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- with sequence listing part of description (Rule 5.2(a))

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24 April 2014

(54) Title: A METHOD FOR COMPREHENSIVE SEQUENCE ANALYSIS USING DEEP SEQUENCING TECHNOLOGY

ID #	NGS		aCGH/Southern Blot		breakpoint		
	Size	Percentage (%)	Size	Percentage (%)	NGS	PCR+ Sequencing	aCGH
A	None	0%	none	0%	N.D.	N.D.	N.D.
B	5,182 bp	82%	~5kb	54%	m.10,901- m.16,082	m.10,901- m.16,082	~m.10,900- ~m.16,100
C	3,361 bp	90%	~3 kb	82%	m.10,053 - m.13,413	m.10,053 -m.13,413	~m.10,025 ~m.13,400
D	13,031 bp	58%	~13kb	N.D.	m.3,271- m.16,301	m.3,271- m.16,301	N.D.
E	4,420 bp	96%	~4.5 kb	N.D.	m.10,952- m.15,371	m.10,952- m.15,371	~m.11,000- ~m.15,300

Fig. 1

(57) Abstract: The present invention relates to a deep sequencing technique used to sequence nuclear and/or mitochondrial DNA. The invention also relates to techniques for quality control in a diagnostic laboratory environment. Specifically, approaches are disclosed to analyze the nuclear and or mitochondrial genomes by massively parallel sequencing, which demonstrates superior sensitivity and specificity of base calls with quantitative information at each nucleotide position of the genome. This method is able to simultaneously detect large deletions, map breakpoints, and quantify deletion heteroplasmy. Sufficient qualitative and quantitative controls for each sample are disclosed for use in a diagnostic setting.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/29266

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2012.01)

USPC - 435/6.1; 702/20

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: 435/6.1; 702/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 435/6.1, 6.11; 702/20, 19 (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

Search terms: mitochondrial DNA (mtDNA); heteroplasmy, spiking mixture, artificially mixed samples, proportional mixing, quantitative control, massively parallel sequencing, deep sequencing, sequencing error rate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TANG et al., Characterization of mitochondrial DNA heteroplasmy using a parallel sequencing system. Biotechniques, April 2010, Vol 48, No 4, Pages 287-296. Especially pg 288 col 2 para 2, pg 292 col 1 para 3, pg 292 col 2 para 2, pg 292 col 3 para 2, pg 292 fig 2.	1-11
X	LI et al., Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. Am J Hum Genet, 13 August 2010, Vol 87, No 2, Pages 237-249. Especially abstract, pg 238 col 2 para 3-4, pg 240 col 2 para 3.	1-11
T	ZHANG et al., Comprehensive 1-Step Molecular Analyses of Mitochondrial Genome by Massively Parallel Sequencing. Clin Chem, ePub 9 July 2012, published ahead of print at doi: 10.1373/clinchem.2011.181438. Especially abstract, pg 2 col 2 para 2, pg 3 col 2 para 3-4, pg 6 table 2.	1-8

☐ 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

"&" document member of the same patent family

Date of the actual completion of the international search

9 August 2012 (09.08.2012)

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24 AUG 2012

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/29266

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:
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 1-11, drawn to a method and kit comprising at least three known sequences of DNA wherein each sequence is at a different known concentration.

Group II: Claims 12-16 and 21-23, drawn to a method comprising receiving and pooling a plurality of samples, sequencing the pooled samples, and demultiplexing the samples.

Group III: Claims 17-20, drawn to a method, system, and non-transitory computer readable-medium comprising receiving at least three parameters corresponding to DNA sequencing.

---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.:
1-11

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 12/29266

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

The inventions listed as Groups I-III 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 shared technical feature of the inventions listed as Groups I-III is a quality control of sequencing data.

This shared technical feature fails to provide a contribution over the prior art, as evidenced by US 2010/0227329 A1 to Cuppens (published 9 September 2010; hereinafter 'Cuppens'). Cuppens discloses the quality control of the sequencing of nucleic acid fragments comprised in a biological sample (para [0027]), wherein the method comprises adding one or more nucleic acids comprising a known sequence to that sample prior to its analytic processing (para [0020]). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

Further, the special technical feature of the inventions listed as Group I is at least three known sequences of DNA wherein each sequence is at a different known concentration. This special technical feature is not shared by the inventions of Groups II and III. The special technical feature of the inventions listed as Group II is a method comprising the steps of pooling a plurality of samples, sequencing the pooled samples, and demultiplexing the samples. This special technical feature is not shared by the inventions of Groups I and III. The special technical feature of the inventions listed as Group III is a processor or computer readable-medium. This special technical feature is not shared by the inventions of Groups I and II.

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-III lack unity with one another.