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(54) Title: HYPERTHERMOPHILIC POLYMERASE ENABLED PROXIMITY EXTENSION ASSAY

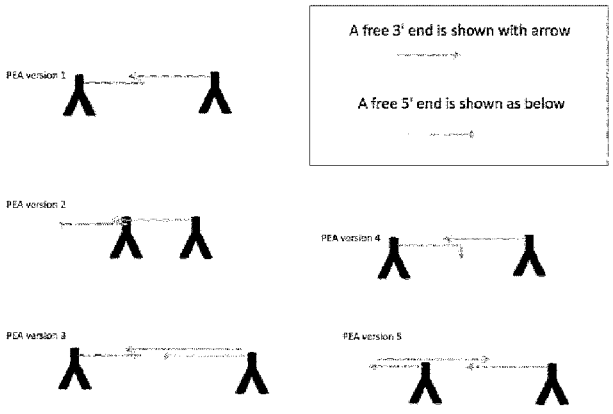

Figure 1

(57) Abstract: The present invention relates to a proximity probe based detection assay ("proximity assay") for an analyte in a sample, specifically a proximity probe extension assay (PEA), an in particular to an improvement in the method to reduce non-specific "background" signals, wherein the improvement comprises the use in such assays of a hyperthermophilic polymerase, said method comprising: (a) contacting said sample with at least one set of at least first and second proximity probes, which probes each comprise an analyte-binding domain and a nucleic acid domain and can simultaneously bind to the analyte; (b) allowing the nucleic acid domains of the proximity probes to interact with each other upon binding of said proximity probes to said analyte, wherein said interaction comprises the formation of a duplex; (c) extending the 3' end of at least one nucleic acid domain of said duplex to generate an extension product, wherein the extension reaction comprises increasing the temperature of assay above room temperature and uses a polymerase enzyme which is characterised as having less than 20% of its maximal enzyme activity at 40°C and having less than 10% of its maximal enzyme activity at 25°C, wherein the optimum temperature for maximal activity of the polymerase is more than 40°C and wherein the polymerase is selected from Pyrococcus furiosus (Pfu) DNA polymerase and Pyrococcus woesei (Pwo) DNA polymerase or a derivative or mutant thereof, preferably wherein said derivative is a sequence- modified derivative; and (d) amplifying and detecting the extension product.

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
20 March 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/051690

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

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   b. (time)

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3. Additional comments:

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N33/53
ADD.

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)
C12Q G01N

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/107743 A1 (OLINK AB [SE]; DZIEGLEWSKA HANNA EVA [GB]; SCHALLMEINER EDITH [SE]) 27 September 2007 (2007-09-27) pages 18,19,25; claims 7,14	21-24
X	WO 2005/123963 A2 (UNIV LELAND STANFORD JUNIOR [US]; FREDRIKSSON SIMON [US]; DAVIS RONALD) 29 December 2005 (2005-12-29) paragraphs [00119], [00124], [00156], [00157]; claims 29-33	21-24
Y	US 2008/131883 A1 (ADAMS THOMAS [US] ET AL) 5 June 2008 (2008-06-05) paragraphs [0010], [0365], [0382], [0388]; figures 1-3,5-7; example 3	1-24

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

[X] 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
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Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schmitt, Anja

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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.
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Y	M. LUNDBERG ET AL: "Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood", NUCLEIC ACIDS RESEARCH, vol. 39, no. 15, 6 June 2011 (2011-06-06), pages e102-e102, XP055022762, ISSN: 0305-1048, DOI: 10.1093/nar/gkr424 the whole document	1-24
Y	WO 2012/007511 A1 (OLINK AB [SE]; FREDRIKSSON SIMON [SE]; TRAN BONNIE [SE]) 19 January 2012 (2012-01-19) page 11 page 39, line 31 - page 42, line 5	1-24
Y	D. A. DI GIUSTO: "Proximity extension of circular DNA aptamers with real-time protein detection", NUCLEIC ACIDS RESEARCH, vol. 33, no. 6, 23 March 2005 (2005-03-23) , pages e64-e64, XP055096915, DOI: 10.1093/nar/gni063 the whole document	1-24
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Y,P	pages 17,18,45; claims 1,7, 10, 15-17, 22-25, 31-35, 39, 45, 48-51; figures 1,8	8,9

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Information on patent family members

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