PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	16 APR 2020
Applicant's or agent's file reference 45AH-288805-WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 19/64910	06 December 2019 (06.12.2019)	07 December 2018 (07.12.2018)

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

IPC - C12N 15/85, C12N 15/87, C12N 15/79 (2020.01)

CPC - C12N 2710/10343, C12N 2800/108, A61K 48/00, C12N 15/86, C12N 2830/46

Applicant
CELLTHEON CORPORATION

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 43*bis*.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 66.1*bis*(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	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	01 April 2020	Lee Young PCT Help Desk Telephone No. 571-272-4300

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Box No. I 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(b)).

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:

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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. 7 and 14

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. 7 and 14 are so unclear that no meaningful opinion could be formed *(specify)*:

claims 7 and 14 are not not drafted in accordance with the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

☐ 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. 7 and 14

☐ 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 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-6 directed to a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a matrix attachment region (MAR) core capable to attach to a mammalian nuclear matrix; and to a cell comprising the same. The recombinant polynucleotide will be searched to the extent that the MAR core encompasses a nucleic acid having at least 90% sequence identity to SEQ ID NO: 1. It is believed that claim 1, limited to a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a matrix attachment region (MAR) core having at least 90% sequence identity to SEQ ID NO: 1 encompass this first named invention, and thus these claims will be searched without fee to the extent that the recombinant polynucleotide comprises said elements. Additional recombinant polynucleotides comprising a MAR will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected oligonucleotides. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a MAR, the MAR comprising a core having at least 90% sequence identity to SEQ ID NO: 5, a 5' flanking region having at least 75% sequence identity to SEQ ID NO: 6 and a 3' flanking region having at least 75% sequence identity to SEQ ID NO: 7 (claims 1-5). Another exemplary election would be a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a MAR, the MAR comprising a core having at least 90% sequence identity to SEQ ID NO: 9, a 5' flanking region having at least 75% sequence identity to SEQ ID NO: 10, a 3' flanking region having at least 75% sequence identity to SEQ ID NO: 11, and (another) MAR having at least 75% sequence identity to SEQ ID NO: 4 (claims 1-7).

Group II+: Claims 8-13, directed to method of transfecting to a cell a coding sequence, comprising contacting the cell with a first polynucleotide comprising the coding sequence and a promoter for initiating transcription of the coding sequence, and a second, unlinked polynucleotide comprising a MAR. Group II+ will be searched upon payment of additional fees. The transfection method may be searched, for example, to the extent that the MAR core encompasses a nucleic acid having at least 90% sequence identity to SEQ ID NO: 1. It is believed that claim 8, reads on this exemplary invention. Additional transfection methods will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected . Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a method of transfecting nucleic acids comprising a first polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a second, unlinked polynucleotide comprising a MAR, the MAR comprising a core having at least 90% sequence identity to SEQ ID NO: 5, a 5' flanking region having at least 75% sequence identity to SEQ ID NO: 6 and a 3' flanking region having at least 75% sequence identity to SEQ ID NO: 7, and a second MAR having at least 75% sequence identity to SEQ ID NO: 4 (claims 8-13).

----------SEE FIRST SUPPLEMENTAL BOX TO CONTINUE----------

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1, limited to a polynucleotide comprising SEQ ID NO: 1

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

1. Statement

Novelty (N)	Claims	1	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	1	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a matrix attachment region (MAR) core nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 1, wherein the MAR core is capable to attach to a mammalian nuclear matrix.

The relevant prior arts on record are given below.

Regarding claim 1, US 2018/0187229 A1 to Selexis SA (hereinafter 'Selexis'). teaches polynucleotides comprising MARs (abstract "purified and isolated DNA sequences having protein production increasing activity and more specifically to the use of matrix attachment regions (MARs)", para [0063] ""MARs", according to a well-accepted model, may mediate the anchorage of specific DNA sequence to the nuclear matrix, generating chromatin loop domains that extend outwards ... they contain a core-unwinding element (CUE) that might represent the nucleation point of strand separation ... Several simple AT-rich sequence motifs have often been found within MAR sequences"), identifying said MARs sequences (para [0047] The sequences SEQ ID Nos 1 to 23 have been identified by scanning human chromosome 1 and 2 using SMAR SCAN, showing that the identification of novel MAR sequences is feasible using the tools reported thereafter whereas SEQ ID No 24 to 27 have been identified by scanning the complete human genome using the combined SMAR SCAN method."), and further teaches multiple specific MAR sequences, and MARs having three regions (para [0052] "particular combinations of elements or fragments of the sequences SEQ ID Nos 1 to 27 and cLysMAR elements or fragments are also envisioned in the present invention, depending on the functional results to be obtained. Elements of the cLysMAR are e.g. the B, K and F regions ... The preferred elements of the cLysMAR used in the present invention are the B, K and F regions. Only one element might be used or multiple copies of the same or distinct elements (multimerized elements) might be used," para [0081] "the cLysMAR element and/or fragment are consisting of at least one nucleotide sequence selected from the B, K and F regions.", para [0249] "In this deletion study, the loss of MAR activity coincided with discrete regions of transition which overlap with the 5'-MAR B-, K- and F-fragment, respectively. In 5' deletions, activity was mostly lost when fragment K and F were removed. 3' deletions that removed the F and b [B] elements had the most pronounced effects."). However, Selexis does not teach a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a matrix attachment region (MAR) core nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 1, wherein the MAR core is capable to attach to a mammalian nuclear matrix.

GenBank submission CT009593.9 (hereinafter 'CT009593.9') teaches a 93184 bp Zebrafish DNA sequence comprising, within nt 70786-70971, a 186 nt sequene that share 100% identity to the applicant's 186 nt SEQ ID NO: 1 (sequence of CT009593.9), however, CT009593.9 does not teach recombinant proteins with matrix attachment regions, nor does CT009593.9 that the 93184 bp Zebrafish DNA sequence comprise said matrix attachment regions, such as the 186 nt SEQ ID NO: 1 of the applicant.

There is no prior art on record that discloses the claimed subject matter, specifically, a recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a matrix attachment region (MAR) core nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 1, wherein the MAR core is capable to attach to a mammalian nuclear matrix.

Thus, claim 1 meets the criteria set out in PCT Article 33(2) and 33(3).

Claim 1 has industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. IV (3) Lack of unity of invention:

Group III+: Claim 15, directed to an isolated chimeric matrix attachment region (MAR), comprising (a) a MAR core, (b) a 5' flanking region and (c) a 3' flanking region. Group III+ will be searched upon payment of additional fees. The chimeric MAR may be searched, for example, to the extent that the MAR encompasses (a) a core nucleic acid sequence having at least 75% sequence identity to SEQ ID NO: 1, (b) a 5 ' flanking region having at least 75% sequence identity to SEQ ID NO: 2 and (c) a 3 ' flanking region having at least 75% sequence identity to SEQ ID NO: 3. It is believed that claim 15, limited to a chimeric MAR having nucleic acid sequences (a)-(c), each having at least 75% sequence identity to SEQ ID NOs: 1-3, respectively, read on this exemplary invention. Additional chimeric MAR nucleic acids will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a a chimeric MAR having nucleic acid sequences (a)-(c), each having at least 75% sequence identity to SEQ ID NOs: 5-7, respectively (Claim 15).

The inventions listed as Groups I+, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the nucleic acid sequences of Group I+ and, accordingly, this group lacks unity a priori.

No technical features are shared between the nucleic acid sequences of Group II+ and, accordingly, this group lacks unity a priori.

No technical features are shared between the nucleic acid sequences of Group III+ and, accordingly, this group lacks unity a priori.

Additionally, even if the inventions listed as Group I+, Group II+ or Group III+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Group I+ requires a (single) recombinant polynucleotide comprising a coding sequence, a promoter configured to initiate the transcription of the coding sequence, and a MAR core, not required by Groups II+ and III+.

Group II+ requires a method for transfecting two polynucleotides, one comprising a coding sequence and promoter, the second comprising one or more MAR sequences, not required by Groups I+ and III+.

Group III+ requires an isolated, chimeric MAR polynucleotide comprising core, a 5' flanking region and a 3' flanking region, each region from a different natural MAR, not required by Groups I+ and II+.

Common Technical Features

The inventions of Groups I+, II+ and III+ share the technical feature of a polynucleotide comprising a MAR that further contains an AT rich core (see SEQ ID NO: 1)and 5' and 3' regions, wherein the core is capable to attach to a mammalian nuclear matrix. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by US 2018/0187229 A1 to Selexis SA (hereinafter 'Selexis').

Selexis teaches polynucleotides comprising MARs (abstract "purified and isolated DNA sequences having protein production increasing activity and more specifically to the use of matrix attachment regions (MARs)", para [0063] ""MARs", according to a well-accepted model, may mediate the anchorage of specific DNA sequence to the nuclear matrix, generating chromatin loop domains that extend outwards ... they contain a core-unwinding element (CUE) that might represent the nucleation point of strand separation ... Several simple AT-rich sequence motifs have often been found within MAR sequences"), identifying said MARs sequences (para [0047] The sequences SEQ ID Nos 1 to 23 have been identified by scanning human chromosome 1 and 2 using SMAR SCAN, showing that the identification of novel MAR sequences is feasible using the tools reported thereafter whereas SEQ ID No 24 to 27 have been identified by scanning the complete human genome using the combined SMAR SCAN method."), and further teaches multiple specific MAR sequences, and MARs having three regions (para [0052] "particular combinations of elements or fragments of the sequences SEQ ID Nos 1 to 27 and cLysMAR elements or fragments are also envisioned in the present invention, depending on the functional results to be obtained. Elements of the cLysMAR are e.g. the B, K and F regions ... The preferred elements of the cLysMAR used in the present invention are the B, K and F regions. Only one element might be used or multiple copies of the same or distinct elements (multimerized elements) might be used," para [0081] " the cLysMAR element and/or fragment are consisting of at least one nucleotide sequence selected from the B, K and F regions.", para [0249] In this deletion study, the loss of MAR activity coincided with discrete regions of transition which overlap with the 5'-MAR B-, K- and F-fragment, respectively. In 5' deletions, activity was mostly lost when fragment K and F were removed. 3' deletions that removed the F and b [B] elements had the most pronounced effects."). Although Selexis does not expressly recite core, 5' and 3' nucleotide sequences claimed by the applicant, Selexis does teach AT rich MAR sequences with 5', middle and 3' regions, further defines active regions, teaches MAR having 3 elements chosen based on their activity and teaches sequence scanning methods to identify MAR in genome sequences. In light of these teachings, it would have been obvious to an artisan of ordinary skill to identify and produce MAR based on the methods of Selexis.

----------SEE NEXT SUPPLEMENTAL BOX TO CONTINUE----------

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. IV (3) Lack of unity of invention:

The inventions of Groups I+ and II+ share the technical feature of a polynucleotide comprising a coding sequence and a promoter.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by Selexis. Selexis teaches DNA sequences (with or without a MAR) (para [0118] "Preferably said purified and isolated DNA sequence comprises a promoter which is operably linked to a gene of interest", para [0134] " the MAR nucleotide sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest. But the invention also envisions the fact that said first and or at least second MAR nucleotide sequences are located on a sequence distinct from the one containing the promoter and the gene of interest.", para [0167] As a particular example of the transfection method, said purified DNA sequence comprising at least one DNA sequence of interest can be introduced in form of multiple unlinked plasmids, comprising a gene of interest operably linked to a promoter, a selectable marker gene, and/or protein production increasing elements such as MAR sequences."),

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II+ and III+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.