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(54) Title: SPECIFIC NUCLEAR-ANCHORED INDEPENDENT LABELING SYSTEM

(57) Abstract: Materials and methods for labeling and isolating particular cell types from mixed cell populations are provided herein. Also provided herein are methods for generating data representing a synthetic genetic sequence configured for labeling at least one cell type by causing expression of a marker in the at least one cell type.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/38520

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/62, C07K 14/47, C12N 15/00, C12N 15/81, C12N 15/67 (2020.01)

CPC - C07K 2319/01, C07K 2319/20, C12N 15/1003, C12N 15/907, G01N 33/5082, G01N 2500/02

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. |
|---|---|---|
| Y<br>-----<br>A | WO 2018/138478 A1 (THE UNIVERSITY OF DURHAM) 2 August 2018 (02.08.2018) Abstract; Claim 7; Claim 23; p31, ln 1-8; p31, ln 1-8; p35, ln 25-33; SEQ ID NO:28; p42, ln 12-15 | 1, 3-8, 16-17<br>----------<br>2 |
| Y<br>----------<br>A | US 2012/0124685 A1 (HENIKOFF et al.) 17 May 2012 (17.05.2012) Abstract; para [0016]; para [0142]; SEQ ID NO 93; para [0189]; para [0222]; para [0376]; para [0380] | 1, 3-8, 16-17<br>----------<br>2 |
| Y | WO 2006/091638 A2 (THE REGENTS OF UNIVERSITY OF CALIFORNIA) 31 August 2006 (31.08.2006) Abstract; p65-66, SEQ ID NO:4 | 5 |
| Y | HIOKI, H. Compartmental organization of synaptic inputs to parvalbumin-expressing GABAergic neurons in mouse primary somatosensory cortex. Anat Sci Int. 3 December 2014, Vol. 90, No. 1, pp 7-21 (Epub p 1-16). Abstract; p 7, Fig. 4 legend | 8 |

☐ 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 |
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| 3 November 2020 | 17 DEC 2020 |
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/38520

**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.:
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:
----Please see continuation in first 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.:
1-8, 16-17

**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**
Information on patent family members

International application No.
PCT/US 20/38520

Continuation of Box No. III. Observations where unity of invention is lacking.

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 1-8, 16-17 directed to a virus particle and nucleic acid construct for cell-type specific expression of a tagged Sun I fusion polypeptide driven by a promoter sequence specific for a selected cell type.

Group II, claims 9-15, 18-19, directed to a virus particle and nucleic acid construct comprising a tagged Sun I fusion polypeptide encoding sequence flanked by lox sequences, wherein the Sun I sequence is arranged in reverse orientation with respect to a promoter.

Group III, claims 20-27, directed to a method for labeling a selected cell type within a population of different cell types.

Group IV, claims 28-34, directed to a method for using a Cre-lox recombination system to label a selected cell type within a population of different cell types.

The inventions listed as Groups I-IV do not relate to a single special technical feature 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:

Group I has the special technical feature of a nucleic acid construct for cell-type specific expression of a tagged Sun I fusion polypeptide driven by a cell type-specific promoter, that is not required by Groups II-IV.

Group II has the special technical feature of a nucleic acid construct comprising a tagged Sun I fusion polypeptide encoding sequence flanked by lox sequences, wherein the Sun I sequence is arranged in reverse orientation with respect to a promoter, that is not required by Groups I, III and IV.

Group III has the special technical feature of a method for labeling a selected cell type within a population of different cell types, that is not required by Groups I, II and IV.

Group IV has the special technical feature of a method for using a Cre-lox recombination system to label a selected cell type within a population of different cell types requiring (ii) a second nucleic acid construct comprising a sequence encoding a Cre recombinase operably linked to a promoter sequence specific for the selected cell type, that is not required by Groups I-III.

Common technical features:

Groups I-IV share the common technical feature of:
(a) a sequence encoding a tagged Sun I fusion polypeptide, wherein the tagged Sun I fusion polypeptide consists essentially of (i) an N-truncated fragment of a SunI gene, wherein the fragment encodes at least a portion of a portion of a Sun I protein, and wherein the portion of the SunI protein is 400 to 600 amino acids in length, and (ii) a sequence encoding a tag polypeptide; and (b) a promoter sequence.

Groups I and III further share the common technical feature of:
(b) a promoter sequence specific for a selected cell type, wherein the promoter sequence is operably linked to the sequence encoding the tagged SunI fusion polypeptide, and is effective to drive expression of the sequence encoding the tagged SunI fusion polypeptide in the selected cell type.

Groups II and IV further share the common technical feature of:
(b) a first lox sequence flanking the 5' end of the sequence encoding the tagged SunI fusion polypeptide and a second lox sequence flanking the 3' end of the sequence encoding the tagged SunI fusion polypeptide, and
(c) a promoter sequence downstream of the lox sequence flanking the 3' end of the sequence encoding the tagged SunI fusion polypeptide, such that the sequence encoding the tagged SunI fusion polypeptide is in reverse orientation with respect to the promoter.

However, these shared technical features do not represent a contribution over prior art, because these shared technical features are rendered obvious by WO 2018/138478 A1 to the University of Durham (hereinafter 'Univ Durham') in view of US 4,959,317 A to Sauer (hereinafter 'Sauer').

------please see continuation on next sheet----------

Continuation of Box No. III. Observations where unity of invention is lacking.

---------continued from previous sheet----------

Univ Durham teaches (a) a sequence encoding a tagged Sun I fusion polypeptide, wherein the tagged Sun I fusion polypeptide consists essentially of (i) an N-truncated fragment of a Sun I gene, wherein the fragment encodes at least a portion of a portion of a Sun I protein, and wherein the portion of the SunI protein is 400 to 600 amino acids in length, and (ii) a sequence encoding a tag polypeptide; and (b) a promoter sequence (Claim 7 - 'The method according to claim 6, wherein the SUN domain is encoded by a nucleotide sequence substantially as set out in SEQ ID No. 9, 11, 13, 15 and/or 17, or a variant or fragment thereof.'; Claim 23 - 'The method according to any one of claims 1 to 20, wherein the agent is a modified SUN protein that consists of a SUN domain.'; p31, ln 1-8, - 'Figure 3 is a schematic diagram of the DN-SUNL construct, which encodes a dominant negative SUNi luminal domain in relation to the full-length Sum protein. Major Sun I protein domains and topologies within the nuclear envelope are indicated. The truncated SUNi protein (lacks the N-terminus, which is found in the nucleoplasm, and the three hydrophobic domains) is fused to the Torsin A signal peptide (SP; the amino acid sequence is given), GFP (green fluorescent protein) and expressed in the endoplasmic reticulum (ER) and nuclear envelope lumen'; p35, ln 25-33, - 'All cloned fragments were sequenced in their entirety. PCDNA3.1 (-) (Invitrogen) was used to engineer the DN-SUNL ... The DN-SUNL comprises torsin-A signal peptide (SP) sequence, sequences encoding GFP (Green Fluorescent Protein), the coiled-coil domain and the SUN-domain of a murine SUNi protein (the SUNi transgene protein lacks the N-terminal domain and the transmembrane domain). The full polypeptide sequence (819 amino acids) of one embodiment of the DN-SUNL is provided herein as SEQ ID No. 28 (previously referred to as SEQ ID No. 40 in patent application GB1701438.2)'; Note, pCDNA3.1 vector contains a CMV promoter for heterologous protein expression). Univ Durham does not expressly teach (b) a promoter sequence specific for a selected cell type. However, since cell-type/tissue specific promoters were well known in the art, it would have been obvious to one of ordinary skill in the art that a cell-type specific promoter of choice could be used to drive the expression in specific cells of the tagged Sun I fusion polypeptide of Univ Durham instead of the CMV promoter, when a cell-type specific expression of the said Sun I fusion polypeptide was desired.

Sauer teaches using a Cre-lox system to generate an inversion of a target gene (Abstract - 'A method for producing site-specific recombination of DNA in eukaryotic cells at regions designated lox sites is disclosed.'; Claim 1 - 'A method for producing site-specific recombination of DNA in eukaryotic cells, comprising,

(a) introducing into the cells a first DNA sequence comprising a first lox site and a second DNA sequence comprising a second lox site, and (b) contacting the lox sites with Cre, thereby producing the site specific recombination.'; Claim 11 - 'A method as defined in claim 4, wherein the first and second lox sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.'). Since use of Cre-lox system for recombining sequences was well known in the art, it would have been obvious to one of ordinary skill in the art that by placing (b) a first lox sequence flanking the 5' end of the sequence encoding the tagged Sun I fusion polypeptide and a second lox sequence flanking the 3' end of the sequence encoding the tagged Sun I fusion polypeptide, and

(c) a promoter sequence downstream of the lox sequence flanking the 3' end of the sequence encoding the tagged Sun I fusion polypeptide, such that the sequence encoding the tagged SunI fusion polypeptide is in reverse orientation with respect to the promoter, one could generate a Cre-inducible recombinant construct in which the Sun I fusion gene is juxtaposed in the correct orientation with respect to the promoter, by excision of lox sites flanking the Sun I fusion gene according to Sauer, to achieve Cre-inducible expression in a desired cell that has both Cre expression vector and the Sun I expression vector.

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.

Therefore, Group I-IV inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.