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- (71) **Applicant (for all designated States except US):** CELLECTIS [FR/FR]; 102, Avenue Gaston Roussel, F-93235 Romainville Cedex (FR).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** SOURDIVE, David [FR/FR]; 19, Rue Louise Michel, F-92300 Levallois-Perret (FR).
- (74) **Agent:** CABINET ORES; 36 rue de St Petersburg, F-75008 Paris (FR).
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DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, 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.

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(54) **Title:** MEGANUCLEASES VARIANTS CLEAVING A DNA TARGET SEQUENCE IN THE NANOG GENE AND USES THEREOF

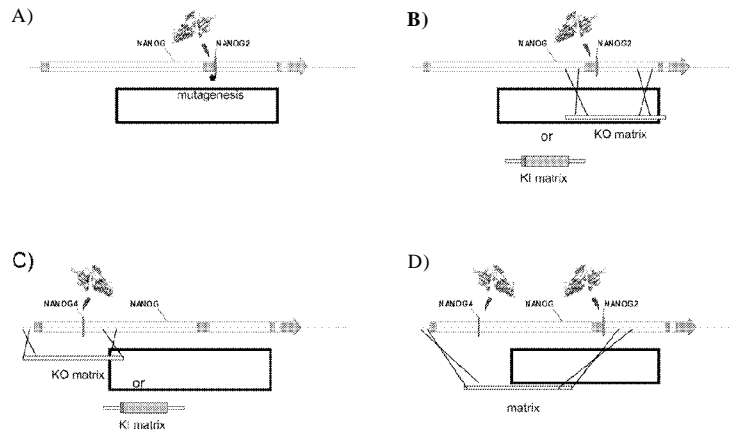


Figure 1

(57) **Abstract:** Meganuclease variants cleaving DNA target sequences of the NANOG gene, vectors encoding such variants, and cells expressing them. Methods of using meganuclease variants recognizing NANOG gene sequences for modifying the NANOG gene sequence or for incorporating a gene of interest or therapeutic gene using the NANOG gene as a landing pad and a safe harbor locus.

WO 2012/004671 A3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/IB2011/001934

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. C12N9/22 ADD.				
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols) C12N				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal , BIOSIS, Sequence Search , WPI Data				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	SCHAMBACH AXEL ET AL: "Generation and genetic modification of induced pluripotent stem cells.", EXPERT OPINION ON BIOLOGICAL THERAPY JUL 2010 LNKD- PUBMED:20528610, vol. 10, no. 7, July 2010 (2010-07) , pages 1089-1103 , XP009153949, ISSN : 1744-7682 page 1093 , left-hand column - page 1094, left-hand column	1-4		
X	----- W0 2006/097784 AI (CELLECTIS [FR] ; PAQUES FREDERIC [FR] ) 21 September 2006 (2006-09-21) cited in the application	5-25, 31-33		
A	----- the whole document	1-4		
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<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.                 </td> <td style="width: 50%; border: none;"> <input checked="" type="checkbox"/> See patent family annex.                 </td> </tr> </table>			<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> 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 document 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  <p style="text-align: center;">12 April 2012</p>		Date of mailing of the international search report  <p style="text-align: center;">20/04/2012</p>		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer  <p style="text-align: center;">Herrmann, Klaus</p>		

INTERNATIONAL SEARCH REPORT

International application No  
PCT/IB2011/001934

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	wo 2009/095742 AI (CELLECTIS [FR] ; GRIZOT SYLVESTRE [FR] ) 6 August 2009 (2009-08-06)	5-25, 31-33
A	the whole document	1-4
A	CHAMBERS IAN ET AL: "Nanog safeguards pluri potency and mediates germline development" , NATURE (LONDON) , vol . 450, no. 7173, December 2007 (2007-12) , page 1230, XP002663241, ISSN: 0028-0836, DOI : DOI : 10.1038/NATURE06403 the whole document	1-25, 31-33
A	HOCKEMEYER DIRK ET AL: "Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases" , NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol . 27, no. 9, 1 September 2009 (2009-09-01) , pages 851-857, XP009143038, ISSN: 1087-0156, DOI : 10.1038/NBT.1562 the whole document	1-25, 31-33
A	FUSAKI NOEMI ET AL: "Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus , an RNA virus that does not integrate into the host genome" , PROCEEDINGS OF THE JAPAN ACADEMY SERIES B PHYSICAL AND BIOLOGICAL SCIENCES, vol . 85, no. 8, October 2009 (2009-10) , pages 348-362 , XP002663242, ISSN: 0386-2208, DOI : DOI : 10.2183/PJAB.85.348 abstract	1-4
A	TSUJI OSAHIKO ET AL: "Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury" , PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol . 107, no. 28, 6 July 2010 (2010-07-06) , pages 12704-12709, XP002663243 , ISSN: 0027-8424, DOI : DOI : 10.1073/PNAS.0910106107 abstract	1-4

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2011/001934

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W <sub>O</sub> 2006097784	A1	21--09--2006	CN 101198694 A
			WO 2006097784 A1
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W <sub>O</sub> 2009095742	A1	06--08--2009	AU 2009208732 A1
			CA 2729124 A1
			CN 101970649 A
			EP 2245147 A1
			JP 2011514144 A
			US 2011179506 A1
			WO 2009095742 A1
			WO 2009095793 A1
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2011/001934

## 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:

see additional 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 an 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. :  
  
1-25, 31-33
  
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.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4

A method to generate clean and/or safe and/or secure iPS and their derivatives at various differentiation stages by using at least one endonuclease.

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2. claims: 5-25, 31-33

A meganuclease that induces a double-strand break in the NANOG gene "and variants", and subject-matter relating thereto.

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3. claims: 26, 27

A cancer or tumor cell into which a reporter gene has been operatively inserted so as to coexpress with the NANOG gene using a meganuclease that induces a double-strand break in the NANOG gene and variants, and subject-matter relating thereto.

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4. claim: 28

A recombinant cell containing the NANOG gene or part of it and the nucleotide sequence of interest.

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5. claims: 29, 30

A cell bank containing cells in which NANOG is knocked-out.

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6. claims: 34-36

Purified iPS cells culture wherein said iPS cells are clean and/or safe and/or secure, and subject-matter relating thereto.

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