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(54) Title: RECOMBINANT CLONE SELECTION SYSTEM

(57) Abstract

Cloning systems useful for the isolation of recombinant nucleic acid are disclosed in which the recombination of cloning-system nucleic acid and foreign nucleic acid is linked to the expression of a moiety on the surface of a host organism, the moiety being a first member of a binding pair. When recombination occurs between the nucleic acid and the foreign nucleic acid, the moiety is expressed on the surface of the host organism. The isolation of recombinant nucleic acid is then performed by attaching a second member of the binding pair to a solid support and contacting the host organism with the support. When the first member of the binding pair is expressed on the surface of the host organism, the host organism binds to the second member of the binding pair attached to the solid support, thereby selectively isolating those organisms. Other aspects of the invention include devices for the isolation of individual cells that differentially express binding moieties on their surface. The devices may be adapted for polynucleotide sequencing and analysis of polynucleoides in the isolated cells. Additional aspects of the invention include methods of using the devices for single cell isolation and analysis.

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/13010

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/70 C12N11/16

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

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.
A	JOURNAL OF BACTERIOLOGY, vol. 155, no. 3, September 1983, pages 1071-1077, XP000612289 KEHOE, M. ET AL.: "Expression of a cloned K88ac adhesion antigen determinant: Identification of a new adhesion cistron and role of a vector-encoded promoter" see the whole document ---	1-7
A	MOLECULAR MICROBIOLOGY, vol. 11, no. 3, 1994, pages 525-536, XP000610997 HULAMAN, T.T ET AL.: "Leucine-responsive regulatory protein, IS1 insertions, and the negative regulator FaeA control the expression of the fae (K88) operon in Escherichia coli" see the whole document ---	1-7

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

[X] Patent family members are listed in annex.

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"E" earlier document but published on or after the international filing date

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
2 April 1997	18. 04. 97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/13010

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 198 474 A (ENDOTRONICS INC) 22 October 1986 see the whole document	8,9
E	WO 96 32486 A (TNO ;LEER ROBERT JAN (NL); POUWELS PIETER HENDRIK (NL); BOERSMA WI) 17 October 1996 see the whole document	8-11
E	WO 96 40943 A (UNIV ROCKEFELLER ;DARZINS ALDIS (US); WHITEHEAD STEPHEN (US); HRUB) 19 December 1996 see the whole document	10,11
X	WO 95 17509 A (MAX PLANCK GESELLSCHAFT ;KLAUSER THOMAS (DE); KRAMER JOACHIM (DE);) 29 June 1995 see the whole document	10-13
X	WO 94 00581 A (VIAGEN OY) 6 January 1994 see the whole document	10-13
A	WO 92 14819 A (DU PONT) 3 September 1992 see abstract	12,13
X	WO 89 04203 A (UNIV NORTH CAROLINA) 18 May 1989 see the whole document	14-41
E	WO 96 36696 A (BREEDERS T) 21 November 1996 see the whole document	14,15
X	WO 93 02360 A (TRANSMED BIOTECH INC) 4 February 1993 see the whole document	14-41
X	WO 91 19567 A (CHIRON CORP) 26 December 1991 see the whole document	14,15

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ernational application No.

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INTERNATIONAL SEARCH REPORT

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

2. ☐ 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:

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:

6 inventions * see continuation-sheet PCT/ISA/210 *

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 searches 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.:

1-7,14-16,20-41

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.:

Remark on Protest

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

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 13010

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1). Claims 1-7

which are directed to a cloning system for expression in a host cell (E. coli) comprising a repressor gene with an insertion site, a promoter for promoting expression of the repressor gene, a surface-expressed moiety gene (type 1 fimbriae) and an operator functionally linked to the expression of the surface-expressed gene and a method for cloning an isolating recombinant nucleic acids by using such a cloning system wherein the host organism additionally is contacted with a solid support having attached thereto a binding moiety (RNase B) capable of specifically binding to the surface expressed moiety.

2). Claims 8-9

which are directed to a different cloning system comprising inter alia a first and a second insertion site located at different positions of the construct and furthermore embrace an expression blocking sequence.

3). Claims 10-11

which differ from the first and second group of claims in that they are directed to a cloning system comprising inter alia a tag moiety sequence being adapted for linking to a foreign nucleic acid and thereby forming an insertion sequence.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

4). Claims 12-13

which differ from the groups of claims listed above in that they are directed to a cloning system comprising inter alia a first and a second promoter as well as a lethal gene.

5). Claims 14-16 and 20-41

which are directed to a host organism capture system comprising a solid support and a plurality of binding moieties attached to the solid support and a method of analysing individual cells of interest, said method comprising contacting a liquid containing a cell population to such a host capture system. The subject-matter of this group of claims is totally different from the residual matter claimed.

6). Claims 17-19

which are directed to a method for fabricating a host capture system including an array of binding moieties bound to a solid support. Concerning this group of claims it has to be noted that there is neither a linkage to group (5) claims nor to any of the claims of the residual groups.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ...nal Application No
PCT/US 96/13010

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 96/13010

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