Abstract: The present invention relates to a method for the highly specific, targeted capture of regions of human genomes and transcriptomes from the blood, i.e. from cell free circulating DNA, exosomes, microRNA, circulating tumor cells, or total blood cells, to allow for the highly sensitive detection of mutation, expression, copy number, translocation, alternative splicing, and methylation changes using combined nuclease, ligation, polymerase, and massively parallel sequencing reactions. The method generates a collection of different circular chimeric single-stranded nucleic acid constructs, suitable for sequencing on multiple platforms. In some embodiments, each construct of the collection comprised a first single stranded segment of original genomic DNA from a host organism and a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism. These chimeric constructs are suitable for identifying and enumerating mutations, copy changes, translocations, and methylation changes. In other embodiments, input mRNA, IncRNA, or miRNA is used to generate circular DNA products that reflect the presence and copy number of specific mRNA's, IncRNA's splice-site variants, translocations, and miRNA.
INTERNATIONAL SEARCH REPORT

International application No. PCT/US15/3724

A. CLASSIFICATION OF SUBJECT MATTER

IPC (s) - C07K 14/005; G01N 33/569; C12N 7/00; (2015.01)

CPC - A61K 2039/53, 39/12, 2039/552; C12Q1/701

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)

IPC(8): C07K 14/005; G01N 33/569; C12N7/00; (2015.01)
CPC: A61K 2039/53, 39/12, 2039/552; C12N 2750/00022; C12Q1/701

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)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Dialog ProQuest; PubMed; Google; Google Scholar: oligonucleotide, "single-stranded."
"nucleic acid;" construct, chimeric, genomic, circular, host, "organism, bacter*." "vir*;" barcode, tag, roll" circle, amplification, hybridiz*, polym*, probe, primer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2013/0315944 A1 (VIRGINIA TECH INTELLECTUAL PROPERTIES, INC.) November 28, 2013; abstract; paragraphs [0003], [0010], [0014], [0019], [0047], [0057], [0058], [0107]</td>
<td>1-8</td>
</tr>
<tr>
<td>Y</td>
<td>US 2009/0099041 A1 (CHURCH, GM et al.) April 16, 2009; abstract; paragraphs [0008], [0009], [0013], [0019], [0039], [0046], [0051], [0076], [0090], [0093], [0115]</td>
<td>1-8</td>
</tr>
<tr>
<td>Y</td>
<td>US 2012/0272272 A1 (BARANY, F et al.) October 25, 2012; abstract; paragraphs [0030], [0039], [0040]-[0042], [0057], [0058], [0092]</td>
<td>3-5, 6/4, 6/5, 7/6/4, 7/6/5, 8/6/4 and 8/6/5</td>
</tr>
</tbody>
</table>

* 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)
  "D" 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
  "S" document member of the same patent family

Date of the actual completion of the international search
06 November 2015 (06.11.2015)

Date of mailing of the international search report
31 March 2016

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Facsimile No. 571-273-8300

Authorized officer
Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774
## INTERNATIONAL SEARCH REPORT

### 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 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.: 1-8

### 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.

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Form PCT/ISA/2 10 (continuation of first sheet (2)) (January 2015)
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 1-8 are directed toward a collection of different circular chimeric single-stranded nucleic acid constructs, each construct comprising: a first single stranded segment of original genomic DNA from a host organism and a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, wherein the nucleotide sequence of both a unique identifier portion and the segment of original genomic DNA distinguishes one chimeric single-stranded nucleic acid construct in the collection from every other chimeric single-stranded nucleic acid construct in the collection.

Group II: Claims 9-66 are directed toward systems comprising a collection of different circular chimeric single-stranded nucleic acid constructs, including a second single stranded nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a first solid support primer-specific portion, a second solid support primer-specific portion.

Group III: Claims 67-82 are directed toward methods for identifying, in a sample, one or more target ribonucleic acid molecules.

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 special technical features include wherein the nucleotide sequence of a segment of original genomic DNA distinguishes one chimeric single-stranded nucleic acid construct in the collection from every other chimeric single-stranded nucleic acid construct in the collection, which is not present in either of Groups II or III, the special technical features of Group II including a solid support primer-specific portion, which is not present in either of Groups I or III, the special technical features of Group III including ligating the 3’ and 5’ ends of each second oligonucleotide probe to form circular ligated products.

Groups I-III share the technical features including: circular nucleic acid constructs; a single stranded synthetic nucleic acid segment that is linked to a single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism; and an identifier sequence or portion. Groups I and II share the technical features including: a collection of different circular chimeric single-stranded nucleic acid constructs, each construct comprising: a first single stranded segment of original genomic DNA from a host organism and a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising an identifier portion; said chimeric single-stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification and/or sequencing. Groups I and III share the technical features including distinguishing. Groups II and III share the technical features including: a patient identifier sequence; one or more oligonucleotides complementary to a portion of a nucleic acid; and hybridizing the oligonucleotides to a complementary nucleic acid.

"""Continued on Next Supplemental Page."""
However, these shared technical features are previously disclosed by US 2013/0315944 A1 (VIRGINIA TECH INTELLECTUAL PROPERTIES, INC.) (hereinafter ‘Virginia Tech’) in view of US 2009/0099041 A1 to Church, et al. (hereinafter ‘Church’).

Virginia Tech discloses the use of circular nucleic acid constructs (infectious nucleic acid molecules of Torque teno sus virus, which are circular, including an artificially introduced genetic marker sequence in intron 1 (circular nucleic acid constructs); paragraphs [0003], [0010], [0014]; a single stranded synthetic nucleic acid segment (an artificially introduced genetic marker sequence in intron 1 of a single-stranded circular nucleic acid; paragraphs [0003], [0010], [0014]) that is linked to a single stranded segment (that is introduced into intron 1 of a single-stranded circular viral genome; paragraphs [0003], [0010], [0014]) and comprises a nucleotide sequence that is exogenous to the host organism (and comprises an artificially introduced marker sequence (and comprises a nucleotide sequence that is exogenous to the host organism); paragraphs [0014], [0040]); and an identifier sequence or portion; paragraphs [0014], [0040]); a collection of different circular chimeric single-stranded nucleic acid constructs (supernatants collected from cells transfected with circular chimeric single-stranded nucleic acid constructs including introduced genetic marker sequences; paragraphs [0003], [0014], [0057]), each construct comprising: a first single stranded segment of original genomic DNA from a host organism (each construct comprising a genomic DNA from a single-stranded DNA virus; paragraph [0003]), [0010]) and a second single stranded synthetic nucleic acid segment (an artificially introduced genetic marker sequence in intron 1 of a single-stranded circular nucleic acid; paragraphs [0003], [0010], [0014]) that is linked to a single stranded segment (that is introduced into intron 1 of a single-stranded circular viral genome; paragraphs [0003], [0010], [0014]) and comprises a nucleotide sequence that is exogenous to the host organism (and comprises an artificially introduced marker sequence; paragraphs [0014], [0040]), said nucleotide sequence comprising an identifier portion (said nucleotide sequence comprising an artificially introduced marker sequence (said nucleotide sequence comprising an identifier portion); paragraph [0014]); said chimeric single-stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification (wherein Torque teno virus genomes may be amplified via rolling-circle amplification (said chimeric single-stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification); paragraph [0003]); distinguishing (paragraph [0058]); one or more oligonucleotides complementary to a portion of a nucleic acid (an oligonucleotide for mutagenesis (one or more oligonucleotides complementary to a portion of a nucleic acid); paragraph [0107]); and hybridizing the oligonucleotide to a complementary nucleic acid (annealing (hybridizing) the oligonucleotide to a complementary nucleic acid; paragraph [0107]); and a method for diagnosing TTsuV infection (a method for diagnosing TTsuV infection; paragraph [0019]). Virginia Tech does not disclose the use of a patient identifier sequence. Church discloses the use of rolling circle probes comprising a barcode sequence to uniquely tag the probes used with respect to a sample from a patient (rolling circle probes comprising a barcode sequence to uniquely tag the probes used with respect to a sample from a patient; paragraph [0008]); a linear, single-stranded DNA which is converted to circular DNA (paragraph [0009]), and amplifying the circular DNA using rolling circle amplification (paragraph [0009]); including the use of primers complementary to a portion of a nucleic acid (focus-specific primers; paragraph [0058]) and hybridizing the primers to the complementary template nucleic acid (amplifying the nucleic acid via PCR using the primers (hybridizing the primers to the complementary template nucleic acid); paragraph [0013]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Virginia Tech, for including the use of a patient-specific barcode as a marker, as previously disclosed by Church, with the single-stranded viral genomes, previously disclosed by Virginia Tech, for enabling tracking of the viral genomes sequenced to the patient of origin, based on the previous disclosure of Church, for enabling the determination of the heterogeneity of the virus present in a particular subject or set of subjects, for diagnosing the presence of the virus in a particular subject, and performing epidemiological studies or assess the mutation rates of the virus in populations.

Since none of the special technical features of the Groups I-III inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Virginia Tech and Church references, unity of invention is lacking.