

CLAIMS

We Claim:

1. A method for facilitating prenatal diagnosis of a genetic disorder from a maternal sample associated with a pregnant woman, the method comprising:
 - generating a first set of target-associated molecules comprising a first target-associated sequence comprising:
 - a first target-associated region with sequence similarity to a first target sequence region of a first target sequence of a biological target, wherein the biological target is associated with the genetic disorder; and
 - a target variation region with sequence dissimilarity to a sequence region of the target sequence;
 - generating a co-amplified spike-in mixture based on co-amplifying the first set of target-associated molecules and first nucleic acid molecules from the maternal sample, wherein the first nucleic acid molecules comprise the target sequence region;
 - Sanger sequencing the co-amplified spike-in mixture to determine at least one chromatogram-related output comprising first peaks associated with the first target-associated region, the target sequence region of the biological target, the target variation region, and the sequence region of the biological target;
 - determining a first set of target-associated abundance metrics, wherein each target-associated abundance metric, of the first set of target-associated abundance metrics, corresponds to a different pair of a base of the first target-associated sequence and a base of the first target sequence, wherein determining the first set of target-associated abundance metrics comprises, for each of the different pairs:
 - determining a peak intensity metric for the base of the first target-associated sequence of the pair, based on the at least one chromatogram-related output;
 - determining a peak intensity metric for the base of the first target sequence of the pair, based on the at least one chromatogram-related output; and
 - determining a target-associated abundance metric of the first set of target-associated abundance metrics, based on the peak intensity metric for the base

of the first target-associated sequence and the peak intensity metric for the base of the first target sequence;

- determining an overall target-associated abundance metric based on the first set of target-associated abundance metrics; and
 - facilitating the prenatal diagnosis of the genetic disorder based on a comparison between the overall target-associated abundance metric and a reference-associated overall abundance metric describing abundance of a biological reference relative reference-associated molecules.
2. The method of Claim 1,
- wherein the genetic disorder comprises at least one of a chromosomal abnormality and a single gene disorder,
 - wherein the first target sequence region is associated with at least one of a first chromosome and a mutation,
 - wherein a reference sequence region of the biological reference is associated with at least one of a second chromosome and a lack of the mutation, and
 - wherein facilitating the prenatal diagnosis of the genetic disorder comprises facilitating the prenatal diagnosis of the at least one of the chromosomal abnormality and the single gene disorder based on the comparison between the overall target-associated abundance metric and the reference-associated overall abundance metric.
3. The method of Claim 2, wherein the genetic disorder comprises the chromosomal abnormality, wherein the first target sequence corresponds to a first loci of the first chromosome, wherein the method further comprises:
- generating a second set of target-associated molecules comprising a second target-associated sequence, wherein the second target-associated sequence comprises:
 - a second target-associated region with sequence similarity to a second target sequence region of a second target sequence,
 - wherein the second target sequence corresponds to a second loci of the first chromosome; and

- determining a second set of target-associated abundance metrics based on the at least one chromatogram-related output, wherein each target-associated abundance metric, of the second set of target-associated abundance metrics, corresponds to a different pair of a base of the second target-associated sequence and a base of the second target sequence,
 - wherein determining the overall target-associated abundance metric comprises determining the overall target-associated abundance metric based on the first set of target-associated abundance metrics and the second set of target-associated abundance metrics, and
 - wherein the reference sequence region of the biological reference is associated with the second chromosome.
4. The method of Claim 1, wherein facilitating the prenatal diagnosis comprises facilitating the prenatal diagnosis of the genetic disorder based on a fetal fraction measurement, the overall target-associated abundance metric, and the reference-associated overall abundance metric.
 5. The method of Claim 1, wherein the target variation region comprises at least one of an insertion and a deletion compared to the target sequence, wherein the at least one chromatogram-related output comprises alignment positions corresponding to the first peaks, wherein, for each of the different pairs, the base of the first target-associated sequence corresponds to a first alignment position that is different from a second alignment position corresponding to the base of the first target sequence, and wherein the alignment positions of the at least one chromatogram-related output comprise the first and the second alignment positions.
 6. A method for facilitating characterization of a medical condition from a sample comprising target molecules, the method comprising:
 - generating a set of target-associated molecules comprising a target-associated sequence comprising:

- a first target-associated region with sequence similarity to a first target sequence region of a target sequence of a biological target, wherein the biological target is associated with the genetic disorder; and
 - a target variation region with sequence dissimilarity to a sequence region of the target sequence;
- performing Sanger sequencing based on the set of target-associated molecules and the target molecules comprising the target sequence, to determine at least one chromatogram-related output comprising a first set of peaks associated with the first target-associated region, the first target sequence region of the biological target, the target variation region, and the sequence region of the biological target;
 - determining a set of target-associated abundance metrics for a set of different pairs of bases based on the first set of peaks of the at least one chromatogram-related output, wherein each different pair of bases, from the set of different pairs of bases, corresponds to a pair of a base of the target-associated sequence and a base of the target sequence; and
 - facilitating the characterization of the medical condition based on the set of target-associated abundance metrics and a set of reference-associated abundance metrics describing abundance of a biological reference relative reference-associated molecules.
7. The method of Claim 6, wherein the target variation region comprises at least one of a substitution, an insertion, and a deletion, relative to the sequence region of the target sequence, and wherein determining the set of target-associated abundance metrics comprises determining the set of target-associated abundance metrics based on the first set of peaks and the at least one of the substitution, the insertion, and the deletion.
 8. The method of Claim 7, wherein the target variation region comprises at least one of the insertion and the deletion, wherein the at least one chromatogram-related output comprises alignment positions corresponding to the first set of peaks, and wherein determining the set of target-associated abundance metrics comprises, for each of the different pairs:

- determining a peak intensity metric, at a first alignment position of the alignment positions, for the base of the first target-associated sequence of the pair, based on the at least one chromatogram-related output;
 - determining a peak intensity metric, at a second alignment position of the alignment positions, for the base of the first target sequence of the pair, based on the at least one chromatogram-related output, wherein the first alignment position is different from the second alignment position, and wherein the alignment positions comprise the first and the second alignment positions; and
 - determining a target-associated abundance metric of the set of target-associated abundance metrics, based on the peak intensity metric for the base of the target-associated sequence and the peak intensity metric for the base of the first target sequence.
9. The method of Claim 8,
- wherein the first alignment position corresponds to a first peak and a second peak of the first set of peaks,
 - wherein the first peak corresponds to an overlapping base of the target-associated sequence,
 - wherein the first peak corresponds to a first target-associated abundance metric of the set of target-associated abundance metrics,
 - wherein the second peak corresponds to an overlapping base of the target sequence, and
 - wherein the second peak corresponds to a second target-associated abundance metric of the set of target-associated abundance metrics.
10. The method of Claim 6,
- wherein the medical condition comprises at least one of a chromosomal abnormality, a single gene disorder, and a cancer condition,
 - wherein the first target sequence region is associated with at least one of a first chromosome and a mutation,

- wherein a reference sequence region of the biological reference is associated with at least one of a second chromosome and a lack of the mutation, and
 - wherein facilitating the characterization of the medical condition comprises facilitating the characterization of the at least one of the chromosomal abnormality, the single gene disorder, and the cancer condition, based on the set of target-associated abundance metrics and the set of reference-associated abundance metrics.
11. The method of Claim 6, further comprising adding at least one sequence region to at least one of the set of target-associated molecules and the target molecules, wherein the at least one sequence region comprises at least one of (a) a second target-associated region with sequence similarity to a second target sequence region, and (b) at least one sequence repeat of at least one of a region of the target-associated sequence and a region of the target sequence.
12. The method of Claim 11,
- wherein adding the at least one sequence region comprises adding the at least one sequence repeat of the at least one of the region of the target-associated sequence and the region of the target sequence,
 - wherein the first set of peaks of the at least one chromatogram-related output correspond to a first sequencing for the first target-associated region, the first target sequence region of the biological target, the target variation region, and the sequence region of the biological target,
 - wherein the at least one chromatogram-related output comprises a second set of peaks corresponding to a second sequencing for the first target-associated region, the first target sequence region of the biological target, the target variation region, and the sequence region of the biological target, and
 - wherein determining the set of target-associated abundance metrics comprises determining the set of target-associated abundance metrics based on the first set of peaks and the second set of peaks.

13. The method of Claim 6, further comprising adding at least one sequence repeat to at least one of the set of target-associated molecules and the target molecules, wherein adding the at least one sequence repeat comprises co-amplifying, with a set of primers comprising a hairpin sequence, the set of target-associated molecules and the target molecules.
14. A method for biological target quantification from a sample comprising target molecules, the method comprising:
 - determining at least one chromatogram-related output associated with a first set of target-associated molecules and a set of target molecules, based on Sanger sequencing associated with the first set of target-associated molecules and the set of target molecules, wherein the first set of target-associated molecules comprises a first target-associated sequence comprising:
 - a first target-associated region with sequence similarity to a first target sequence region of a first target sequence of a biological target, wherein the set of target molecules comprises the first target sequence region; and
 - a target variation region with sequence dissimilarity to a sequence region of the first target sequence;
 - determining a first set of target-associated abundance metrics for different sets of bases based on the at least one chromatogram-related output, wherein each different set of bases, from the different sets of bases, comprises at least one base of the first target-associated sequence and at least one base of the first target sequence; and
 - determining a first overall abundance metric describing a first abundance of the biological target for the sample, based on the first set of target-associated abundance metrics.
15. The method of Claim 14,
 - wherein determining the at least one chromatogram-related output comprises determining the at least one chromatogram-related output based on the Sanger sequencing associated with the first set of target-associated molecules, the set of target molecules, and a second set of target-associated molecules,

- wherein the second set of target-associated molecules comprises a second target-associated sequence comprising a second target-associated region with sequence similarity to a second target sequence region of a second target sequence, and
 - wherein the method further comprises determining a second set of target-associated abundance metrics for different sets of bases of the second target-associated sequence and the second target sequence, based on the at least one chromatogram-related output.
16. The method of Claim 15, wherein the first target sequence corresponds to a first loci of a chromosome, wherein the second target sequence corresponds to a second loci of the chromosome, and wherein the method further comprises facilitating characterization of a chromosomal abnormality based on the first overall abundance metric.
17. The method of Claim 14, wherein the biological target is associated with a medical condition, and wherein the method further comprises facilitating characterization of the medical condition based on a first comparison between the first overall abundance metric and an overall reference-associated abundance metric describing abundance of a biological reference.
18. The method of Claim 17,
- wherein the medical condition comprises at least one of a single gene disorder and a cancer condition,
 - wherein the first overall abundance metric is associated with a first time period,
 - wherein the method further comprises determining a second overall abundance metric describing a second abundance of the biological target, wherein the second overall abundance metric is associated with a second time period, and
 - wherein facilitating the characterization of the medical condition comprises facilitating the characterization of the medical condition based on the first comparison and the second overall abundance metric.
19. The method of Claim 14,
- wherein the Sanger sequencing comprises:

- Sanger sequencing of a first sample comprising the first set of target-associated molecules; and
 - Sanger sequencing of a second sample comprising the set of target molecules,
 - wherein determining the at least one chromatogram-related output comprises:
 - determining a first chromatogram-related output associated with the first set of target-associated molecules, based on the Sanger sequencing of the first sample; and
 - determining a second chromatogram-related output associated with the set of target molecules, based on the Sanger sequencing of the second sample, and
 - wherein determining the first set of target-associated abundance metrics comprises determining the first set of target-associated abundance metrics based on the first chromatogram-related output and the second chromatogram-related output.
20. The method of Claim 14, wherein determining the first set of target-associated abundance metrics based on the at least one chromatogram-related output comprises determining the first set of target-associated abundance metrics for the different sets of bases based on at least one of peak intensities, peak areas, peak metrics for bases sharing a base type, and peak metrics for bases with different base types.