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[Continued on next page]

(54) Title: MICROBIAL ENRICHMENT PRIMERS

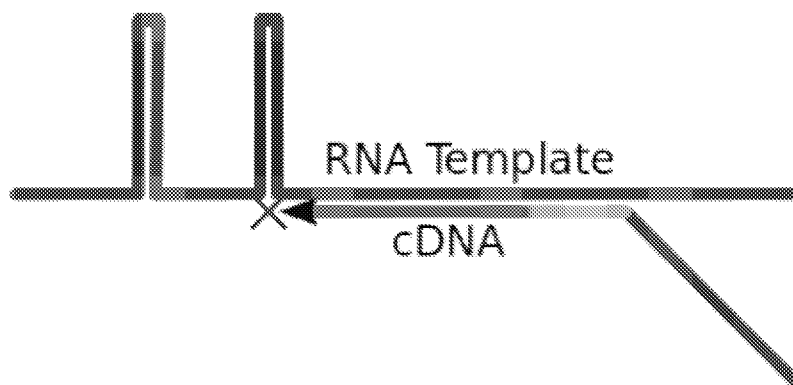


FIGURE 1

(57) Abstract: A primer set, referred to as the Microbial Enrichment Primers (MEP) that generates cDNA libraries depleted of ribosomal and mitochondrial sequences is provided. The primer set is useful in a method that enriches for the presence of microbial nucleic acids from samples. The method enhances sensitivity of microbe detection.



TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *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))*

**(88) Date of publication of the international search report:**

19 July 2012

**Published:**

— *with international search report (Art. 21(3))*

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/59783

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07H 21/00, C12P 19/34, C12Q 1/68 (2012.01)

USPC - 536/24.33, 435/91.2

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)

USPC: 536/24.33, 435/91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPC: 536/24.33, 24.32; 435/91.2, 6.12, 6.15, 6.1 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Electronic data bases: PubWEST (USPT, EPAB, JPAB, PGPB); Google Scholar; GenCore sequence search (NT)

Search terms: oligonucleotide, hexamer, 6mer, primer, amplification, target population, random, non-random, microbial enrichment primers

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0187969 A1 (CASTLE et al.) 7 August 2008 (07.08.2008). Especially para [0009], SEQ ID NOS: 1, 49, 109, 139, 200,346,462,483,600,621,721,829,919.	1
Y	US 2010/0029511 A1 (RAYMOND et al.) 4 February 2010 (04.02.2010). Especially para [0008], [0014], SEQ ID NOS: 594,650	1
Y	US 2003/0224357 A1 (SANTALUCIA et al.) 4 December 2003 (04.12.2003). Especially SEQ ID NO: 13	1
Y	US 2010/0120022 A1 (AYALON-SOFFER et al.) 13 May 2010 (13.05.2010). Especially SEQ ID NO: 3645	1
Y	US 6,068,991A (LIU et al.) 30 May 2000 (30.05.2000). Especially SEQ ID NO: 9	1
Y	US 5,770,402A (BEUTLER et al.) 23 June 1998 (23.06.1998) Especially SEQ ID NO: 3	1
Y	US 2003/0229044 A1 (STEINMAN et al.) 11 December 2003 (11.12.2003). Especially SEQ ID NO: 37	1

☐ Further documents are listed in the continuation of Box C.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"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)

"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

"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

"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

"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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

3 May 2012 (03.05.2012)

Date of mailing of the international search report

**18 MAY 2012**

Name and mailing address of the ISA/US

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/59783

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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.: 6-10, 15-25, 29-33, and 37-42  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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: Claims 1-5, drawn to a composition comprising 20 nucleic acid sequences, wherein the nucleic acid sequences are hexamers comprising the sequences of SEQ ID NOs: 1-20.

Group II+: Claims 1-5, drawn to a comprising 20 or more nucleic acid sequences, wherein the nucleic acid sequences are hexamers selected from any of SEQ ID NOs: 21-1662. If Applicant elects to have this group searched, Applicant must specify the specific hexamer nucleic acid sequence to be searched. Each unique hexamer sequence constitutes an inventive concept.

---please see continuation on extra 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 additional fees, this Authority did not invite payment of additional fees.
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.:  
Claims 1-5 restricted to SEQ ID NOs: 1-20 (i.e. Claim 1)

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/59783

Continuation of Box No III Observations where unity of invention is lacking

Group III: Claims 11-14, drawn to a method for designing a primer set for amplification of microbial nucleic acids.

Group IV: Claims 26-28, drawn to a method for amplifying a microbial nucleic acid.

Group V: Claims 34-36, drawn to a composition comprising 800 or more nucleic acids having the structure H-Na-ST as set forth in claim 34.

The inventions listed as Groups I-V 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:

The special technical feature of the inventions listed as Groups I-V is a primer for amplifying a microbial nucleic acid. This special technical feature fails to provide a contribution over the prior art, as evidenced by US 2009/0081675 A1 to Colston et al. (published 26 March 2009; hereinafter 'Colston'). Colston discloses primers for detecting target microbial nucleic acid (abstract), the primers capable of amplifying target microbial sequences (para [0024]). Colston further teaches primer sets that may include hexamers (para [0187]-[0188], [0169]). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

Another special technical feature of the inventions listed as Groups I and II+ is a hexamer sequence. This special technical feature fails to provide a contribution over Colston, which discloses hexamer sequences (para [0187]-[0188]). Further, significant structural similarities cannot readily be ascertained among each unique hexamer nucleic acid sequence. Without significant structural similarities, the hexamer nucleic acid sequences do not have a shared special technical feature. In the absence of a shared special technical feature, the inventions lack unity with one another.

Further, the special technical features of the inventions listed as Group III are the method steps for designing a primer set for amplification of microbial nucleic acids. This special technical feature is not shared by the inventions of Groups I-II+ and IV-V. The special technical features of the inventions listed as Group IV are the method steps for amplifying a microbial nucleic acid. This special technical feature is not shared by the inventions of Groups I-III and V. The special technical features of the inventions listed as Group V is a set of 800 or more nucleic acids having the structure H-Na-ST as set forth in claim 34. This special technical feature is not shared by the inventions of Groups I-IV.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. Without a shared special technical feature, the inventions of Groups I-V lack unity with one another.