



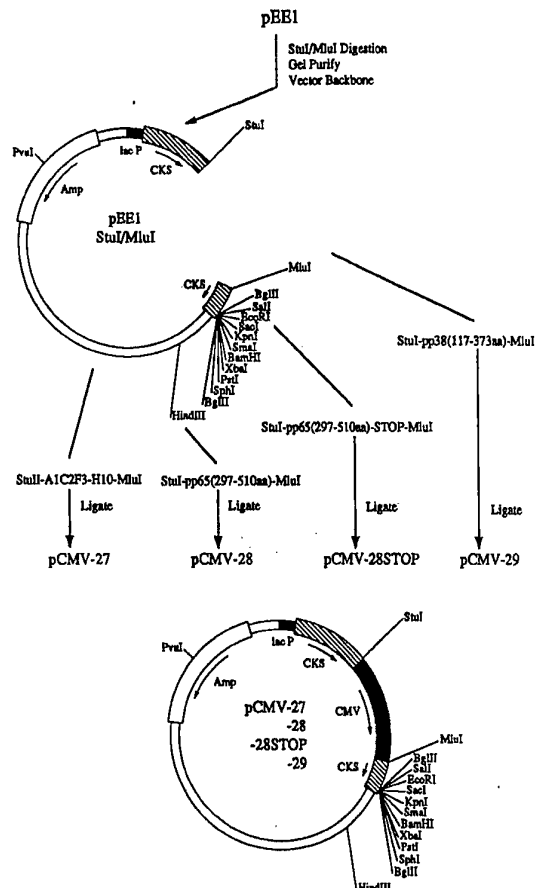
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<p>(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).</p>	<p>(88) Date of publication of the international search report: 19 December 1996 (19.12.96)</p>	
<p>(72) Inventors: MAINE, Gregory, T.; 5743 Constitution Avenue, Gurnee, IL 60031 (US). CHOVAN, Linda, E.; 7851 18th Avenue, Kenosha, WI 53143 (US).</p>		
<p>(74) Agents: DANCKERS, Andreas, M. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).</p>		

(54) Title: EMBEDDED EXPRESSION OF HETEROLOGOUS PROTEINS

(57) Abstract

A method for expressing a heterologous protein in a prokaryotic host cell, comprising the steps of: (a) providing a DNA vector having: (1) a control region operatively linked to a first genetic element encoding a carrier protein capable of expression in the host cell, and (2) a second genetic element encoding the heterologous protein, the second element being embedded within the first element such that the first and second elements are contiguous and in the same reading frame; (b) transforming the host cell with the DNA vector; and (c) expressing a fusion protein of the heterologous protein and the carrier protein, wherein the heterologous protein is joined at its N-terminus to a first domain of the carrier protein and at its C-terminus to a second domain of the carrier protein, and also a method for confirming intact expression of heterologous proteins, as well as fusion proteins, DNA constructs, plasmid vectors and transformed host cells relating to the above methods.



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A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/72 C12N15/62 C12N15/38 C12N15/54 C12N9/12 C12N1/21 C07K14/045 //(C12N1/21,C12R1:19)		
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 6 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	EP,A,0 355 737 (BEHRINGWERKE AG) 28 February 1990 see the whole document ---	1-5, 13, 14, 21-24, 26-28, 36, 38, 39
X	WO,A,92 13955 (GENETICS INST) 20 August 1992 see the whole document ---	1, 4, 13, 14, 24, 27, 36, 38, 39
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Date of the actual completion of the international search 29 October 1996		Date of mailing of the international search report 08. 11. 96
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 Hornig, H

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PCT/US 96/06841

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENE, vol. 85, no. 1, 21 December 1989, pages 115-124, XP000085752 HEDEGAARD L ET AL: "TYPE 1 FIMBRIAE OF ESCHERICHIA COLI AS CARRIERS OF HETEROLOGOUS ANTIGENIC SEQUENCES" see the whole document ---	1,3,4, 13,14, 24,26, 27,36, 38,39
A	EP,A,0 331 961 (ABBOTT LAB) 13 September 1989 cited in the application see the whole document ---	1-39
P,X	WO,A,95 16044 (GENETICS INST) 15 June 1995 see the whole document ---	1,4,13, 14,24, 27,36, 38,39
P,X	WO,A,96 01321 (ABBOTT LAB ;LANDINI MARIA PAOLA (IT); RIPALTI ALESSANDRO (IT); MAI) 18 January 1996 see page 45, line 7 - page 49, line 13 see page 49, line 15 - page 54, line 14 see page 54, line 22 - page 55, line 4; figures 12-16B -----	1,3-24, 26-39

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/06841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0355737	28-02-90	DE-A- 3828666	01-03-90
		AU-B- 614174	22-08-91
		AU-A- 4016089	01-03-90
		JP-A- 2135095	23-05-90

WO-A-9213955	20-08-92	US-A- 5270181	14-12-93
		AU-B- 663489	12-10-95
		AU-A- 1467192	07-09-92
		CA-A- 2093643	07-08-92
		EP-A- 0574506	22-12-93
		JP-B- 2513978	10-07-96
		US-A- 5292646	08-03-94

EP-A-0331961	13-09-89	AT-T- 131876	15-01-96
		AU-A- 3120689	14-09-89
		CA-A- 1335358	25-04-95
		DE-D- 68925138	01-02-96
		DE-T- 68925138	13-06-96
		ES-T- 2083957	01-05-96
		JP-A- 2035089	05-02-90
		JP-B- 8013273	14-02-96
		US-A- 5124255	23-06-92
		US-A- 5312737	17-05-94
		US-A- 5322769	21-06-94

WO-A-9516044	15-06-95	AU-A- 1399495	27-06-95

WO-A-9601321	18-01-96	AU-A- 2418895	25-01-96
