(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 December 2012 (20.12.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/174119 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2012/042263

(22) International Filing Date:
13 June 2012 (13.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/498,443 17 June 2011 (17.06.2011) US
61/550,779 24 October 2011 (24.10.2011) US

(71) Applicant: **LIFE TECHNOLOGIES CORPORATION** [US/US]; 5791 Van Allen Way, Carlsbad, CA 92008 (US).

(72) Inventors; and
(71) Applicants : **JI, Yongmei** [CN/US]; 5451 South East View Park, Chicago, IL 60615 (US). **SHIH, Sheung-Mei ("Rita")** [CN/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **DEGORICIJA, Lovorka** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **ZHOU, Zhaohui** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **CUMMINGS, Craig** [US/US]; 16 Driftwood Court, Pacifica, CA 92008 (US). **FURTADO, Manohar** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **BRZOSKA, Pius** [DE/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **BURRELL, Angela** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **LEONG, Harrison** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **FANG, Xingwang** [CN/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US). **BOUNPHENG, Mangkey** [US/US]; 10305 Maydell Street, Austin, TX 78748 (US). **SHAH, Rohan** [US/US]; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US).

(74) Agent: **SUBRAMONY, Priya D.**; c/o Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR DETECTION OF CRONOBACTER SPP. AND CRONOBACTER SPECIES AND STRAINS

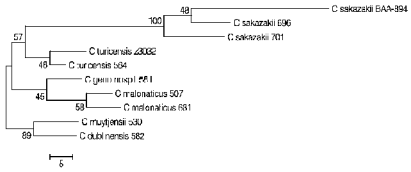

FIG. 1C

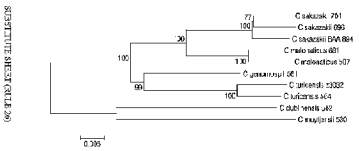

FIG. 1A

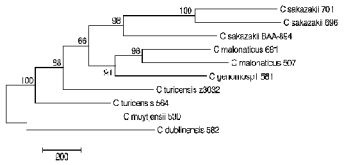

FIG. 1B

(57) Abstract: Disclosed are genomic sequences for nine strains of C*ronobacter spp*. (C. *sakazakii* - 696, 701, 680; C. *malonaticus* - 507, 681; C. *turicensis* - 564; C. *muytjensii* - 530; C. *dublinensis* - 582; C. *genomospl* - 581) and compositions, methods, and kits for detecting, identifying and distinguishing C*ronobacter spp*. strains from each other and from non-C*ronobacter spp*. strains. Some embodiments describe isolated nucleic acid compositions unique to certain C*ronobacter* strains as well as compositions that are specific to all C*ronobacter spp*. Primer and probe compositions and methods of use of primers and probes are also provided. Kits for identification of C*ronobacter spp*. are also described. Some embodiments relate to computer software methods for setting a control based threshold for analysis of PCR

WO 2012/174119 A3

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, 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).

Published:

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

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

(88) Date of publication of the international search report:
11 April 2013

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042263

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
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

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, Sequence Search, 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.
Y	EP 2 287 333 A1 (BIOTECON DIAGNOSTICS GMBH [DE]) 23 February 2011 (2011-02-23) the whole document	1,2, 16-35

-/--

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

[X] See patent family annex.

* 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2012	02/01/2013

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	Mundel, Christophe

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUCEROVA EVA ET AL: "Genome Sequence of Cronobacter sakazakii BAA-894 and Comparative Genomic Hybridization Analysis with Other Cronobacter Species", PLOS ONE, vol. 5, no. 3, E9556, March 2010 (2010-03) , pages 1-10, XP002683596, ISSN: 1932-6203 abstract Core Genome; page 3 C. sakazakii Unique Genes; page 3 table 2 Sequencing of C. sakazakii ATCC BAA-894 and Assembly.; page 8	1,2, 16-35

Y	ALMEIDA C ET AL: "Development and application of a novel peptide nucleic acid probe for the specific detection of Cronobacter genomospecies (Enterobacter sakazakii) in powdered infant formula", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 75, no. 9, 1 May 2009 (2009-05-01), pages 2925-2930, XP002646481, AMERICAN SOCIETY FOR MICROBIOLOGY, US ISSN: 0099-2240, DOI: 10.1128/AEM.02470-08 abstract Cronobacter probe design and theoretical evaluation.; page 2925 table 1	1,2, 16-35

Y	YAN XIANGHE ET AL: "Comprehensive Approaches to Molecular Biomarker Discovery for Detection and Identification of Cronobacter spp. (Enterobacter sakazakii) and Salmonella spp.", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 77, no. 5, March 2011 (2011-03), pages 1833-1843, XP002683597, abstract page 1833, right-hand column, line 6 - line 15	1,2, 16-35

	-/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIU Y ET AL: "Real time PCR using TaqMan and SYBR Green for detection of Enterobacter sakazakii in infant formula", JOURNAL OF MICROBIOLOGICAL METHODS, vol. 65, no. 1, 1 April 2006 (2006-04-01), pages 21-31, XP027926806, ELSEVIER, AMSTERDAM, NL ISSN: 0167-7012 [retrieved on 2006-04-01] abstract 2.4 Real time PCR detection of E. sakazakii using SYBR Green; page 23 3.2.2 . Specificity of primers and probes.; page 26 - page 27	1,2, 16-35
Y	DERZELLE SYLVIANE ET AL: "A robotic DNA purification protocol and real-time PCR for the detection of Enterobacter sakazakii in powdered infant formulae", BMC MICROBIOLOGY, vol. 6, no. 1, 100, 13 December 2006 (2006-12-13), pages 1-12, XP021022423, BIOMED CENTRAL, LONDON, GB ISSN: 1471-2180, DOI: 10.1186/1471-2180-6-100 abstract page 2, right-hand column, line 3 - line 8 page 6, left-hand column, line 6 - line 18 Conclusion; page 8	1,2, 16-35
Y	SEO K H ET AL: "Rapid, specific detection of Enterobacter sakazakii in infant formula using a real-time PCR assay", JOURNAL OF FOOD PROTECTION, vol. 68, no. 1, 1 January 2005 (2005-01-01), pages 59-63, XP009107590, INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION, US ISSN: 0362-028X abstract tables 1, 2	1,2, 16-35

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEHNER ANGELIKA ET AL: "16S rRNA gene based analysis of Enterobacter sakazakii strains from different sources and development of a PCR assay for identification", BMC MICROBIOLOGY, vol. 4, no. 1, 43, 25 November 2004 (2004-11-25), pages 1-7, XP021002594, BIOMED CENTRAL, LONDON, GB ISSN: 1471-2180, DOI: 10.1186/1471-2180-4-43 abstract	1,2, 16-35
Y	BALDWIN ADAM ET AL: "Multilocus sequence typing of Cronobacter sakazakii and Cronobacter malonaticus reveals stable clonal structures with clinical significance which do not correlate with biotypes", BMC MICROBIOLOGY, vol. 9, no. 1, 23 October 2009 (2009-10-23), pages 1-9, XP021062327, BIOMED CENTRAL, LONDON, GB ISSN: 1471-2180, DOI: 10.1186/1471-2180-9-223 abstract page 2, right-hand column, line 1 - line 25 table 1	1,2, 16-35
Y	LIU Y ET AL: "PCR and oligonucleotide array for detection of Enterobacter sakazakii in infant formula", MOLECULAR AND CELLULAR PROBES, vol. 20, no. 1, 1 February 2006 (2006-02-01), pages 11-17, XP024930022, ACADEMIC PRESS, LONDON, GB ISSN: 0890-8508, DOI: 10.1016/J.MCP.2005.08.004 [retrieved on 2006-02-01] abstract 3.1. Design of the specific primers and probes for detection of E. sakazakii; page 13 page 16, left-hand column, line 50 - line 56	1,2, 16-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/042263

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.:
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:

see additional 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 an 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.:

see additional sheet(s)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2(completely); 1, 16-35(partially)

   An isolated nucleic acid sequence having SEQ ID NO: 1, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

   ---

2. claims: 3(completely); 1, 16-35(partially)

   An isolated nucleic acid sequence having SEQ ID NO: 2, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

   ---

3. claims: 4(completely); 1, 16-35(partially)

   An isolated nucleic acid sequence having SEQ ID NO: 3, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

   ---

4. claims: 5(completely); 1, 16-35(partially)

   An isolated nucleic acid sequence having SEQ ID NO: 4, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

   ---

5. claims: 6(completely); 1, 16-35(partially)

   An isolated nucleic acid sequence having SEQ ID NO: 5, fragments thereof, at least 25 contiguous nucleotide

thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

6. claims: 7(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 6, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

7. claims: 8(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 7, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

8. claims: 9(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 8, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

9. claims: 10(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 9, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

10. claims: 11(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 10, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

11. claims: 12(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 11, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

12. claims: 13(completely); 1, 16-35(partially)

An isolated nucleic acid sequence having SEQ ID NO: 12, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

13. claims: 1, 15-34(all partially)

An isolated nucleic acid sequence having SEQ ID NO: 13, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

14. claims: 1, 15-34(all partially)

An isolated nucleic acid sequence having SEQ ID NO: 14, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in

a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

15. claims: 1, 15-35(all partially)

An isolated nucleic acid sequence having SEQ ID NO: 15, fragments thereof, at least 25 contiguous nucleotide thereof, complements thereof or sequence comprising at least 90% nucleic acid sequence identity thereto. Uses thereof in a method of distinguishing an organism belonging to a Cronobacter spp from a non-Cronobacter spp strain, in methods for detecting Cronobacter spp in a sample, in kits for the detection of Cronobacter spp.

---

16. claims: 36-38(completely); 22-27, 35(partially)

An isolated nucleic acid sequence comprising SEQ ID NO: 16-1278, complements thereof, fragments thereof and labeled derivative thereof. Uses of said nucleic acids in a method for detecting a Cronobacter spp in a sample, in a kit for the detection of Cronobacter spp

---

17. claims: 39-41

A method for setting a control based threshold CBT comprising executing a set of computer readable instructions by a computer system interfacing with a PCR instrument.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/042263

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2287333 A1	23-02-2011	EP 2287333 A1	23-02-2011
		US 2012190028 A1	26-07-2012
		WO 2011015356 A1	10-02-2011