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(54) Title: METHODS AND COMPOSITIONS FOR SYNTHESIS OF NUCLEIC ACID MOLECULES USING MULTIPLE RECOGNITION SITES

(57) Abstract: The present invention provides compositions and methods for recombinational cloning. The compositions include vectors having multiple recombination sites and/or multiple topoisomerase recognition sites. The methods permit the simultaneous cloning of two or more different nucleic acid molecules. In some embodiments the molecules are fused together while in other embodiments the molecules are inserted into distinct sites in a vector. The invention also generally provides for linking or joining through recombination a number of molecules and/or compounds (e.g., chemical compounds, drugs, proteins or peptides, lipids, nucleic acids, carbohydrates, etc.) which may be the same or different. The invention also provides host cells comprising nucleic acid molecules of the invention or prepared according to the methods of the invention, and also provides kits comprising the compositions, host cells and nucleic acid molecules of the invention, which may be used to synthesize nucleic acid molecules according to the methods of the invention.

INTERNATIONAL SEARCH REPORT

International application No.

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| A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C12Q 1/68; C12N 1/21, 15/00, 15/11, 15/63; C12P 19/34 US CL : 435/6, 69.1, 91.1, 91.2, , 252.3, 320.1; 536/23.1 | | | |
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| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | | |
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| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | |
| Category * Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim | No. | | |
| X WO 02/16594 A2 (CHESTNUT et al.) 28 February 2002 (28.02.2002), pages 1-7 and 10- 1-11, 13, 14, and 12. | 16- | | |
| X,T US 2003/0220249 A1 (HACKETT et al) 27 November 2003 (27.11.2003), section [0195], [0217], and [0238]. | | | |
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| Further documents are listed in the continuation of Box C. See patent family annex. | | | |
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| Date of the actual completion of the international search Date of mailing of the international search report March 2004 (04 03 2004) | | | |
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| Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Authorized officer Young J. Kim | | | |
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| Continuation of B. FIELDS SEARCHED Item 3: | |
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| (1011, 001 01, 010, 010, 010, 010) | |
| search terms: recombination, topoisomerase recognition, restriction sites, vector | ncDNAGW_DT/cc\ nENTD DT/cc\ ncDNA |
| search terms: recombination, topoisomerase recognition, restriction sites, vector | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- |
| search terms: recombination, topoisomerase recognition, restriction sites, vector DEST41, pENTR/D-TOPO, pENTR/SD/D-TOPO, pcDNA3.2/V5/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| search terms: recombination, topoisomerase recognition, restriction sites, vector DEST41, pENTR/D-TOPO, pENTR/SD/D-TOPO, pcDNA3.2/V5/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
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| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/VS/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| search terms: recombination, topoisomerase recognition, restriction sites, vector DEST41, pENTR/D-TOPO, pENTR/SD/D-TOPO, pcDNA3.2/V5/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/VS/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/VS/GWD-TOPO | , pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA- , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PcDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PcDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PcDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
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| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PcDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PcDNA3.2/VS/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | , pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
| DES141, PENTR/D-10PO, PENTR/SD/D-10PO, PCDNA3.2/V5/GWD-TOPO | pcDNA6.2/V5/GWD-TOPO, and cloning. |
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