PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference		FOR FURTHER ACTION
45AH-288805-WO		see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 19/64910	06 December 2019 (06.12.2019)	07 December 2018 (07.12.2018)
Applicant		
CELLTHEON CORPORATION		

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 6 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 43.6*bis*(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. 1

☒ 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.

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Box No. I **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:

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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.: 7, 14
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:

---------- see extra sheet ----------

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.:
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

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.

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A. CLASSIFICATION OF SUBJECT MATTER

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

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
See Search History document

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.
A	US 2018/0187229 A1 (SELEXIS SA) 05 July 2018 (05.07.2020) abstract, para [0047], [0052], [0063], [0249]	1
A	GenBank submission CT009593.9, 24 March 2009 [online]. [Retrieved on 1 April 2020]. Retrieved from the internet <URL:https://www.ncbi.nlm.nih.gov/nuccore/CT009593 > entire document, nt 70786-70971	1

☐ 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
"D" document cited by the applicant in the international application
"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	Date of mailing of the international search report
01 April 2020	16 APR 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

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Information on patent family members

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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).

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+.

********** See Next Extra Sheet to continue **********

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continuation of previous extra sheet:

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.

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.