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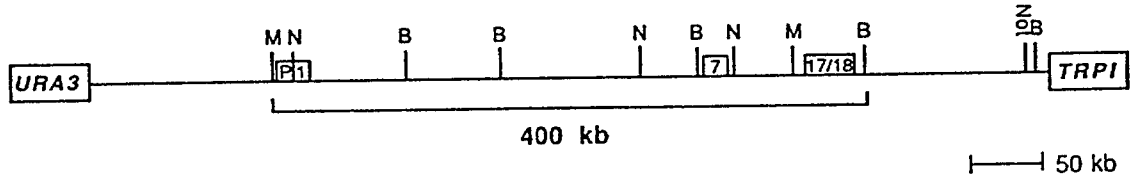

(57) Abstract

This invention provides a method for the efficient introduction of cloned, very high molecular weight DNA into the germline of mice, whereby large genes can be expressed appropriately in transgenic mice. The β-amyloid precursor protein (APP) is known to be a complex gene consisting of 18 exons with total size estimates greater than 170 kb encoding three major RNA splicing forms. According to this invention, a neomycin resistance cassette is introduced into one of the arms of a 650 kb yeast artificial chromosome (YAC) which contains the entire unrearranged APP gene within 400 kb. Following gel purification, the YAC is introduced into embryonic stem (ES) cells by lipid mediated transfection. Neomycin resistant ES lines are isolated with the human APP gene stably integrated in an unrearranged state and expressing properly initiated and spliced full length human APP mRNA and APP human protein. Mouse chimeras generated from these ES lines transmit the YAC to their offspring, generating novel APP YAC transgenic mice. These transgenic mice express human APP gene products at significant levels in brain and peripheral tissues that mirror the expression of endogenous mouse APP gene products. This procedure will have great utility for transgenic studies of gene expression involving large genes and gene complexes.

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 94/03619

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/87 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 5 C12N C07K 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO,A,93 05165 (AGRICULTURE AND FOOD RESEARCH COUNCIL ET AL.) 18 March 1993 see page 4, line 19 - page 5, line 35 and claims. ---	1-5 6-12
X	SCIENCE vol. 259 , 1993 pages 1904 - 1907 W.M. STRAUSS ET AL.; 'Germ line transmission of a yeast artificial chromosome spanning the murine alpha-1(I) collagen locus'	1-5
Y	see the whole document. --- -/--	6-12

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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	Yeats, S

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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.
O,Y	J. CELL. BIOCHEM. SUPPL. 0 (16 PART E) 1992 page 212 B.T. LAMB AND J.H. GEARHART 'Characterization and manipulation of yeast artificial chromosomes containing the human amyloid precursor protein gene' & Keystone Symposium on advances in understanding neurodegenerative disorders, Big Sky, Montana, USA, March 28 - April 4, 1992.	1-12
Y	EP,A,0 518 583 (IMPERIAL CHEMICAL INDUSTRIES PLC) 16 December 1992 see page 11, lines 32-56 and claims.	1-12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
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