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(54) Title: IMPROVED METHOD FOR HOMOLOGOUS RECOMBINATION

(57) Abstract: The present invention discloses a method to construct eukaryotic cells having a target sequence in a chromosomal DNA sequence replaced by a replacement sequence of interest comprising: - modifying a parent eukaryotic cell with a preference for NHR to provide a eukaryotic cell having an increased HR/NHR ratio as compared to the parent cell, - providing two sets of DNA molecules of which the first set comprises DNA molecules each comprising a first non-functional fragment of the replacement sequence of interest flanked at its 5'-side by a DNA sequence substantially homologous to a sequence of the chromosomal DNA flanking the target sequence and the second set comprises DNA molecules each comprising a second non-functional fragment of the DNA replacement sequence of interest overlapping with the first non-functional fragment and flanked at its 3'-side by a DNA sequence substantially homologous to a sequence of the chromosomal DNA flanking the target sequence, wherein the first and second non-functional fragments become functional upon recombination; - transforming the modified eukaryotic cells with both sets of DNA molecules; and - growing the cells to obtain transformed progeny cells having a DNA molecule from the first set recombined with a DNA molecule from the second set into a DNA molecule comprising a functional replacement sequence and having the target sequence replaced by the functional replacement sequence.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

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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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EPO-Internal, WPI Data, Sequence Search, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/013979 A (INVITROGEN CORP [US]; UNIV CALIFORNIA [US]; BERTOLINI LUCIANA R [US];) 1 February 2007 (2007-02-01) the whole document	1-9
Y	WO 2005/095624 A (DSM IP ASSETS BV [NL]; DEKKER PETRUS JACOBUS THEODORU [NL]; BERG VAN D) 13 October 2005 (2005-10-13) the whole document	1-9
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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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.
Y	NIELSEN ET AL: "Efficient PCR-based gene targeting with a recyclable marker for <i>Aspergillus nidulans</i> " FUNGAL GENETICS AND BIOLOGY, SAN DIEGO, CA, US, vol. 43, no. 1, January 2006 (2006-01), pages 54-64, XP005234491 ISSN: 1087-1845 the whole document -----	1-9
A	NINOMIYA Y ET AL: "Highly efficient gene replacements in <i>Neurospora</i> strains deficient for nonhomologous end-joining" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 101, no. 33, 17 August 2004 (2004-08-17), pages 12248-12253, XP002346025 ISSN: 0027-8424 the whole document -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

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WO 2005095624	A	13-10-2005	AU	2005229437 A1	13-10-2005
			CA	2559827 A1	13-10-2005
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