CLAIMS

1. A method for analyzing nucleic acid molecules in a sample of polynucleotides, comprising:

a) adding a subset of fragment size control molecules to the nucleic acid molecules in the sample of polynucleotides, wherein the fragment size control molecules are nucleic acid molecules, thereby producing a first spike-in sample;

b) extracting nucleic acids from the first spike-in sample;

c) processing at least a subset of the extracted nucleic acids, thereby producing a processed sample, wherein the processing comprises partitioning, tagging, and amplifying at least a subset of the first spike-in sample, wherein the partitioning comprises fractionating the nucleic acid molecules into a plurality of partitioned sets based on methylation level of the nucleic acid molecules;

d) enriching for at least a subset of the processed sample, thereby producing an enriched sample;

e) sequencing at least a subset of the enriched sample to generate a plurality of sequence reads; and

f) analyzing the plurality of sequence reads to generate a plurality of fragment size scores of the subset of fragment size control molecules.

2. A system, comprising a controller comprising or capable of accessing, computer readable media comprising non-transitory computer-executable instructions which, when executed by at least one electronic processor perform a method comprising:

a) adding a subset of fragment size control molecules to the nucleic acid molecules in a sample of polynucleotides, wherein the fragment size control molecules are nucleic acid molecules, thereby producing a first spike-in sample;

b) extracting nucleic acids from the first spike-in sample;

c) processing at least a subset of the extracted nucleic acids, thereby producing a processed sample, wherein the processing comprises partitioning, tagging, and amplifying at least a subset of the first spike-in sample, wherein the partitioning comprises fractionating the nucleic acid molecules into a plurality of partitioned sets based on methylation level of the nucleic acid molecules;

d) enriching for at least a subset of the processed sample, thereby producing an enriched sample;

e) sequencing at least a subset of the enriched sample to generate a plurality of sequence reads; and

f) analyzing the plurality of sequence reads to generate a plurality of fragment size scores of the subset of fragment size control molecules.

3. The method of claim 1, or the system of claim 2, wherein the method further comprises:

prior to c), adding a second subset of fragment size control molecules, thereby producing a second spike-in sample;

prior to d), adding a third subset of fragment size control molecules, thereby producing a third spike-in sample; and/or

prior to e), adding a fourth subset of fragment size control molecules, thereby producing a fourth spike-in sample.

4. The method of claim 1, or the system of claim 2, wherein the method further comprises:

prior to c), adding a second subset of fragment size control molecules to the extracted nucleic acids, thereby producing a second spike-in sample; wherein in step c) at least a subset of the second spike-in sample is processed;

prior to d), adding a third subset of fragment size control molecules to the processed sample, thereby producing a third spike-in sample; wherein in step d) at least a subset of the third spike-in sample is enriched; and

prior to e), adding a fourth subset of fragment size control molecules to at least a subset of the enriched sample, thereby producing a fourth spike-in sample; wherein in step e) the fourth spike-in sample is sequenced;

and wherein step f) generates a plurality of fragment size scores of the first subset of fragment size control molecules, the second subset of fragment size control molecules, the third subset of fragment size control molecules, and/or the fourth subset of fragment size control molecules.

5. The method or system of any one of the preceding claims, wherein the method further comprises comparing the plurality of fragment size scores with a plurality of fragment size thresholds; the method optionally further comprising:

optimizing the analysis of nucleic acid molecules in the sample of polynucleotides based on the plurality of fragment size scores;

correcting for fragment size bias in the analysis of nucleic acid molecules in the sample of polynucleotides using the plurality of fragment size scores; and/or

classifying the method as (i) being a success, if at least one of the plurality of fragment size scores is within a corresponding fragment size threshold of the plurality of fragment size thresholds; or (ii) being unsuccessful, if at least one of the plurality of fragment size scores is not within the corresponding fragment size threshold of the plurality of fragment size thresholds.

6. The method or system of any of the preceding claims, wherein the subset of fragment size control molecules comprises a plurality of fragment size control molecules comprising a fragment size region, optionally

wherein the fragment size control molecules further comprises an identifier region, for example wherein the identifier region (i) is on one or both sides of the fragment size region and/or (ii) comprises a molecular barcode; and/or

wherein the subset comprises at least one group of fragment size control molecules, for example (i) wherein the fragment size regions of the plurality of fragment size control molecules in a group are of same length, or wherein the length of the fragment size region in a first group of fragment size control molecules is different from the length of the fragment size region in a second group of fragment size control molecules, and/or (ii) wherein the fragment size regions of the fragment size control molecules in a group comprise the same oligonucleotide sequence, or wherein the fragment size regions of the fragment size control molecules in a group comprise at least two distinguishable oligonucleotide sequences.

7. The method or system of any one of the preceding claims, wherein the fragment size control molecules comprise one or more primer binding sites, for example wherein the primer binding site is in the identifier region.

8. The method or system of claim 6 or claim 7, wherein

the fragment size regions of the fragment size control molecules in a first subset of fragment size control molecules comprises an oligonucleotide sequence distinguishable from the oligonucleotide sequence of the fragment size regions of the fragment size control molecules in a second subset of fragment size control molecules; and/or

the length of the fragment size region is (i) at least 10 bp, at least 50 bp, at least 60 bp, at least 70 bp, at least 80 bp, at least 90 bp, at least 100 bp, at least 120 bp, at least 150 bp, at least 200 bp, at least 250 bp, at least 300 bp, at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, or at least 1000 bp; or (ii) between 10 bp and 1000 bp.

9. The method or system of any one of claims 3-8, wherein

each of the subsets of the fragment size control molecules is in equimolar concentration, or each of the subsets of the fragment size control molecules is in non-equimolar concentration; and/or

each of the groups of the fragment size control molecules in the subset is in equimolar concentration, or each of the groups of the fragment size control molecules in the subset is in non-equimolar concentration.

10. The method or system of any one of the preceding claims, wherein the tagging comprises attaching a set of tags to the nucleic acids to produce a population of tagged nucleic acids, wherein the tagged nucleic acids comprise one or more tags, optionally wherein

the set of tags used in a first partitioned set of a plurality of partitioned sets resulting from the partitioning is different from the set of tags used in a second partitioned set of the plurality of partitioned sets; and/or

the set of tags is attached to the nucleic acids by ligation of adapters to the nucleic acids, wherein the adapters comprise one or more tags.

11. The method or system of any one of the preceding claims, wherein the concentration of the fragment size control molecules is between 1 attomole and 10 picomoles.

12. The method or system of any one of the preceding claims, wherein the sample of polynucleotides is a sample of cell-free polynucleotides; for example wherein the sample of cell-free polynucleotides

is selected from the group consisting of a sample of cell-free DNA, and a sample of cell-free RNA; or

is a sample of cell-free DNA, for example wherein the cell-free DNA is between 1 ng and 500 ng.

13. The method or system of any one of the preceding claims, further comprising generating a report which optionally includes information on, and/or information derived from, the analysis of the nucleic acid molecules, optionally further comprising communicating the report to a third party, such as the subject from whom the sample derived or a health care practitioner.

14. The method or system of any of the preceding claims, wherein the fragment size control molecules

are synthetic molecules; or

are generated as amplicons by PCR amplification.

15. The method or system of any of the preceding claims, wherein the fragment size control molecules comprise a nucleic acid sequence corresponding to a non-human genome.

16. The method or system of any of claims 1 to 14, wherein the plurality of fragment size scores is estimated based on the number of fragment size control molecules in the subset of fragment size control molecules.