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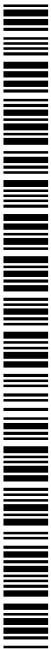
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(71) Applicant: GENZYME CORPORATION [US/US];  
One Kendall Square, Cambridge, MA 02139 (US).

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(72) Inventor: YEW, Nelson; 25 Rockdale Hill Circle, West Upton, MA 01568 (US).



(54) Title: EXPRESSION VECTORS CONTAINING HYBRID UBIQUITIN PROMOTERS

(57) **Abstract:** Sustained transgene expression will be required for the vast majority of genetic diseases being considered for gene therapy. The initially high levels of expression attained with plasmid DNA (pDNA) vectors containing viral promoters, such as that from cytomegalovirus (CMV), decline precipitously to near background levels within 2 to weeks. We have constructed pDNA vectors containing the human cellular ubiquitin B (Ub) promoter and evaluated their expression in the mouse lung. Cationic lipid-pDNA complexes were instilled intranasally (IN) or injected intravenously (IV) into immunodeficient BALB/c mice. Chloramphenicol acetyltransferase (CAT) reporter gene expression from the Ub promoter was initially very low at day 2 post-administration but by day 35 exceeded the level of expression attained from a CMV promoter vector by 4- to 9-fold. Appending a portion of the CMV enhancer 5' of the Ub promoter (CMV-Ub) increased CAT expression to nearly that of the CMV promoter and expression persisted in the lung for at least three months, with 50% of day 2 levels remaining at day 84. In the liver, expression from the CMV-Ub hybrid promoter was sustained for 42 days. Since previous studies have shown that eliminating immunostimulatory CpG motifs in pDNA vectors reduces their toxicity, we constructed a CpG deficient version of the CMV-Ub vector expressing alphagalactosidase A, the enzyme that is deficient in Fabry disease, a lysosomal storage disorder. After IN or IV administration, levels of alpha-galactosidase A from this vector were not only undiminished but increased 500% to 1500% by day 35. These results suggest that CpG-reduced plasmid vectors containing a CMV-Ub hybrid promoter may provide the long-term expression and efficacy required for a practical gene therapeutic.

WO 02/024932 A3

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/28870

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 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)

IPC 7 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, WPI Data, PAJ, CHEM ABS Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 063 598 A (BERGEMANN KLAUS ET AL) 16 May 2000 (2000-05-16) page 3, line 66 -page 4, line 15 page 25; claims 1,4,6,15 page 26; claim 16	1-4,6-11
Y	---	5
Y	WO 00 14262 A (GENZYME CORP) 16 March 2000 (2000-03-16) cited in the application page 65; claim 1 figure 6	5
A	WO 98 32869 A (BAVARIAN NORDIC RES INST AS ;JOHANSEN TEIT E (DK); NEUROSEARCH AS) 30 July 1998 (1998-07-30) ---	-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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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

Seroz, T

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SCHORPP M ET AL: "The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 24, no. 9, 1 May 1996 (1996-05-01), pages 1787-1788, XP002121787 ISSN: 0305-1048 cited in the application</p> <p>-----</p> <p>YEW N S ET AL: "Reduced inflammatory response to plasmid DNA vectors by elimination" MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, USA, vol. 1, no. 3, March 2000 (2000-03), pages 255-262, XP001078874 ISSN: 1525-0016</p> <p>-----</p>	
A		

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 6063598	A 16-05-2000	DE CA WO EP JP	19539493 A1 2234071 A1 9715664 A1 0857211 A1 11513572 T	30-04-1997 01-05-1997 01-05-1997 12-08-1998 24-11-1999
WO 0014262	A 16-03-2000	AU EP WO US	5911899 A 1109924 A2 0014262 A2 2002042383 A1	27-03-2000 27-06-2001 16-03-2000 11-04-2002
WO 9832869	A 30-07-1998	WO EP	9832869 A1 0961830 A1	30-07-1998 08-12-1999