

CELL-FREE DNA SIZE DETECTION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/024,489, filed May 13, 2020, U.S. Provisional Application No. 63/054,617, filed July 21, 2020, U.S. Provisional Application No. 63/065,375, filed August 13, 2020, and U.S. Provisional Application No. 63/106,741, filed October 28, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Detection and analysis of cell-free nucleic acids has emerged as important method for studying health of an individual. For example, increased levels of cell-free nucleic acids are observed in individuals that have cancer. In addition, analysis of cell-free nucleic acids in a pregnant person allow for non-invasive prenatal screening for chromosomal abnormalities in a fetus.

SUMMARY

[0003] Provided herein are methods of cell-free DNA size detection and analysis. Disclosed herein is a method wherein a more significant size difference by using single strand library prep than double stranded library prep. For example, a difference in fragment size between late stage cancer and healthy may be 29bp using a single strand DNA library prep, and 12bp using double stranded prep.

[0004] Further disclosed herein is a method wherein a more significant is observed size difference between differentially methylated molecules between cancer and healthy cell-free DNA.

[0004a] In one aspect, provided herein is a method for nucleic acid analysis, comprising: (a) preparing a first single-stranded deoxynucleic acid (DNA) library from a first plurality of nucleic acid molecules, said first plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) preparing a second single-stranded DNA library from a second plurality of nucleic acid molecules, said second plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a control; (c) using said first single-stranded DNA library to measure a first size distribution for at least a subset of said first plurality of nucleic acid molecules; (d) using said second single-stranded DNA library to measure a second size distribution for at least a subset of said second plurality of nucleic acid molecules; (e) using said first size distribution and said second size distribution to determine a difference in size between said first size distribution and said second size distribution.

[0004b] In another aspect, provided herein is a method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature; (c) measuring a size for said individual nucleic acid molecule; (d)

associating said genomic feature with a disease based on said size of said individual nucleic acid molecule.

[0004c] In another aspect, provided herein is a method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature; (c) identifying at least a 5' end or a 3' end for said individual nucleic acid molecule; (d) associating said genomic feature with a disease based on said 5' end or said 3' end of said individual nucleic acid molecule.

[0005] In one aspect, provided herein are methods for nucleic acid analysis, comprising: (a) preparing a first single-stranded deoxynucleic acid (DNA) library from a first plurality of nucleic acid molecules, the first plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) preparing a second single-stranded DNA library from a second plurality of nucleic acid molecules, the second plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a control; (c) using the first single-stranded DNA library to measure a first size distribution for at least a subset of the first plurality of nucleic acid molecules; (d) using the second single-stranded DNA library to measure a second size distribution for at least a subset of the second plurality of nucleic acid molecules; (e) using the first size distribution and the second size distribution to determine a difference in size between the first size distribution and the second size distribution. In some cases, (a) comprises denaturing the first plurality of nucleic acid molecules. In some cases, (b) comprises denaturing the second plurality of nucleic acid molecules. In some cases, (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of the first plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA. In some cases, (b) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of the