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(54) Title: METHODS AND NUCLEIC ACID VECTORS FOR RAPID EXPRESSION AND SCREENING OF CDNA CLONES

(57) Abstract: Recombinant DNA vectors and methods for cloning and expressing nucleic acid molecules by using a combination of site-specific recombination and end-to-end joining or linking of nucleic acid molecules, such as endonuclease restriction digestion and ligation. The DNAs, vectors and methods can be used for inserting, exchanging, transferring a variety of DNA segment (s) both *in vitro* and *in vivo*. Also disclosed are linker molecules and methods using these linkers the can be used for cloning a gene of interest into an expression vector in one-step. The linker sequences comprise adapter sequences for cloning purposes, as well as eukaryotic and prokaryotic ribosome binding sites for increase translation efficiency.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/23362

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68; C12N 15/00, 5/00; C07H 21/02, 2104

US CL : 435/6, 320.1, 325; 536/23.1, 24.1

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)

U.S. : 435/6, 320.1, 325; 536/23.1, 24.1

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 practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,143,557 A (HARTLEY et al.) 07 November 2000 (07.11.2000), column 4, lines 45-60, column 4, line 65 to column 5, line 10, column 5, lines 13-20, Figure 1; column 11, lines 50-55, column 13, line 56 to column 16, line 10, column 18.	1-24 and 35
A	US 4,959,317 A (SAUER) 25 September 1990 (25.09.1990), see entire document.	1-24 and 35
A	US 5,348,886 A (LEE et al.) 20 September 1994 (20.09.1994), see entire document.	1-24 and 35
A	US 5,783,386 A (JACOBS, JR. et al.) 21 July 1998 (21.07.1998), see entire document.	1-24 and 35

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/23362

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim 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. Claim 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:
Please See Continuation Sheet

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.: 1-24 and 35
- Remark on Protest The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-24 and 35, drawn to a method for transferring a nucleic acid fragment from one Parent molecule to another Parent molecule.

Group II, claim(s) 25-30 and 33 and 34, drawn to a method of amplifying a nucleic acid and a kit comprising oligonucleotide primers involved therein.

Group III, claim(s) 31 and 32, drawn to a method of producing a vector.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: 37 CFR 1.475 which governs Lack of Unity practice in International and National Stage Application recites that an international or a national application containing claims to different categories of invention will be considered to have unity of invention *if the claims are drawn to only one of the following combination of categories:*

- 1) a product and a process specially adapted for the manufacture of said product; or
- 2) a product and process of use of said product; or
- 3) a product and a process specially adapted for the manufacture of said product; and a use of said product; or
- 4) a process and an apparatus or means specifically designed for carrying out the said process; or
- 5) a product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Instant application contains three methods which employ different steps to achieve different outcomes as recited in the different Groups. 37 CFR 1.475 states that if an application contains claims to "more or less than one of the combination of categories of invention set forth in paragraph (recited above), unity of invention might not be present. Therefore, the additional methods of Groups II and III are determined to be outside of the combination of categories characterized under unity of invention practice and therefore, are grouped separately.

Continuation of B. FIELDS SEARCHED Item 3:

US Patent Databases (USPT, USPGP, EPO, JPO, DERWENT, IBM-TDB)

search terms: recombination, vector, recombination sites, atfb, attp, atfl, attr, functional/regulatory region/element, ligation, ligase, overhang, sticky ends, parent DNA/molecules.