Gene Therapy vectors, which are especially useful for cystic fibrosis, and methods for using the vectors are disclosed. In preferred embodiments, the vectors are adenovirus-based. Advantages of adenovirus-based vectors for gene therapy are that they appear to be relatively safe and can be manipulated to encode the desired gene product and at the same time are inactivated in terms of their ability to replicate in a normal lytic viral life cycle. Additionally, adenovirus has a natural tropism for airway epithelia. Therefore, adenovirus-based vectors are particularly preferred for respiratory gene therapy applications such as gene therapy for cystic fibrosis. In one embodiment, the adenovirus-based gene therapy vector comprises an adenovirus 2 serotype genome in which the E1a and E1b regions of the genome, which are involved in early stages of viral replication have been deleted and replaced by genetic material of interest (e.g., DNA encoding the cystic fibrosis transmembrane regulator protein). In another embodiment, the adenovirus-based therapy vector is a pseudo-adenovirus (PAV). PAVs contain no potentially harmful viral genes, have a theoretical capacity for foreign material of nearly 36 kb, may be produced in reasonably high titers and maintain the tropism of the parent adenovirus for dividing and non-dividing human target cell types.
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TJ Tajikistan
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### A. CLASSIFICATION OF SUBJECT MATTER

| IPC 5 | C12N15/86 | C12N15/12 | A61K48/00 |

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 5 | C12N | C07K | A61K |

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

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>P, X</td>
<td>CELL., vol. 75, no. 2, 22 October 1993, CAMBRIDGE, NA US pages 207 - 216 ZABNER, J. ET AL. 'Adenovirus-mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with Cystic Fibrosis' see the whole document ---</td>
<td>1-5, 8, 18</td>
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<td>P, X</td>
<td>FR, A, 2 688 514 (CNRS) 17 September 1993 see page 2, line 25 - page 3, line 5 ---</td>
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</table>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

**Special categories of cited documents:**

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- "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:** 30 May 1994

**Date of mailing of the international search report:** 4-10-1994

**Name and mailing address of the ISA:** European Patent Office, P. B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31) 070 340-2040, Tx. 31 651 epo nl, Fax (+31) 070 340-3016

**Authorized officer:** CHAMONNET, F.
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<tr>
<td>X</td>
<td>NUCLEIC ACIDS RESEARCH., vol.11, no.24, 1983, ARLINGTON, VIRGINIA US pages 8735 - 8745 SASSONE-CORSI, P. ET AL. 'Far upstream sequences are required for efficient transcription from the adenovirus-2 E1A transcription unit' see the whole document</td>
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<td>X</td>
<td>EP,A,0 185 573 (INSERM) 25 June 1986 see the whole document</td>
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<td>CELL., vol.68, no.1, 10 January 1992, CAMBRIDGE, MA US pages 143 - 155 ROSENFELD, M.A. ET AL. 'In vivo transfer of the human Cystic Fibrosis Transmembrane Conductance Regulator gene to the airway epithelium' see the whole document</td>
<td>1-5,8,18</td>
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<tr>
<td>Y</td>
<td>EP,A,0 446 017 (GENZYME CORPORATION) 11 September 1991 cited in the application see page 21 - page 23; claims 21,28-30,65,67</td>
<td>1-5,8,18</td>
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INTERNATIONAL SEARCH REPORT

PCT/US93/11667

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. X Claims Nos.; because they relate to subject matter not required to be searched by this Authority, namely:
   
   Remark: Although claims 18, 24, 25 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. X 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:

   Obscurities: claim 6 refers to "sequens shown in figure 17". However "figure 17 shows an example of UV fluorescence from an agarose electrophoresis (p7, 1.1)"

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 annex

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. X 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.:

   1-5, 7, 8, 18 (completely); 11, 14, 24, 25 (partially)

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest.

□ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet [1]) (July 1992)
LACK OF UNITY OF INVENTION

1. Claims 1-5,7,8,18 (completely); 11,14,24,25 (partially):
   Adenovirus-2 based vectors deleted for Ela and Elb genes

2. Claims 9,10,12,13,15,16 (completely); 11,14,22-25 (partially):
   Adenoviral vectors deleted for all E4 open reading frames
   except 6 or 3

3. Claims 17,19-21 (completely); 22,23 (partially):
   Gene therapy for Cystic Fibrosis by administering
   to the pulmonary airways of a patient a vector
   encoding CFTR gene
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Form PCT ISA/210 (patent family annex) (July 1992)