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(54) Title: PLURIPOTENT EMBRYONIC STEM (ES) CELL LINES, IMPROVED METHODS FOR THEIR PRODUCTION, AND THEIR USE FOR GERM LINE TRANSMISSION AND FOR THE GENERATION OF GENETICALLY MODIFIED ANIMALS

(57) Abstract: The invention relates to a novel composition for maintaining and growing pluripotent and germ line competent mouse embryonic stem cells. The composition includes high glucose DMEM, non-essential amino acids, glutamine, beta-mercaptoethanol and fetal bovine serum or the equivalents thereof, which is conditioned by an immortalized rabbit fibroblast cell line transduced with genomic rabbit Leukemia Inhibitory Factor (LIF). The invention further relates to the use of the composition for producing embryonic stem cell lines and to the use of these cell lines in the production of transgenic animals.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/07396

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C12N5/06 A01K67/027

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)

IPC 7 C12N A01K

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, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 602 307 A (BAYLOR COLLEGE OF MEDICINE, HOUSTON US) 11 February 1997 (1997-02-11) column 27, line 9 - line 14 ---	1,2,4, 7-13
X	US 5 690 926 A (HOGAN BRIGID L M) 25 November 1997 (1997-11-25) column 8, line 27 - line 29 --- -/--	1,2,4, 7-13

Further documents are listed in the continuation of box C.

Patent family members are listed in 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)
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- *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
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- *&* document member of the same patent family

Date of the actual completion of the international search

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21.05.02

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/07396

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>SLAGER H G ET AL: "SECRETION OF TRANSFORMING GROWTH FACTOR-BETA ISOFORMS BY EMBRYONIC STEM CELLS: ISOFORM AND LATENCY ARE DEPENDENT ON DIRECTION OF DIFFERENTIATION" JOURNAL OF CELLULAR PHYSIOLOGY, NEW YORK, NY, US, vol. 156, no. 2, 1 August 1993 (1993-08-01), pages 247-256, XP000644712 ISSN: 0021-9541 the whole document</p> <p style="text-align: center;">---</p>	<p>1,2, 10-13</p>
X	<p>DATABASE WPI Derwent Publications Ltd., London, GB; AN 1998-210272 XP002189758 TAKEDA CHEM IND LTD: "Transgenic animals having inactivated LDL receptor gene", 1998 abstract & JP 10 056915 A (TAKEDA CHEM IND LTD) 3 March 1998 (1998-03-03) abstract</p> <p style="text-align: center;">---</p>	<p>1,2,4, 7-13</p>
Y	<p>RAMIREZ-SOLIS R ET AL: "GENE TARGETING IN EMBRYONIC STEM CELLS" METHODS IN ENZYMOLOGY, ACADEMIC PRESS INC, SAN DIEGO, CA, US, vol. 225, 1992, pages 855-878, XP000918540 ISSN: 0076-6879 page 859</p> <p style="text-align: center;">---</p>	<p>1-13, 15-28</p>
Y	<p>WO 99 09141 A (BIOTRANSPLANT INC) 25 February 1999 (1999-02-25) the whole document</p> <p style="text-align: center;">---</p>	<p>1-13, 15-28</p>
X	<p>MOREADITH R W ET AL: "GENE TARGETING IN EMBRYONIC STEM CELLS: THE NEW PHYSIOLOGY AND METABOLISM" JOURNAL OF MOLECULAR MEDICINE, SPRINGER VERLAG, DE, vol. 75, 1997, pages 208-216, XP002917335 ISSN: 0946-2716 figure 2</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	<p>14,15, 17-19</p>

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/07396

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>THOMPSON S ET AL: "GERM LINE TRANSMISSION AND EXPRESSION OF A CORRECTED HPRT GENE PRODUCED BY GENE TARGETING IN EMBRYONIC STEM CELLS" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 56, no. 2, 27 January 1989 (1989-01-27), pages 313-321, XP001061551 ISSN: 0092-8674 page 320, left-hand column, paragraph 4 -right-hand column, paragraph 2 ---</p>	<p>14,15, 17-19, 22-28</p>
X	<p>ZIJLSTRA ET AL: "Germ-line transmission of a disrupted beta2-microglobulin gene produced by homologous recombination in embryonic cells" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB. vol. 342, no. 248, 23 November 1989 (1989-11-23), pages 435-438, XP002113106 ISSN: 0028-0836 figure 2 -----</p>	<p>14,15, 17-19, 22-28</p>

INTERNATIONAL SEARCH REPORT

international application No.
PCT/EP 01/07396

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.
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.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- 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: 1-13, 16, 20 all completely and 17-19,
21-28 all partially

ES cell culture media conditioned by fibroblasts producing LIF, process of culturing ES cells in such media, ES cell lines obtainable by such process and their use.

2. Claims: 14, 15 all completely and 17-19,
21-28 all partially

ES cells with germline transmission capability and their use

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/07396

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5602307	A	11-02-1997	NONE	
US 5690926	A	25-11-1997	US 5453357 A US 5670372 A	26-09-1995 23-09-1997
WO 9909141	A	25-02-1999	AU 8827998 A WO 9909141 A1	08-03-1999 25-02-1999