

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2016/079276

International filing date (day/month/year)
30.11.2016

Priority date (day/month/year)
02.12.2015

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/01 A61K31/7068 C07H19/06 A61L31/16 A01H1/06 A01H3/04

Applicant
UNIVERSITÄT BASEL

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 usually 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:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0
Fax: +49 89 2399 - 4465

Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

Kurz, Birgit

Telephone No. +49 89 2399-0



Box No. I Basis of the 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 43bis.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 13ter.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 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.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:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>4-11, 15-17, 19</u>
	No: Claims	<u>1-3, 12-14, 18</u>
Inventive step (IS)	Yes: Claims	<u>4-7, 9-11, 17</u>
	No: Claims	<u>1-3, 8, 12-16, 18, 19</u>
Industrial applicability (IA)	Yes: Claims	<u>1-19</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The present application discloses that treatment of Arabidopsis wild-type seedlings with 40 μ M zebularine and 5 μ g/ml α -amanitin combined with heat stress leads to a high accumulation of extrachromosomal DNA of the heat responsive ONSEN transposable element and insertion of the new copies of said transposon in the genome of the progeny plants, which show increased heat tolerance.

Reference is made to the following documents:

- D1 DHAVAL VARSHNEY ET AL: "SINE transcription by RNA polymerase III is suppressed by histone methylation but not by DNA methylation", NATURE COMMUNICATIONS, vol. 6, 23 March 2015, page 6569, XP055272268, DOI: 10.1038/ncomms7569

- D2 TUNCAY BAUBEC ET AL: "Effective, homogeneous and transient interference with cytosine methylation in plant genomic DNA by zebularine", THE PLANT JOURNAL, vol. 57, no. 3, 1 February 2009 (2009-02-01), pages 542-554, XP055271538, GB ISSN: 0960-7412, DOI: 10.1111/j.1365-313X.2008.03699.x

- D3 VLADIMIR V. CAVRAK ET AL: "How a Retrotransposon Exploits the Plant's Heat Stress Response for Its Activation", PLOS GENETICS, vol. 10, no. 1, 30 January 2014 (2014-01-30), page e1004115, XP055271558, DOI: 10.1371/journal.pgen.1004115

- D4 W. MATSUNAGA ET AL: "The effects of heat induction and the siRNA biogenesis pathway on the transgenerational transposition of ONSEN, a copia-like retrotransposon in Arabidopsis thaliana", PLANT AND CELL PHYSIOLOGY, vol. 53, no. 5, 14 December 2011, pages 824-833, XP055271560, UK ISSN: 0032-0781, DOI: 10.1093/pcp/pcr179

- D7 BUCHER ETIENNE ET AL: "Epigenetic control of transposon transcription and mobility in Arabidopsis", CURRENT OPINION IN PLANT BIOLOGY, vol. 15, no. 5, 2012, pages 503-510, XP028958933, ISSN: 1369-5266, DOI: 10.1016/J.PBI.2012.08.006

- D8 S. VISPE ET AL: "Triptolide is an inhibitor of RNA polymerase I and II-dependent transcription leading predominantly to down-regulation of short-lived mRNA", MOLECULAR CANCER THERAPEUTICS, vol. 8, no. 10, 1 October 2009, pages 2780-2790, XP055272275, US ISSN: 1535-7163, DOI: .1158/1535-7163.MCT-09-0549

D9 H. SAZE ET AL: "DNA Methylation in Plants: Relationship to Small RNAs and Histone Modifications, and Functions in Transposon Inactivation", PLANT AND CELL PHYSIOLOGY, vol. 53, no. 5, 1 February 2012 (2012-02-01), pages 766-784, XP055272283, UK ISSN: 0032-0781, DOI: 10.1093/pcp/pcs008

- 1 The methods of independent claims 1 and 8 in their present formulation encompass the treatment of humans, the use of human germ cells and breeding of plants and animals (including humans). No unified criteria exist in the PCT-contracting states concerning this subject-matter and the applicant is informed that in some PCT-contracting states the presently claimed subject-matter might not be patentable.

The search has been carried out for all claims and has been based on the alleged effects of the compounds/treatments.

In case that the methods are not meant to encompass human beings, the independent claims have to be limited accordingly (e.g. by referring to non-human eukaryotic cells/organisms).

- 2 Claim 1 is directed to a method for the mobilization of a transposable element in a eukaryotic cell, said method comprising the steps of:

a) providing a eukaryotic cell comprising a transposable element
b) contacting said cell with an inhibitor of transcription,
thereby yielding a eukaryotic cell with a mobilized transposable element.

- 2.1 D1 discloses a method for mobilising SINE retrotransposons in animal cells. The cells are treated with α -amanitin (inhibitor of transcription), caetocin (inhibitor of histone H5K9 methylation) or 5-azacytidine (inhibitor of DNA methylation).

D1 discloses on page 5 the use of α -amanitin and concomitant inhibition of pol II. An effect on mobilisation of transposable elements (TEs) is not reported. However, the method steps required in present claim 1 and the method steps carried out in D1 are identical. It follows that either the effect obtained is identical, leading to an objection for lack of novelty (Article 33(2) PCT) of claims 1-3, 12-14 and 18 and an objection for lack of inventive step (Article 33(3) PCT) of claims 15, 16 and 19, or the effect is not identical, leading to an objection for lack of disclosure according to Article 5 PCT. If the

method steps carried out in D1 are identical to those in claim 1 without leading to the claimed effect, then the method is not disclosed in a manner sufficiently clear and complete to be carried out by a person skilled in the art.

Concerning inventive step it is noted that identification of the correct concentration/ratio is a routine procedure.

- 3 Present claim 8 is directed to a method for increasing genetic and/or epigenetic variation in a population of eukaryotic organisms comprising providing the organism, contacting it with an inhibitor of DNA methylation and/or an inhibitor of transcription, and propagating the organism.
- 3.1 In general terms, the mobilisation of TEs is a method for increasing genetic and/or epigenetic variation. The step of propagating the organisms is not explicitly disclosed in any of the cited documents, so that claim 8 is considered novel and complying with the requirements of Article 33(2) PCT. However, mobilisation of TEs with zebularine (i.e. an inhibitor of DNA methylation) is known from D2 (pages 547 and Figure 6). In view of the disclosure of D2 present claims 8, 12 and 16 are considered to lack inventive step in the sense of Article 33(3) PCT. Mobilisation of TEs by demethylation is generally known in the art (see page 1, line 31 to page 2, line 9 of the description of the present application; D7, page 506; D9, page 776 and 777), as well as suitable chemicals (D1, D2, D8, D9).

Re Item VIII

Certain observations on the international application

- 4 A method for mobilising the ONSEN transposon which can be detected in the progeny plants by subjecting wild-type Arabidopsis seedlings to zebularine, α -amanitin and heat stress in the disclosed concentration and ratio of the compounds may potentially comply with the requirements of the PCT.

However, this is not reflected in the independent claims. Claims 1 and 8 are directed in general terms to a method for mobilising any transposon in any organism by simply contacting a transposon containing cell with a transcription inhibitor (claim 1) or a transcription inhibitor and/or an inhibitor of DNA methylation.

The application as filed only provides experimental support for a method, wherein zebularine and α -amanitin are applied in combination.

Furthermore, as shown in D1, mere DNA demethylation is not sufficient to allow transposition of all kinds of transposons to take place in all kinds of cells, while e.g. D3 and D4 show that in plants for transposons linked to specific kinds of stress (e.g. ONSEN) the exposure of the plants to this stress (e.g. heat stress) is required to activate the element. Consequently, the subject-matter of claims 1 and 8 is neither supported by the description nor sufficiently disclosed in the sense of Articles 6 and 5 PCT.

- 5 Mobilisation of transposons by the claimed method in organisms other than plants is not supported by the present application (Article 6 PCT). Example 5 merely discloses DNA demethylation in human cells, but no TE mobilisation.
- 6 Claim 18 lacks clarity (Article 6 PCT), as it refers back to claim 1 and at the same time requires presence of an inhibitor of transcription and an inhibitor of DNA methylation. This combination is only disclosed in claim 2.