticidal protein can optionally have insecticidal activity against a fall armyworm insect pest or colony that has resistance to another insecticidal agent, including another insecticidal protein (such as, *e.g.*, a *Bt* protein). In embodiments, the chimeric insecticidal protein has insecticidal activity against a fall armyworm colony that is resistant to a Vip3A protein (*e.g.*, a Vip3Aa, including without limitation maize event MIR162) or a Cry1F protein (*e.g.*, Cry1Fa, including without limitation maize event TC1507). In embodiments, the chimeric insecticidal protein has enhanced activity against the resistant fall armyworm colony as compared with one or more of the parent molecules, *e.g.*, BT-0029, BT-0022, a Cry1Fa, a Cry1Ia, a Cry1If, a Cry1Ka and/or a Cry1Ca.

[00212] The invention also encompasses antibodies that specifically bind to a chimeric insecticidal protein of the invention. The antibody can optionally be a monoclonal antibody or a polyclonal antiserum. In embodiments, the antibody is selective for the chimeric protein and does not bind to one or more of the parent molecules (*e.g.*, BT-0029, BT-0022, Cry1Fa, and the like), and can be used to distinguish the chimeric protein from the parent protein. Such antibodies may be produced using standard immunological techniques for production of polyclonal antisera and, if desired, immortalizing the antibody-producing cells of the immunized host for sources of monoclonal antibody production. Techniques for producing antibodies to any substance of interest are well known, *e.g.*, as described in Harlow and Lane (1988. *Antibodies a laboratory manual*. pp. 726. Cold Spring Harbor Laboratory) and as in Goding (*Monoclonal Antibodies: Principles & practice*. 1986. Academic Press, Inc., Orlando, FL). The present invention also encompasses an insecticidal protein that cross-reacts with an antibody, particularly a monoclonal antibody, raised against one or more of the chimeric insecticidal proteins of the present invention.

[00213] The antibodies according to the invention are useful, *e.g.*, in immunoassays for determining the amount or presence of a chimeric insecticidal protein of the invention or an antigenically related polypeptide, *e.g.*, in a biological sample. Such assays are also useful in quality-controlled production of compositions containing one or more of the chimeric insecticidal proteins of the invention or an antigenically related polypeptide. In addition, the antibodies can be used to assess the efficacy of recombinant production of one or more of the chimeric proteins of the invention or an antigenically related polypeptide, as well as for screening expression libraries for the presence of a nucleotide sequence encoding one or more of the chimeric proteins of the invention or an antigenically related polypeptide. Antibodies further find use as affinity ligands for purifying or isolating any one or more of the proteins of the invention or an antigenically related polypeptide.

Nucleic acids, Expression Cassettes, and Vectors.

[00214] As a further aspect, the invention provides nucleic acids encoding the polypeptides of the invention, including modified polypeptides and toxin fragments as described herein.

[00215] According to some embodiments, the invention provides a nucleic acid molecule comprising a nucleotide sequence that comprises, consists essentially of, or consists of: (a) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID Nos: 20-35, or a toxin fragment thereof; (b) a nucleotide sequence encoding an amino acid sequence that is substantially identical to the amino acid sequence of (a); (c) a nucleotide sequence that anneals under stringent hybridization conditions to the nucleotide sequence of (a) or (b); or (d) a nucleotide sequence that differs from the nucleotide sequences of (a), (b) or (c) due to the degeneracy of the genetic code.

[00216] In embodiments, the nucleic acid molecule comprises a nucleotide sequence that comprises, consists essentially of, or consists of: (a) a nucleotide sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 14, or SEQ ID NO: 16, or a toxin-encoding fragment thereof; (b) a nucleotide sequence that is substantially identical to the nucleotide sequence of (a); (c) a nucleotide sequence that anneals under stringent hybridization conditions to the nucleotide sequence of (a) or (b); or (d) a nucleotide sequence that differs from the nucleotide sequences of (a), (b) or (c) due to the degeneracy of the genetic code. Optionally, the nucleotide sequence comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 14 or SEQ ID NO: 16.

[00217] In embodiments, the nucleotide sequence is a partially or completely synthetic sequence, *e.g.*, that has codons optimized for expression in a host organism, *e.g.*, in a bacterium host or a plant host (for example, a transgenic monocot plant host or a transgenic dicot plant host). Non-limiting examples

nucleotide sequences that are codon-optimized for expression in a maize plant include SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 11.

[00218] In representative embodiments, for expression in transgenic plants, the nucleotide sequences of the invention are modified and/or optimized. For example, although in many cases genes from microbial organisms can be expressed in plants at high levels without modification, low expression in transgenic plants may result from microbial nucleotide sequences having codons that are not preferred in plants. It is known in the art that living organisms have specific preferences for codon usage, and the codons of the nucleotide sequences described in this invention can be changed to conform with plant preferences, while maintaining the amino acids encoded thereby. Furthermore, it is known in the art that high expression in plants, for example corn plants, can be achieved from coding sequences that have at least about 35% GC content, or at least about 45%, or at least about 50%, or at least about 60%. Microbial nucleotide sequences that have low GC contents may express poorly in plants. Although certain nucleotide sequences can be adequately expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray et al. Nucl. Acids Res. 17:477-498 (1989)). In addition, in embodiments, the nucleotide sequence is modified to remove illegitimate splice sites that may cause message truncation. Such modifications to the nucleotide sequences can be made using well known techniques of site directed mutagenesis, PCR, and synthetic gene construction using the methods described, for example, in US Patent Nos. 5,625,136; 5,500,365 and 6,013,523.

[00219] In some embodiments, the invention provides synthetic coding sequences or polynucleotide made according to the procedure disclosed in U.S. Pat. No. 5,625,136. In this procedure, maize preferred codons, *i.e.*, the single codon that most frequently encodes that amino acid in maize, are used. The maize preferred codon for a particular amino acid can be derived, for example, from known gene sequences from maize. For example, maize codon usage for 28 genes from maize

plants is found in Murray et al., Nucleic Acids Research 17:477-498 (1989). It is recognized that codons optimized for expression in one plant species will also function in other plant species but possibly not at the same level as the plant species for which the codons were optimized. In this manner, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of a nucleotide sequence may be optimized or synthetic. That is, a polynucleotide may comprise a nucleotide sequence that is part native sequence and part codon optimized sequence.

[00220] In representative embodiments, a polynucleotide of the invention is an isolated polynucleotide. In embodiments, a polynucleotide of the invention is a recombinant polynucleotide.

[00221] In embodiments, the invention further provides a nucleic acid molecule comprising a polynucleotide of the operably associated with a promoter (*e.g.*, a heterologous promoter). Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and/or tissue-specific promoters. In particular aspects, a promoter useful with the invention is a promoter capable of initiating transcription of a nucleotide sequence in a plant cell, *e.g.*, in a cell of a monocot (*e.g.*, maize or rice) or dicot (*e.g.*, soybean, cotton) plant.

[00222] In embodiments, a heterologous promoter is a plant-expressible promoter (*e.g.*, monocot expressible or dicot expressible). For example, without limitation, the plant-expressible promoter can be selected from the following promoters: ubiquitin, cestrum yellow virus, corn TrpA, OsMADS 6, maize H3 histone, bacteriophage T3 gene 9 5' UTR, corn sucrose synthetase 1, corn alcohol dehydrogenase 1, corn light harvesting complex, corn heat shock protein, maize mtl, pea small subunit RuBP carboxylase, rice actin, rice cyclophilin, Ti plasmid mannopine synthase, Ti plasmid nopaline synthase, petunia chalcone isomerase, bean glycine rich protein 1, potato patatin, lectin, CaMV 35S and S-E9 small subunit RuBP carboxylase promoter.

[00223] Although many promoters from dicotyledons have been shown to be operational in monocotyledons and vice versa, in embodiments, dicotyledonous

promoters are selected for expression in dicotyledons, and monocotyledonous promoters for expression in monocotyledons. However, there is no restriction to the provenance of selected promoters; it is sufficient that they are operational in driving the expression of the nucleotide sequences in the desired cell.

[00224] The choice of promoter can vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. Thus, for example, expression of the nucleotide sequences of the invention can be in any plant and/or plant part, (*e.g.*, in leaves, in stalks or stems, in ears, in inflorescences (*e.g.*, spikes, panicles, cobs, *etc.*), in roots, seeds and/or seedlings, and the like). For example, where expression in a specific tissue or organ is desired, a tissue-specific or tissue-preferred promoter can be used (*e.g.*, a root specific/preferred promoter). In contrast, where expression in response to a stimulus is desired a promoter inducible by stimuli or chemicals can be used. Where continuous expression at a relatively constant level is desired throughout the cells of a plant a constitutive promoter can be chosen.

[00225] Promoters useful with the invention include, but are not limited to, those that drive expression of a nucleotide sequence constitutively, those that drive expression when induced, and those that drive expression in a tissue- or developmentally-specific manner. These various types of promoters are known in the art.

[00226] Suitable constitutive promoters include, for example, CaMV 35S promoter (Odell et al., *Nature* 313:810-812, 1985); Arabidopsis At6669 promoter (see PCT Publication No. W004081173A2); maize Ubi 1 (Christensen et al., *Plant Mol. Biol.* 18:675-689, 1992); rice actin (McElroy et al., *Plant Cell* 2:163-171, 1990); pEMU (Last et al., *Theor. Appl. Genet.* 81:581-588, 1991); CaMV 19S (Nilsson et al., *Physiol. Plant* 100:456-462, 1997); GOS2 (de Pater et al., *Plant J* November; 2(6):837-44, 1992); ubiquitin (Christensen et al., *Plant Mol. Biol.* 18: 675-689, 1992); Rice cyclophilin (Bucholz et al., *Plant Mol Biol.* 25(5):837-43, 1994); Maize H3 histone (Lepetit et al., *Mol. Gen. Genet.* 231: 276-285, 1992); Actin 2 (An et al., *Plant J.* 10(1):107-121, 1996), constitutive root tip CT2 promoter (SEQ ID NO:1535; see also PCT application No. IL/2005/000627) and Synthetic Super MAS (Ni et al., *The*

Plant Journal 7: 661-76, 1995). Other constitutive promoters include those in U.S. Pat. Nos. 5,659,026, 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142.

[00227] Tissue-specific or tissue-preferential promoters useful for the expression of the polypeptides of the invention in plants, optionally maize, include those that direct expression in root, pith, leaf or pollen. Suitable tissue-specific promoters include, but not limited to, leaf-specific promoters (such as described, for example, by Yamamoto et al., Plant J. 12:255-265, 1997; Kwon et al., Plant Physiol. 105:357-67, 1994; Yamamoto et al., Plant Cell Physiol. 35:773-778, 1994; Gotor et al., Plant J. 3:509-18, 1993; Orozco et al., Plant Mol. Biol. 23:1129-1138, 1993; and Matsuoka et al., Proc. Natl. Acad. Sci. USA 90:9586-9590, 1993), seed-preferred promoters (*e.g.*, from seed specific genes; Simon, et al., Plant Mol. Biol. 5: 191, 1985; Scofield, et al., J. Biol. Chem. 262: 12202, 1987; Baszczynski, et al., Plant Mol. Biol. 14: 633, 1990), Brazil Nut albumin (Pearson et al., Plant Mol. Biol. 18: 235-245, 1992), legumin (Ellis, et al. Plant Mol. Biol. 10: 203-214, 1988), Glutelin (Takaiwa, et al., Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, et al., FEBS Letts. 221: 43-47, 1987), Zein (Matzke et al., Plant Mol Biol, 143).323-32 1990), napA (Stalberg, et al., Planta 199: 515-519, 1996), Wheat SPA (Albanietal, Plant Cell, 9: 171-184, 1997), sunflower oleosin (Cummins, et al., Plant Mol. Biol. 19: 873-876, 1992)], endosperm specific promoters (*e.g.*, wheat LMW and HMW, glutenin-1 (Mol Gen Genet 216:81-90, 1989; NAR 17:461-2), wheat a, b and g gliadins (EMB03:1409-15, 1984), Barley ltrl promoter, barley B1, C, D hordein (Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996), Barley DOF (Mena et al., The Plant Journal, 116(1): 53-62, 1998), Biz2 (EP99106056.7), Synthetic promoter (Vicente-Carbajosa et al., Plant J. 13: 629-640, 1998), rice prolamin NRP33, rice -globulin Glb-1 (Wu et al., Plant Cell Physiology 39(8) 885-889, 1998), rice alpha-globulin REB/OHP-1 (Nakase et al. Plant Mol. Biol. 33: 513-S22, 1997), rice ADP-glucose PP (Trans Res 6:157-68, 1997), maize ESR gene family (Plant J 12:235-46, 1997), sorghum gamma-kafirin (Plant Mol. Biol 32:1029-35, 1996)], embryo specific promoters (*e.g.*, rice OSH1; Sato et al., Proc. Nati. Acad. Sci. USA, 93: 8117-8122),

KNOX (Postma-Haarsma et al., Plant Mol. Biol. 39:257-71, 1999), rice oleosin (Wu et al., J. Biochem., 123:386, 1998)] flower-specific promoters, for example, AtPRP4, chalcone synthase (chsA) (Van der Meer, et al., Plant Mol. Biol. 15, 95-109, 1990), LAT52 (Twell et al., Mol. Gen. Genet. 217:240-245; 1989), apetala-3, and promoters specific for plant reproductive tissues (*e.g.*, OsMADS promoters; U.S. Patent Publication 2007/0006344).

[00228] Examples of promoters suitable for preferential expression in green tissue include many that regulate genes involved in photosynthesis and many of these have been cloned from both monocotyledons and dicotyledons. One such promoter is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, Plant Molec. Biol. 12:579-589 (1989)). Another promoter for root specific expression is that described by de Framond (FEBS 290:103-106 (1991) or US Patent No. 5,466,785). Another promoter useful in the invention is the stem specific promoter described in U.S. Pat. No. 5,625,136, which naturally drives expression of a maize trpA gene.

[00229] In addition, promoters functional in plastids can be used. Non-limiting examples of such promoters include the bacteriophage T3 gene 9 5' UTR and other promoters disclosed in U.S. Patent No. 7,579,516. Other promoters useful with the invention include but are not limited to the S-E9 small subunit RuBP carboxylase promoter and the Kunitz trypsin inhibitor gene promoter (Kti3).

[00230] In some embodiments of the invention, inducible promoters can be used. Thus, for example, chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Regulation of the expression of nucleotide sequences of the invention via promoters that are chemically regulated enables the polypeptides of the invention to be synthesized only when the crop plants are treated with the inducing chemicals. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of a chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Examples

of such technology for chemical induction of gene expression is detailed in published application EP 0 332 104 and US Patent No. 5,614,395.

[00231] Chemical inducible promoters are known in the art and include, but are not limited to, the maize *In2-2* promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco *PR-1* a promoter, which is activated by salicylic acid (*e.g.*, the PR1a system), steroid steroid-responsive promoters (*see, e.g.*, the glucocorticoid-inducible promoter in Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88, 10421-10425 and McNellis *et al.* (1998) *Plant J.* 14, 247-257) and tetracycline-inducible and tetracycline-repressible promoters (*see, e.g.*, Gatz *et al.* (1991) *Mol. Gen. Genet.* 227, 229-237, and U.S. Patent Numbers 5,814,618 and 5,789,156, Lac repressor system promoters, copper-inducible system promoters, salicylate-inducible system promoters (*e.g.*, the PR1a system), glucocorticoid-inducible promoters (Aoyama *et al.* (1997) *Plant J.* 11:605-612), and ecdysone-inducible system promoters.

[00232] Other non-limiting examples of inducible promoters include ABA- and turgor-inducible promoters, the auxin-binding protein gene promoter (Schwob *et al.* (1993) *Plant J.* 4:423-432), the UDP glucose flavonoid glycosyl-transferase promoter (Ralston *et al.* (1988) *Genetics* 119:185-197), the MPI proteinase inhibitor promoter (Cordero *et al.* (1994) *Plant J.* 6:141-150), and the glyceraldehyde-3-phosphate dehydrogenase promoter (Kohler *et al.* (1995) *Plant Mol. Biol.* 29:1293-1298; Martinez *et al.* (1989) *J. Mol. Biol.* 208:551-565; and Quigley *et al.* (1989) *J. Mol. Evol.* 29:412-421). Also included are the benzene sulphonamide-inducible (US Patent No. 5,364,780) and alcohol-inducible (Int'l Patent Application Publication Nos. WO 97/06269 and WO 97/06268) systems and glutathione S-transferase promoters. Likewise, one can use any of the inducible promoters described in Gatz (1996) *Current Opinion Biotechnol.* 7:168-172 and Gatz (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:89-108. Other chemically inducible promoters useful for directing the expression of the nucleotide sequences of this invention in plants are disclosed

in US Patent 5,614,395. Chemical induction of gene expression is also detailed in EP 0 332 104 (to Ciba-Geigy) and U.S. Patent 5,614,395.

[00233] Another category of promoters useful in the invention are wound inducible promoters. Numerous promoters have been described that are expressed at wound sites and also at the sites of phytopathogen infection. Ideally, such a promoter should only be active locally at the sites of insect invasion, and in this way the insecticidal proteins only accumulate in cells that need to synthesize the insecticidal proteins to kill the invading insect pest. Examples of promoters of this kind include those described by Stanford et al. *Mol. Gen. Genet.* 215:200-208 (1989), Xu et al. *Plant Molec. Biol.* 22:573-588 (1993), Logemann et al. *Plant Cell* 1:151-158 (1989), Rohrmeier & Lehle, *Plant Molec. Biol.* 22:783-792 (1993), Firek et al. *Plant Molec. Biol.* 22:129-142 (1993), and Warner et al. *Plant J.* 3:191-201 (1993).

[00234] In embodiments a nucleic acid of the invention can comprise, consist essentially of, or consist of an expression cassette, or can be comprised within an expression cassette.

[00235] An expression cassette comprising a polynucleotide of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one other of its other components. An expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, the expression cassette is heterologous with respect to the host, *i.e.*, the particular nucleic acid sequence of the expression cassette does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event.

[00236] In addition to the promoters operatively associated with the nucleotide sequences of the invention, an expression cassette of this invention can also include other regulatory elements. Regulatory elements include, but are not limited to, enhancers, introns, translation leader sequences, termination signals, and polyadenylation signal sequences. Examples of suitable transcription terminator signals are available and known in the art (*e.g.*, tml from CaMV, E9 from *rbcS*). Any

available terminator known to function in plants can be used in the context of this invention.

[00237] Numerous other sequences can be incorporated into expression cassettes described in this invention. These include sequences that have been shown to enhance expression such as intron sequences (*e.g.*, from Adh1 and bronzel) and viral leader sequences (*e.g.*, from TMV, MCMV and AMV).

[00238] For more efficient initiation of translation, sequences adjacent to the initiating methionine may be modified. For example, they can be modified by the inclusion of sequences known to be effective in plants. Joshi has suggested an appropriate consensus for plants (NAR 15:6643-6653 (1987)) and Clonetech suggests a further consensus translation initiator (1993/1994 catalog, page 210). These consensus sequences are suitable for use with the nucleotide sequences of this invention. The sequences are incorporated into constructions comprising the nucleotide sequences, up to and including the ATG (while leaving the second amino acid unmodified), or alternatively up to and including the GTC subsequent to the ATG (with the possibility of modifying the second amino acid of the transgene).

[00239] In embodiments, it may be desired to target expression of the polypeptides of the present invention to a specific cellular location in the plant cell. In some cases, localization in the cytosol may be desirable, whereas in other cases, localization in some subcellular organelle may be preferred. Any mechanism for targeting gene products, *e.g.*, in plants, can be used to practice this invention, and such mechanisms are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. Sequences have been characterized which cause the targeting of gene products to other cell compartments. For example, amino terminal sequences can be responsible for targeting a protein of interest to a cell compartment, such as, a vacuole, mitochondrion, peroxisome, protein bodies, endoplasmic reticulum, chloroplast, starch granule, amyloplast, apoplast or cell wall of a plant cell (*e.g.* Unger et. al. Plant Molec. Biol. 13: 411-418 (1989); Rogers et. al. (1985) Proc. Natl. Acad. Sci. USA 82: 6512-651; U.S. Pat. No. 7,102,057; WO 2005/096704).

Optionally, the signal sequence may be an N-terminal signal sequence from waxy, an N-terminal signal sequence from gamma-zein, a starch binding domain, a C-terminal starch binding domain, a chloroplast targeting sequence, which imports the mature protein to the chloroplast (Comai et. al. (1988) J. Biol. Chem. 263: 15104-15109; van den Broeck, et. al. (1985) Nature 313: 358-363; U.S. Pat. No. 5,639,949) or a secretion signal sequence from aleurone cells (Koehler & Ho, Plant Cell 2: 769-783 (1990)). Additionally, amino terminal sequences in conjunction with carboxyl terminal sequences are responsible for vacuolar targeting of gene products and can be used with the present invention (Shinshi et. al. (1990) Plant Molec. Biol. 14: 357-368). In one embodiment, the signal sequence selected includes the known cleavage site, and the fusion constructed takes into account any amino acids after the cleavage site(s), which are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or, alternatively, replacement of some amino acids within the transgene sequence. These construction techniques are well known in the art and are equally applicable to any cellular compartment.

[00240] It will be recognized that the above-described mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell-targeting goal under the transcriptional regulation of a promoter that has an expression pattern different to that of the promoter from which the targeting signal derives.

[00241] An expression cassette of the invention also can include a nucleotide sequence for a selectable marker, which can be used to select a transformed plant, plant part and/or plant cell. Many examples of suitable selectable markers are known in the art and can be used in the expression cassettes described herein.

[00242] Examples of selectable markers include, but are not limited to, a nucleotide sequence encoding neo or nptII, which confers resistance to kanamycin, G418, and the like (Potrykus et al. (1985) Mol. Gen. Genet. 199:183-188); a nucleotide sequence encoding bar, which confers resistance to phosphinothricin; a nucleotide

sequence encoding an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which confers resistance to glyphosate (Hinchee et al. (1988) Biotech. 6:915-922); a nucleotide sequence encoding a nitrilase such as *bxn* from *Klebsiella ozaenae* that confers resistance to bromoxynil (Stalker et al. (1988) Science 242:419-423); a nucleotide sequence encoding an altered acetolactate synthase (ALS) that confers resistance to imidazolinone, sulfonyleurea or other ALS-inhibiting chemicals (EP Patent Application No. 154204); a nucleotide sequence encoding a methotrexate-resistant dihydrofolate reductase (DHFR) (Thillet et al. (1988) J. Biol. Chem. 263:12500-12508); a nucleotide sequence encoding a dalapon dehalogenase that confers resistance to dalapon; a nucleotide sequence encoding a mannose-6-phosphate isomerase (also referred to as phosphomannose isomerase (PMI)) that confers an ability to metabolize mannose (US Patent Nos. 5,767,378 and 5,994,629); a nucleotide sequence encoding an altered anthranilate synthase that confers resistance to 5-methyl tryptophan; or a nucleotide sequence encoding *hph* that confers resistance to hygromycin. One of skill in the art is capable of choosing a suitable selectable marker for use in an expression cassette of this invention.

[00243] Additional selectable markers include, but are not limited to, a nucleotide sequence encoding β -glucuronidase or *uidA* (GUS) that encodes an enzyme for which various chromogenic substrates are known; an R-locus nucleotide sequence that encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta et al., "Molecular cloning of the maize R-nj allele by transposon-tagging with Ac" 263-282 In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium (Gustafson & Appels eds., Plenum Press 1988)); a nucleotide sequence encoding β -lactamase, an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin) (Sutcliffe (1978) Proc. Natl. Acad. Sci. USA 75:3737-3741); a nucleotide sequence encoding *xyle* that encodes a catechol dioxygenase (Zukowsky et al. (1983) Proc. Natl. Acad. Sci. USA 80:1101-1105); a nucleotide sequence encoding tyrosinase, an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone, which in turn condenses to form melanin (Katz et al. (1983) J.

Gen. Microbiol. 129:2703-2714); a nucleotide sequence encoding β -galactosidase, an enzyme for which there are chromogenic substrates; a nucleotide sequence encoding luciferase (lux) that allows for bioluminescence detection (Ow et al. (1986) Science 234:856-859); a nucleotide sequence encoding aequorin which may be employed in calcium-sensitive bioluminescence detection (Prasher et al. (1985) Biochem. Biophys. Res. Comm. 126:1259-1268); or a nucleotide sequence encoding green fluorescent protein (Niedz et al. (1995) Plant Cell Reports 14:403-406). One of skill in the art can choose a suitable selectable marker for use in an expression cassette of this invention.

[00244] In some embodiments, an expression cassette of the invention also can include polynucleotides that encode other desired traits in addition to the chimeric insecticidal proteins of the invention. Examples of such other polynucleotides include that those encode a polypeptide or a dsRNA for the other desired trait(s) of interest. Such expression cassettes comprising the “stacked” traits may be used, *e.g.*, to create plants, plant parts or plant cells having a desired phenotype with the stacked traits (*i.e.*, molecular stacking). Such stacked combinations in plants can also be created by other methods including, but not limited to, cross breeding plants by any conventional methodology (*i.e.*, a breeding stack). If stacked by genetically transforming the plants, the nucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The additional nucleotide sequences can be introduced simultaneously in a co-transformation protocol with a nucleotide sequence, nucleic acid molecule, nucleic acid construct, or composition of this invention, provided by any combination of expression cassettes. For example, if two nucleotide sequences will be introduced, they can be incorporated in separate cassettes (*trans*) or can be incorporated on the same cassette (*cis*). Expression of polynucleotides can be driven by the same promoter or by different promoters. It is further recognized that polynucleotides can be stacked at a desired genomic location using a site-specific

recombination system. *See, e.g.*, Int'l Patent Application Publication Nos. WO 99/25821; WO 99/25854; WO 99/25840; WO 99/25855 and WO 99/25853.

[00245] In representative embodiments, the expression cassette can also include an additional coding sequence for one or more polypeptides or double stranded RNA molecules (dsRNA) of interest for an agronomic trait (*e.g.*, an agronomic trait that is primarily of benefit to a seed company, grower or grain processor). A polypeptide of interest can be any polypeptide encoded by a nucleotide sequence of interest. Non-limiting examples of polypeptides of interest that are suitable for production in plants include those resulting in agronomically important traits such as herbicide resistance (also sometimes referred to as "herbicide tolerance"), virus resistance, bacterial pathogen resistance, insect resistance, nematode resistance, or fungal resistance. *See, e.g.*, U.S. Patent Nos. 5,569,823; 5,304,730; 5,495,071; 6,329,504; and 6,337,431. In embodiments, the polypeptide of interest can be one that increases plant vigor or yield (including traits that allow a plant to grow at different temperatures, soil conditions and levels of sunlight and precipitation), or one that allows identification of a plant exhibiting a trait of interest (*e.g.*, a selectable marker, seed coat color, *etc.*). Various polypeptides of interest, as well as methods for introducing these polypeptides into a plant, are described, for example, in US Patent Nos. 4,761,373; 4,769,061; 4,810,648; 4,940,835; 4,975,374; 5,013,659; 5,162,602; 5,276,268; 5,304,730; 5,495,071; 5,554,798; 5,561,236; 5,569,823; 5,767,366; 5,879,903; 5,928,937; 6,084,155; 6,329,504 and 6,337,431; as well as US Patent Publication No. 2001/0016956. *See also*, on the World Wide Web at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/.

[00246] Polynucleotides conferring resistance/tolerance to an herbicide that inhibits the growing point or meristem, such as an imidazalinone or a sulfonylurea can also be suitable in some embodiments of the invention. Exemplary polynucleotides in this category code for mutant ALS and AHAS enzymes as described, *e.g.*, in U.S. Patent Nos. 5,767,366 and 5,928,937. U.S. Patent Nos. 4,761,373 and 5,013,659 are directed to plants resistant to various imidazalinone or sulfonamide herbicides. U.S. Patent No. 4,975,374 relates to plant cells and plants containing a nucleic acid

encoding a mutant glutamine synthetase (GS) resistant to inhibition by herbicides that are known to inhibit GS, *e.g.*, phosphinothricin and methionine sulfoximine. U.S. Patent No. 5,162,602 discloses plants resistant to inhibition by cyclohexanedione and aryloxyphenoxypropanoic acid herbicides. The resistance is conferred by an altered acetyl coenzyme A carboxylase (ACCase).

[00247] Polypeptides encoded by nucleotides sequences conferring resistance to glyphosate are also suitable for the invention. *See, e.g.*, U.S. Patent No. 4,940,835 and U.S. Patent No. 4,769,061. U.S. Patent No. 5,554,798 discloses transgenic glyphosate resistant maize plants, which resistance is conferred by an altered 5-enolpyruvyl-3-phosphoshikimate (EPSP) synthase gene.

[00248] Polynucleotides coding for resistance to phosphono compounds such as glufosinate ammonium or phosphinothricin, and pyridinoxy or phenoxy propionic acids and cyclohexones are also suitable. *See*, European Patent Application No. 0 242 246. *See also*, U.S. Patent Nos. 5,879,903, 5,276,268 and 5,561,236.

[00249] Other suitable polynucleotides include those coding for resistance to herbicides that inhibit photosynthesis, such as a triazine and a benzonitrile (nitrilase). *See*, U.S. Patent No. 4,810,648. Additional suitable polynucleotides coding for herbicide resistance include those coding for resistance to 2,2-dichloropropionic acid, sethoxydim, haloxyfop, imidazolinone herbicides, sulfonylurea herbicides, triazolopyrimidine herbicides, s-triazine herbicides and bromoxynil. Also suitable are polynucleotides conferring resistance to a protox enzyme, or that provide enhanced resistance to plant diseases; enhanced tolerance of adverse environmental conditions (abiotic stresses) including but not limited to drought, excessive cold, excessive heat, or excessive soil salinity or extreme acidity or alkalinity; and alterations in plant architecture or development, including changes in developmental timing. *See, e.g.*, U.S. Patent Publication No. 2001/0016956 and U.S. Patent No. 6,084,155.

[00250] Additional suitable polynucleotides include those coding for pesticidal (*e.g.*, insecticidal) polypeptides. These polypeptides may be produced in amounts sufficient to control, for example, insect pests (*i.e.*, insect controlling amounts). In

embodiments, the polypeptide is a lepidopteran-active, coleopteran-active, hemipteran-active and/or dipteran-active polypeptide, or any combination thereof. It is recognized that the amount of production of a pesticidal polypeptide in a plant necessary to control insects or other pests may vary depending upon the cultivar, type of pest, environmental factors and the like. Polynucleotides useful for additional insect or pest resistance include, for example, those that encode toxins identified in *Bacillus* organisms. Polynucleotides comprising nucleotide sequences encoding *Bacillus thuringiensis* (Bt) Cry proteins from several subspecies have been cloned and recombinant clones have been found to be toxic to lepidopteran, dipteran and coleopteran insect larvae. Examples of such *Bt* insecticidal proteins include the Cry proteins such as Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, Cry1D, Cry1Ea, Cry1Fa, Cry3A, Cry9A, Cry9B, Cry9C, and the like, as well as vegetative insecticidal proteins such as Vip1, Vip2, Vip3, and the like, and any combination of the foregoing *Bt* insecticidal proteins. A full list of *Bt*-derived proteins can be found on the worldwide web at *Bacillus thuringiensis* Toxin Nomenclature Database maintained by the University of Sussex (see also, Crickmore et al. (1998) Microbiol. Mol. Biol. Rev. 62:807-813).

[00251] In embodiments, an additional polypeptide is an insecticidal polypeptide derived from a non-*Bt* source, including without limitation, an alpha-amylase, a peroxidase, a cholesterol oxidase, a patatin, a protease, a protease inhibitor, a urease, an alpha-amylase inhibitor, a pore-forming protein, a chitinase, a lectin, an engineered antibody or antibody fragment, a *Bacillus cereus* insecticidal protein, a *Xenorhabdus* spp. (such as *X. nematophila* or *X. bovienii*) insecticidal protein, a *Photorhabdus* spp. (such as *P. luminescens* or *P. asymobiotica*) insecticidal protein, a *Brevibacillus* spp. (such as *B. laterosporous*) insecticidal protein, a *Lysinibacillus* spp. (such as *L. sphearicus*) insecticidal protein, a *Chromobacterium* spp. (such as *C. subtsugae* or *C. piscinae*) insecticidal protein, a *Yersinia* spp. (such as *Y. entomophaga*) insecticidal protein, a *Paenibacillus* spp. (such as *P. propylaea*) insecticidal protein, a *Clostridium* spp. (such as *C. bifermentans*) insecticidal protein, a *Pseudomonas* spp. (such as *P. fluorescens*) and a lignin.

[00252] Polypeptides that are suitable for production in plants further include those that improve or otherwise facilitate the conversion of harvested plants or plant parts into a commercially useful product, including, for example, increased or altered carbohydrate content or distribution, improved fermentation properties, increased oil content, increased protein content, improved digestibility, and increased nutraceutical content, *e.g.*, increased phytosterol content, increased tocopherol content, increased stanol content or increased vitamin content. Polypeptides of interest also include, for example, those resulting in or contributing to a reduced content of an unwanted component in a harvested crop, *e.g.*, phytic acid, or sugar degrading enzymes. By “resulting in” or “contributing to” is intended that the polypeptide of interest can directly or indirectly contribute to the existence of a trait of interest (*e.g.*, increasing cellulose degradation by the use of a heterologous cellulase enzyme).

[00253] In some embodiments, the polypeptide contributes to improved digestibility for food or feed. Xylanases are hemicellulolytic enzymes that improve the breakdown of plant cell walls, which leads to better utilization of the plant nutrients by an animal. This leads to improved growth rate and feed conversion. Also, the viscosity of the feeds containing xylan can be reduced. Heterologous production of xylanases in plant cells also can facilitate lignocellulosic conversion to fermentable sugars in industrial processing.

[00254] Numerous xylanases from fungal and bacterial microorganisms have been identified and characterized (*see, e.g.*, U.S. Patent No. 5,437,992; Coughlin et al. (1993) “Proceedings of the Second TRICEL Symposium on *Trichoderma reesei* Cellulases and Other Hydrolases” Espoo; Souminen and Reinikainen, eds. (1993) Foundation for Biotechnical and Industrial Fermentation Research 8:125-135; U.S. Patent Publication No. 2005/0208178; and PCT Publication No. WO 03/16654). In particular, three specific xylanases (XYL-I, XYL-II, and XYL-III) have been identified in *T. reesei* (Tenkanen et al. (1992) *Enzyme Microb. Technol.* 14:566; Torronen et al. (1992) *Bio/Technology* 10:1461; and Xu et al. (1998) *Appl. Microbiol. Biotechnol.* 49:718).

[00255] In other embodiments, a polypeptide useful for the invention can be a polysaccharide degrading enzyme. Plants of this invention producing such an enzyme may be useful for generating, for example, fermentation feedstocks for bioprocessing. In some embodiments, enzymes useful for a fermentation process include alpha amylases, proteases, pullulanases, isoamylases, cellulases, hemicellulases, xylanases, cyclodextrin glycotransferases, lipases, phytases, laccases, oxidases, esterases, cutinases, granular starch hydrolyzing enzyme and other glucoamylases.

[00256] Polysaccharide-degrading enzymes include: starch degrading enzymes such as alpha-amylases (EC 3.2.1.1), glucuronidases (E.C. 3.2.1.131); exo-1,4-alpha-D glucanases such as amyloglucosidases and glucoamylase (EC 3.2.1.3), beta-amylases (EC 3.2.1.2), alpha-glucosidases (EC 3.2.1.20), and other exo-amylases; starch debranching enzymes, such as a) isoamylase (EC 3.2.1.68), pullulanase (EC 3.2.1.41), and the like; b) cellulases such as exo-1,4-3-cellobiohydrolase (EC 3.2.1.91), exo-1,3-beta-D-glucanase (EC 3.2.1.39), beta-glucosidase (EC 3.2.1.21); c) L-arabinases, such as endo-1,5-alpha-L-arabinase (EC 3.2.1.99), alpha-arabinosidases (EC 3.2.1.55) and the like; d) galactanases such as endo-1,4-beta-D-galactanase (EC 3.2.1.89), endo-1,3-beta-D-galactanase (EC 3.2.1.90), alpha-galactosidase (EC 3.2.1.22), beta-galactosidase (EC 3.2.1.23) and the like; e) mannanases, such as endo-1,4-beta-D-mannanase (EC 3.2.1.78), beta-mannosidase (EC 3.2.1.25), alpha-mannosidase (EC 3.2.1.24) and the like; f) xylanases, such as endo-1,4-beta-xylanase (EC 3.2.1.8), beta-D-xylosidase (EC 3.2.1.37), 1,3-beta-D-xylanase, and the like; and g) other enzymes such as alpha-L-fucosidase (EC 3.2.1.51), alpha-L-rhamnosidase (EC 3.2.1.40), levanase (EC 3.2.1.65), inulanase (EC 3.2.1.7), and the like. In one embodiment, the alpha-amylase is the synthetic alpha-amylase, Amy797E, described in US Patent No. 8,093,453.

[00257] Further enzymes which may be used with the invention include proteases, such as fungal and bacterial proteases. Fungal proteases include, but are not limited to, those obtained from *Aspergillus*, *Trichoderma*, *Mucor* and *Rhizopus*, such

as *A. niger*, *A. awamori*, *A. oryzae* and *M. miehei*. In some embodiments, the polypeptides of this invention can be cellobiohydrolase (CBH) enzymes (EC 3.2.1.91). In one embodiment, the cellobiohydrolase enzyme can be CBH1 or CBH2.

[00258] Other enzymes useful with the invention include, but are not limited to, hemicellulases, such as mannases and arabinofuranosidases (EC 3.2.1.55); ligninases; lipases (*e.g.*, E.C. 3.1.1.3), glucose oxidases, pectinases, xylanases, transglucosidases, alpha 1,6 glucosidases (*e.g.*, E.C. 3.2.1.20); esterases such as ferulic acid esterase (EC 3.1.1.73) and acetyl xylan esterases (EC 3.1.1.72); and cutinases (*e.g.*, E.C. 3.1.1.74).

[00259] Double stranded RNA (dsRNA) molecules useful with the invention include, but are not limited to those that suppress target pest (*e.g.*, insect) genes. In embodiments, the dsRNA targets a gene in a lepidopteran, coleopteran, hemipteran or dipteran insect pest, or any combination of the foregoing. As used herein the words "gene suppression", when taken together, are intended to refer to any of the well-known methods for reducing the levels of protein produced as a result of gene transcription to mRNA and subsequent translation of the mRNA. Gene suppression is also intended to mean the reduction of protein expression from a gene or a coding sequence including posttranscriptional gene suppression and transcriptional suppression. Posttranscriptional gene suppression is mediated by the homology between all or a part of a mRNA transcribed from a gene or coding sequence targeted for suppression and the corresponding double stranded RNA used for suppression, and refers to the substantial and measurable reduction of the amount of available mRNA available in the cell for binding by ribosomes. The transcribed RNA can be in the sense orientation to effect what is called co-suppression, in the anti-sense orientation to effect what is called anti-sense suppression, or in both orientations producing a dsRNA to effect what is called RNA interference (RNAi). Transcriptional suppression is mediated by the presence in the cell of a dsRNA, a gene suppression agent, exhibiting substantial sequence identity to a promoter DNA sequence or the complement thereof to effect what is referred to as promoter trans

suppression. Gene suppression may be effective against a native plant gene associated with a trait, *e.g.*, to provide plants with reduced levels of a protein encoded by the native gene or with enhanced or reduced levels of an affected metabolite. Gene suppression can also be effective against target genes in plant pests that may ingest or contact plant material containing gene suppression agents, specifically designed to inhibit or suppress the expression of one or more homologous or complementary sequences in the cells of the pest. Such genes targeted for suppression can encode an essential protein, the predicted function of which is selected from the group consisting of muscle formation, juvenile hormone formation, juvenile hormone regulation, ion regulation and transport, digestive enzyme synthesis, maintenance of cell membrane potential, amino acid biosynthesis, amino acid degradation, sperm formation, pheromone synthesis, pheromone sensing, antennae formation, wing formation, leg formation, development and differentiation, egg formation, larval maturation, digestive enzyme formation, haemolymph synthesis, haemolymph maintenance, neurotransmission, cell division, energy metabolism, respiration, and apoptosis.

[00260] In embodiments, the nucleic acids of the invention can further comprise, consist essentially of, or consist of a vector. In embodiments, the polynucleotides and expression cassettes of the invention are comprised within a vector. Vectors for use in transformation of plants and other organisms are well known in the art. Non-limiting examples of general classes of vectors include a plasmid, phage vector, phagemid vector, cosmid vector, fosmid, bacteriophage, artificial chromosome, or a viral vector. In embodiments, the vector is plant vector, *e.g.*, for use in transformation of plants. In embodiments, the vector is a bacterial vector, *e.g.*, for use in transformation of bacteria. Suitable vectors for plants, bacteria and other organisms are known in the art.

Transgenic Plants, Plant Parts, Plant Cells, Seed.

[00261] The invention also encompasses a transgenic non-human host cell comprising a polynucleotide, a nucleic acid molecule, an expression cassette, a

vector, or a polypeptide of the invention. The transgenic non-human host cell can include, but is not limited to, a plant cell (including a monocot cell and/or a dicot cell), a yeast cell, a bacterial cell or an insect cell. Accordingly, in some embodiments, the invention provides a bacterial cell selected from the genera *Bacillus*, *Brevibacillus*, *Clostridium*, *Xenorhabdus*, *Photorhabdus*, *Pasteuria*, *Escherichia*, *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Salmonella*, *Pasteurella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, or *Alcaligenes*. Thus, for example, as biological insect control agents, the chimeric insecticidal proteins of the invention can be produced by expression of a polynucleotide encoding the same in a bacterial cell. For example, in some embodiments, a *Bacillus thuringiensis* cell comprising a polynucleotide encoding a chimeric insecticidal protein of the invention is provided.

[00262] In embodiments, the transgenic plant cell is a dicot plant cell or a monocot plant cell. In additional embodiments, the dicot plant cell is a soybean cell, sunflower cell, tomato cell, cole crop cell, cotton cell, sugar beet cell or a tobacco cell. In further embodiments, the monocot cell is a barley cell, maize cell, oat cell, rice cell, sorghum cell, sugar cane cell or wheat cell. In embodiments, the invention provides a plurality of dicot cells or monocot cells comprising a polynucleotide expressing a chimeric insecticidal protein of the invention. In embodiments, the plurality of cells are juxtaposed to form an apoplast and are grown in natural sunlight. In embodiments, the transgenic plant cell cannot regenerate a whole plant.

[00263] In embodiments of the invention, an insecticidal protein of the invention is expressed in a higher organism, for example, a plant. In this case, transgenic plants expressing effective amounts of the insecticidal protein protect themselves from plant pests such as insect pests. When an insect starts feeding on such a transgenic plant, it ingests the expressed insecticidal protein. This can deter the insect from further biting into the plant tissue or may even harm or kill the insect. In embodiments, a polynucleotide of the invention is inserted into an expression cassette, which is then stably integrated in the genome of the plant. In other

embodiments, the polynucleotide is included in a non-pathogenic self-replicating virus.

[00264] In some embodiments of the invention, a transgenic plant cell comprising a nucleic acid molecule or polypeptide of the invention is a cell of a plant part, a plant organ or a plant culture (each as described herein) including, but not limited to, a root, a leaf, a seed, a flower, a fruit, a pollen cell, organ or plant culture, and the like, or a callus cell or culture.

[00265] A transgenic plant or plant cell in accordance with the invention may be a monocot or dicot plant or plant cell and includes, but is not limited to, corn (maize), soybean, rice, wheat, barley, rye, oat, sorghum, millet, sunflower, safflower, sugar beet, cotton, sugarcane, oilseed rape, alfalfa, tobacco, peanut, vegetable (including, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, carrot, eggplant, cucumber, radish, spinach, potato, tomato, asparagus, onion, garlic, melon, pepper, celery, squash, pumpkin, zucchini, and the like), fruit (including, apple, pear, quince, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, and the like), a specialty plant or plant cell (such as *Arabidopsis*), or a woody plant or plant cell (such as coniferous and/or deciduous trees). In embodiments, a plant or plant cell of the of the invention is a crop plant or plant cell such as maize, sorghum, wheat, sunflower, tomato, a crucifer, pepper, potato, cotton, rice, soybean, sugar beet, sugarcane, tobacco, barley, oilseed rape plant or plant cell, and the like.

[00266] The invention further provides a part of a transgenic plant of the invention. Optionally, the plant part comprises a chimeric insecticidal protein of the invention and/or a nucleic acid encoding the same.

[00267] The invention further provides a seed of a transgenic plant of the invention or a seed that produces the transgenic plant of the invention. Optionally, the seed comprises a chimeric insecticidal protein of the invention and/or a nucleic acid encoding the same.

[00268] Additional embodiments of the invention include harvested products produced from the transgenic plants, plant parts or seed of the invention, as well as a

processed product produced from a harvested product. A harvested product can be a whole plant or any plant part, as described herein. Thus, in some embodiments, non-limiting examples of a harvested product include a seed, a fruit, a flower or part thereof (*e.g.*, an anther, a stigma, and the like), a leaf, a stem, and the like. In other embodiments, a processed product includes, but is not limited to, a flour, meal, oil, starch, cereal, and the like produced from a harvested seed or other plant part of the invention. . Optionally, the harvested product or the processed product comprises a chimeric insecticidal protein of the invention and/or a nucleic acid encoding the same.

[00269] In other embodiments, the invention provides an extract from a transgenic plant, plant part or of the invention, optionally wherein the extract comprises a chimeric insecticidal protein of the invention and/or a nucleic acid encoding the same. Extracts from plants or plant parts can be made according to procedures well known in the art (See, de la Torre et al., *Food, Agric. Environ.* 2(1):84-89 (2004); Guidet, *Nucleic Acids Res.* 22(9): 1772-1773 (1994); Lipton et al., *Food Agric. Immun.* 12:153-164 (2000)).

[00270] The chimeric insecticidal protein can function in the plant part, plant cell, plant organ, seed, harvested product, processed product or extract, and the like, as an insect control agent. In other words, the chimeric insecticidal protein can continue to perform the insecticidal function it had in the transgenic plant. The nucleic acid can function to express the chimeric insecticidal protein. As an alternative to encoding the insecticidal protein of the invention, the nucleic acid can function to identify a transgenic plant part, plant cell, plant organ, seed, harvested product, processed product or extract of the invention.

[00271] In embodiments, a transgenic plant, plant part, plant cell, plant organ, or seed of the invention is hemizygous for a polynucleotide or expression cassette of the invention. In embodiments, a transgenic plant, plant part, plant cell, plant organ, or seed of the invention is homozygous for a polynucleotide or expression cassette of the invention.

[00272] In embodiments, a transgenic plant, plant part, plant cell, plant organ, seed, harvested product, processed product or extract has increased resistance to one or more insect pests (*e.g.*, a lepidopteran pest, such as fall armyworm) as compared with a suitable control that does not comprise a nucleic acid encoding an insecticidal protein of the invention.

Plant Transformation.

[00273] Procedures for transforming plants are well known and routine in the art and are described throughout the literature. Non-limiting examples of methods for transformation of plants include transformation via bacterial-mediated nucleic acid delivery (*e.g.*, via *Agrobacterium*), viral-mediated nucleic acid delivery, silicon carbide or nucleic acid whisker-mediated nucleic acid delivery, liposome mediated nucleic acid delivery, microinjection, microparticle bombardment, calcium-phosphate-mediated transformation, cyclodextrin-mediated transformation, electroporation, nanoparticle-mediated transformation, sonication, infiltration, PEG-mediated nucleic acid uptake, as well as any other electrical, chemical, physical (mechanical) or biological mechanism that results in the introduction of nucleic acid into the plant cell, including any combination thereof. General guides to various plant transformation methods known in the art include Miki et al. ("Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B. R. and Thompson, J. E., Eds. (CRC Press, Inc., Boca Raton, 1993), pages 67-88) and Rakowoczy-Trojanowska (*Cell. Mol. Biol. Lett.* 7:849-858 (2002)).

[00274] For *Agrobacterium*-mediated transformation, binary vectors or vectors carrying at least one T-DNA border sequence are generally suitable, whereas for direct gene transfer (*e.g.*, particle bombardment and the like) any vector is suitable and linear DNA containing only the construction of interest can be used. In the case of direct gene transfer, transformation with a single DNA species or co-transformation can be used (Schocher et al., *Biotechnology* 4:1093- 1096 (1986)). For both direct gene transfer and *Agrobacterium*-mediated transfer, transformation is

usually (but not necessarily) undertaken with a selectable marker that may be a positive selection (e.g., Phosphomannose Isomerase), provide resistance to an antibiotic (e.g., kanamycin, hygromycin or methotrexate) or a herbicide (e.g., glyphosate or glufosinate). However, the choice of selectable marker is not critical to the invention.

[00275] *Agrobacterium*-mediated transformation is a commonly used method for transforming plants because of its high efficiency of transformation and because of its broad utility with many different species. *Agrobacterium*-mediated transformation typically involves transfer of the binary vector carrying the foreign DNA of interest to an appropriate *Agrobacterium* strain that may depend on the complement of vir genes carried by the host *Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (Uknes et al. (1993) Plant Cell 5:159-169). The transfer of the recombinant binary vector to *Agrobacterium* can be accomplished by a triparental mating procedure using *Escherichia coli* carrying the recombinant binary vector, a helper *E. coli* strain that carries a plasmid that is able to mobilize the recombinant binary vector to the target *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to *Agrobacterium* by nucleic acid transformation (Höfgen & Willmitzer (1988) Nucleic Acids Res. 16:9877).

[00276] Dicots as well as monocots may be transformed using *Agrobacterium*. Methods for *Agrobacterium*-mediated transformation of rice include well known methods for rice transformation, such as those described in any of the following: European patent application EP 1198985 A1, Aldemita and Hodges (Planta 199: 612-617, 1996); Chan et al. (Plant Mol Biol 22 (3): 491-506, 1993), Hiei et al. (Plant J 6 (2): 271-282, 1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol 14(6): 745-50, 1996) or Frame et al. (Plant Physiol 129(1): 13-22, 2002), which disclosures are incorporated by reference herein as if fully set forth. Said methods are further described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S. D. Kung and R. Wu, Academic Press (1993)

128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The nucleic acids or the construct to be expressed is preferably cloned into a vector, which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). *Agrobacteria* transformed by such a vector can then be used in known manner for the transformation of plants, such as plants used as a model, like *Arabidopsis* or crop plants such as, by way of example, tobacco plants, for example by immersing bruised leaves or chopped leaves in an *Agrobacterial* solution and then culturing them in suitable media. The transformation of plants by means of *Agrobacterium tumefaciens* is described, for example, by Hagen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F. F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S. D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

[00277] Transformation of a plant by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows methods well known in the art. Transformed tissue is regenerated on selection medium carrying an antibiotic or herbicide resistance marker between the binary plasmid T-DNA borders.

[00278] As discussed previously, another method for transforming plants, plant parts and plant cells involves propelling inert or biologically active particles at plant tissues and cells. See, e.g., US Patent Nos. 4,945,050; 5,036,006 and 5,100,792.

Generally, this method involves propelling inert or biologically active particles at the plant cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the nucleic acid of interest. Alternatively, a cell or cells can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., a dried yeast cell, a dried bacterium or a bacteriophage, each containing one or more nucleic acids sought to be introduced) also can be propelled into plant tissue.

[00279] In other embodiments, a polynucleotide of the invention can be directly transformed into the plastid genome. A major advantage of plastid transformation is that plastids are generally capable of expressing bacterial genes without substantial modification, and plastids are capable of expressing multiple open reading frames under control of a single promoter. Plastid transformation technology is extensively described in U.S. Patent Nos. 5,451,513, 5,545,817, and 5,545,818, in PCT application no. WO 95/16783, and in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91, 7301-7305. The basic technique for chloroplast transformation involves introducing regions of cloned plastid DNA flanking a selectable marker together with the gene of interest into a suitable target tissue, *e.g.*, using biolistics or protoplast transformation (*e.g.*, calcium chloride or PEG mediated transformation). The 1 to 1.5 kb flanking regions, termed targeting sequences, facilitate homologous recombination with the plastid genome and thus allow the replacement or modification of specific regions of the plastome. Initially, point mutations in the chloroplast 16S rRNA and *rps12* genes conferring resistance to spectinomycin or streptomycin can be utilized as selectable markers for transformation (Svab, Z., Hajdukiewicz, P., and Maliga, P. (1990) Proc. Natl. Acad. Sci. USA 87, 8526-8530; Staub, J. M., and Maliga, P. (1992) Plant Cell 4, 39-45). The presence of cloning sites between these markers allows creation of a plastid targeting vector for introduction of foreign genes (Staub, J.M., and Maliga, P. (1993) EMBO J. 12, 601-606). Substantial increases in transformation frequency can be obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial *aadA* gene encoding the spectinomycin-cleaving enzyme aminoglycoside- 3'- adenylyltransferase (Svab, Z., and Maliga, P. (1993) Proc. Natl. Acad. Sci. USA 90, 913-917). Previously, this marker had been used successfully for high-frequency transformation of the plastid genome of the green alga *Chlamydomonas reinhardtii* (Goldschmidt-Clermont, M. (1991) Nucl. Acids Res. 19:4083-4089). Other selectable markers useful for plastid transformation are known in the art and encompassed within the scope of the invention. Typically, approximately 15-20 cell division cycles following

transformation are required to reach a homoplastidic state. Plastid expression, in which genes are inserted by homologous recombination into all of the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear-expressed genes to permit expression levels that can readily exceed 10% of the total soluble plant protein. In one embodiment, a polynucleotide of the invention can be inserted into a plastid-targeting vector and transformed into the plastid genome of a desired plant host. Thus, plants homoplastic for plastid genomes containing a nucleotide sequence of the invention can be obtained, which are capable of high expression of the polynucleotide.

[00280] Methods of selecting for transformed, transgenic plants, plant cells or plant tissue culture are routine in the art and can be employed in the methods of the invention provided herein. For example, a recombinant vector of the invention also can include an expression cassette comprising a nucleotide sequence for a selectable marker, which can be used to select a transformed plant, plant part or plant cell.

[00281] Further, as is well known in the art, intact transgenic plants can be regenerated from transformed plant cells, plant tissue culture or cultured protoplasts using any of a variety of known techniques. Plant regeneration from plant cells, plant tissue culture or cultured protoplasts is described, for example, in Evans et al. (Handbook of Plant Cell Cultures, Vol. 1, MacMilan Publishing Co. New York (1983)); and Vasil I. R. (ed.) (Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I (1984), and Vol. II (1986)).

[00282] Additionally, the genetic properties engineered into the transgenic seeds and plants, plant parts, or plant cells of the invention described above can be passed on by sexual reproduction or vegetative growth and therefore can be maintained and propagated in progeny plants. Generally, maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as harvesting, sowing or tilling.

[00283] A polynucleotide therefore can be introduced into the plant, plant part or plant cell in any number of ways that are well known in the art, as described above.

Therefore, no particular method for introducing one or more polynucleotides into a plant is relied upon, rather any method that allows the one or more polynucleotides to be stably integrated into the genome of the plant can be used. Where more than one polynucleotides is to be introduced, the respective polynucleotides can be assembled as part of a single nucleic acid molecule, or as separate nucleic acid molecules, and can be located on the same or different nucleic acid molecules. Accordingly, the polynucleotides can be introduced into the cell of interest in a single transformation event, in separate transformation events, or, for example, in plants, as part of a breeding protocol.

[00284] Once a desired polynucleotide has been transformed into a particular plant species, it may be propagated in that species or moved into other varieties of the same species, particularly including commercial varieties, using traditional breeding techniques.

Insecticidal Compositions.

[00285] In some embodiments, the invention provides an insecticidal composition comprising a chimeric insecticidal protein of the invention in an agriculturally acceptable carrier. As used herein an “agriculturally-acceptable carrier” can include natural or synthetic, organic or inorganic material which is combined with the active protein to facilitate its application to or in the plant, or part thereof. Examples of agriculturally acceptable carriers include, without limitation, powders, dusts, pellets, granules, sprays, emulsions, colloids, and solutions. Agriculturally-acceptable carriers further include, but are not limited to, inert components, dispersants, surfactants, adjuvants, tackifiers, stickers, binders, or combinations thereof, that can be used in agricultural formulations. Such compositions can be applied in any manner that brings the pesticidal proteins or other pest control agents in contact with the pests. Accordingly, the compositions can be applied to the surfaces of plants or plant parts, including seeds, leaves, flowers, stems, tubers, roots, and the like. In

other embodiments, a plant producing an insecticidal protein of the invention in planta is an agriculturally- acceptable carrier of the expressed insecticidal protein. In embodiments, the compositions and agriculturally-acceptable carriers of the invention exclude transgenic plants.

[00286] In further embodiments, the insecticidal composition comprises a bacterial cell or a transgenic bacterial cell of the invention, wherein the bacterial cell or transgenic bacterial cell produces an insecticidal protein of the invention. Such an insecticidal composition can be prepared by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of *Bacillus thuringiensis* (*Bt*), including a transgenic *Bt* culture. In embodiments, a composition of the invention may comprise at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least 99% by weight a polypeptide of the invention. In additional embodiments, the composition comprises from about 1% to about 99% by weight of the insecticidal protein of the invention.

[00287] The insecticidal proteins of the invention can be used in combination with other pest control agents to increase pest target spectrum and/or for the prevention or management of insect resistance. Furthermore, the use of the insecticidal proteins of the invention in combination with an insecticidal agent which has a different mode of action or target a different receptor in the insect gut has particular utility for the prevention and/or management of insect resistance.

[00288] Therefore, in some embodiments, the invention provides a composition that controls one or more plant pests (*e.g.*, an insect pest such as a lepidopteran insect pest, a coleopteran insect pest, a hemipteran insect pest and/or a dipteran insect pest), wherein the composition comprises a first pest control agent, which is a chimeric insecticidal protein of the invention and at least a second pest control agent that is different from the first pest control agent. In other embodiments, the composition is a formulation for topical application to a plant. In still other

embodiments, the composition is a transgenic plant. In further embodiments, the composition is a combination of a formulation topically applied to a transgenic plant. In some embodiments, the formulation comprises the first pest control agent, which is a chimeric insecticidal protein of the invention when the transgenic plant comprises the second pest control agent. In other embodiments, the formulation comprises the second pest control agent when the transgenic plant comprises the first pest control agent, which is a chimeric insecticidal protein of the invention.

[00289] In some embodiments, the second pest control agent can be one or more of a chemical pesticide, such as an insecticide, a *Bacillus thuringiensis* (Bt) insecticidal protein, and/or a non-Bt pesticidal agent including without limitation a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Brevibacillus laterosporus* insecticidal protein, a *Bacillus sphaericus* insecticidal protein, a protease inhibitor (both serine and cysteine types), a lectin, an alpha-amylase, a peroxidase, a cholesterol oxidase, or a double stranded RNA (dsRNA) molecule.

[00290] In other embodiments, the second pest control agent is one or more chemical pesticides, which is optionally a seed coating. Non-limiting examples of chemical pesticides include pyrethroids, carbamates, neonicotinoids, neuronal sodium channel blockers, insecticidal macrocyclic lactones, gamma-aminobutyric acid (GABA) antagonists, insecticidal ureas and juvenile hormone mimics. In other embodiments, the chemical pesticide is one or more of abamectin, acephate, acetamiprid, amidoflumet (S-1955), avermectin, azadirachtin, azinphos-methyl, bifenthrin, binfenazate, buprofezin, carbofuran, chlorfenapyr, chlorfluazuron, chlorpyrifos, chlorpyrifos-methyl, chromafenozide, clothianidin, cyfluthrin, beta-cyfluthrin, cyhalothrin, lambda-cyhalothrin, cypermethrin, cyromazine, deltamethrin, diafenthiuron, diazinon, diflubenzuron, dimethoate, diofenolan, emamectin, endosulfan, esfenvalerate, ethiprole, fenothicarb, fenoxycarb, fenpropathrin, fenproximate, fenvalerate, fipronil, flonicamid, flucythrinate, tau-fluvalinate, flufenerim (UR-50701), flufenoxuron, fonophos, halofenozide, hexaflumuron, imidacloprid, indoxacarb, isofenphos, lufenuron, malathion, metaldehyde, methamidophos, methidathion, methomyl, methoprene, methoxychlor,

monocrotophos, methoxyfenozide, nithiazin, novaluron, noviflumuron (XDE-007), oxamyl, parathion, parathion-methyl, permethrin, phorate, phosalone, phosmet, phosphamidon, pirimicarb, profenofos, pymetrozine, pyridalyl, pyriproxyfen, rotenone, spinosad, spiromesifin (BSN 2060), sulprofos, tebufenozide, teflubenzuron, tefluthrin, terbufos, tetrachlorvinphos, thiacloprid, thiamethoxam, thiodicarb, thiosultap-sodium, tralomethrin, trichlorfon and triflumuron, aldicarb, oxamyl, fenamiphos, amitraz, chinomethionat, chlorobenzilate, cyhexatin, dicofol, dienochlor, etoxazole, fenazaquin, fenbutatin oxide, fenpropathrin, fenpyroximate, hexythiazox, propargite, pyridaben and tebufenpyrad. In still other embodiments, the chemical pesticide is selected from one or more of cypermethrin, cyhalothrin, cyfluthrin and beta-cyfluthrin, esfenvalerate, fenvalerate, tralomethrin, fenothicarb, methomyl, oxamyl, thiodicarb, clothianidin, imidacloprid, thiacloprid, indoxacarb, spinosad, abamectin, avermectin, emamectin, endosulfan, ethiprole, fipronil, flufenoxuron, triflumuron, diofenolan, pyriproxyfen, pymetrozine and amitraz.

[00291] In additional embodiments, the second pest control agent can be one or more of any number of *Bacillus thuringiensis* insecticidal proteins including but not limited to a Cry protein, a vegetative insecticidal protein (VIP) and insecticidal chimeras of any of the preceding insecticidal proteins. In other embodiments, the second pest control agent is a Cry protein selected from: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Af, Cry1Ag, Cry1Ah, Cry1Ai, Cry1Aj, Cry1Ba, Cry1Bb, Cry1Bc, Cry1Bd, Cry1Be, Cry1Bf, Cry1Bg, Cry1Bh, Cry1Bi, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Dc, Cry1Dd, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Ga, Cry1Gb, Cry1Gc, Cry1Ha, Cry1Hb, Cry1Hc, Cry1Ia, Cry1Ib, Cry1Ic, Cry1Id, Cry1Ie, Cry1If, Cry1Ig, Cry1Ja, Cry1Jb, Cry1Jc, Cry1Jd, Cry1Ka, Cry1La, Cry1Ma, Cry1Na, Cry1Nb, Cry2Aa, Cry2Ab, Cry2Ac, Cry2Ad, Cry2Ae, Cry2Af, Cry2Ag, Cry2Ah, Cry2Ai, Cry2Aj, Cry2Ak, Cry2Al, Cry2Ba, Cry3Aa, Cry3Ba, Cry3Bb, Cry3Ca, Cry4Aa, Cry4Ba, Cry4Ca, Cry4Cb, Cry4Cc, Cry5Aa, Cry5Ab, Cry5Ac, Cry5Ad, Cry5Ba, Cry5Ca, Cry5Da, Cry5Ea, Cry6Aa, Cry6Ba, Cry7Aa, Cry7Ab, Cry7Ac, Cry7Ba, Cry7Bb, Cry7Ca, Cry7Cb, Cry7Da, Cry7Ea, Cry7Fa, Cry7Fb, Cry7Ga, Cry7Gb, Cry7Gc, Cry7Gd, Cry7Ha, Cry7Ia, Cry7Ja, Cry7Ka, Cry7Kb, Cry7La, Cry8Aa,

Cry8Ab, Cry8Ac, Cry8Ad, Cry8Ba, Cry8Bb, Cry8Bc, Cry8Ca, Cry8Da, Cry8Db, Cry8Ea, Cry8Fa, Cry8Ga, Cry8Ha, Cry8Ia, Cry8Ib, Cry8Ja, Cry8Ka, Cry8Kb, Cry8La, Cry8Ma, Cry8Na, Cry8Pa, Cry8Qa, Cry8Ra, Cry8Sa, Cry8Ta, Cry9Aa, Cry9Ba, Cry9Bb, Cry9Ca, Cry9Da, Cry9Db, Cry9Dc, Cry9Ea, Cry9Eb, Cry9Ec, Cry9Ed, Cry9Ee, Cry9Fa, Cry9Ga, Cry10Aa, Cry11Aa, Cry11Ba, Cry11Bb, Cry12Aa, Cry13Aa, Cry14Aa, Cry14Ab, Cry15Aa, Cry16Aa, Cry17Aa, Cry18Aa, Cry18Ba, Cry18Ca, Cry19Aa, Cry19Ba, Cry19Ca, Cry20Aa, Cry20Ba, Cry21Aa, Cry21Ba, Cry21Ca, Cry21Da, Cry21Ea, Cry21Fa, Cry21Ga, Cry21Ha, Cry22Aa, Cry22Ab, Cry22Ba, Cry22Bb, Cry23Aa, Cry24Aa, Cry24Ba, Cry24Ca, Cry25Aa, Cry26Aa, Cry27Aa, Cry28Aa, Cry29Aa, Cry29Ba, Cry30Aa, Cry30Ba, Cry30Ca, Cry30Da, Cry30Db, Cry30Ea, Cry30Fa, Cry30Ga, Cry31Aa, Cry31Ab, Cry31Ac, Cry31Ad, Cry32Aa, Cry32Ab, Cry32Ba, Cry32Ca, Cry32Cb, Cry32Da, Cry32Ea, Cry32Eb, Cry32Fa, Cry32Ga, Cry32Ha, Cry32Hb, Cry32Ia, Cry32Ja, Cry32Ka, Cry32La, Cry32Ma, Cry32Mb, Cry32Na, Cry32Oa, Cry32Pa, Cry32Qa, Cry32Ra, Cry32Sa, Cry32Ta, Cry32Ua, Cry33Aa, Cry34Aa, Cry34Ab, Cry34Ac, Cry34Ba, Cry35Aa, Cry35Ab, Cry35Ac, Cry35Ba, Cry36Aa, Cry37Aa, Cry38Aa, Cry39Aa, Cry40Aa, Cry40Ba, Cry40Ca, Cry40Da, Cry41Aa, Cry41Ab, Cry41Ba, Cry42Aa, Cry43Aa, Cry43Ba, Cry43Ca, Cry43Cb, Cry43Cc, Cry44Aa, Cry45Aa, Cry46Aa, Cry46Ab, Cry47Aa, Cry48Aa, Cry48Ab, Cry49Aa, Cry49Ab, Cry50Aa, Cry50Ba, Cry51Aa, Cry52Aa, Cry52Ba, Cry53Aa, Cry53Ab, Cry54Aa, Cry54Ab, Cry54Ba, Cry55Aa, Cry56Aa, Cry57Aa, Cry57Ab, Cry58Aa, Cry59Aa, Cry59Ba, Cry60Aa, Cry60Ba, Cry61Aa, Cry62Aa, Cry63Aa, Cry64Aa, Cry65Aa, Cry66Aa, Cry67Aa, Cry68Aa, Cry69Aa, Cry69Ab, Cry70Aa, Cry70Ba, Cry70Bb, Cry71Aa, Cry72Aa, Cry73Aa, or any combination of the foregoing. In embodiments, the Cry protein is a Cry1Fa, for example, as represented by maize event TC1507.

[00292] In further embodiments, the second pest control agent is one or more Vip3 vegetative insecticidal proteins selected from Vip3Aa1, Vip3Aa2, Vip3Aa3, Vip3Aa4, Vip3Aa5, Vip3Aa6, Vip3Aa7, Vip3Aa8, Vip3Aa9, Vip3Aa10, Vip3Aa11, Vip3Aa12, Vip3Aa13, Vip3Aa14, Vip3Aa15, Vip3Aa16, Vip3Aa17, Vip3Aa18, Vip3Aa19, Vip3Aa20, Vip3Aa21, Vip3Aa22, Vip3Aa23, Vip3Aa24, Vip3Aa25, Vip3Aa26,

Vip3Aa27, Vip3Aa28, Vip3Aa29, Vip3Aa30, Vip3Aa31, Vip3Aa32, Vip3Aa33, Vip3Aa34, Vip3Aa35, Vip3Aa36, Vip3Aa37, Vip3Aa38, Vip3Aa39, Vip3Aa40, Vip3Aa41, Vip3Aa42, Vip3Aa43, Vip3Aa44, Vip3Ab1, Vip3Ab2, Vip3Ac1, Vip3Ad1, Vip3Ad2, Vip3Ae1, Vip3Af1, Vip3Af2, Vip3Af3, Vip3Ag1, Vip3Ag2, Vip3Ag3, HM117633, Vip3Ag4, Vip3Ag5, Vip3Ah1, Vip3Ba1, Vip3Ba2, Vip3Bb1, Vip3Bb2, Vip3Bb3, or any combination of the foregoing. In embodiments, the Vip3 protein is Vip3Aa (US Patent No. 6,137,033), for example, as represented by corn event MIR162 (US Patent No. 8,232,456; US Patent No. 8,455,720; and US Patent No. 8,618,272).

[00293] In embodiments, the second pest control agent may be derived from sources other than *B. thuringiensis*. For example, the second pest control agent can be an alpha-amylase, a peroxidase, a cholesterol oxidase, a patatin, a protease, a protease inhibitor, a urease, an alpha-amylase inhibitor, a pore-forming protein, a chitinase, a lectin, an engineered antibody or antibody fragment, a *Bacillus cereus* insecticidal protein, a *Xenorhabdus* spp. (such as *X. nematophila* or *X. bovienii*) insecticidal protein, a *Photorhabdus* spp. (such as *P. luminescens* or *P. asymbiotica*) insecticidal protein, a *Brevibacillus* spp. (such as *B. laterosporous*) insecticidal protein, a *Lysinibacillus* spp. (such as *L. sphearicus*) insecticidal protein, a *Chromobacterium* spp. (such as *C. subtsugae* or *C. piscinae*) insecticidal protein, a *Yersinia* spp. (such as *Y. entomophaga*) insecticidal protein, a *Paenibacillus* spp. (such as *P. propylaea*) insecticidal protein, a *Clostridium* spp. (such as *C. bifermentans*) insecticidal protein, a *Pseudomonas* spp. (such as *P. fluorescens*) and a lignin. In other embodiments, the second agent may be at least one insecticidal protein derived from an insecticidal toxin complex (Tc) from *Photorhabdus*, *Xenorhabdus*, *Serratia*, or *Yersinia*. In other embodiments, the insecticidal protein may be an ADP-ribosyltransferase derived from an insecticidal bacteria, such as *Photorhabdus* spp. In other embodiments, the insecticidal protein may be a VIP protein, such as VIP1 and/or VIP2 from *B. cereus*. In still other embodiments, the insecticidal protein may be a binary toxin derived from an insecticidal bacteria, such as ISP1A and ISP2A from *B. laterosporous* or BinA and

BinB from *L. sphaericus*. In still other embodiments, the insecticidal protein may be engineered or may be a hybrid or chimera of any of the preceding insecticidal proteins.

[00294] In some embodiments, the second pesticidal agent can be non-proteinaceous, for example, an interfering RNA molecule such as a dsRNA, which can be expressed transgenically or applied as part of a composition (*e.g.*, using topical methods). An interfering RNA typically comprises at least a RNA fragment against a target gene, a spacer sequence, and a second RNA fragment which is complementary to the first, so that a double-stranded RNA structure can be formed. RNA interference (RNAi) occurs when an organism recognizes double-stranded RNA (dsRNA) molecules and hydrolyzes them. The resulting hydrolysis products are small RNA fragments of about 19–24 nucleotides in length, called small interfering RNAs (siRNAs). The siRNAs then diffuse or are carried throughout the organism, including across cellular membranes, where they hybridize to mRNAs (or other RNAs) and cause hydrolysis of the RNA. Interfering RNAs are recognized by the RNA interference silencing complex (RISC) into which an effector strand (or “guide strand”) of the RNA is loaded. This guide strand acts as a template for the recognition and destruction of the duplex sequences. This process is repeated each time the siRNA hybridizes to its complementary-RNA target, effectively preventing those mRNAs from being translated, and thus “silencing” the expression of specific genes from which the mRNAs were transcribed. Interfering RNAs are known in the art to be useful for insect control (see, for example, publication WO2013/192256, incorporated by reference herein). An interfering RNA designed for use in insect control produces a non-naturally occurring double-stranded RNA, which takes advantage of the native RNAi pathways in the insect to trigger down-regulation of target genes that may lead to the cessation of feeding and/or growth and may result in the death of the insect pest. The interfering RNA molecule may confer insect resistance against the same target pest as the protein of the invention, or may target a different pest. The targeted insect plant pest may feed by chewing, sucking, or piercing. Interfering RNAs are known in the art to be useful for insect control. In

embodiments, the dsRNA useful for insect control is described in US Provisional Application Nos. 62/371,259, 62/371,261, or 62/371,262, filed on August 5, 2016. In embodiments, the dsRNA useful for insect control is described in U.S. Patent Nos. 9,238,8223, 9,340, 797, or 8,946,510. In embodiments, the dsRNA useful for insect control is described in U.S. Patent Application Nos. 12/868,994, 13/831, 230, 14/207,313, or 14/207318. In other embodiments, the interfering RNA may confer resistance against a non-insect plant pest, such as a nematode pest or a virus pest.

[00295] In still further embodiments, the first insect control agent, which is a chimeric insecticidal protein of the invention and the second pest control agent are co-expressed in a transgenic plant. This co-expression of more than one pesticidal principle in the same transgenic plant can be achieved by genetically engineering a plant to contain and express the nucleic acid sequences encoding the insect control agents. For example, the co-expression of more than one pesticidal agent in the same transgenic plant can be achieved by making a single recombinant vector comprising coding sequences of more than one pesticidal agent in a “molecular stack” and genetically engineering a plant to contain and express all the pesticidal agents in the transgenic plant. Such molecular stacks may be also be made by using mini-chromosomes as described, for example in US Patent 7,235,716. Alternatively, a plant, Parent 1, can be genetically engineered for the expression of the chimeric insecticidal protein of the invention. A second plant, Parent 2, can be genetically engineered for the expression of a second pest control agent. By crossing Parent 1 with Parent 2, progeny plants are obtained which express both insect control agents from Parents 1 and 2.

[00296] In other embodiments, the invention provides a stacked transgenic plant resistant to plant pest infestation comprising a nucleic acid (*e.g.*, DNA) sequence encoding a dsRNA for suppression of an essential gene in a target pest and a nucleic acid *e.g.*, (DNA) sequence encoding a chimeric insecticidal protein of the invention exhibiting insecticidal activity against the target pest. It has been reported that dsRNAs are ineffective against certain lepidopteran pests (Rajagopol et al. 2002. J. Biol. Chem. 277:468-494), likely due to the high pH of the midgut which

destabilizes the dsRNA. Therefore, in some embodiments where the target pest is a lepidopteran pest, a chimeric insecticidal protein of the invention acts to transiently reduce the midgut pH which serves to stabilize the co-ingested dsRNA rendering the dsRNA effective in silencing the target genes.

[00297] Transgenic plants or seed comprising and/or expressing an insecticidal protein of the invention can also be treated with an insecticide or insecticidal seed coating as described in U. S. Patent Nos. 5,849,320 and 5,876,739. In embodiments, where both the insecticide or insecticidal seed coating and the transgenic plant or seed of the invention are active against the same target insect, for example a lepidopteran pest (*e.g.*, fall armyworm), the combination is useful (i) in a method for further enhancing activity of the composition of the invention against the target insect, and/or (ii) in a method for preventing development of resistance to the composition of the invention by providing yet another mechanism of action against the target insect. Thus, in embodiments, the invention provides a method of enhancing control of a lepidopteran insect population comprising providing a transgenic plant or seed of the invention and applying to the plant or the seed an insecticide or insecticidal seed coating to a transgenic plant or seed of the invention.

[00298] Even where the insecticide or insecticidal seed coating is active against a different insect, the insecticide or insecticidal seed coating is useful to expand the range of insect control, for example by adding an insecticide or insecticidal seed coating that has activity against coleopteran insects to a transgenic seed of the invention, which, in some embodiments, has activity against lepidopteran insects, the coated transgenic seed produced controls both lepidopteran and coleopteran insect pests.

Methods of Making and Using the Chimeric Insecticidal Proteins, Nucleic Acids, and Transgenic Plants.

[00299] The invention also encompasses methods of producing an insect-resistant (*e.g.*, a lepidopteran insect-resistant) transgenic plant. In representative embodiments, the method comprises: introducing into a plant a polynucleotide,

expression cassette or vector of the invention comprising a nucleotide sequence that encodes a chimeric insecticidal protein of the invention (including toxin fragments and modified forms that are substantially identical to the polypeptides specifically disclosed herein), wherein the nucleotide sequence is expressed in the plant to produce the chimeric insecticidal protein of the invention, thereby conferring to the plant resistance to the insect pest, and producing an insect-resistant transgenic plant (*e.g.*, as compared with a suitable control plant, such as a plant that does not comprise the polynucleotide, expression cassette or vector of the invention and/or does not express a polypeptide of the invention).

[00300] In embodiments, the method of introducing the polynucleotide, expression cassette or vector of the invention into the plant comprises first transforming a plant cell with the polynucleotide, expression cassette or vector and regenerating a transgenic plant therefrom, where the transgenic plant comprises the polynucleotide, expression cassette or vector and expresses the chimeric insecticidal protein of the invention.

[00301] Alternatively, or additionally, the introducing step can comprise crossing a first plant comprising the polynucleotide, expression cassette or vector with a second plant (*e.g.*, a different plant from the first plant, for example, a plant that does not comprise the polynucleotide, expression cassette or vector) and, optionally, producing a progeny plant that comprises the polynucleotide, expression cassette or vector and expresses a chimeric insecticidal protein of the invention, thereby resulting in increased resistance to at least one insect pest. Thus, a transgenic plant of the invention encompasses a plant that is the direct result of a transformation event and the progeny thereof (of any generation) that comprise the polynucleotide, expression cassette or vector and optionally expresses the chimeric insecticidal protein resulting in increased resistance to at least one insect pest.

[00302] The invention further provides a method of identifying a transgenic plant of the invention, the method comprising detecting the presence of a polynucleotide, expression cassette, vector or chimeric insecticidal protein of the invention in a plant (or a plant cell, plant part, and the like derived therefrom), and thereby identifying the

plant as a transgenic plant of the invention based on the presence of the polynucleotide, expression cassette, vector or chimeric insecticidal protein of the invention.

[00303] The invention further provides a method of producing a transgenic plant with increased resistance to at least one insect pest (*e.g.*, a least one lepidopteran pest), the method comprising: planting a seed comprising a polynucleotide, expression cassette or vector of the invention, and growing a transgenic plant from the seed, where the transgenic plant comprises the polynucleotide, expression cassette or vector and produces the chimeric insecticidal protein.

[00304] In embodiments, transgenic plants produced by the methods of the invention comprise a polynucleotide, expression cassette or vector of the invention. In embodiments, a transgenic plant produced by the methods of the invention comprise a chimeric insecticidal protein of the invention and, optionally have increased resistance to at least one insect pest.

[00305] The methods of producing a transgenic plant described herein optionally comprise a further step of harvesting a seed from the transgenic plant, where the seed comprises the polynucleotide, expression cassette or vector and produces the chimeric insecticidal protein. Optionally, the seed produces a further transgenic plant that comprises the polynucleotide, expression cassette or vector and produces the chimeric insecticidal protein, and thereby has increased resistance to at least one insect pest.

[00306] The invention further provides plant parts, plant cells, plant organs, plant cultures, seed, plant extracts, harvested products and processed products of the transgenic plants produced by the methods of the invention.

[00307] As a further aspect, the invention also provides a method of producing seed, the method comprising: providing a transgenic plant that comprises a polynucleotide, expression cassette or vector of the invention, and harvesting a seed from the transgenic plant, wherein the seed comprises the polynucleotide, expression cassette, vector and produces the chimeric insecticidal protein. Optionally, the seed produces a further transgenic plant that comprises the polynucleotide, expression

cassette or vector and produces the chimeric insecticidal protein, and thereby has increased resistance to at least one insect pest. In representative embodiments, the step of providing the transgenic plant comprises planting a seed that produces the transgenic plant.

[00308] The invention further provides a method of producing a hybrid plant seed, the method comprising: crossing a first inbred plant, which is a transgenic plant comprising a polynucleotide, expression cassette or vector of the invention, and optionally expressing a chimeric insecticidal protein of the invention with a different inbred plant (*e.g.*, an inbred plant that does not comprise a polynucleotide, expression cassette or vector of the invention) and allowing hybrid seed to form. Optionally, the method further comprises harvesting a hybrid seed. In embodiments, the hybrid seed comprises the polynucleotide, expression cassette or vector of the invention, and in embodiments may further comprise a chimeric insecticidal protein of the invention and have increased resistance to an insect pest. In embodiments, the hybrid seed produces a transgenic plant that comprises the polynucleotide, expression cassette or vector of the invention, expresses the chimeric insecticidal protein of the invention, and has increased resistance to at least one insect pest.

[00309] In some embodiments, a transgenic plant of the invention is resistant to at least one lepidopteran insect pest (as described herein). In embodiments, the transgenic plant controls a fall armyworm insect pest or colony that is resistant to a Vip3A (*e.g.*, a Vip3Aa protein, for example, as expressed in maize event MIR162) and/or Cry1F protein (*e.g.*, a Cry1Fa protein, for example, as expressed in maize event TC1507).

[00310] In further embodiments, a method of controlling at least one insect pest (*e.g.*, at least one lepidopteran insect pest, such as fall armyworm) comprises providing a chimeric insecticidal protein of the invention. In embodiments, the method comprises delivering (*e.g.*, orally delivering) to the insect pest or an environment thereof an effective amount of a chimeric insecticidal protein of the invention. Generally, to be effective, the polypeptide is orally ingested by the insect. However, the chimeric insecticidal protein can be delivered to the insect in many recognized

ways. The ways to deliver a protein orally to an insect include, but are not limited to, providing the protein (1) in a transgenic plant, wherein the insect eats (ingests) one or more parts of the transgenic plant, thereby ingesting the polypeptide that is expressed in the transgenic plant; (2) in a formulated protein composition(s) that can be applied to or incorporated into, for example, insect growth media; (3) in a protein composition(s) that can be applied to the surface, for example, sprayed, onto the surface of a plant part, which is then ingested by the insect as the insect eats one or more of the sprayed plant parts; (4) a bait matrix; or (5) any other art-recognized protein delivery system. Thus, any method of oral delivery to an insect can be used to deliver the toxic proteins of the invention. In some particular embodiments, the chimeric insecticidal protein of the invention is delivered orally to an insect, for example, where the insect ingests one or more parts of a transgenic plant of the invention.

[00311] In other embodiments, the insecticidal protein of the invention is delivered orally to an insect, wherein the insect ingests one or more parts of a plant sprayed with a composition comprising the insecticidal protein of the invention. Delivering the composition of the invention to a plant surface can be done using any method known to those of skill in the art for applying compounds, compositions, formulations and the like to plant surfaces. Some non-limiting examples of delivering to or contacting a plant or part thereof include spraying, dusting, sprinkling, scattering, misting, atomizing, broadcasting, soaking, soil injection, soil incorporation, drenching (*e.g.*, root, soil treatment), dipping, pouring, coating, leaf or stem infiltration, side dressing or seed treatment, and the like, and combinations thereof. These and other procedures for contacting a plant or part thereof with a compound(s), composition(s) or formulation(s) are well-known to those of skill in the art.

[00312] In further embodiments, the invention provides a method of controlling a lepidopteran insect pest that is resistant to a Vip3A protein (*e.g.*, a Vip3Aa protein, for example, as expressed by maize event MIR162) and/or a Cry1F protein (*e.g.* a Cry1Fa protein, for example, as expressed by maize event TC1507) comprising delivering to the lepidopteran insect or an environment thereof an effective amount

of a chimeric insecticidal protein or composition of the invention. In representative embodiments, the resistant insect pest is a resistant fall armyworm insect pest or colony.

[00313] In other embodiments, the invention provides a method of preventing the development of resistance in a population of a target lepidopteran insect pest to a Vip3A (*e.g.*, a Vip3Aa protein, for example, as expressed by maize event MIR162) and/or Cry1F (*e.g.* a Cry1Fa protein, for example, as expressed by maize event TC1507) protein expressed in a transgenic plant, the method comprising delivering to the target population a transgenic plant comprising a polynucleotide comprising a nucleotide sequence encoding a Vip3A protein and/or a nucleotide sequence encoding a Cry1F protein; and a polynucleotide expression cassette or vector of the invention expressing a chimeric insecticidal protein of the invention. In some embodiments, the target lepidopteran insect pest is fall army worm. According to foregoing embodiments, the transgenic plant can comprise a breeding stack of two or more of the insecticidal traits, a molecular stack of two or more of the insecticidal traits, or a combination of both.

[00314] In some embodiments, the invention encompasses a method of providing a farmer with a means of controlling an insect pest (*e.g.*, a lepidopteran pest, such as fall armyworm), the method comprising supplying or selling to the farmer plant material such as a seed, the plant material comprising a polynucleotide, expression cassette, vector capable of expressing a chimeric insecticidal protein of the invention. In embodiments, the plant material comprises the chimeric insecticidal protein of the invention and, optionally, has increased resistance to at least one insect pest. In embodiments, the plant material is a seed, and a plant grown from the seed comprises the polynucleotide, expression cassette or vector of the invention, expresses a chimeric insecticidal protein of the invention, and has increased resistance to the at least one insect pest.

[00315] In addition to providing compositions, the invention provides methods of producing a chimeric insecticidal protein toxic to a lepidopteran pest. Such a method comprises, culturing a transgenic non-human host cell that comprises a

polynucleotide, expression cassette or vector of the invention that expresses a chimeric insecticidal protein of the invention under conditions in which the host cell produces the chimeric insecticidal protein that is toxic to the lepidopteran pest. In some embodiments, the transgenic non-human host cell is a plant cell. In some other embodiments, the plant cell is a maize cell. In other embodiments, the conditions under which the plant cell or maize cell are grown include natural sunlight. In other embodiments, the transgenic non-human host cell is a bacterial cell. In still other embodiments, the transgenic non-human host cell is a yeast cell.

[00316] In some embodiments, the methods of the invention provide control of at least one lepidopteran insect pest, including without limitation, one or more of the following: *Ostrinia* spp. such as *O. nubilalis* (European corn borer) and/or *O. furnacalis* (Asian corn borer); *Plutella* spp. such as *P. xylostella* (diamondback moth); *Spodoptera* spp. such as *S. frugiperda* (fall armyworm), *S. littoralis* (Egyptian cotton leafworm), *S. ornithogalli* (yellowstriped armyworm), *S. praefica* (western yellowstriped armyworm), *S. eridania* (southern armyworm) and/or *S. exigua* (beet armyworm); *Agrotis* spp. such as *A. ipsilon* (black cutworm), *A. segetum* (common cutworm), *A. gladiaria* (claybacked cutworm), and/or *A. orthogonia* (pale western cutworm); *Striacosta* spp. such as *S. albicosta* (western bean cutworm); *Helicoverpa* spp. such as *H. zea* (corn earworm), *H. punctigera* (native budworm), and/or *H. armigera* (cotton bollworm); *Heliothis* spp. such as *H. virescens* (tobacco budworm); *Diatraea* spp. such as *D. grandiosella* (southwestern corn borer) and/or *D. saccharalis* (sugarcane borer); *Trichoplusia* spp. such as *T. ni* (cabbage looper); *Sesamia* spp. such as *S. nonagroides* (Mediterranean corn borer) and/or *S. calamistis* (pink stem borer); *Pectinophora* spp. such as *P. gossypiella* (pink bollworm); *Cochylis* spp. such as *C. hospes* (banded sunflower moth); *Manduca* spp. such as *M. sexta* (tobacco hornworm) and/or *M. quinquemaculata* (tomato hornworm); *Elasmopalpus* spp. such as *E. lignosellus* (lesser cornstalk borer); *Pseudoplusia* spp. such as *P. includens* (soybean looper); *Anticarsia* spp. such as *A. gemmatilis* (velvetbean caterpillar); *Plathypena* spp. such as *P. scabra* (green cloverworm); *Pieris* spp. such as *P. brassicae* (cabbage butterfly), *Papaipema* spp.

such as *P. nebris* (stalk borer); *Pseudaletia* spp. such as *P. unipuncta* (common armyworm); *Peridroma* spp. such as *P. saucia* (variegated cutworm); *Keiferia* spp. such as *K. lycopersicella* (tomato pinworm); *Artogeia* spp. such as *A. rapae* (imported cabbageworm); *Phthorimaea* spp. such as *P. operculella* (potato tuberworm); *Chrysodeixis* spp. Such as *C. includes* (soybean looper); *Feltia* spp. such as *F. ducens* (dingy cutworm); *Chilo* spp. such as *C. suppressalis* (striped stem borer), *Cnaphalocrocis* spp. such as *C. medinalis* (rice leaffolder), or any combination of the foregoing. In embodiments, the methods of the invention provide control of a fall armyworm insect pest or colony that is resistant to a Vip3A (e.g., a Vip3Aa protein, for example, as expressed in maize event MIR162) and/or Cry1F protein (e.g., a Cry1Fa protein, for example, as expressed in maize event TC1507).

[00317] The invention also provides for uses of the chimeric insecticidal proteins, nucleic acids, transgenic plants, plant parts, seed and insecticidal compositions of the invention, for example, to control an insect pest, such as a lepidopteran pest (as described herein).

[00318] In embodiments, the invention provides a method of using a polynucleotide, expression cassette, vector or host cell of the invention to produce an insecticidal composition for controlling an insect pest (e.g., a lepidopteran insect pest).

[00319] In embodiments, the invention provides a method of using a polynucleotide, expression cassette or vector of the invention to produce a transgenic seed, where the transgenic seed grows a transgenic plant with increased resistance to an insect pest.

[00320] As another aspect, the invention also contemplates the use of a transgenic plant of the invention to produce a transgenic seed, which is optionally a hybrid seed.

[00321] In embodiments, the invention provides a method of using a chimeric insecticidal protein, polynucleotide, expression cassette, vector, transgenic plant or insecticidal composition of the invention to prevent the development of resistance in a population of a target lepidopteran insect pest to a Vip3A protein and/or Cry1F protein (each as described herein).

[00322] Statements of the invention include without limitation:

[00323] 1. A chimeric insecticidal protein that is toxic to a lepidopteran insect pest comprising in an N-terminal to C-terminal direction: (a) an N-terminal region of a first Cry1 protein, which is an N-terminal region of a BT-0029 protein of SEQ ID NO:2 or an amino acid sequence that is at least 90% identical thereto, fused to (b) a C-terminal region of a different Cry1 protein; wherein a crossover position between the first Cry1 protein and the different Cry1 protein is located in conserved block 3.

[00324] 2. The chimeric insecticidal protein according to statement 1, wherein the different Cry1 protein is a Cry1F, a Cry1G, a Cry1I or a Cry1K protein.

[00325] 3. The chimeric insecticidal protein according to statement 1, wherein the different Cry1 protein is a Cry1Fa protein.

[00326] 4. The chimeric insecticidal protein according to statement 1, wherein the different Cry1 protein is a Cry1Ia or a Cry1If protein.

[00327] 5. The chimeric insecticidal protein according to statement 1, wherein the different Cry1 protein is a BT-0022 protein of SEQ ID NO: 1.

[00328] 6. The chimeric insecticidal protein according to statement 1, wherein the C-terminal region of the different Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from position 464 to 602 of SEQ ID NO: 8 or an amino acid sequence that is at least 80% identical thereto.

[00329] 7. The chimeric insecticidal protein according to statement 1, wherein the C-terminal region of the different Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from position 498 to 636 of SEQ ID NO: 1 or an amino acid sequence that is at least 80% identical thereto.

[00330] 8. The chimeric insecticidal protein according to any one of statements 1 to 7, wherein the N-terminal region of the first Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from position 1 to 458 of SEQ ID NO: 2 or an amino acid sequence that is at least 90% identical thereto.

[00331] 9. The chimeric insecticidal protein according to any one of statements 1 to 8, wherein the chimeric insecticidal protein further comprises at the

N-terminus a peptidyl fragment that is cleaved from the protoxin after ingestion by the lepidopteran insect pest.

[00332] 10. The chimeric insecticidal protein according to any one of statements 1 to 9, wherein the chimeric insecticidal protein further comprises at the C-terminus a pro-toxin tail from a Cry protein that is cleaved from the protoxin after ingestion by the lepidopteran insect pest.

[00333] 11. The chimeric insecticidal protein according to any one of statements 1 to 10, wherein the chimeric insecticidal protein comprises: (a) the amino acid sequence from position 1 to 597 of SEQ ID NO: 3 or a toxin fragment thereof, or (b) an amino acid sequence that is at least 80% identical to the amino acid sequence of (a).

[00334] 12. The chimeric insecticidal protein according to any one of statements 1 to 11, wherein the chimeric insecticidal protein comprises: (a) the amino acid sequence of SEQ ID NO: 3 or a toxin fragment thereof; or (b) an amino acid sequence that is at least 80% identical to the amino acid sequence of (a).

[00335] 13. The chimeric insecticidal protein according to any one of statements 1 to 10, wherein the chimeric insecticidal protein comprises: (a) the amino acid sequence from positions 1 to 597 of SEQ ID NO: 9 or a toxin fragment thereof; or (b) an amino acid sequence that is at least 80% identical to the amino acid sequence of (a).

[00336] 14. The chimeric insecticidal protein according to any one of statements 1 to 10 or 13, wherein the chimeric insecticidal protein comprises: (a) the amino acid sequence of SEQ ID NO: 9 or a toxin fragment thereof; or (b) an amino acid sequence that is at least 80% identical to the amino acid sequence of (a).

[00337] 15. The chimeric insecticidal protein according to any one of statements 1 to 14, wherein the chimeric insecticidal protein comprises the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 9.

[00338] 16. The chimeric insecticidal protein according to any one of statements 1 to 15, wherein the chimeric insecticidal protein has insecticidal activity against one or more of *Spodoptera frugiperda* (fall armyworm), *Chrysodeixis*

includes (soybean looper), *Diatraea saccharalis* (sugarcane borer), *Diatraea grandiosella* (southwest corn borer), and *Agrotis ipsilon* (black cutworm).

[00339] 17. The chimeric insecticidal protein according to any one of statements 1 to 16, wherein the chimeric insecticidal protein has insecticidal activity against a *Spodoptera frugiperda* insect pest or colony with resistance to a Vip3A protein and/or a Cry1F protein.

[00340] 18. A polynucleotide comprising a nucleotide sequence encoding the chimeric insecticidal protein according to any one of statements 1 to 17.

[00341] 19. The polynucleotide according to statement 18, wherein the polynucleotide is codon optimized for expression in a plant.

[00342] 20. The polynucleotide according to statement 18 or statement 19, wherein the polynucleotide comprises a nucleotide sequence that comprises: (a) a nucleotide sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 14 or a toxin-encoding fragment thereof; (b) a nucleotide sequence that is substantially identical to the nucleotide sequence of (a); or (c) a nucleotide sequence that anneals under stringent hybridization conditions to the nucleotide sequence of (a) or (b); or (d) a nucleotide sequence that differs from the nucleotide sequence of (a), (b) or (c) due to the degeneracy of the genetic code.

[00343] 21. The polynucleotide according to statement 18 or statement 19, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 14.

[00344] 22. A nucleic acid molecule comprising the polynucleotide according to any one of statements 18 to 21 operably associated with a heterologous promoter.

[00345] 23. The nucleic acid molecule according to statement 22, wherein the heterologous promoter is a plant expressible promoter.

[00346] 24. A vector comprising the polynucleotide according to any one of statements 18 to 21 or the nucleic acid molecule according to statement 22 or statement 23.

- [00347]** 25. A transgenic cell comprising the polynucleotide according to any one of statements 18 to 21, the nucleic acid molecule according to statement 22 or statement 23, or the vector according to statement 24.
- [00348]** 26. The transgenic cell according to statement 25, wherein the cell is a transgenic bacterial cell.
- [00349]** 27. The transgenic cell according to statement 25, wherein the cell is a transgenic plant cell.
- [00350]** 28. The transgenic plant cell according to statement 27, wherein the plant cell is: (i) a monocot cell, optionally a barley cell, a maize cell, an oat cell, a rice cell, a sorghum cell, a sugarcane cell or a wheat cell; or (ii) a dicot cell, optionally a soybean cell, a sunflower cell, a tomato cell, a cole crop cell, a cotton cell, a sugar beet cell or a tobacco cell.
- [00351]** 29. A transgenic plant comprising the transgenic plant cell according to statement 27 or statement 28.
- [00352]** 30. The transgenic plant according to statement 29, wherein the plant is: (i) a monocot plant, optionally a barley plant, a maize plant, an oat plant, a rice plant, a sorghum plant, a sugarcane plant or a wheat plant; or (ii) a dicot plant, optionally a soybean plant, a sunflower plant, a tomato plant, a cole crop plant, a cotton plant, a sugar beet plant or a tobacco plant.
- [00353]** 31. The transgenic plant according to statement 28 or statement 29, wherein the transgenic plant comprises a further nucleotide sequence encoding a second insect control agent.
- [00354]** 32. A transgenic plant part of the transgenic plant according to any one of statements 29 to 31, wherein the transgenic plant part comprises the chimeric insecticidal protein.
- [00355]** 33. A transgenic seed of the transgenic plant according to any one of statements 29 to 31.
- [00356]** 34. A harvested product derived from the transgenic plant according to any one of statements 29 to 31, the transgenic plant part according to statement 32,

or the transgenic seed according to statement 33, wherein the harvested product comprises the chimeric insecticidal protein.

[00357] 35. A processed product derived from the harvested product according to statement 34, wherein the processed product is a flour, a meal, an oil, a starch, or a product derived from any of the foregoing.

[00358] 36. An insecticidal composition comprising the chimeric insecticidal protein according to any one of statements 1 to 17 and an agriculturally acceptable carrier.

[00359] 37. The insecticidal composition according to statement 36, wherein the agriculturally acceptable carrier is a powder, a dust, a pellet, a granule, a spray, an emulsion, a colloid or a solution.

[00360] 38. The insecticidal composition according to statement 36 or statement 37, wherein the composition is a desiccated, lyophilized, homogenized, extracted, filtrated, centrifuged and/or sedimented composition and/or is a bacterial culture concentrate.

[00361] 39. The insecticidal composition according to any one of statements 36 to 38, wherein the composition comprises a transgenic bacterial cell that produces the chimeric insecticidal protein.

[00362] 40. The insecticidal composition according to any one of statements 36 to 39, wherein the composition comprises from about 1% to about 99% by weight of the chimeric insecticidal protein.

[00363] 41. The insecticidal composition according to any one of statements 36 to 40, wherein the composition comprises a second insect control agent.

[00364] 42. A method of producing a transgenic plant with increased resistance to a lepidopteran insect pest, the method comprising introducing into a plant the polynucleotide of any one of statements 18 to 21, the nucleic acid molecule of statement 22 or statement 23, or the vector of statement 24, wherein the chimeric insecticidal protein is expressed in the plant, thereby producing a transgenic plant with increased resistance to an insect pest.

- [00365]** 43. The method according to statement 42, wherein the introducing step comprises: (i) transforming a plant cell with the polynucleotide, nucleic acid molecule or vector and regenerating a transgenic plant; or (ii) crossing a first plant comprising the polynucleotide, nucleic acid molecule or vector with a second plant.
- [00366]** 44. The method according to statement 42 or statement 43, wherein the method further comprises obtaining a progeny plant from the transgenic plant, wherein the progeny plant comprises the polynucleotide, the nucleic acid molecule or the vector and has increased resistance to an insect pest.
- [00367]** 45. A method of producing a transgenic plant with increased resistance to a lepidopteran insect pest, the method comprising: (a) planting a seed comprising the polynucleotide according to any one of statements 18 to 21, the nucleic acid molecule according to statement 22 or statement 23, or the vector according to statement 24; and (b) growing a transgenic plant from the seed, wherein the transgenic plant comprises the polynucleotide, nucleic acid molecule or vector and produces the chimeric insecticidal protein.
- [00368]** 46. The method according to statement 45, wherein the method further comprises: (c) harvesting a seed from the transgenic plant of (b), wherein the harvested seed comprises the chimeric insecticidal protein.
- [00369]** 47. A method of producing a seed, the method comprising: (a) providing a transgenic plant that comprises the polynucleotide according to any one of statements 18 to 21, the nucleic acid molecule according to statement 22 or statement 23, or the vector according to statement 24; and (b) harvesting a seed from the transgenic plant of (a), wherein the harvested seed comprises the chimeric insecticidal protein.
- [00370]** 48. A method of producing a hybrid plant seed, the method comprising: (a) crossing a first inbred plant, which is a transgenic plant comprising the polynucleotide according to any one of statements 18 to 21, the nucleic acid molecule according to statement 22 or statement 23, or the vector according to statement 24, with a different inbred plant; and (b) allowing a hybrid seed to form.

- [00371]** 49. A method of controlling a lepidopteran insect pest, the method comprising delivering to the insect pest or an environment thereof a composition comprising an effective amount of the chimeric insecticidal protein of any one of statements 1 to 17 or the insecticidal composition of any one of statements 36 to 41.
- [00372]** 50. A method of controlling a lepidopteran insect pest resistant to a Vip3A protein and/or a Cry1F protein, the method comprising delivering to the resistant lepidopteran insect pest or an environment thereof the insecticidal protein of any one of statements 1 to 17 or the insecticidal composition of any one of statements 36 to 41.
- [00373]** 51. A method of reducing the development of resistance to a Vip3A protein and/or a Cry1F protein in a population of a target lepidopteran insect pest, the method comprising delivering to the target population or an environment thereof a transgenic plant comprising: (i) the polynucleotide according to any one of statements 18 to 21, the nucleic acid molecule according to statement 22 or statement 23, or the vector according to statement 24; and (ii) a polynucleotide comprising a nucleotide sequence encoding a Vip3A protein and/or a nucleotide sequence encoding a Cry1F protein; wherein the chimeric insecticidal protein and the Vip3A protein and/or the Cry1F protein are produced in the transgenic plant.
- [00374]** 52. The method according to any one of statements 42 to 51, wherein the lepidopteran insect pest includes one or more of *Spodoptera frugiperda* (fall armyworm), *Chrysodeixis includes* (soybean looper), *Diatraea saccharalis* (sugarcane borer), *Diatraea grandiosella* (southwest corn borer) and *Agrotis ipsilon* (black cutworm).
- [00375]** 53. The method according to any one of statements 42 to 52, wherein the lepidopteran insect pest includes a *Spodoptera frugiperda* (fall armyworm) that is resistant to a Vip3A protein and/or a Cry1F protein.
- [00376]** 54. The method according to any one of statements 42 to 53, wherein the plant is: (i) a monocot plant, optionally a barley plant, a maize plant, an oat plant, a rice plant, a sorghum plant, a sugarcane plant or a wheat plant; or (ii) a dicot

plant, optionally a soybean plant, a sunflower plant, a tomato plant, a cole crop plant, a cotton plant, a sugar beet plant or a tobacco plant.

[00377] 55. The method according to any one of statements 42 to 54, wherein the plant is a maize plant.

[00378] The invention will now be described with reference to the following examples. It will be appreciated by those skilled in the art that these examples do not limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Other embodiments of the invention may be practiced without departing from the spirit and the scope of the invention, the scope of which is defined by the disclosure and the appended claims.

EXAMPLES

Example 1. Discovery of a Chimeric BT-0029 Protein, Bt29-Bt22 with Insecticidal Activity against Fall Armyworm

[00379] Two lepidopteran-active proteins from *Bacillus thuringiensis* (Bt), BT-0022 (SEQ ID NO: 1; nearest known Cry family member Cry1If) and BT-0029 (SEQ ID NO:2; nearest known Cry family member Cry1Gb), have been disclosed in PCT/US16/038947.

[00380] As shown in Table 1 below, the insect spectrum data for BT-0022 and BT-0029 indicate that BT-0022 has no fall armyworm (FAW *Spodoptera frugiperda*) activity, and BT-0029 has only weak FAW activity. Other lepidopteran insect pests tested were: European corn borer (ECB; *Ostrinia nubilalis*), black cutworm (BCW; *Agrotis ipsilon*), corn earworm (CEW; *Helicoverpa zea*), sugarcane borer (SCB; *Diatraea saccharalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), western bean cutworm (WBCW; *Striacosta albicosta*), soybean looper (SBL; *Pseudoplusia includens*), velvet bean caterpillar (VBC; *Anticarsia gemmatilis*), and tobacco budworm (TBW; *Heliothis virescens*).

Table 1. Insecticidal activity of BT-0022 and BT-0029 against various Lepidopteran insects.

	% Mortality									
	ECB	BCW	FAW	CEW	SCB	SWCB	WBCW	SBL	VBC	TBW
BT-0022	100	100	0	20 (M)	92	100	0	100	0	42 (S)
BT-0029	0	25	25	8	33	17	ND	100	100	ND

M – Medium-sized larvae

S- Small-sized larvae

ND – Not Determined

[00381] A protein engineering approach was used in an effort to try to enhance the FAW activity of BT-0029. Using BT-0029 as a template, six engineered proteins were designed by replacing domain III (DIII) of BT-0029 with a domain III from a different Cry protein. Table 2 illustrates six chimeric proteins with their domain composition information. Table 3 provides the sequence information for the full-length proteins.

Table 2. Engineered BT-0029 proteins by domain swap

Name	Domain I	Domain II	Domain III	Protoxin Tail
Bt29-1Ab	BT-0029	BT-0029	Cry1Ab	BT-0029
Bt29-Bt22	BT-0029	BT-0029	BT-0022	BT-0029
Bt29-Bt67	BT-0029	BT-0029	BT-0067	BT-0029
Bt29-Bt51	BT-0029	BT-0029	BT-0051	BT-0029
Bt29-8D	BT-0029	BT-0029	Cry8D	BT-0029
Bt29-3A	BT-0029	BT-0029	Cry3A	BT-0029

Table 3. Full-length protein sequence information.

Name	Closest Cry Family Member	Sequence
BT-0029	Cry1Gb	SEQ ID NO: 2
Cry1Ab	Not applicable	Genbank accession No. AAA22330.1
BT-0022	Cry1If	SEQ ID NO: 1
Bt-0067	Cry9Eb	WO2016/094165
Bt-0051	Cry9Ca	WO2016/094159
Cry8D	Not applicable	Genbank accession No. BAF93483.1
Cry3A	Not applicable	Genbank accession No. AAA22336.1
Cry1Ca	Not applicable	Genbank accession No. AF362020.1

[00382] cDNAs encoding the engineered proteins were synthesized by Genewiz (South Plainfield, NJ) and cloned into a *Bacillus thuringiensis* (*Bt*) expression vector containing a Cry1Ac promoter and no terminator. The plasmid DNAs were introduced into *Bt* strain AB227 via an electroporation-mediated transformation procedure. All six engineered proteins were produced as crystal proteins in *Bt*. The crystal proteins were purified from the cultures and dissolved in a buffer containing 50 mM Na₂CO₃ pH 11.0, 2 mM DTT. Each soluble protein was then evaluated in an insect bioassay.

[00383] Briefly, insecticidal activity of the chimeric proteins was examined by an artificial diet based insect bioassay in which the solubilized crystal proteins were overlaid on the surface of artificial insect diet, with a final concentration of 2 - 3.2 µg/cm². The buffer (50 mM Na₂CO₃ pH 11.0, 2 mM DTT) used for solubilizing the *Bt* crystal proteins served as the negative control. Full-length Cry1Fa protein (SEQ ID NO: 8) was used as a positive control for FAW activity. Each chimeric protein was tested in duplicate. Insect mortality was assessed on day 7.

[00384] The results are shown in Table 4. When the chimeric proteins were tested in the insect bioassay, the Bt29-Bt22 chimera (SEQ ID NO: 3) showed strong insecticidal activity against FAW, whereas two other chimeric proteins (Bt29-Bt67 and Bt29-3A) showed only minor activity to the pest. The Bt29-1Ab chimera did not demonstrate any detectable FAW activity.

[00385] For this test, not enough protein of the Bt29-Bt51 and Bt29-8D chimeras were produced to evaluate insecticidal activity.

Table 4: Insecticidal Activity against Fall Armyworm

Treatment	FAW % Mortality	Treatment	FAW % Mortality
Buffer (negative control)	8	Bt29-Bt67	17
Cry1Fa (full-length; positive control)	100	Bt29-3A	25
Bt29-Bt22	100	Bt29-3A	33
Bt29-Bt22	83	Bt29-1Ab	0
Bt29-Bt67	8	Bt29-1Ab	0
Bt29-Bt67	17		

Example 2: Insecticidal Activity of Chimeric BT-0029 Proteins against Various Lepidopteran Pests

[00386] Four of the chimeric proteins described in Example 1 (Bt29-3A, Bt29-Bt22, Bt29-1Ab and Bt29-Bt67) were examined for their insecticidal activities against the following lepidopteran pests using art-recognized artificial diet bioassay methods: sugarcane borer (SCB; *Diatraea saccharalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), soybean looper (SBL; *Pseudoplusia includens*), corn earworm (CEW; *Helicoverpa zea*), tobacco budworm (TBW; *Heliothis virescens*), black cutworm (BCW; *Agrotis ipsilon*) and European corn borer (ECB; *Ostrinia nubilalis*).

[00387] As shown in Table 5, all four chimeric proteins tested retained the strong SBL activity of native BT-0029. Bt29-Bt22 and Bt29-Bt67 had weak SCB activity comparable to native BT-0029, whereas Bt29-3A and Bt29-1Ab essentially lost all SCB activity. A slightly increased SWCB activity was observed in Bt29-Bt67 compared to full-length BT-0029. Fairly consistent with the results in Table 1 for native BT-0029, only weak BCW activity was observed for Bt29-3A, Bt29-Bt22 and Bt29-Bt67, and none of the chimeras had CEW, TBW or ECB activity.

Table 5: Insecticidal Activity of BT-0029 chimeric proteins against Lepidopteran Pests

Treatment	% Mortality						
	SCB	SWCB	SBL	CEW	TBW	BCW	ECB
Buffer (negative control)	0	17	0	0	0	8	0
Cry1Fa (full-length; positive control)	25	42	100	73	100	42	100
Bt29-3A	0	42	100	0	0	8	0
Bt29-3A	8	25	100	0	0	25	0
Bt29-Bt22	50	17	100	0	0	17	0
Bt29-Bt22	33	67	100	0	0	25	0
Bt29-Bt67	25	58	100	0	0	33	0
Bt29-Bt67	42	73	100	0	0	8	0
Bt29-1Ab	8	0	100	0	0	0	0
Bt29-1Ab	0	0	100	0	0	0	0
Bt29-1Fa	100	100	100	0	0	0	75

Example 3: Bt29-1F Possesses Insecticidal Activity Against Fall Armyworm

[00388] To examine additional chimeric proteins, a second set of BT-0029

chimeric proteins was designed and tested (Table 6). Bt29-1Fa is a chimeric protein in which domain III of BT-0029 was replaced with domain III of Cry1Fa. The full-length amino acid sequence of Cry1Fa is provided as SEQ ID NO: 8 (*see also* Genbank accession number AAB00376.1). The amino acid sequence of the Bt29-1Fa chimera is provided as SEQ ID NO: 9, wherein the Cry1Fa sequence is amino acids 459 to 597.

[00389] Bt29-1Ka is a chimeric protein in which domain III of BT-0029 was replaced with domain III of Cry1Ka. The full-length amino acid sequence of Cry1Ka is provided as SEQ ID NO: 12 (*see also* Genbank accession number AAB00376.1). The amino acid sequence of the Bt29-1Ka chimera is also provided as SEQ ID NO: 13, wherein the Cry1Ka sequence is amino acids 459 to 597. A second version of a Bt29-1Ka chimeric protein (designated herein as Bt29-1Kav2) was made by moving the cross-over junction between the Cry1Ka sequence and the BT-0029 sequence in the C-terminus of domain III from amino acid 598 (the cross-over in the first Bt29-1Ka chimera) to amino acid 610.. The amino acid sequence of the Bt29-1Kav2 chimera is provided as SEQ ID NO: 15, wherein the Cry1Ka sequence is amino acids 459 to 609.

[00390] Bt29-1Ca is a chimeric protein in which domain III of Bt-0029 was replaced with domain III of Cry1Ca. The full-length amino acid sequence of Cry1Ca is provided as SEQ ID NO: 17 (*see also* Genbank accession number AF362020.1). The amino acid sequence of the Bt29-1Ca chimera is provided as SEQ ID NO: 18, wherein the the Cry1Ca sequence is amino acids 467 to 617.

Table 6. Engineered BT-0029 proteins by domain III swap

Name	Domain I	Domain II	Domain III	Protoxin Tail
Bt29-1Fa	BT-0029	BT-0029	Cry1Fa	BT-0029
Bt29-1Ka	BT-0029	BT-0029	Cry1Ka	BT-0029
Bt29-1Ca	BT-0029	BT-0029	Cry1Ca	BT-0029

[00391] Insecticidal activity of the Bt29-1Fa (SEQ ID NO: 9), Bt29-1Ka (SEQ ID NO: 13, Bt29-1Kav2 (SEQ ID NO: 16) and Bt29-1Ca (SEQ ID NO: 18) chimeras were examined by an artificial diet based insect bioassay, in which the solubilized proteins were overlaid on the surface of artificial insect diet, with a final concentration of 2 µg/cm². The buffer (50 mM Na₂CO₃ pH 10.5, 2 mM DTT) used for solubilizing the *Bt* crystal proteins served as the negative control. Full-length Cry1Fa protein (SEQ ID NO: 8) was used as a positive control for FAW activity. Each chimeric protein was tested in triplicate. Insecticidal activity was assessed as effective mortality on day 7 (larvae that have growth inhibition and are moribund are scored as effectively dead).

[00392] As shown in Table 7, the chimeric protein designated Bt29-1Fa demonstrated strong activity against FAW.

[00393] In this test, insufficient Bt29-1Ka protein was produced to evaluate in the bioassay. Surprisingly, the Bt29-1Kav2 chimera produced protein and was active against fall armyworm (see Table 7).

Table 7: Insecticidal Activity of Bt29-1Fa hybrid against Fall Armyworm

Treatment	FAW % Mortality
Bt29-1Fa	100
Bt29-1Fa	100
Bt29-1Fa	100
Bt29-1Ca	100
Bt29-1Kav2	75
Cry1Fa (full length; positive control)	100
Buffer (negative control)	8

Example 4: Bt29-Bt22 and Bt29-1Fa Possess Insecticidal Activity against Cry1F-resistant Fall Armyworm

[00394] To determine if the toxicity of the BT-0029 chimeric proteins to FAW is through a mode of action (MOA) distinct from Cry1Fa, the two chimeric proteins, Bt29-Bt22 and Bt29-1Fa were produced as described in Example 1, and the crystal

proteins were isolated and purified. Purified crystals were dissolved in Buffer 1 (50 mM Na₂CO₃/NaHCO₃, pH 11, 2 mM DTT), and the purity of the dissolved protein preparation was monitored using a Bio-Rad Experion system (BioRad, Hercules, CA).

[00395] The purified proteins were tested for efficacy against a strain of FAW that is resistant to the insecticidal toxin Cry1Fa. A diet-overlay assay was performed with various doses of each purified toxin (125, 500 and 2000 ng/cm²), essentially as described in Example 1. Vip3A protein (positive control) was dissolved in PBS and the other proteins in Buffer 1 (50 mM Na₂CO₃/NaHCO₃, pH 11, 2 mM DTT). The two negative control treatments were PBS and Buffer 1. A FAW population that is susceptible to either Cry1Fa or Vip3A, i.e. FAW susceptible strain, was also tested. Each protein was tested in duplicates. Insecticidal activity was assessed as effective mortality on day 7 (larvae that have growth inhibition and are moribund are counted as effectively dead).

[00396] As shown in Table 8, the susceptible FAW larvae were controlled by Cry1Fa, Vip3A, Bt29-Bt22 and Bt29-1Fa, even at the lowest concentration. In contrast, the Cry1F-resistant FAW strain was not controlled by any dose of Cry1Fa tested, demonstrating the strain is resistant to this toxin (Table 9). Surprisingly, both Bt29-Bt22 and Bt29-1Fa were highly efficacious in controlling the Cry1F-resistant FAW even at a very low dose of 125 ng/cm² as shown in Table 9, suggesting that the mode of action of these two chimeric proteins against resistant FAW is different from the mode of action of a Cry1Fa protein.

Table 8: Insecticidal Activity of Bt29-Bt22 and Bt29-1Fa against susceptible Fall Armyworm at various concentrations

Treatment	Dose (ng/cm ²)	% Mortality
Vip3Aa	125	100
Vip3Aa	500	100
Vip3Aa	2000	100
Cry1Fa	125	100
Cry1Fa	500	100

Cry1Fa	2000	100
Bt29-Bt22	125	90
Bt29-Bt22	500	96
Bt29-Bt22	2000	100
Bt29-1Fa	125	92
Bt29-1Fa	500	100
Bt29-1Fa	2000	100
Buffer 1		0
PBS		0

Table 9: Insecticidal Activity of Bt29-Bt22 and Bt29-1Fa against Cry1Fa-resistant Fall Armyworm

Treatment	Dose (ng/cm ²)	% Mortality
Vip3Aa	125	100
Vip3Aa	500	100
Vip3Aa	2000	100
Cry1Fa	125	0
Cry1Fa	500	0
Cry1Fa	2000	0
Bt29-Bt22	125	100
Bt29-Bt22	500	100
Bt29-Bt22	2000	100
Bt29-1Fa	125	100
Bt29-1Fa	500	100
Bt29-1Fa	2000	100
Buffer 1		0
PBS		0

Example 5: Bt29-Bt22 and Bt29-1Fa Possess Insecticidal Activity Against Vip3A-resistant Fall Armyworm

[00397] To determine if the toxicity of the BT-0029 chimeric proteins is through a different MOA from Vip3A protein, the Bt29-Bt22 and Bt29-1Fa proteins were produced as described in Example 1, and the crystal proteins were isolated and purified. Purified crystals were dissolved in Buffer 1 (50 mM Na₂CO₃/NaHCO₃, pH 11, 2 mM DTT), and the purity of the dissolved protein preparation was monitored using a Bio-Rad Experion system (BioRad, Hercules, CA).

[00398] The purified proteins were tested for efficacy against a strain of FAW that is resistant to the insecticidal toxin Vip3A. A diet-overlay assay was performed with various doses of each purified toxin (125, 500 and 2000 ng/cm²) essentially as described in Example 1. Vip3A protein was dissolved in PBS and the other proteins in Buffer 1 (50 mM Na₂CO₃/NaHCO₃, pH 11, 2 mM DTT). The two negative control treatments were PBS and Buffer 1. Cry1Fa proteins was used as a positive control for the Vip3A-resistant FAW strain. Each protein was tested in duplicate. Insecticidal activity was assessed as effective mortality on day 7 (larvae that have growth inhibition and are moribund are scored as effectively dead).

[00399] The Vip3A-resistant FAW strain was not controlled by any dose of Vip3A tested, demonstrating that the strain is resistant to this toxin (Table 10). In contrast, both Bt29-Bt22 and Bt29-1Fa demonstrated a high degree of efficacy against the Vip3A-resistant FAW suggesting that the mode of action of these two chimeric proteins against resistant FAW is different from the mode of action of a Vip3A protein.

Table 10: Insecticidal Activity of Bt29-Bt22 and Bt29-1Fa against Vip3A-resistant Fall Armyworm

Treatment	Dose (ng/cm ²)	% Mortality
Vip3Aa	125	0
Vip3Aa	500	0
Vip3Aa	2000	0
Cry1Fa	125	100
Cry1Fa	500	100
Cry1Fa	2000	100
Bt29-Bt22	125	100
Bt29-Bt22	500	100
Bt29-Bt22	2000	100
Bt29-1Fa	125	100
Bt29-1Fa	500	100
Bt29-1Fa	2000	100
Buffer 1		0
PBS		0

Example 6. Truncations in Protoxin Tail Region

[00400] It is well-known that the protoxin form of *Bt* Cry proteins is processed at both the N- and C-terminal ends to produce the mature toxin. Six C-terminal truncated forms of the Bt29-Bt22 (SEQ ID NO: 3) chimera, which comprises a BT-0029 protoxin tail region, were made as shown in Figure 3. In all truncated versions, designated herein as Bt29-Bt22Tr1 (SEQ ID NO: 20), Bt29-Bt22Tr2 (SEQ ID NO: 21), Bt29-Bt22Tr3 (SEQ ID NO: 22), Bt29-Bt22Tr4 (SEQ ID NO: 23), Bt29-Bt22Tr5 (SEQ ID NO: 24), Bt29-Bt22Tr6 (SEQ ID NO: 25), domain III of BT-0022 (ending with PVTA), and at least the first 6 amino acids of the BT-0029 protoxin tail (TFEAEY) were retained. Those skilled in the art will appreciate that the end of domain III and the beginning of the protoxin tail has not been delineated with precision for BT-0029 or the chimeras described herein. The core domain III shown in Figures 1A and 1B are based on alignments with other Cry proteins in which the domain III region has been delineated.

[00401] The Bt29-Bt22Tr1, -Tr2, -Tr4, and -Tr5 truncated forms all showed FAW activity assessed as growth inhibition (not mortality) in insect bioassays when tested as *E. coli* lysates. The Bt29-Bt22Tr1 and -Tr4 forms were the most promising, demonstrating the highest activity in this assay (67% and 75% growth inhibition, respectively).

[00402] A second Bt29-Bt22 full-length chimera was made wherein the protoxin tail region is from BT-0022 (Cry1I) instead of BT-0029 (Cry1G). Six C-terminal truncated forms of this full-length chimera, presented herein as SEQ ID Nos:26-31, were made and tested against fall armyworm and soybean looper. Cry1Fa was used as the positive control. Results are shown in Table 11.

Table 11. Insecticidal activity of Bt29-Bt22 with truncated BT-0022 protoxin tail against fall armyworm and soybean looper.

Chimera	SEQ ID NO:	Effective Mortality (%)	
		FAW	SBL
BT29BT22-TL22v1	26	75	100
BT29BT22-TL22v2	27	63	100

BT29BT22-TL22v3	28	50	100
BT29BT22-TL22v4	29	58	100
BT29BT22-TL22v5	30	54	100
BT29BT22-TL22v6	31	83	100
Buffer	NA	0	0
Cry1Fa	8	100	100

[00403] Four truncated forms of the Bt29-1Fa (SEQ ID NO: 9), which comprises the BT-0029 protoxin tail region, presented herein as Bt29-1FaTr1 (SEQ ID NO:32), Bt29-1FaTr2 (SEQ ID NO: 33), Bt29-1FaTr3 (SEQ ID NO: 34) and Bt29-1FaTr4 (SEQ ID NO:35), were produced in an E. coli expression system and lysates were tested against fall armyworm. Results are shown in Table 12.

Table 12. Insecticidal activity of truncated Bt29-1Fa chimeric proteins.

Chimeric Protein	SEQ ID NO:	% FAW Mortality
Bt29-1FaTr1	32	17
Bt29-1FaTr2	33	8
Bt29-1FaTr3	34	42
Bt29-1FaTr4	35	58
Cry11Fa	8	100
Buffer	NA	0
pET29	NA	0

Example 7. Expression and Activity of Chimeric Bt29-Bt22 and Bt29-Cry1Fa Proteins in Maize Plants

[00404] Prior to expression in plants, synthetic polynucleotides comprising a nucleotide sequence encoding a Bt29-Bt22 and a Bt29-1Fa chimera were synthesized on an automated gene synthesis platform (Genscript, Inc., Piscataway, NJ). For this example, a first and second expression cassette was made comprising a maize ubiquitin promoter (Ubi1) operably linked to either the Bt29-Bt22 or Bt29-1Fa chimeric coding sequence which was operably linked to a maize ubiquitin terminator and a third expression cassette was made comprising a Ubi1 promoter

operably linked to a phosphomannose isomerase (PMI) coding sequence which was operably linked to a Ubi terminator. Expression of PMI allows for positive selection of transgenic plants on mannose. For plant transformation, the first and third expression cassettes and the second and third expression cassettes were cloned into a suitable vector for *Agrobacterium*-mediated maize transformation.

- [00405]** Transformation of immature maize embryos is performed essentially as described in Negrotto et al., 2000, Plant Cell Reports 19: 798 803. Briefly, *Agrobacterium* strain LBA4404 (pSB1) comprising an expression vector expressing Bt29-Bt22 or Bt29-Cry1Fa is grown on YEP (yeast extract (5 g/L), peptone (10g/L), NaCl (5g/L), 15g/l agar, pH 6.8) solid medium for 2- 4 days at 28°C. Approximately 0.8×10^9 *Agrobacterium* cells are suspended in LS-inf media supplemented with 100 μ M As. Bacteria are pre-induced in this medium for approximately 30-60 minutes.
- [00406]** Immature embryos from an inbred maize line are excised from 8-12 day old ears into liquid LS-inf + 100 μ M As. Embryos are rinsed once with fresh infection medium. *Agrobacterium* solution is then added and embryos are vortexed for 30 seconds and allowed to settle with the bacteria for 5 minutes. The embryos are then transferred scutellum side up to LSAs medium and cultured in the dark for two to three days. Subsequently, between approximately 20 and 25 embryos per petri plate are transferred to LSDc medium supplemented with cefotaxime (250 mg/l) and silver nitrate (1.6 mg/l) and cultured in the dark at approximately 28°C for 10 days.
- [00407]** Immature embryos, producing embryogenic callus are transferred to LSD1M0.5S medium. The cultures are selected on this medium for approximately 6 weeks with a subculture step at about 3 weeks. Surviving calli are transferred to Reg1 medium supplemented with mannose. Following culturing in the light (16 hour light/ 8 hour dark regiment), green tissues are then transferred to Reg2 medium without growth regulators and incubated for about 1-2 weeks. Plantlets are transferred to Magenta GA-7 boxes (Magenta Corp, Chicago Ill.) containing Reg3 medium and grown in the light. After about 2-3 weeks, plants are tested for the presence of the PMI genes and the chimeric *Bt cry* gene by PCR. Positive plants from the PCR assay are transferred to a greenhouse for further evaluation.

[00408] Transgenic plants were evaluated for copy number (determined by Taqman analysis), protein expression level (determined by ELISA), and efficacy against insect species of interest in leaf excision bioassays. Specifically, plant tissue (leaf or silks) was excised from single copy events (V3-V4 stage) and infested with neonate larvae of a target pest, then incubated at room temperature for 5 days. Leaf disks from transgenic plants expressing each chimeric *Bt* protein were tested against fall armyworm (*Spodoptera frugiperda*, FAW).

[00409] Results confirm that the transgenic plants express the chimeric proteins of the invention and are active against insect pests. Protein expression in the transgenic events for the Bt29-Bt22 chimera ranged from about 25-125 ng/mg total soluble protein (TSP) and for the Bt29-1Fa chimera ranged from about 25-290 ng/mg TSP. About 90% and 92% of the transgenic events expressing the Bt29-Bt22 and Bt29-1Fa chimeras, respectively, produced 100% mortality and larval growth inhibition to fall armyworm.

[00410] The above examples clearly illustrate the advantages of the invention. Although the present invention has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the claimed invention except as and to the extent that they are included in the accompanying claims.

What is claimed is:

1. A chimeric insecticidal protein that is toxic to a lepidopteran insect pest comprising in an N-terminal to C-terminal direction:

(a) an N-terminal region of a first Cry1 protein, which is an N-terminal region of a BT-0029 protein of SEQ ID NO:2 or an amino acid sequence that is at least 90% identical thereto, fused to

(b) a C-terminal region of a different Cry1 protein;

wherein a crossover position between the first Cry1 protein and the different Cry1 protein is located in conserved block 3.

2. The chimeric insecticidal protein according to claim 1, wherein the different Cry1 protein is a) a Cry1F, a Cry1G, a Cry1I, a Cry1K or a Cry1C protein; or b) is a Cry1Fa, a Cry1Ia, a Cry1If, a Cry1Ka or a Cry1Ca; or c) is a BT-0022 protein of SEQ ID NO:1.

3. The chimeric insecticidal protein according to claim 1, wherein the C-terminal region of the different Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from a) position 464 to 602 of SEQ ID NO: 8 or an amino acid sequence that is at least 80% identical thereto; or b) position 498 to 636 of SEQ ID NO: 1 or an amino acid sequence that is at least 80% identical thereto.

4. The chimeric insecticidal protein according to any one of claims 1 to 3, wherein the N-terminal region of the first Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from position 1 to 458 of SEQ ID NO: 2 or an amino acid sequence that is at least 90% identical thereto.

5. The chimeric insecticidal protein according to any one of claims 1 to 4, wherein the chimeric insecticidal protein further comprises at the C-terminus a pro-toxin tail from a Cry protein that is cleaved from the protoxin after ingestion by the lepidopteran insect pest.

6. The chimeric insecticidal protein according to any one of claims 1 to 5, wherein the chimeric insecticidal protein comprises:

- (a) the amino acid sequence from position 1 to 597 of SEQ ID NO: 3 or a toxin fragment thereof, or
- (b) the amino acid sequence of SEQ ID NO: 3 or a toxin fragment thereof; or
- (c) the amino acid sequence from positions 1 to 597 of SEQ ID NO: 9 or a toxin fragment thereof; or
- (d) the amino acid sequence of SEQ ID NO: 9 or a toxin fragment thereof; or
- (e) an amino acid sequence that is at least 80% identical to the amino acid sequence of (a), (b), (c) or (d).

7. The chimeric insecticidal protein according to any one of claims 1 to 6, wherein the chimeric insecticidal protein has insecticidal activity against a *Spodoptera frugiperda* insect pest with resistance to a Vip3A protein or a Cry1F protein.

8. A polynucleotide comprising (a) a nucleotide sequence encoding the chimeric insecticidal protein according to any one of claims 1 to 7; or (b) the nucleotide sequence of (a) that is codon optimized for expression in a plant.

9. The polynucleotide according to claim 8, wherein the polynucleotide comprises a nucleotide sequence that comprises:

- (a) a nucleotide sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 14 or a toxin-encoding fragment thereof;
- (b) a nucleotide sequence that is substantially identical to the nucleotide sequence of (a);
- (c) a nucleotide sequence that anneals under stringent hybridization conditions to the nucleotide sequence of (a) or (b); or
- (d) a nucleotide sequence that differs from the nucleotide sequence of (a), (b) or (c) due to the degeneracy of the genetic code.

10. A nucleic acid molecule comprising the polynucleotide according to claim 8 or 9 operably associated with a heterologous plant expressible promoter.
11. A vector comprising the nucleic acid molecule according to claim 10.
12. A transgenic cell comprising the polynucleotide according to claims 8 or 9, wherein the transgenic cell is a bacterial cell or a plant cell.
13. The transgenic plant cell according to claim 12, wherein the plant cell is:
 - (a) a monocot cell, optionally a barley cell, a maize cell, an oat cell, a rice cell, a sorghum cell, a sugarcane cell or a wheat cell; or
 - (b) a dicot cell, optionally a soybean cell, a sunflower cell, a tomato cell, a cole crop cell, a cotton cell, a sugar beet cell or a tobacco cell.
14. A transgenic plant comprising the transgenic plant cell according to claim 13, wherein the plant is:
 - (a) a monocot plant, optionally a barley plant, a maize plant, an oat plant, a rice plant, a sorghum plant, a sugarcane plant or a wheat plant; or
 - (b) a dicot plant, optionally a soybean plant, a sunflower plant, a tomato plant, a cole crop plant, a cotton plant, a sugar beet plant or a tobacco plant.
15. The transgenic plant according to claim 14, wherein the transgenic plant further comprises a nucleotide sequence encoding a second insect control agent.
16. A transgenic plant part of the transgenic plant according to claim 14, wherein the transgenic plant part comprises the chimeric insecticidal protein.
17. A transgenic seed of the transgenic plant according to claim 14.

18. A harvested product derived from the transgenic plant according to claim 14, wherein the harvested product comprises the chimeric insecticidal protein.

19. A processed product derived from the harvested product according to claim 18, wherein the processed product is a flour, a meal, an oil, a starch, or a product derived from any of the foregoing.

20. An insecticidal composition comprising the chimeric insecticidal protein according to any one of claims 1 to 17 and an agriculturally acceptable carrier.

21. A method of producing a transgenic plant with increased resistance to a lepidopteran insect pest, the method comprising:

(a) introducing into a plant by transforming a plant cell with the polynucleotide of claims 8 or 9; or crossing a first plant comprising the polynucleotide, with a second plant, wherein the chimeric insecticidal protein is expressed in the plant, thereby producing a transgenic plant with increased resistance to an insect pest; and optionally obtaining a progeny plant from the transgenic plant, wherein the progeny plant comprises the polynucleotide and has increased resistance to an insect pest; or

(b) planting a seed comprising the polynucleotide and growing a transgenic plant from the seed, wherein the transgenic plant comprises the polynucleotide and produces the chimeric insecticidal protein.

22. The method according to claim 21, wherein the method further comprises harvesting a seed from the transgenic plant of (b), wherein the harvested seed comprises the chimeric insecticidal protein.

23. A method of producing a seed, the method comprising:

(a) providing a transgenic plant that comprises the polynucleotide according to claims 8 or 9; and

(b) harvesting a seed from the transgenic plant of (a), wherein the harvested seed comprises the chimeric insecticidal protein.

24. A method of producing a hybrid plant seed, the method comprising:

(a) crossing a first inbred plant, which is a transgenic plant comprising the polynucleotide according to claims 8 or 9; and

(b) allowing a hybrid seed to form.

25. A method of controlling a lepidopteran insect pest, the method comprising delivering to the insect pest or an environment thereof a composition comprising an effective amount of the chimeric insecticidal protein of any one of claims 1 to 7, wherein the lepidopteran insect pest is susceptible or resistant to a Vip3 protein or a Cry1F protein .

26. A method of reducing the development of resistance to a Vip3A protein or a Cry1F protein in a population of a target lepidopteran insect pest, the method comprising delivering to the target population or an environment thereof a transgenic plant comprising:

(i) the polynucleotide according to claims 8 or 9; and

(ii) a polynucleotide comprising a nucleotide sequence encoding a Vip3A protein or a nucleotide sequence encoding a Cry1F protein;

wherein the chimeric insecticidal protein and the Vip3A protein or the Cry1F protein are produced in the transgenic plant.

BT-0029	1 --MEINQN-----Q--CVPYNCLNPESEILNVAIFS-----SEQVAEIHUKITRLILENFLPGGSFAFLGLFDLIWGIFNEDQWSAF	1
BT-0022	1 --MKSQN--MHQSLSNNAIVDKNFTGSLNNTNTELQNFHEG--IEPFVSVSTIQIGIAGKILGNLGVFPAGQVSLYSFILGELWPKGKQWEIF	1
Cry1Fa	1 --MENNQN-----Q--CVPYNCLNPESEILNVAIFS-----GRPLDISLSTRFLSEFPVGVGVAFLGLFDLIWGFIITPSDWSLF	1
Cry1Ka	1 MNSNRKNENEIINALSIPAVSNHSAQMDLSPDARIEDSLCVAEGNIDPFVSASTVQTGISIAGRIILGVLPVFPAGQLASFYSFLVGELWPSGRDPWEIF	1
BT-0029	75 LRQVEELIN-QRITFARGQAIQRLVGFGRSYDEYIILAKEWENDPDNPASKERVTRFRRTDDALLTGVLMAIPGFEALTSVYAQSANLHLALLRDA	75
BT-0022	96 MEHVEELIN-QKISITARNKALADLKLGDALAVYHESLESWIKNRNTRTSVKSQYITLEMFVQSLSFSAVSGEEVPLLPPIYAQAANLHLLLRDA	96
Cry1Fa	75 LLQIEQLIE-QRIETELERNRAITTLRGLADSYEIIYIEALREWEANPNNAQLREDVRIRFANTDDALITAINNFTLTSTFEIPLLSVYVQAANLHLLLRDA	75
Cry1Ka	101 MEHVEQIVRQQQITDSVRDITAIARLEGLGRGYSYQQALETWLDNRNDARSRIIRERYIALELDITTAIPFSIRNEEVPLLMVYAQAANLHLLLRDA	101
BT-0029	174 VFFGERWGLTQTININDLYSRKNSIRDYTNHCVRFYNIGLGNLNVIRP-----EYRFQRELTISVLDLVALFPNYDIRTYPIPTKSQLTREIYTDPIISP	174
BT-0022	195 SIFGKEWGLSDSEISTFYNRQVERTSDYSDHCTKWFDTGLNRLKGSNAEIIWKYNQFRRDMTLMVLDLVALFQSYDTHMYPKIKTTAQLTREYVYTAIGTV	195
Cry1Fa	174 VSFGQGWGLDIA TVNNHYNRLINLIHRYTKHCLDTYNQGLENLRGNTTROWARENQFRRDLTLTVLDLVALFPNYDVRTYPIQISSQLTREIYTSVIED	174
Cry1Ka	201 SLFGSEWGMSSADVNYXQEQIRYITEEYSNHCVQWYNTGLNRLRGTTAETWTWRYNQFRRDLTLGVLDLVALFPNYDVRTYPIPTTAQLTREYIDPENGTV	201
BT-0029	270 GAQAG-----YTLQDVLREPHLMDFELNRLIIYTGEYRGIRHWAGHEVESRTGMMNIRFPFLYGTAAETAEPTRFITPSTFPGLNLFYRTL	270
BT-0022	295 HPHPSFTITWYNNNAFSAIEAAVIRSPHLLDFLEQVTIYLLSRWSNTQYMMWGG-HKLEFRITGG-TLNTSTQSTNTSINPVTLPFTSRDIYRT	295
Cry1Fa	274 SPVSAN-----IFGNFRAEFQVPRPHLMDFMNSLFTVTAETVRSQTVWGGHLVSS-RNTAGNINFPSTYGVNPGGAIWIADEDPRP-----FYRTL	274
Cry1Ka	301 AGP-----NNSWFRNGASFAIENAIIRQPHLYDFLTLNLTITYRRS-QVGTIIMNLWAG-HRITFNRIQGGSTSEMVMYGAITNPVSVSDIPEVNRDVYRT	301
BT-0029	355 SAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSLDQPLEGES--SLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTN	355
BT-0022	393 ESLAGLNLFLTQPVNGVPRVDFHWKFVTHP-IASDNFYYPGYAGIGTQLQDSENEILPPTTGQPNYESYSHRLSHIGLISASHVKALVSWTHRSADRTN	393
Cry1Fa	360 SDPVFVRGGFNPVYVGLRGVAFQQTG--TNHTRFERNSGTIDSLDEIPPDQNSCAPWNDYSHVLNHVTFVRWPGEISGSDSWRAPMFSWTHRSATPTN	360
Cry1Ka	394 VSLAGGLGSLSGIRYGLIRVDFDMIFRNHPDIVTGLFYHPGHAGIATQVKDSDELPPETTEQPNYRAFSHLLSHISMGPTTQDVPPVYSWTHOSADRTN	394
BT-0029	453 TIDPDVITQIPLVKAFLNLSGATIVKGPFGTGGDILRRTNVGSFGDMRVNITAPLSQRYRVIRYASTDLOFYTNINGTTINIGNFSSTMDSGDDLQYQ	453
BT-0022	492 TIHSDSITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTSGGPFASNVNLDWNLSQLRYRARIYASTNLNRMVYTIAGERIFAGQFNKTMNTGDPPLTFQ	492
Cry1Fa	458 TIDPERITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTSGGPFAYTIVNINQOLPQRYRARIYASTNLRIYVTVAGERIFAGQFNKTMNTGDPPLTFQ	458
Cry1Ka	494 TINSDRITQIPLVKAHTLQSGTIVVKGPGFTGGDILRRTSGGPFASNVNLDWNLSQLRYRARIYASTNLRIYVTVAGERIFAGQFNKTMNTGDPPLTFQ	494
BT-0029	553 RFRVAGFTTPTTFSDANSTFTTIGAF'SSSNNEVYIDRIEFVPAEVTFEAEYDLEKAQKAVNALFTSSNQIGLKTDTV-DYHIDKVSNLNVECLSDEFCLDE	553
BT-0022	592 SFSYATIDTAFTEPTKASSLTGVGADTFSSGNEVYVDRFELIPVTATLEAVTDLERAQKAVHLEFTSTNPGGLKTDVAKDHYTNTISKSVQSVFRCRCSE	592
Cry1Fa	558 SFSYATINTAFTFPMSSQSSFTIGADTFSSGNEVYIDRIEFELIPVTATFEAEYDLEKAQKAVNALFTSINQIGIKTDVT-DYHIDQVSNLVDCLSDEFCLDE	558
Cry1Ka	594 SFSYATINTAFTFPERSSSLTIGADTFSSGNEVYVDRFELIQVTATFEAESDLERARKAVNALFTSTINPRGLKTDVT-DYHIDQVSNLVECLSDEFCLDK	594

CB3

Domain III

Domain III

Fig. 1A

BT-0029	652	KRELSEKVKHAKRLCDERNLLQDPNFRGINRQPDGRWGSTDITIQQGDDVFRENVTPLPGTFDECYPTLYQKIDESKLKAYTRYELRGYIEDSQDLEI
BT-0022	692	TR-----IYRWGYPSSKKEYWYIWGYTSKY-----
Cry1Fa	657	KRELSEKVKHAKRLSDERNLLQDPNFKGINRQLDRGWRGSTDITIQRGDDVFRENVTPLPGTFDECYPTLYQKIDESKLKPYTRYQLRGYIEDSQDLEI
Cry1Ka	693	KRELLEEVKYAKRLSDERNLLQDPTFTSISQQTDRGWIGSTGTSIQGGDDIFKENYVRLPGTVDECYPTLYQKIDESQLKSYTRYQLRGYIEDSQDLEI
BT-0029	752	YLIRYNAKHETVNPVPGTGSIMPLSAQSPIGKCGEPNRCATHLEWNPDLDCSCRDGGEKCAHSHHFSLDIDVGCTDLNEDLGVMVIFKIKTQDGHARLGNL
BT-0022	716	-----
Cry1Fa	757	YLIRYNAKHETVNLGIGSLWPLSVQSPIRKCGEPNRCAPHLEWNPDLDCSCRDGGEKCAHSHHFSLDIDVGCTDLNEDLDVMVIFKIKTQDGHARLGNL
Cry1Ka	793	YLIRYNAKHETLSVPGIESPWPSSGVYPSGRCGEPNRCAPRIEWNPDLDSCRYGECVHHSHHFSLDIDVGCTDLNEDLGVMVIFKIKTQDGHAKLGNL
BT-0029	852	EFLEEKPLVGEALARVKRAEKKWRDKREKLEETNIVYKEAKKSVDALEFVNSQYDRLQADTNIA--IHAADKRVHSIREAYLPELSVIPGVNAAIFEELEG
BT-0022	716	-----
Cry1Fa	857	EFLEEKPLVGEALARVKRAEKKWRDKREKLEETNIVYKEAKESVDALFVNSQYDQLQADTNIAMIHAAADKRVHRIREAYLPELSVIPGVNVDIFEELKG
Cry1Ka	893	EFIEEKPLLKALS RVKRAEKKWRDKYEKLQLETKRVYTEAKESVDALFVDSQYDKLQANTNIGIIHGADKQVHRIREPILSELPLVIP SINAAIFEELEG
BT-0029	952	RIFTAYSLYDARNVIKNGDFNNGLS CWNVKGHV DVEEQNNHRSVLVVPWEAEVSQEV RVCPRGRGYILRV TAYKEGYGEGCVTIHEIEDNTDELKFSNCV
BT-0022	716	-----
Cry1Fa	957	RIFTAFFLYDARNVIKNGDFNNGLS CWNVKGHV DVEEQNNHRSVLVVPWEAEVSQEV RVCPRGRGYILRV TAYKEGYGEGCVTIHEIENNTDELKFSNCV
Cry1Ka	993	HIFKAYSLYDARNVIKNGDFNNGLS CWNVKGHV DVQQN-HHRSVLVLSEWAEVSQKVRVCPDRGYILRV TAYKEGYGEGCVTIHEFEDNTDVLKFRNFV
BT-0029	1052	EEEIYPNNITVCNDYITATQEEYEGTYTSRNR-----GYDGAYESSN SVPADYASAYEEKAYTDGRRDNTCESNRGYDYTPLPAGYVTKELEYFPETDK
BT-0022	710	-----
Cry1Fa	1057	EEEVYPNNITVCNDYTANQEEYGGAYTSRNR-----GYDETYGNSN SVPADYASVYEEKSYTDGRRDNPCESNRGYDYTPLPAGYVTKELEYFPETDK
Cry1Ka	1092	EEEVYPNNITVCNDYTTNQSAEGSTDACNSYNRGYEDGYENRYEPNPSAPVNYTPTVEEGMYTDTQGYNHCVSDRGYRNHTPLPAGYVTLELEYFPETE Q
BT-0029	1146	VWIEIGETEGTFFIVDSVELLMEE
BT-0022	716	-----
Cry1Fa	1151	VWIEIGETEGTFFIVDSVELLMEE
Cry1Ka	1192	VWIEIGETEGTFFIVGSVELLMEE

Fig.1B

BT-0029	1	MEINNQCVPYNCLNNPESIEILNVAIFSSEQVAEIHKLKTRILILENPLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEELINQRIITEFARGQAIQRLVG
BT29-BT22	1	MEINNQCVPYNCLNNPESIEILNVAIFSSEQVAEIHKLKTRILILENPLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEELINQRIITEFARGQAIQRLVG
BT29-1Fa	1	MEINNQCVPYNCLNNPESIEILNVAIFSSEQVAEIHKLKTRILILENPLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEELINQRIITEFARGQAIQRLVG
BT29-1Ka	1	MEINNQCVPYNCLNNPESIEILNVAIFSSEQVAEIHKLKTRILILENPLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEELINQRIITEFARGQAIQRLVG
BT-0029	101	FGRSYDEYILALKEWENDPNPASKERVTRFRRTDDALLTGVPVPLMAIPGFELATLSVYAQSANLHLALLRDVFFGGERWGLTQTININDLSRLKNSIRD
BT29-BT22	101	FGRSYDEYILALKEWENDPNPASKERVTRFRRTDDALLTGVPVPLMAIPGFELATLSVYAQSANLHLALLRDVFFGGERWGLTQTININDLSRLKNSIRD
BT29-1Fa	101	FGRSYDEYILALKEWENDPNPASKERVTRFRRTDDALLTGVPVPLMAIPGFELATLSVYAQSANLHLALLRDVFFGGERWGLTQTININDLSRLKNSIRD
BT29-1Ka	101	FGRSYDEYILALKEWENDPNPASKERVTRFRRTDDALLTGVPVPLMAIPGFELATLSVYAQSANLHLALLRDVFFGGERWGLTQTININDLSRLKNSIRD
BT-0029	201	YTNHCVRFFYNIGLGNLKVIRPEYYRFQRELTISVLDLVALFPNVDIRTYPIPTKSQLTREIYTDPIISPQAQAGYTLQDVLRPHLMDFLNRLIIYTGEY
BT29-BT22	201	YTNHCVRFFYNIGLGNLKVIRPEYYRFQRELTISVLDLVALFPNVDIRTYPIPTKSQLTREIYTDPIISPQAQAGYTLQDVLRPHLMDFLNRLIIYTGEY
BT29-1Fa	201	YTNHCVRFFYNIGLGNLKVIRPEYYRFQRELTISVLDLVALFPNVDIRTYPIPTKSQLTREIYTDPIISPQAQAGYTLQDVLRPHLMDFLNRLIIYTGEY
BT29-1Ka	201	YTNHCVRFFYNIGLGNLKVIRPEYYRFQRELTISVLDLVALFPNVDIRTYPIPTKSQLTREIYTDPIISPQAQAGYTLQDVLRPHLMDFLNRLIIYTGEY
BT-0029	301	RGIRHWAGHEVESSRTGMMTNIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGFIQPNNGEQLYRVRGTLDSL
BT29-BT22	301	RGIRHWAGHEVESSRTGMMTNIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGFIQPNNGEQLYRVRGTLDSL
BT29-1Fa	301	RGIRHWAGHEVESSRTGMMTNIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGFIQPNNGEQLYRVRGTLDSL
BT29-1Ka	301	RGIRHWAGHEVESSRTGMMTNIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGFIQPNNGEQLYRVRGTLDSL
BT-0029	401	DQLPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAFLNLSHGATIVKPGFTGGDILRRINVSFGDMR
BT29-BT22	401	DQLPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAFLNLSHGATIVKPGFTGGDILRRINVSFGDMR
BT29-1Fa	401	DQLPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAFLNLSHGATIVKPGFTGGDILRRINVSFGDMR
BT29-1Ka	401	DQLPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAFLNLSHGATIVKPGFTGGDILRRINVSFGDMR
BT-0029	501	VNITAPLSQRYRARIYASTTDLQFYTNINGTTINIGNFSSTMSDGLQYGRFVRVAGFTTPTFTSDAMSTFTIGAFSSNNVEYIDRIEFVPAEVTFE
BT29-BT22	501	VNLDWNLSQRYRARIYASTTDLQFYTNINGTTINIGNFSSTMSDGLQYGRFVRVAGFTTPTFTSDAMSTFTIGAFSSNNVEYIDRIEFVPAEVTFE
BT29-1Fa	501	VNINGQLPQRYRARIYASTTDLQFYTNINGTTINIGNFSSTMSDGLQYGRFVRVAGFTTPTFTSDAMSTFTIGAFSSNNVEYIDRIEFVPAEVTFE
BT29-1Ka	501	VNLDNLSQRYRARIYASTTDLQFYTNINGTTINIGNFSSTMSDGLQYGRFVRVAGFTTPTFTSDAMSTFTIGAFSSNNVEYIDRIEFVPAEVTFE
BT-0029	601	AEYDLEKAQKAVNALFSSNQIGLKTDTVTDYHIDKVSNLVECLSEDFCLDEKRELSEKVKHAKRLCERNLLQDPNFRGINRQPDGRGWRGSTDITIQGGD
BT29-BT22	601	AEYDLEKAQKAVNALFSSNQIGLKTDTVTDYHIDKVSNLVECLSEDFCLDEKRELSEKVKHAKRLCERNLLQDPNFRGINRQPDGRGWRGSTDITIQGGD
BT29-1Fa	601	AEYDLEKAQKAVNALFSSNQIGLKTDTVTDYHIDKVSNLVECLSEDFCLDEKRELSEKVKHAKRLCERNLLQDPNFRGINRQPDGRGWRGSTDITIQGGD
BT29-1Ka	601	AEYDLEKAQKAVNALFSSNQIGLKTDTVTDYHIDKVSNLVECLSEDFCLDEKRELSEKVKHAKRLCERNLLQDPNFRGINRQPDGRGWRGSTDITIQGGD

Fig.2A

BT-0029	701	DVFKENYVTLPGTFDECYPTLYQKIDESKLYKAYTRYELRGYIEDSQDLEIYLIRYNAKHETVNVPGTGS_WPLSAQSPIGKCGEPNRCATHLEWNPDL
BT29-BT22	701	DVFKENYVTLPGTFDECYPTLYQKIDESKLYKAYTRYELRGYIEDSQDLEIYLIRYNAKHETVNVPGTGS_WPLSAQSPIGKCGEPNRCATHLEWNPDL
BT29-1Fa	701	DVFKENYVTLPGTFDECYPTLYQKIDESKLYKAYTRYELRGYIEDSQDLEIYLIRYNAKHETVNVPGTGS_WPLSAQSPIGKCGEPNRCATHLEWNPDL
BT29-1Ka	701	DVFKENYVTLPGTFDECYPTLYQKIDESKLYKAYTRYELRGYIEDSQDLEIYLIRYNAKHETVNVPGTGS_WPLSAQSPIGKCGEPNRCATHLEWNPDL
BT-0029	801	CSCRDGEKCAHSHHFSLDIDVGCTDLNEDLGWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLELETNIVYKEAKKSVDALE
BT29-BT22	801	CSCRDGEKCAHSHHFSLDIDVGCTDLNEDLGWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLELETNIVYKEAKKSVDALE
BT29-1Fa	801	CSCRDGEKCAHSHHFSLDIDVGCTDLNEDLGWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLELETNIVYKEAKKSVDALE
BT29-1Ka	801	CSCRDGEKCAHSHHFSLDIDVGCTDLNEDLGWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLELETNIVYKEAKKSVDALE
BT-0029	901	VNSQYDRLQADTNIAIIHAADKRVHSIREAYLPELSIPGVNAAIFEELEGRIFTAYSLSYDARNVIKNGDFNNGLSCHWNVKGHVDEEQNNHRSVLVVP
BT29-BT22	901	VNSQYDRLQADTNIAIIHAADKRVHSIREAYLPELSIPGVNAAIFEELEGRIFTAYSLSYDARNVIKNGDFNNGLSCHWNVKGHVDEEQNNHRSVLVVP
BT29-1Fa	901	VNSQYDRLQADTNIAIIHAADKRVHSIREAYLPELSIPGVNAAIFEELEGRIFTAYSLSYDARNVIKNGDFNNGLSCHWNVKGHVDEEQNNHRSVLVVP
BT29-1Ka	901	VNSQYDRLQADTNIAIIHAADKRVHSIREAYLPELSIPGVNAAIFEELEGRIFTAYSLSYDARNVIKNGDFNNGLSCHWNVKGHVDEEQNNHRSVLVVP
BT-0029	1001	WEAEVSQEVRCVPCGRGYILRVITAYKEGYGEGCVTIHEIEDNTDELKFSNCVEEIIYPNNTVTCNDYTATQEEYEGTYSRNRGYDGAYESNSSVPADYAS
BT29-BT22	1001	WEAEVSQEVRCVPCGRGYILRVITAYKEGYGEGCVTIHEIEDNTDELKFSNCVEEIIYPNNTVTCNDYTATQEEYEGTYSRNRGYDGAYESNSSVPADYAS
BT29-1Fa	1001	WEAEVSQEVRCVPCGRGYILRVITAYKEGYGEGCVTIHEIEDNTDELKFSNCVEEIIYPNNTVTCNDYTATQEEYEGTYSRNRGYDGAYESNSSVPADYAS
BT29-1Ka	1001	WEAEVSQEVRCVPCGRGYILRVITAYKEGYGEGCVTIHEIEDNTDELKFSNCVEEIIYPNNTVTCNDYTATQEEYEGTYSRNRGYDGAYESNSSVPADYAS
BT-0029	1101	AYEEKAYTDGRRDNTCESNRGYGDTPLPAGYVTKLEYFPETDKVWIEIGETEGTIFVDSVELLLMEE
BT29-BT22	1101	AYEEKAYTDGRRDNTCESNRGYGDTPLPAGYVTKLEYFPETDKVWIEIGETEGTIFVDSVELLLMEE
BT29-1Fa	1101	AYEEKAYTDGRRDNTCESNRGYGDTPLPAGYVTKLEYFPETDKVWIEIGETEGTIFVDSVELLLMEE
BT29-1Ka	1101	AYEEKAYTDGRRDNTCESNRGYGDTPLPAGYVTKLEYFPETDKVWIEIGETEGTIFVDSVELLLMEE

Fig. 2B

SEQ ID NO: 3
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 20
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 21
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 22
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 23
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 24
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE
 SEQ ID NO: 25
 1 MEINNQCVPYNCLNPESEILNVAIFSSSEQVAEIHKLITRILILENFLPGGSFAFGLFDLIWGFNEDQWSAFLRQVEE

 Bt29-Bt22
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDA.LTGVPLMAIPGFELATLSVYA
 SEQ ID NO: 20
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA
 SEQ ID NO: 21
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA
 SEQ ID NO: 22
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA
 SEQ ID NO: 23
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA
 SEQ ID NO: 24
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA
 SEQ ID NO: 25
 81 LINQRITFARGQAIQRLVGFGRSYDEYILALKEWENDPDNPASKERVTRFRRTTDDALLTGVPPLMAIPGFELATLSVYA

 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 3
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 20
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 21
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 22
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 23
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 24
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL
 SEQ ID NO: 25
 161 QSANLHLALLRDVFFGERWGLTQTNINDLYSRLKNSIRDYTNHCVRFYNIGLGNLNVIRPEYYRFQRELTISVLDLVAL

 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 3
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 20
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 21
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 22
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 23
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 24
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT
 SEQ ID NO: 25
 241 FPNYDIRTYPIPTKSQTLTREIYTDPIISPGAQAGYTLQDVLRPHLMDFLNRLIIYTGEYRGIRHWAGHEVESRTGMMT

Fig. 3A

SEQ ID NO: 3	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 20	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 21	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 22	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 23	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 24	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 25	321	NIRFPLYGTAATAEPTRFITPSTFPGLNLFYRILSAPIFRDEPGANIIIRYRTSLVEGVGF IQPNNGEQLYRVRGTLDSL
SEQ ID NO: 3	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 20	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 21	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 22	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 23	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 24	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 25	401	DQPLEGESSLTEYSHRLCHVRFAQSLRNAEPLDYARVPMFSWTHRSATPTNTIDPDVITQIPLVKAHTLQSGTTVVKGP
SEQ ID NO: 3	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 20	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 21	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 22	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 23	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 24	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 25	481	GFTGGDILRRTSGGPFASFNVNLDWNLSQRYRARIYASTTNLRMYVTIAGERIFAGQFNKTMNTGDPDLTFQSFYSYATID
SEQ ID NO: 3	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEKAQKAVNALFTSSNQIGLKTDTVTDYHIDKVSNLV
SEQ ID NO: 20	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEKAQKAVNALFTSSNQIGLKTDTVTDYHIDKVSNLV
SEQ ID NO: 21	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEKAQKAVNALFTSSNQIGLKTDTVTDYHIDKV----
SEQ ID NO: 22	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEKAQKAVNALFTSSNQI-----
SEQ ID NO: 23	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEKAQK-----
SEQ ID NO: 24	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEYDLEK-----
SEQ ID NO: 25	561	TAFTFPTKASSLTVGADTFSSGNEVYVDRFELIPVTATFEAEY-----

Fig. 3B

Bt29-Bt22	961	DARNV I K N G D F N N G L S C W N V K G H V D V E E Q N N H R S V L V P E W E A E V S Q E V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I E D
SEQ ID NO:20	653	-----
SEQ ID NO:21	637	-----
SEQ ID NO:22	623	-----
SEQ ID NO:23	611	-----
SEQ ID NO:24	608	-----
SEQ ID NO:25	604	-----
Bt29-Bt22	1041	N T D E L K F S N C V E E E I Y P N N T V T C N D Y T A T Q E E Y E G T Y T S R N R G Y D G A Y E S N S S V P A D Y A S A Y E E K A Y T D G R R D N T C E S N R
SEQ ID NO:20	653	-----
SEQ ID NO:21	637	-----
SEQ ID NO:22	623	-----
SEQ ID NO:23	611	-----
SEQ ID NO:24	608	-----
SEQ ID NO:25	604	-----
Bt29-Bt22	1121	G Y G D Y T P L P A G Y V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V E L L L M E E
SEQ ID NO:20	653	-----
SEQ ID NO:21	637	-----
SEQ ID NO:22	623	-----
SEQ ID NO:23	611	-----
SEQ ID NO:24	608	-----
SEQ ID NO:25	604	-----

Fig. 3D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13^{ter}. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13^{ter}. 1(a)).
 - ☐ on paper or in the form of an image file (Rule 13^{ter}. 1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-3, 8, and 9 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/063722

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01H 5/00; A01H 5/10; A01N 63/02; C07K 14/325; C12N 5/10; C12N 15/32 (2018.01)

CPC - A01N 37/46; A01N 65/22; A01N 65/44; C07K 14/325 (2018.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 514/4.5; 530/300; 530/350 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 8,735,560 B1 (ENGLISH et al) 27 May 2014 (27.05.2014) entire document	1-4
Y	US 2015/0148288 A1 (VESTARON CORPORATION) 28 May 2015 (28.05.2015) entire document	1-4
A	WO 2011/084631 A1 (DOW AGROSCIENCES LLC et al) 14 July 2011 (14.07.2011) entire document	1-4
A	WO 2016/061377 A2 (MONSANTO TECHNOLOGY LLC) 21 April 2016 (21.04.2016) entire document	1-4
A	WO 2016/061197 A1 (PIONEER HI-BRED INTERNATIONAL, INC.) 21 April 2016 (21.04.2016) entire document	1-4
P, A	WO 2017/003811 A1 (SYNGENTA PARTICIPATIONS AG et al) 05 January 2017 (05.01.2017) entire document	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 January 2018

Date of mailing of the international search report

16 FEB 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: GREGORY W. WARREN
SYNGENTA CROP PROTECTION, LLC
9 DAVIS DRIVE
RESEARCH TRIANGLE PARK, NC 27709

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

16 FEB 2018

Applicant's or agent's file reference
81090-WO-REG

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2017/063722

International filing date (day/month/year)

29 November 2017

Priority date (day/month/year)

12 December 2016

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A01H 5/00; A01H 5/10; A01N 63/02; C07K 14/325; C12N 5/10; C12N 15/32 (2018.01)

CPC - A01N 37/46; A01N 65/22; A01N 65/44; C07K 14/325 (2018.01)

Applicant SYNGENTA PARTICIPATIONS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion

25 January 2018

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2017/063722

Box No. 1 Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed.
- ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

- ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
- ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

SEQ ID NOs: 1-3, 8, and 9 were searched.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2017/063722

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-26

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 5-26 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 5-26 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 5-26

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2017/063722

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4 lack an inventive step under PCT Article 33(3) as being obvious over English et al. (hereinafter English) in view of Vestaron Corporation (hereinafter Vestaron).

Regarding Claim 1, English discloses a chimeric insecticidal protein that is toxic to a lepidopteran insect pest (lepidopteran active toxin proteins, Title; the insecticidal portions of these insecticidal Bt toxins can be coupled, fused, linked connected, or mated to a supplemental Cry protein domain III, the resulting protein fusion or chimera exhibiting improved insecticidal activity, Col. 5, Lns. 17-21) comprising in an N-terminal to C-terminal direction: (a) an N-terminal region of a first Cry1 protein (an insect inhibitory protein comprising a Cry1 polypeptide, wherein said Cry1 polypeptide comprises a first domain III... linkage... to the C-terminus of said first domain III polypeptide sequence, Col. 5, Lns. 55-61) (b) a C-terminal region of a different Cry1 protein (a supplemental Cry protein domain III from that or any other three domain Cry toxin is operably linked to the first three domain Cry toxin, Col. 5, Lns. 49-51); wherein a crossover position between the first Cry1 protein and the different Cry1 protein is located in conserved block 3 (relative to the conserved homology blocks present in each category and class of three domain toxin proteins. In this case, the breakpoint to be used for insertion of a supplemental Cry protein domain III is considered to be within the amino acid segment that is positioned between any domain II and domain III of any particular three domain Cry toxin, Col. 6, Lns. 3-9).

English fails to explicitly disclose an N-terminal region of a BT-0029 protein of SEQ ID NO:2 or an amino acid sequence that is at least 90% identical thereto, fused to (b).

Vestaron is in the field of insecticidal proteins from bacillus thuringiensis (Abstract) and teaches an N-terminal region of a BT-0029 protein that is at least 90% identical to SEQ ID NO:2 (The sequence listing includes Bt sequences SEQ. ID. NO:s 33-533. These sequences include examples of Bt protein Cry and Cyt protein sequences, Para. [0469]; Vestaron's SEQ ID NO: 193 shares 97.6% identity with SEQ ID NO: 2 of the instant application).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify English with the teaching of Vestaron for the purpose of disrupting insect gut membranes allowing for adventitious infection (sepsis) of the insect by gut flora (Vestaron, Para. [0385]).

Regarding Claim 2, modified English discloses the chimeric insecticidal protein according to claim 1. English further discloses wherein the different Cry1 protein is Cry1 F (said Cry1 polypeptide is... Cry1F, Col. 2, Lns. 63-64).

Regarding Claim 3, modified English discloses the chimeric insecticidal protein according to claim 1.

English fails to explicitly disclose wherein the C-terminal region of the different Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from a) position 464 to 602 of SEQ ID NO: 8 or an amino acid sequence that is at least 80% identical thereto; or b) position 498 to 636 of SEQ ID NO: 1 or an amino acid sequence that is at least 80% identical thereto.

Vestaron teaches a C-terminal region of the different Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from a) position 464 to 602 of SEQ ID NO: 8 (The sequence listing includes Bt sequences SEQ. ID. NO:s 33-533. These sequences include examples of Bt protein Cry and Cyt protein sequences, Para. [0469]; Vestaron's SEQ ID NO: 182 shares 100 identity with SEQ ID NO: 8 (and positions 464-602 of SEQ ID NO: 8) of the instant application).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify English with the teaching of Vestaron for the purpose of disrupting insect gut membranes allowing for adventitious infection (sepsis) of the insect by gut flora (Vestaron, Para. [0385]).

Regarding Claim 4, modified English discloses the chimeric insecticidal protein according to claim 1. English further discloses the N-terminal region of the first Cry1 protein (an insect inhibitory protein comprising a Cry1 polypeptide, wherein said Cry1 polypeptide comprises a first domain III... linkage... to the C-terminus of said first domain III polypeptide sequence, Col. 5, Lns. 55-61).

English fails to explicitly disclose wherein the Cry1 protein comprises an amino acid sequence corresponding to an amino acid sequence from position 1 to 458 of SEQ ID NO: 2 or an amino acid sequence that is at least 90% identical thereto.

Vestaron teaches a Cry1 protein comprises an amino acid sequence that is at least 90% identical to the amino acid sequence from position 1 to 458 of SEQ ID NO: 2 (The sequence listing includes Bt sequences SEQ. ID. NO:s 33-533. These sequences include examples of Bt protein Cry and Cyt protein sequences, Para. [0469]; Vestaron's SEQ ID NO: 193 shares 97.6% identity with SEQ ID NO: 2 [and shares 100% local identity to amino acid positions 1-458 of SEQ ID NO: 2] of the instant application).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify English with the teaching of Vestaron for the purpose of disrupting insect gut membranes allowing for adventitious infection (sepsis) of the insect by gut flora (Vestaron, Para. [0385]).

Claims 1-4 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.