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- (74) Agent: **PRINS, A., W.**; Vereenigde, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).
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- (71) Applicant (for all designated States except US): **INTRO-GENE B.V.** [NL/NL]; Wassenaarseweg 72, NL-2333 AL Leiden (NL).
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- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **HATEBOER, Guus** [NL/NL]; Romeinlaan 55, NL-2105 SP Heemstede (NL). **VERHULST, Karina, Cornelia** [NL/NL]; Haarlemmerstraat 30b, NL-2312 GA Leiden (NL). **SCHOUTEN, Govert, Johan** [NL/NL]; Da Costastraat 82, NL-2321 AR Leiden (NL). **UYTDEHAAG, Alphonsus, Gerardus, Cornelis, Maria** [NL/NL]; Park Arenberg 41, NL-3731 EP De Bilt (NL). **BOUT, Abraham** [NL/NL]; Coymansstraat 24, NL-2751 AR Moerkapelle (NL).
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(54) Title: RECOMBINANT PROTEIN PRODUCTION IN A HUMAN CELL USING SEQUENCES ENCODING ADENOVIRUS E1 PROTEIN

(57) Abstract: The present invention provides methods and compositions for the production of recombinant proteins in a human cell line, using sequences encoding at least one E1 protein of an adenovirus where the cells does not encode a structural adenoviral protein from its genome. The methods and compositions are particularly useful for generating stable expression of human recombinant proteins of interest that are modified post-translationally, e.g. by glycosylation. Such proteins may have advantageous properties in comparison with their counterparts produced in non-human systems like Chinese Hamster Ovary (CHO) cells.

INTERNATIONAL SEARCH REPORT

Internat I Application No

PCT/NL 00/00247

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/85 C07K14/505 C12N15/86

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 C07K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 03163 A (FOND NAT TRANSFUSION SANGUINE) 18 February 1993 (1993-02-18) the whole document ---	1-17, 55-78
X	FALLAUX, FRITS J. ET AL: "New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses." HUMAN GENE THERAPY, (SEPT. 1, 1998) VOL. 9, NO. 13, PP. 1909-1917., XP002115715 the whole document ---	1-17, 55-78
Y	WO 97 00326 A (FALLAUX FRITS JACOBUS ;UNIV LEIDEN (NL); BOUT ABRAHAM (NL); EB ALE) 3 January 1997 (1997-01-03) the whole document ---	1-17, 55-78
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☒ 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)

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

20 July 2000

Date of mailing of the international search report

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

INTERNATIONAL SEARCH REPORT

Internati . Application No

PCT/NL 00/00247

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOUT, A. ET AL: "Production of RCA-free batches of E1-deleted recombinant adenoviral vectors on PER. C6" NUCLEIC ACIDS SYMP. SER. (1998), 38(ADVANCES IN GENE TECHNOLOGY: MOLECULAR BIOLOGY IN THE CONQUEST OF DISEASE), 35-36, 1998, XP002115716 the whole document	1-17, 55-78
Y	--- BOUTL, A. (1) ET AL: "A novel packaging cell line (PER. C6) for efficient production of RCA-free batches of E1-deleted recombinant adenoviral vectors." CANCER GENE THERAPY, (NOV.-DEC., 1997) VOL. 4, NO. 6 CONF. SUPPL., PP. S32-S33. MEETING INFO.: SIXTH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER SAN DIEGO, CALIFORNIA, USA NOVEMBER 20-22, 1997, XP002115717 the whole document	1-17, 55-78
Y	--- BOUT, A. (1) ET AL: "Improved helper cells for RCA-free production of E1-deleted recombinant adenovirus vectors." CANCER GENE THERAPY, (1996) VOL. 3, NO. 6 CONF. SUPPL., PP. S24. MEETING INFO.: FIFTH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER SAN DIEGO, CALIFORNIA, USA NOVEMBER 14-16, 1996, XP002115718 the whole document	1-17, 55-78
Y	--- GRAND R J A ET AL: "MODULATION OF THE LEVEL OF EXPRESSION OF CELLULAR GENES IN ADENOVIRUS 12-INFECTED AND TRANSFORMED HUMAN CELLS." EMBO (EUR MOL BIOL ORGAN) J, (1986) 5 (6), 1253-1260., XP002115719 the whole document	1-17, 55-78
P,X	--- US 5 994 128 A (FALLAUX FRITS JACOBUS ET AL) 30 November 1999 (1999-11-30) the whole document	1-17, 55-78
A	--- GRAND, ROGER J. A. (1) ET AL: "The high levels of p53 present in adenovirus early region 1-transformed human cells do not cause up-regulation of MDM2 expression." VIROLOGY, (1995) VOL. 210, NO. 2, PP. 323-334., XP002115720 the whole document --- -/--	

INTERNATIONAL SEARCH REPORT

Internat / Application No

PCT/NL 00/00247

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YU, DIHUA ET AL: "Enhanced c-erbB-2/neu expression in human ovarian cancer cells correlates with more severe malignancy that can be suppressed by E1A" CANCER RES. (1993), 53(4), 891-8,1993, XP002115721 the whole document ---	
A	WO 95 29994 A (UNIV MICHIGAN ;US HEALTH (US)) 9 November 1995 (1995-11-09) the whole document ---	
A	EP 0 185 573 A (PASTEUR INSTITUT ;CENTRE NAT RECH SCIENT (FR); INST NAT SANTE RECH) 25 June 1986 (1986-06-25) the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00247

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:

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.:
1,6,55, 64-75 {compl.} and claims 3-5, 7-17, 56-63 and 76-78 {partially}

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

1. Claims: 1, 6, 55, 64-75 {completely} and claims 3-5, 7-17, 56-63 and 76-78 {partially}

A method of producing at least one proteinaceous substance in a cell, comprising providing a eukaryotic cell having a sequence encoding at least one E1 protein of an adenovirus, where the cell does not encode a structural adenoviral protein from its genome, use of a human cell encoding said E1 protein for the production of viral or recombinant proteins and a human cell comprising said E1 protein.

2. Claims: 2 {completely} and claims 3-5, 7-17, 56-63, 77 and 78 {partially}

A method of enhancing production of a recombinant proteinaceous substance in a cell, comprising providing a eukaryotic cell having a sequence encoding the proteinaceous substance, whereby the coding sequence is under the control of a CMV-promoter or an E1A promoter and where the cell is provided with E1A activity and use of a human cell in said method.

3. Claims: 18-54 {completely} and claims 76, 77 and 78 {partially}

A recombinant mammalian cell immortalized by the presence of a least one adenoviral E1 protein and comprising a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin.

4. Claims: 79, 80 and 81 {completely}

Use of an adenoviral E1B protein having anti-apoptotic activity for enhancing the production of a proteinaceous substance in a eukaryotic cell.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 00/00247

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9303163 A	18-02-1993	DE 69125292 D DE 69125292 T DK 596881 T EP 0596881 A JP 6508982 T	24-04-1997 25-09-1997 13-10-1997 18-05-1994 13-10-1994
WO 9700326 A	03-01-1997	AU 6018296 A CA 2222140 A EP 0833934 A JP 11507552 T US 5994128 A US 6033908 A	15-01-1997 03-01-1997 08-04-1998 06-07-1999 30-11-1999 07-03-2000
US 5994128 A	30-11-1999	AU 6018296 A CA 2222140 A EP 0833934 A JP 11507552 T WO 9700326 A US 6033908 A	15-01-1997 03-01-1997 08-04-1998 06-07-1999 03-01-1997 07-03-2000
WO 9529994 A	09-11-1995	US 5610043 A CA 2187099 A US 5716830 A US 5814452 A	11-03-1997 09-11-1995 10-02-1998 29-09-1998
EP 0185573 A	25-06-1986	FR 2573436 A AT 76428 T CA 1266627 A DE 3586092 A JP 2610015 B JP 61158795 A	23-05-1986 15-06-1992 13-03-1990 25-06-1992 14-05-1997 18-07-1986