CLAIMS

1. A method of detecting genomic alteration and/or detecting gene expression and/or quantifying a level of gene expression using RNA in a biological sample, comprising:

(a) extracting RNA from the biological sample and converting the RNA to complementary DNA (cDNA);

(b) performing a plurality of multiplexed PCR reactions on the converted cDNA using

(I) a plurality of forward and reverse primer pairs specific to a plurality of **target genes that are capable of undergoing genomic alteration**,

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 to a sequence spanning an exon-exon junction of each control housekeeping gene,

wherein 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 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; or

(iii) each forward and each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of control housekeeping genes is complementary to consecutive sequences 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 comprises a barcode sequence on its 5' end, wherein the barcode sequence of each reverse primer corresponding to each control housekeeping gene is different, and/or

(III) a plurality of primer sets specific to a plurality of **target genes related to protein expression**,

wherein each primer set comprises a plurality of forward and reverse primer pairs specific to each target gene related to protein expression, wherein:

(i) each forward primer of the of the plurality of forward and reverse primer pairs specific to each target gene related to protein expression is complementary to a sequence spanning an exon-exon junction of each target gene related to protein expression,

wherein each reverse primer of the of the plurality of forward and reverse primer pairs specific to each target gene related to protein expression is complementary to a sequence about 100 base pairs downstream of the sequence spanning the exon-exon junction of each target gene related to protein expression,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to each target gene related to protein expression comprises a barcode sequence on its 5' end, wherein the barcode sequence of each reverse primer corresponding to each target gene related to protein expression is different,

(ii) each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes related to protein expression is complementary to a sequence spanning an exon-exon junction of each target gene related to protein expression,

wherein each forward primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes related to protein expression is complementary to a sequence about 100 base pairs

downstream of the sequence spanning the exon-exon junction of each target gene related to protein expression,

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

(iii) each forward and each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes related to protein expression is complementary to consecutive sequences spanning an exon-exon junction of each target gene related to protein expression,

wherein each reverse primer of the plurality of forward and reverse primer pairs specific to the plurality of target genes related to protein expression comprises a barcode sequence on its 5' end, wherein the barcode sequence of each reverse primer corresponding to each target gene related to protein expression is different,

thereby generating a plurality of amplicons;

(c) purifying the plurality of amplicons from step (b);

(d) amplifying the purified product from step (c) by using universal indexed adapter primers to generate a sequencing library;

(e) purifying the sequencing library obtained from step (d);

(f) subjecting the purified sequencing library from step (e) to multiplex sequencing on a next-generation sequencing platform to obtain a plurality of sequencing reads;

(g) deriving a consensus read of each sequence from the plurality of sequencing reads obtained from step (f);

(h) performing a sequence alignment of the consensus read obtained from step (g) to a reference genome,

(I) if the sequence alignment results in a partial alignment to the reference genome of an exon from a first gene and a partial alignment to the reference genome of an exon from a second gene, then:

(i) determining the sequence alignment as a split read,

(ii) counting/enumerating the number of split reads from step (h)(I)(i) that supports a fusion junction, and

(iii) if the number of split reads from step (h)(I)(ii) is two or more, then determining the first gene and the second gene as fusion partners,

(II) if the sequence alignment results in an alignment to the reference genome of the control housekeeping gene, then:

(i) determining the sequence alignment as a consensus read of the control housekeeping gene,

(ii) counting/enumerating consensus read pairs of the control housekeeping gene from step (h)(II)(i), and

(iii) determining the level of gene expression of the control housekeeping gene,

(III) if the sequence alignment results in an alignment to the reference genome of the target gene related to protein expression,

(i) determining the sequence alignment as a consensus read of the target gene related to protein expression,

(ii) counting/enumerating consensus read pairs of the target gene related to protein expression from step (h)(III)(i), and

(iii) determining the level of gene expression of the target gene related to protein expression;

(i) determining presence or absence of the genomic alteration and/or determining presence or absence of the gene expression and/or quantifying the level of the gene expression based on the sequence alignment from step (h).

2. The method of claim 1, wherein the RNA is selected from the group consisting of cell-free RNA (cfRNA) and RNA encapsulated within tissues and/or cells.

3. The method of claim 1 or 2, wherein the biological sample is selected from the group consisting of a liquid sample, a tissue sample, and a cell sample

4. The method of claim 3, wherein the liquid sample is a bodily fluid, wherein optionally the bodily fluid is selected from the group consisting of blood, bone marrow, cerebral spinal fluid, peritoneal fluid, pleural fluid, lymph fluid, ascites, serous fluid, sputum, lacrimal fluid, stool, urine, saliva, ductal fluid from breast, gastric juice and pancreatic

juice, wherein optionally the bodily fluid is blood, and wherein optionally the blood is plasma.

5. The method of claim 3, wherein the tissue sample is a frozen tissue sample or a fixed tissue sample, and wherein optionally the fixed tissue sample is a Formalin-Fixed Paraffin-Embedded (FFPE) tissue sample.

6. The method of any one of claims 1 to 5, wherein the biological sample is to be obtained from a subject having or suspected of having cancer.

7. The method of claim 6, wherein the cancer is selected from the group consisting of leukemia, lung cancer, colorectal cancer, breast cancer, pancreatic cancer, prostate cancer, nasopharyngeal cancer, liver cancer, cholangiocarcinoma, esophageal cancer, urothelial cancer, and gastrointestinal cancer.

8. The method of claim 6 or 7, wherein the cancer is selected from the group consisting of metastatic prostate cancer, metastatic lung cancer, metastatic breast cancer, and leukemia.

9. The method of any one of claims 1 to 8, wherein the amount of RNA used in step (a) is from 6 ng to 100 ng.

10. The method of any one of claims 1 to 9, wherein step (a) is performed using a reverse transcription kit, wherein the reverse transcription kit comprises a buffer for performing reverse transcription, a reverse transcriptase enzyme and a plurality of random primers.

11. The method of any one of claims 1 to 10, wherein the plurality of multiplexed PCR reactions performed on the converted cDNA comprises 3 to 15 PCR cycles.

12. The method of any one of claims 1 to 11, wherein the barcode sequence is an oligonucleotide comprising 10 to 16 random nucleotides.

13. The method of any one of claims 1 to 12, wherein the barcode sequence is an oligonucleotide comprising 10 random nucleotides.

14. The method of any one of claims 1 to 13, wherein the target gene that is capable of undergoing genomic alteration comprises an exon from a gene known to undergo fusion fused to an exon from a partner gene of the gene known to undergo fusion.

15. The method of claim 14, wherein the gene known to undergo fusion is selected from the group consisting of ALK receptor tyrosine kinase, RET proto-oncogene, ROS proto-oncogene 1, fibroblast growth factor receptor 1 (FGFR1), fibroblast growth factor receptor 2 (FGFR2), fibroblast growth factor receptor 3 (FGFR3), neurotrophic receptor tyrosine kinase 1 (NTRK1), neurotrophic receptor tyrosine kinase 2 (NTRK2), neurotrophic receptor tyrosine kinase 3 (NTRK3), neuregulin 1 (NRG1), B-Raf proto-oncogene, serine/threonine kinase (BRAF), transmembrane serine protease 2 (TMPRSS2), MET proto-oncogene, receptor tyrosine kinase (MET), epidermal growth factor receptor (EGFR), estrogen receptor 1 (ESR1), platelet derived growth factor receptor alpha (PDGFRA), androgen receptor (AR), BCR activator of RhoGEF and GTPase (BCR), core-binding factor subunit beta (CBFB), lysine methyltransferase 2A (KMT2A), nucleophosmin 1 (NPM1), PML nuclear body scaffold (PML), and RUNX family transcription factor 1 (RUNX1).

16. The method of claim 14, wherein the partner gene of the gene known to undergo fusion is selected from the group consisting of EMAP like 4 (EML4), kinesin family member 5B (KIF5B), coiled-coil domain containing 6 (CCDC6), CD74 molecule (CD74), transforming acidic coiled-coil containing protein 3 (TACC3), ezrin EZR), ETS transcription factor ERG (ERG), ArfGAP with GTPase domain, ankyrin repeat and PH domain 3 (AGAP3), A-kinase anchoring protein 9 (AKAP9), KIAA1549, tropomyosin 3 (TPM3), translocated promoter region, nuclear basket protein (TPR), trafficking from ER to golgi regulator (TFG), lamin A/C (LMNA), BicC family RNA binding protein 1 (BICC1), RAD51 recombinase (RAD51), CD47 molecule (CD47), Yes1 associated transcriptional regulator (YAP1), ETS variant transcription factor 1 (ETV1), ETS variant transcription factor 4 (ETV4), ETS variant transcription factor 5 (ETV5), ETS variant transcription factor 6 (ETV6), factor interacting with PAPOLA and CPSF1 (FIP1L1), centriolin (CNTRL), ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1), AF4/FMR2 family member 1 (AFF1), MDS1 and EVI1 complex locus (MECOM), MLLT3 super elongation complex subunit (MLLT3), myosin heavy chain

11 (MYH11), PBX homeobox 1 (PBX1), retinoic acid receptor alpha (RARA), and RUNX1 partner transcriptional co-repressor 1 (RUNX1T1).

17. The method of any one of claims 1 to 16, wherein the number of the plurality of forward and reverse primer pairs specific to the plurality of **target genes that are capable of undergoing genomic alteration**, the plurality of forward and reverse primer pairs specific to the plurality of **control housekeeping genes**, and the plurality of primer sets specific to a plurality of **target genes related to protein expression** is at least 300.

18. The method of any one of claims 1 to 17, wherein the length of the plurality of amplicons generated in step (b) is 90 to 110 base pairs.

19. The method of any one of claims 1 to 18, wherein the purification in step (c) and/or (e) is performed using a plurality of paramagnetic beads; and wherein optionally the paramagnetic beads are selected from the group consisting of AMPure XP beads, SPRI beads, and dynabeads.

20. The method of any one of claims 1 to 19, wherein step (g) 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

(g)(III) performing consensus calling for each barcode cluster to obtain the consensus read of each sequence.

21. The method of claim 1-13 and 17-20, wherein the step of determining presence or absence of the genomic alteration and/or determining presence or absence of the gene expression and/or quantifying the level of the gene expression, further comprises performing variant calling of the sequence alignment from step (h).

22. The method of claim 21, wherein the step of variant calling comprises:

(i) identifying differences between a consensus read and a reference genome based on the sequence alignment from step (h); and

(ii) determining the read count of sequence alignments comprising genomic alteration.

23. The method of claim 21 or 22, wherein the genomic alteration is selected from the group consisting of insertions, deletions, and single nucleotide variants, wherein optionally the insertion is a duplication.