

## CLAIMS

### WHAT IS CLAIMED IS:

1. A method for evaluating partitioning of nucleic acid molecules in a sample of polynucleotides based on epigenetic state, comprising:
  - a. partitioning nucleic acid molecules based on epigenetic state from at least a subset of the sample of polynucleotides into a plurality of partitioned sets;
  - b. enriching at least a subset of molecules from the plurality of partitioned sets to generate a set of enriched molecules using probes targeting one or more regions of interest, wherein the set of enriched molecules comprises a group of nucleic acid molecules from the sample of polynucleotides, wherein the group of nucleic acid molecules from the sample of polynucleotides comprises a set of endogenous control molecules;
  - c. sequencing at least a subset of the set of enriched molecules to produce a plurality of sequencing reads;
  - d. analyzing at least a subset of the set of sequencing reads to generate one or more epigenetic partition scores for the set of endogenous control molecules; and
  - e. comparing the one or more epigenetic partition scores with one or more epigenetic partition cut-offs, wherein the one or more epigenetic partition score comprises
    - i) a fraction or percentage of a number of hypermethylated epigenetic-control nucleic acid molecules and/or hypermethylated control molecules in a partitioned set, and the epigenetic partition cut-off is greater than the epigenetic partition score;
    - ii) a fraction or percentage of a number of hypomethylated epigenetic-control nucleic acid molecules and/or hypomethylated control molecules in a partitioned set and the epigenetic partition cut-off is greater than the epigenetic partition score;
    - iii) 0 CG score, wherein the epigenetic partition cut-off for the 0 CG score is 0.01%, 0.02%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 5%, at least 5% or at least 10%;
    - iv) hypo score, wherein the epigenetic partition cut-off for the hypo score is 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 7% or at least 10%;
    - v) methyl-half, wherein the epigenetic partition cut-off for the methyl-half is 5, 10, 15, 20, 25, 30, 35 or 40 mCGs; or
    - vi) methyl-5, wherein the epigenetic partition cut-off for the methyl-5 is 5, 10, 20, 30, 40 or 50 mCGs.
2. The method of claim 1, further comprising an initial step of adding a set of epigenetic-control nucleic acid molecules to the nucleic acid molecules in the sample of polynucleotides, thereby producing a spiked-in sample, and wherein
  - step a. is performed on the spiked-in sample;

within step b., the set of enriched molecules further comprises a group of epigenetic-control nucleic acid molecules; and

step d. additionally generates one or more epigenetic partition scores for the epigenetic-control nucleic acid molecules.

3. The method of claim 2, wherein

i) the set of epigenetic-control nucleic acid molecules comprises two or more subsets of epigenetic-control nucleic acid molecules, wherein a subset of the two or more subsets of epigenetic-control nucleic acid molecules comprises a plurality of epigenetic-control nucleic acid molecules comprising an epigenetic modification region, optionally wherein

a) each subset of epigenetic-control nucleic acid molecules is in equimolar concentration; or each subset of epigenetic-control nucleic acid molecules is in non-equimolar concentration; and/or

b) the epigenetic modification region of the plurality of epigenetic-control nucleic acid molecules in the two or more subsets comprises identical nucleic acid sequence; or the epigenetic modification region of the plurality of epigenetic-control nucleic acid molecules in a first subset comprises a nucleic acid sequence distinguishable from the nucleic acid sequence of the epigenetic modification region of the plurality of epigenetic-control nucleic acid molecules in a second subset;

ii) the epigenetic-control nucleic acid molecule further comprises an identifier region, for example wherein the identifier region is on one or both sides of the epigenetic modification region of the epigenetic-control nucleic acid molecules, optionally wherein the identifier region of the epigenetic-control nucleic acid molecules

a) comprises a molecular barcode;

b) further comprises at least one epigenetic state barcode; and/or

c) comprises one or more primer binding sites;

iii) the epigenetic modification region of the epigenetic-control nucleic acid molecules in at least one subset comprises at least one nucleotide with epigenetic modification; and/or

iv) the plurality of partitioned sets comprises nucleic acid molecules of the spiked-in sample partitioned based on the methylation level of the nucleic acid molecules.

4. The method of any one of the preceding claims, wherein the partitioning comprises partitioning the nucleic acid molecules based on a differential binding affinity of the nucleic acid molecules to a binding agent that preferentially binds to nucleic acid molecules comprising nucleotides with epigenetic modification.

5. The method of any one of the preceding claims, further comprising tagging the nucleic acid molecules in a partitioned set of the plurality of partitioned sets with a set of tags to produce a population of tagged nucleic acid molecules, wherein the tagged nucleic acid molecules comprise one or more tags; optionally wherein

the set of tags used in a first partitioned set of the plurality of partitioned sets is different from the set of tags used in a second partitioned set of the plurality of partitioned sets; and further optionally

the set of tags are attached to the nucleic acid molecules by ligation of adapters to the nucleic acid molecules, wherein the adapters comprise one or more tags.

6. The method of any one of the preceding claims, further comprising

f. classifying the method as (i) being a success, if the one or more epigenetic partition scores of the epigenetic-control nucleic acid molecules and/or the set of endogenous control molecules is within the corresponding epigenetic partition cut-offs; or (ii) being unsuccessful, if at least one of the one or more epigenetic partition scores of the epigenetic control molecules and/or the set of endogenous control molecules is outside the corresponding epigenetic partition cut-off.

7. The method of any one of the preceding claims, wherein the epigenetic state comprises:

- i) an epigenetic modification comprising DNA methylation;
- ii) a nucleotide with an epigenetic modification comprising a 5-methylcytosine; or
- iii) a methylation level of the nucleic acid molecules.

8. The method of any one of the preceding claims, wherein the partitioned set is

- i) hypermethylated partitioned set; or
- ii) hypomethylated partitioned set.

9. The method of any one of the preceding claims, wherein the sequencing of the plurality of enriched molecules is performed by a nucleic acid sequencer, for example wherein the nucleic acid sequencer is a next generation sequencer.

10. The method of any one of the preceding claims, wherein the sample of polynucleotides is

- i) selected from the group consisting of a sample of DNA, a sample of RNA, a sample of polynucleotides, a sample of cell-free DNA, and a sample of cell-free RNA; and/or
- ii) a cell-free DNA, optionally wherein the cell-free DNA is between 1 ng and 500 ng.

11. The method of any one of the preceding claims, further comprising

i) generating a report which optionally includes information on, and/or information derived from, the partitioning of nucleic acid molecules; and optionally

ii) communicating the report to a third party, such as the subject from whom the sample derived or a health care practitioner.