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(54) Title: METHODS FOR IDENTIFICATION AND SELECTION OF HUMAN EMBRYONIC STEM CELL DERIVED CELLS

(57) Abstract: A nucleic acid construct is disclosed, the nucleic acid comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, the nucleic acid sequence being operably linked to a human tissue specific promoter. A method of lineage tracing of human stem cells and isolated human embryonic stem cell comprising the nucleic acid construct are also disclosed.



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INTERNATIONAL SEARCH REPORT

International application No  
PCT/IL2008/000500

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C12N5/06 C12N15/85

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)  
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, EMBASE, WPI Data, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>DATABASE BIOSIS [Online]                      BIOSCIENCES INFORMATION SERVICE,                      PHILADELPHIA, PA, US;                      August 2007 (2007-08),                      HUBER I. ET AL.: "Identification and selection of cardiomyocytes during human embryonic stem cell differentiation"                      XP002496938                      Database accession no. PREV200700536999                      abstract                      &amp; FASEB JOURNAL,                      vol. 21, no. 10, August 2007 (2007-08),                      pages 2551-2563,                      ISSN: 0892-6638</p> <p style="text-align: center;">----- -/--</p>	1,2, 14-20

Further documents are listed in the continuation of Box C.       See patent family annex.

\* Special categories of cited documents :

<p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*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)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p>	<p>*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</p> <p>*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</p> <p>*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.</p> <p>*&amp;* document member of the same patent family</p>
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Date of the actual completion of the international search  30 September 2008	Date of mailing of the international search report  15/01/2009
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  Bladier, Cecile

## INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2008/000500

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>US 2003/003533 A1 (FRANZ WOLFGANG M [DE])            2 January 2003 (2003-01-02)            page 2, paragraph 13            page 3, paragraphs 28,33,34,38,39            page 4, paragraphs 62,64,66            example 1            claims 1,3,9,13,15,18,19,29,31</p>	1,2, 14-20
X	<p>US 2003/035794 A1 (FRANZ WOLFGANG-M [DE]            ET AL) 20 February 2003 (2003-02-20)            page 3, paragraph 25; figures 1,2</p>	1,2
X	<p>BREMER S. ET AL.: "Establishment of an            embryotoxicity assay with green            fluorescence protein-expressing embryonic            cell-derived cardiomyocytes"            ATLA. ALTERNATIVES TO LABORATORY ANIMALS,            LONDON, GB,            vol. 27, no. 3,            1 January 1999 (1999-01-01), pages            471-484, XP009049954            ISSN: 0261-1929            page 472, left-hand column, paragraph 3 -            page 472, right-hand column, paragraph 1            figure 1</p>	1,2
A	<p>HENDERSON S.A. ET AL.: "STRUCTURE,            ORGANIZATION, AND EXPRESSION OF THE RAT            CARDIAC MYOSIN LIGHT CHAIN-2 GENE.            IDENTIFICATION OF A 250-BASE PAIR FRAGMENT            WHICH CONFERS CARDIAC-SPECIFIC EXPRESSION"            JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN            SOCIETY OF BIOCHEMICAL BIOLOGISTS,            BIRMINGHAM,; US,            vol. 264, no. 30,            25 October 1989 (1989-10-25), pages            18142-18148, XP002030651            ISSN: 0021-9258            cited in the application            the whole document</p>	
A	<p>ZHU H. ET AL.: "A CONSERVED 28-BASE-PAIR            ELEMENT HF-1 IN THE RAT CARDIAC MYOSIN            LIGHT-CHAIN-2 GENE CONFERS            CARDIAC-SPECIFIC AND            ALPHA-ADRENERGIC-INDUCIBLE EXPRESSION IN            CULTURED NEONATAL RAT MYOCARDIAL CELLS"            MOLECULAR AND CELLULAR BIOLOGY,            vol. 11, no. 4, 1991, pages 2273-2281,            XP002496936            ISSN: 0270-7306            cited in the application            the whole document</p>	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IL2008/000500

## 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 allsearchable 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.:
  
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.:

see annex

### 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 (partially); 2 (totally); 14-20 (partially)

A nucleic acid construct comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, said nucleic acid sequence being operably linked to a human tissue specific promoter, wherein said construct further comprises an additional polynucleotide comprising a nucleic acid sequence encoding an antibiotic resistance moiety, said nucleic acid sequence being operably linked to a constitutive promoter. A method of lineage tracing of human stem cells using said construct, an isolated cell population generated by said method as well as medical uses of said cell population.

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2. claims: 1, 3 (partially); 4 (totally); 14-20 (partially)

A nucleic acid construct comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, said nucleic acid sequence being operably linked to a human myosin light-chain-2 (MLC-2v) promoter. A method of lineage tracing of human stem cells using said construct, an isolated cell population generated by said method as well as medical uses of said cell population.

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3. claims: 1, 3 (partially); 5 (totally); 14-20 (partially)

A nucleic acid construct comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, said nucleic acid sequence being operably linked to a human atrial natriuretic peptide (ANP) promoter. A method of lineage tracing of human stem cells using said construct, an isolated cell population generated by said method as well as medical uses of said cell population.

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4. claims: 1 (partially), 6-7 (totally), 14-20 (partially)

A nucleic acid construct comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, said nucleic acid sequence being operably linked to a human tissue specific promoter, wherein said construct comprises a lentivirus backbone. A method of lineage tracing of human stem cells using said construct, an isolated cell population generated by said method as well as medical uses of said cell population.

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5. claims: 1 (partially), 8-13 (totally)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An isolated human embryonic stem cell comprising a nucleic acid construct comprising a polynucleotide comprising a nucleic acid sequence encoding a detectable expression product, said nucleic acid sequence being operably linked to a human tissue specific promoter. A purified cell population comprising said human embryonic stem cell.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2008/000500

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 2003003533	A1	02-01-2003	AU 5622201 A	03-10-2001
			DE 10014690 A1	18-10-2001
			WO 0171006 A2	27-09-2001
			EP 1218526 A2	03-07-2002
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			EP 0862644 A2	09-09-1998
			JP 2000500017 T	11-01-2000