What is claimed is:

1. A method of detecting one or more cancer-associated alterations and/or determining site of origin of a cancer-associated alteration within a biological sample comprising nucleic acids, comprising the steps of:

(a) extracting nucleic acid from the biological sample obtained from a subject;

(b) performing a plurality of multiplexed PCR reactions on the nucleic acid using:

a plurality of target capture primer pairs specific to a plurality of target genes comprising one or more cancer-associated alteration,

wherein each target capture primer pair comprises a forward target capture primer and a reverse target capture primer specific to a target gene comprising one or more cancer-associated alteration,

wherein each forward target capture primer and each reverse target capture primer comprises a target-specific sequence at its 3' end that is specific to the target gene, a barcode sequence linker, and an adapter-specific sequence at its 5' end,

thereby generating a plurality of amplicons comprising a plurality of target gene sequences corresponding to the plurality of target genes comprising one or more cancer-associated alteration;

(c) sequencing the plurality of amplicons from step (b) to obtain a plurality of sequencing reads, wherein optionally, the sequencing is multiplex sequencing on a next-generation sequencing platform; and

(d) comparing the plurality of sequencing reads comprising one or more cancer-associated alteration with one or more pre-determined reference cancer-associated alteration profile to compute one or more likelihood score, wherein the one or more likelihood score indicates the probability of a cancer-associated alteration originating from a specific site of origin,

to thereby detect the presence of one or more cancer-associated alterations and/or determine the site of origin of a cancer-associated alteration.

2. The method of claim 1, wherein prior to performing step (c), the method comprises further performing a step of removing target capture primers that have not been incorporated into the plurality of amplicons generated in step (b) using at least one nuclease, thereby generating a purified mixture of the plurality of amplicons comprising the plurality of target gene sequences, optionally wherein the at least one nuclease used to

remove the target capture primers that have not been incorporated into amplicons is an endonuclease and/or an exonuclease.

3. The method of claim 2, further comprising the steps of:

(e) amplifying the plurality of amplicons in the purified mixture as defined in claim 2 by using universal indexed adapter primers to generate a sequencing library, wherein each amplicon of the sequencing library comprises two barcode sequences;

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

(g) mapping the plurality of sequencing reads obtained from step (c) of claim 1 to a first reference genome;

(h) grouping the sequencing reads where the barcode sequences of the sequencing reads are identical into a consensus cluster;

(i) performing a sequence alignment of each consensus cluster obtained from step (h) with all consensus clusters having at least one overlapping barcode sequence, then determining the presence of a consensus base in each sequence alignment result to generate a consensus sequencing read;

(j) mapping each of the consensus sequencing read from step (i) with a second reference genome; and

(k) identifying the differences between the consensus sequencing read and the reference genome from step (j) to identify consensus sequencing reads comprising one or more cancer-associated alteration.

4. The method of any one of the preceding claims, wherein step (d) further comprises optimizing the one or more likelihood score based on a clinico-demographic profile of the subject.

5. The method of any one of the preceding claims, wherein the nucleic acid is cell-free nucleic acid or genomic nucleic acid, wherein optionally the cell-free nucleic acid is cell-free DNA (cfDNA) or cell-free RNA (cfRNA), wherein optionally the genomic nucleic acid is genomic DNA (gDNA) or genomic RNA (gRNA), wherein optionally the nucleic acid comprises cfDNA, cfRNA, gDNA, gRNA, or a combination thereof, and wherein optionally the cfDNA is circulating tumor DNA (ctDNA).

6. The method of any one of the preceding claims, wherein the one or more cancer-associated alteration is selected from a group comprising of single-nucleotide variations, insertions, deletions, genomic copy number alterations, deletions of homopolymeric regions, total mutation (or variant) load, detection of microbial DNA sequences, detection of polymorphisms or single-nucleotide variations in nucleic acid sequences, DNA structural rearrangements giving rise to variant RNA molecules, and incorporation of viral DNA, wherein optionally the variant RNA molecules are selected from the group consisting of structural variants of RNA molecules and copy number alterations of RNA molecules, wherein optionally the viral DNA is Epstein–Barr virus (EBV), hepatitis B virus (HBV), and/or Human papillomavirus (HPV).

7. The method of any one of the preceding claims, wherein the biological sample is a liquid sample, optionally wherein the liquid sample is bodily fluid, optionally wherein the bodily fluid is blood, urine, or saliva, and wherein optionally the blood is plasma or buffy coat.

8. The method of any one of the preceding claims, wherein if the biological sample is blood, then step (a) further comprises separating the biological sample into a plasma component and a buffy coat component, prior to extracting cfDNA or cfRNA from the plasma component and gDNA or gRNA from buffy coat component, wherein optionally the plurality of multiplexed PCR reactions as defined in step (b) performed on the plasma component and the buffy coat component are independent of each other, and wherein optionally the cfDNA and gDNA to be used for step (b) harbor cancer-associated alteration(s) that arise independently from clonal hematopoiesis.

9. The method of any one of the preceding claims, wherein the cancer-associated alterations detected are present at distinct sites in the subject’s body.

10. The method of any one of claims 4-9, wherein the clinico-demographic profile comprises nationality, gender, age, or a combination thereof.

11. The method of any one of the preceding claims, wherein each of the one or more pre-determined reference cancer-associated alteration profile as defined in step (d) correlates with one or more cancer type.

12. The method of any one of the preceding claims, comprising simultaneously identifying presence of one or more cancer type in the subject.

13. The method of claim 11 or 12, wherein the one or more cancer type is an early-stage cancer, localized cancer or metastatic cancer, wherein optionally the one or more cancer is a solid tumor or a hematological cancer, and wherein optionally the one or more cancer is selected from the group consisting of lung cancer, liver cancer, breast cancer, colorectal cancer, prostate cancer, nasopharyngeal cancer, pancreas cancer, bile duct cancer, acute myeloid leukemia, chronic myeloid leukemia, head and neck cancer, oropharynx cancer, skin cancer, eye cancer, urinary tract cancer, lymphoplasmacytic lymphoma, cervix cancer, nasopharyngeal cancer, stomach cancer, myeloproliferative neoplasm, endometrium cancer, lymphoma, kidney cancer, and gastrointestinal stromal tumour.

14. The method of any one of the preceding claims, wherein the subject does not display symptoms of cancer and/or the subject has not previously been diagnosed with cancer, wherein optionally computation of the one or more likelihood score as defined in step (d) is determined using data from a public database, data from a combination of public databases, curation of data for genetic alterations including the presence of viral DNA, or a combination thereof.

15. A kit for detecting one or more cancer-associated alterations and/or determining site of origin of a cancer-associated alteration within a biological sample according to the method of claim 1, comprising a plurality of target capture primer pairs specific to a plurality of target genes comprising one or more cancer-associated alteration as defined in claim 1(b), and instructions for use in the method of claims 1-14, wherein optionally the kit further comprises:

- a buffer for performing a plurality of multiplexed PCR reactions,
- a DNA polymerase,
- a plurality of deoxynucleoside triphosphates (dNTPs), and
- a reagent capable of removing excess primers.