wherein each forward primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes that are capable of undergoing genomic alteration is complementary to a sequence located about 50 base pairs upstream of an exonic junction of each target gene that is capable of undergoing genomic alteration,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes that are capable of undergoing genomic alteration is complementary to a sequence located about 50 base pairs downstream of an exonic junction of each target gene that is capable of undergoing genomic alteration, that is the same or different from the exonic junction targeted by the forward primer,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes that are capable of undergoing genomic alteration comprises a barcode sequence on its 5' end, wherein the barcode sequence of each reverse primer corresponding to each target gene that is capable of undergoing genomic alteration is different, and/or

(II) a plurality of forward and reverse primer pairs specific to a plurality of **control housekeeping genes**, wherein:

(i) each forward primer of the plurality of forward and reverse primer pairs specific to the plurality of control housekeeping genes is complementary to a sequence spanning an exon-exon junction of each control housekeeping gene,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of control housekeeping genes is complementary to a sequence about 100 base pairs downstream of the sequence spanning the exon-exon junction of each control housekeeping gene,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of control housekeeping genes comprises a barcode sequence on its 5' end, wherein the barcode sequence of each reverse primer corresponding to each control housekeeping gene is different;

(ii) each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of control housekeeping genes is complementary

as disclosed in step (b)(III) span an exon-exon junction, wherein the exon length is about 100 base pairs.

[0036] In one example, each reverse primer of the plurality of forward and reverse primer pairs specific to a plurality of **target genes that are capable of undergoing genetic alteration** as disclosed in step (b)(I), each reverse primer of the plurality of forward and reverse primer pairs specific to a plurality of **control housekeeping genes** as disclosed in step (b)(II), and each reverse primer of the plurality of forward and reverse primer pairs specific to a plurality of **target genes related to protein expression** as disclosed in step (b)(III) comprise a barcode sequence on its 5' end, wherein each barcode sequence is different. As used herein, the term "barcode sequence" refers to an encoded molecule or barcode that includes variable amount of information within the nucleic acid sequence. For example, the barcode sequence is a tag that can be read out using any of a variety of sequence identification techniques, for example, nucleic acid sequencing, probe hybridization-based assay, and the like. In some examples, the barcode sequence is used in the method as described herein to tag different converted cDNA sequences of target regions of a sample, such that when the barcode sequence tags to the converted DNA sequences of target regions, each different converted cDNA sequence of target region would then have a unique barcode sequence that is attached to it and read out with the converted cDNA sequence of target region from the sample.

[0037] The barcode sequence allows the pooled analysis of multiple unique target sequences, where the resulting sequence information from the pool can be later attributed back to each starting target sequence. That is, after the process of amplification, the barcode sequence is used to group amplicons to form a family of amplicons having the same barcode sequence. In some examples, the barcode sequence is an overhang that does not complement any sequence within the target region. As each reverse primer carries on its 5' end a randomly assigned barcode sequence as disclosed herein, the barcode sequence allows individual cDNA molecules to be tagged uniquely in the step of sequencing library formation.

[0038] In one example, the barcode sequence is an oligonucleotide comprising 10 to 16 random nucleotides, or 10 to 15 random nucleotides, or 10 to 13 random nucleotides, or 10 random nucleotides, or 11 random nucleotides, or 12 random nucleotides, or 13 random nucleotides, or 14 random nucleotides, or 15 random nucleotides, or 16 random nucleotides. In one example, the barcode sequence is an oligonucleotide comprising 10 to 16 random nucleotides. In one example, the barcode sequence is an oligonucleotide comprising 10 random nucleotides. In one specific example, the barcode sequence is an oligonucleotide comprising 10 random nucleotides which can be represented as NNNNNNNNNN (SEQ ID NO: 1).

[0065] In one example, the universal indexed adapter primers as disclosed in step (d) of the method of the first aspect comprise:

a forward primer comprising the sequence of AATGATACGGCGACCACCGAGATCTACACCTAGCGCTACACTCTTTCCCTACACG ACGCTCTTCCGATC*T (SEQ ID NO: 2); and

a reverse primer comprising the sequence of CAAGCAGAAGACGGCATACGAGATAACCGCGGGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T,(SEQ ID NO: 3),

wherein "*" represents a phosphorothioate bond, and wherein the underlined sequences are the barcode sequences. The plurality of sequencing library formed is then purified, as disclosed in step (e) of the first aspect.

[0066] In one example, the purification of the plurality of sequencing library is performed using an agent such as paramagnetic beads. In one example, the paramagnetic beads are selected from the group consisting of AMPure XP beads, SPRI beads, and Dynabeads. In one example, the paramagnetic beads are AMPure XP beads.

[0067] The purified plurality of sequencing library is then subjected to multiplex sequencing on a next-generation sequencing platform, as disclosed in step (f) of the first aspect, to obtain a plurality of sequencing reads.

[0068] In one example, the plurality of sequencing library is sequenced on NextSeq 550, NovaSeq 6000, or BGI MGISEQ-2000, DNBSEQ-G400, DNBSEQ-T7.

[0069] In one example, the plurality of sequencing library is qualified using the Agilent High Sensitivity DNA Screentape and quantified using KAPA Library Quantification Kit. In one example, the plurality of sequencing library is qualified by determining the size profile of the sequencing library which if successful will have a typical size profile of multiple prominent peaks greater than 200 base pairs (for example, as shown in **Fig. 15**).

[0070] Subsequently, a plurality of consensus reads is derived from each sequence of the plurality of sequencing reads obtained from step (f), as disclosed in step (g) of the first aspect.

[0071] In one example, step (g) of the first aspect further comprises:

(g)(I) detecting the presence of the barcode sequence from each sequencing read,

(g)(II) performing cluster reassignment for the plurality of sequencing reads having the same barcode sequence to generate a plurality of barcode clusters, wherein each barcode cluster contains reads from the same amplicon and with the same barcode sequence, and

10. The method of the present disclosure uses RNA and not DNA as the sample input for detection of genomic alteration events. This allows for detection of genomic alteration events that would have been excluded in a typical DNA-based detection assay. Examples of such genomic alterations include:

- Copy number gains in DNA leading to overexpression of RNA;
- Structural rearrangements involving very long introns of two or more genes; and
- Changes in gene expression patterns corresponding to drug response or resistance.

SEQUENCE LISTING

Table of other sequences

SEQ ID NO	Sequence name	Sequence
1	Barcode sequence	NNNNNNNNNN
2	Universal indexed forward primer	AATGATACGGCGACCACCGAG ATCTACACCTAGCGCTACACT CTTTCCCTACACGACGCTCTTCCGATC*T
3	Universal indexed reverse primer	CAAGCAGAAGACGGCATACGA GATAACCGCGGGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC*T