CLAIMS

1. A method of detecting a genetic variation in a genetic sample from a subject, comprising

contacting first and second probe sets to the genetic sample, wherein the first probe set comprises a first labeling probe and a first tagging probe, and the second probe set comprises a second labeling probe and a second tagging probe,

hybridizing at least parts of the first and second probe sets to first and second nucleic acid regions of interest in nucleotide molecules of the genetic sample, respectively,

ligating the first probe set at least by ligating the first labeling probe and the first tagging probe,

ligating the second probe set at least by ligating the second labeling probe and the second tagging probe,

optionally amplifying the ligated probe sets,

immobilizing the tagging probes to a pre-determined location on a substrate, wherein

the first and second labeling probes and/or the amplified labeling probes thereof ligated to the immobilized tagging probes comprise first and second labels, respectively,

the first and second labels are different,

the immobilized labels are optically resolvable on a per label basis,

the immobilized first and second tagging probes and/or the amplified tagging probes thereof comprise first and second tags, respectively, and

the immobilizing step is performed by immobilizing the tags to the predetermined location,

counting (i) a first number of the first label immobilized to the substrate, and (ii) a second number of the second label immobilized to the substrate, and

comparing the first and second numbers to determine the genetic variation in the genetic sample.

2. The method according to claim 1, wherein the method comprises amplifying the ligated probe sets with or without labeling the probes during the amplification.

3. The method according to claim 1, wherein

each of the first and second labeling probes comprises a forward or reverse priming sequence, and each of the first and second tagging probes comprises a corresponding reverse or forward priming sequence and a tagging nucleotide sequence as a tag,

the method comprises amplifying the ligated probe sets,

the amplifying step comprises amplifying (i) the ligated first labeling and tagging probes with first forward and reverse primers hybridizing to the forward and reverse priming sequences, respectively, wherein the first forward or reverse primer hybridizing to the first labeling probe comprises the first label, and (ii) the ligated second labeling and tagging probes with second forward and reverse primers hybridizing to the forward and reverse priming sequences, respectively, wherein the second forward or reverse primer hybridizing to the second labeling probe comprises the second label,

the amplified tagging nucleotide sequences of the tagging probes are immobilized to a pre-determined location on a substrate, wherein the amplified tagging nucleotide sequences of the first and second tagging probes are the first and second tags,

the first number is the number of the first label in the amplified first probe set immobilized to the substrate, and the second number is the number of the second label in the amplified second probe set immobilized to the substrate.

4. The method according to any of claims 1-3, wherein the determined genetic variation indicates metastasis, presence, absence, and/or risk of cancer, phamacokinetic variability, drug toxicity, transplant rejection, or aneuploidy in the subject.

5. The method according to any of claims 1-4, wherein
the subject is a pregnant subject,
the genetic variation is trisomy 21 in the fetus of the pregnant subject,
the first nucleic acid region of interest is located in chromosome 21, and
the second nucleic acid region of interest is not located in the chromosome 21.

6. The method according to any of claims 1-5, wherein the genetic sample is selected from the group consisting of whole blood, serum, plasma, urine, saliva, sweat, fecal matter, and tears.

7. The method according to any of claims 1-6, wherein the genetic sample is a cell free DNA sample, and the method further comprises isolating the cell free DNA sample from a blood sample of the subject.

8. The method according to any of claims 1-7, wherein the counting step comprises an optical analysis.

9. The method according to any of claims 1-8, wherein the counting step comprises reading the substrate in first and second imaging channels that correspond to the first and second labels,

respectively, and producing one or more images of the substrate, wherein the first and second labeling probes are resolvable in the one or more images.

10. The method according to any of claims 1-9, wherein the immobilized labels of the same type are separated by a distance of at least 250 nm in both dimensions.

11. The method according to any of claims 1-10, wherein the labels are fluorescent dyes.

12. The method according to any of claims 1-11, wherein the tags are the same, and are configured to be immobilized to the same locations on the substrate directly or indirectly.

13. The method according to any of claims 1-12, wherein the substrate comprises a binding partner that contacts and immobilizes the tags.

14. The method according to any of claims 1-13, wherein the immobilizing step comprises hybridizing at least a part of the tag or tagging nucleotide sequence to a corresponding nucleotide molecule immobilized on the substrate.

15. The method according to any of claims 1-14, wherein the tagging probes are immobilized to an array of multiple pre-determined locations on a substrate.

16. The method according to any of claims 1-15, wherein

the first and second labeling probes are hybridized to the first and second nucleic acid regions of interest in nucleotide molecules of the genetic sample, respectively;

the first and second tagging probes are hybridized to the first and second nucleic acid regions of interest in nucleotide molecules of the genetic sample, respectively;

the first labeling probe is hybridized to a region adjacent to where the first tagging probe is hybridized; and

the second labeling probe is hybridized to a region adjacent to where the second tagging probe is hybridized.

17. The method according to any of claims 1-16, wherein the subject is a pregnant subject, the genetic variation is a genetic variation in the fetus of the pregnant subject, and the method further comprises

contacting allele A and B probe sets that are allele-specific to the genetic sample, wherein the allele A probe set comprises an allele A labeling probe and an allele A tagging probe, and the allele

B probe set comprises an allele B labeling probe and an allele B tagging probe,

hybridizing at least a part of each of the allele A and allele B probe sets to a nucleic acid region of interest in nucleotide molecules of the genetic sample, the nucleic acid region of interest comprising a predetermined single nucleotide polymorphism (SNP) site for which a maternal allelic profile differs from a fetal allelic profile at the SNP site, wherein

the at least a part of the allele A probe set hybridizes to a first allele at the SNP site, the at least a part of the allele B probe set hybridizes to a second allele at the SNP site, and the first and second alleles are different from each other,

ligating the allele A and B probe sets at least by ligating (i) the allele A labeling and tagging probes, and (ii) the allele B labeling and tagging probes,

optionally amplifying the ligated probe sets,

immobilizing the tagging probes to a pre-determined location on a substrate, wherein

the allele A and allele B labeling probes and/or the amplified labeling probes thereof ligated to the immobilized tagging probes comprise allele A and allele B labels, respectively,

the allele A and allele B labels are different, and

the immobilized labels are optically resolvable on a per label basis,

counting the numbers of the allele A and allele B labels, and

determining whether a proportion of a fetal material in the genetic sample is sufficient to detect the genetic variation in the fetus based on the numbers of the allele A and allele B labels.

18. The method according to any of claims 1-17, wherein the method excludes sequencing of the nucleic acid region(s) of interest and/or the probes.

19. The method according to any of claims 1-18, wherein the method excludes bulk array readout or analog quantification.

20. The method according to any one of claims 1-19, wherein each of the ligated probe sets independently comprises a naturally-occurring oligonucleotide sequence.

21. The method according to any one of claims 1-20, wherein the comparing step comprises obtaining a ratio of the first and second numbers.

22. The method according to any one of claims 1-21, wherein the substrate comprises wells or raised regions.

23. The method according to any one of claims 1-22, wherein each of the first and second probe

sets comprises two or more labels.

24. The method according to any one of claims 1-23, wherein the genetic variation indicates the size, growth rate, aggressiveness, stage, prognosis, and/or diagnosis of a tumor.

25. The method according to any one of claims 1-24, wherein the ligated first and second probe sets produce circular molecules.

26. The method according to any one of claims 1-25, wherein each of the first and second probe sets and/or amplified probe sets thereof comprises two or more labels.