

[0010] In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is at least 10 times greater than a quantity of material within an input sample for use with traditional sequence capture methods. In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is at least 100 times greater than a quantity of material within an input sample for use with traditional sequence capture methods. In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is at least 250 times greater than a quantity of material within an input sample for use with traditional sequence capture methods. In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is at least 500 times greater than a quantity of material within an input sample for use with traditional sequence capture methods. In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is at least 1000 times greater than a quantity of material within an input sample for use with traditional sequence capture methods. In some embodiments, the quantity of genomic material within the input sample for use with the disclosed methods is about 1000 times greater than a quantity of material within an input sample for use with traditional sequence capture methods.

[0011] In one aspect of the present disclosure is a method of sequencing genomic material within a sample comprising: homogenizing a tumor sample and/or lymph node sample to provide a homogenized sample; isolating at least 0.5 micrograms of genomic material from the homogenized sample; preparing the at least 0.5 micrograms of isolated genomic material for sequencing; and sequencing the prepared genomic material, wherein the method comprises at least one pre-capture or post-capture amplification step, wherein an aggregate number of amplification cycles conducted during the at least one pre-capture or post-capture amplification step is 1 to 4 cycles. In some embodiments, the method does not comprise any amplification steps prior to sequencing. In some embodiments, the method comprises at least one pre-capture or post-capture amplification step, wherein an aggregate number of amplification cycles conducted during the at least one pre-capture or post-capture amplification step is at most 4 cycles. In some embodiments, the aggregate number of amplification cycles is 3.

In some embodiments, the aggregate number of amplification cycles is 2. In some embodiments, the preparing of the at least 0.5 micrograms of isolated genomic material for sequencing comprises hybridizing the at least 0.5 micrograms of isolated genomic to capture probes and capturing the hybridized

genomic material. In some embodiments, an amount of captured genomic material ranges from about 90ng to about 900ng. In some embodiments, 1 or 2 amplification cycles are performed on the captured genomic material. In some embodiments, the homogenized sample comprises a representative sampling of cells. In some embodiments, at least 1 microgram of genomic material is isolated from the homogenized samples. In some embodiments, at least 5 micrograms of genomic material is isolated from the homogenized samples. In some embodiments, at least 10 micrograms of genomic material is isolated from the homogenized samples.

[0012] In another aspect of the present disclosure is a method of sequencing DNA within a sample comprising isolating at least 0.5 micrograms of DNA from a blood sample; preparing the at least 0.5 micrograms of isolated DNA for sequencing, and sequencing the prepared DNA, wherein the preparing of the at least 0.5 micrograms of isolated DNA for sequencing comprises hybridizing the at least 0.5 micrograms of isolated genomic to capture probes and capturing the hybridized genomic material, wherein the method comprising 1 to 4 amplification cycles prior to sequencing. In some embodiments, the method comprises 0 amplification steps prior to sequencing. In some embodiments, the preparing of the at least 0.5 micrograms of isolated DNA for sequencing comprises hybridizing the at least 0.5 micrograms of isolated genomic to capture probes and capturing the hybridized genomic material. In some embodiments, an amount of captured genomic material ranges from about 90ng to about 900ng. In some embodiments, 1 or 2 amplification cycles are performed on the captured genomic material. In some embodiments, at least 1 microgram of DNA is isolated from the blood sample.

[0013] In another aspect of the present disclosure is a method of targeted representational sequencing comprising: (i) homogenizing at least a portion of a tumor, one or more whole or partial lymph nodes, or any combination thereof to provide a homogenized sample; (ii) extracting genomic material from the homogenized sample; (iii) capturing the extracted genomic material onto beads; and (iv) sequencing the captured genomic material; wherein the targeted representational sequencing comprises performing 1 to 4 amplification cycles prior to sequencing of the captured genomic material. In some embodiments, the at most 3 amplification cycles may be

conducted prior to capture of the extracted genomic material or after capture of the extracted genomic material, or any combination thereof. In some embodiments, no pre-capture amplification cycles are conducted. In some embodiments, an amount of captured genomic material ranges from about 90ng to about 900ng. In some embodiments, from

1 to 3 amplification cycles are performed following capture of the extracted genomic material, but prior to sequencing. In some embodiments, at least 0.5 micrograms of genomic material is extracted from the homogenized sample. In some embodiments, at least 100 times more genomic material is derived from the homogenized sample as compared with an amount of input material used in a sequencing method requiring more than 4 amplification cycles.

[0014] The present disclosure provides a method of sequencing DNA within a sample comprising: providing at least 0.5 micrograms of input genomic material, the at least 0.5 micrograms of genomic material derived from a tumor sample, a lymph node sample, or a blood sample, isolating DNA from the input genomic sample, preparing the isolated DNA for sequencing, and sequencing the prepared DNA, wherein the method does not comprise any amplification steps. The present disclosure is a method of sequencing DNA within a sample comprising: isolating at least 0.5 micrograms of genomic material from an input sample, the input sample derived from a tumor sample, a lymph node sample, a blood sample or any combination thereof; preparing the isolated genomic material for sequencing; and sequencing the prepared genomic material, wherein the method does not comprise any amplification steps. In some embodiments, the at least 0.5 micrograms of input genomic material is derived from multiple histological and/or biopsy specimens. In some embodiments, the at least 0.5 micrograms of input genomic material is derived from a homogenized tumor sample. In some embodiments, the at least 0.5 micrograms of input genomic material is derived from a homogenized lymph node sample. In some embodiments, the at least 0.5 micrograms of input genomic material is a representative sampling of the tumor sample, lymph node sample, or blood sample from which it is derived. In some embodiments, the sequencing is performed using a next-generation sequencing method. In some embodiments, sequencing is performed using a synthesis sequencing methodology.

[0015] In another aspect of the present disclosure is a method of reducing PCR-introduced mutations during sequencing comprising isolating DNA from a sample comprising a sufficient amount of genomic material; preparing the isolated DNA for sequencing; and sequencing the prepared DNA, wherein the method comprises

1 to 3 amplification cycles prior to sequencing. In some embodiments, the method comprises 1 or 2 amplification cycles prior to sequencing. In some embodiments, sufficient amount of input genomic material is an amount such that no pre-capture amplification cycles are utilized. In some embodiments, the sample is derived from a patient suspected of having cancer. In some embodiments, the sample is derived from a

[0019] The present disclosure provides a sequence capture method where a step of removing PCR-duplicate reads prior to sequencing is eliminated, the sequence capture method comprising providing an input sample comprising at least 0.5 micrograms of genomic material. In some embodiments, the input sample comprises at least 1 microgram of genomic material. In some embodiments, the input sample comprises at least 5 micrograms of genomic material. In some embodiments, the input sample comprises at least 10 micrograms of genomic material.

[0020] The present disclosure provides a sequencing method where PCR-introduced mutations are virtually eliminated, the sequencing method comprising capturing at least 0.05 micrograms of genomic material. In some embodiments, about 0.05 micrograms of genomic material are provided after capture of the genomic material. In some embodiments, 1 or 2 post-capture amplification cycles are performed prior to sequencing.

[0021] The present disclosure provides a method of treating cancer by identifying cancer subtypes responsive to a particular treatment or active pharmaceutical ingredient, wherein the cancer subtype is identified by sequencing an input sample comprising a representative sampling of a tumor, lymph node, or blood; the input sample comprising a sufficient quantity of genomic material, and wherein the step of sequencing requires at most 3 amplification cycles. In another aspect, the present disclosure refers to a particular treatment or active pharmaceutical ingredient, wherein the cancer subtype is identified by sequencing an input sample comprising a representative sampling of a tumor, lymph node, or blood, the input sample comprising a sufficient quantity of genomic material, and wherein 1 to 4 amplification cycles are conducted prior to sequencing.

[0022] As noted herein, traditional sequencing workflows may introduce certain biases. In some instances, a PCR-induced bias in the information content of an amplified DNA sample may be maintained when the sample is sequenced using next-generation sequencing (NGS) methods. The application of NGS to a population of amplified DNA fragments thus results in two drawbacks, namely (1) a large number of sequencing reads are expended in the redundant sequencing of copies of the same original fragment, which is not cost effective, and (2) the numerical biases introduced by the amplification process can lead to misrepresentation of the

information present in the original unamplified sample, and this is especially important when the primary purpose of the targeted sequencing assay is to accurately determine the presence and relative frequency of different DNA sequences in a sample. An additional drawback with PCR amplification of DNA fragments prior to NGS is that the PCR process can

CLAIMS

1. A method of sequencing genomic material within a sample comprising: homogenizing a tumor sample and/or lymph node sample to provide a homogenized sample; isolating at least 0.5 micrograms of genomic material from the homogenized sample; preparing the at least 0.5 micrograms of isolated genomic material for sequencing; and sequencing the prepared genomic material, wherein the method comprises at least one pre-capture or post-capture amplification step, wherein an aggregate number of amplification cycles conducted during the at least one pre-capture or post-capture amplification step is 1 to 4 cycles.
2. The method of claim 1, wherein the aggregate number of amplification cycles is 3.
3. The method of claim 1, wherein the aggregate number of amplification cycles is 2.
4. The method of claim 1, wherein the preparing of the at least 0.5 micrograms of isolated genomic material for sequencing comprises hybridizing the at least 0.5 micrograms of isolated genomic to capture probes and capturing the hybridized genomic material.
5. The method of claim 4, wherein an amount of captured genomic material ranges from 90ng to 900ng.
6. The method of claim 4, wherein 1 or 2 amplification cycles are performed on the captured genomic material.
7. The method of claim 1, wherein the homogenized sample comprises a representative sampling of cells.
8. The method of claim 1, wherein at least 1 microgram of genomic material is isolated from the homogenized sample.
9. The method of claim 1, wherein at least 5 micrograms of genomic material is isolated from the homogenized sample.
10. The method of claim 1, wherein at least 10 micrograms of genomic material is isolated from the homogenized sample.

11. A method of sequencing DNA within a sample comprising isolating at least 0.5 micrograms of DNA from a blood sample; preparing the at least 0.5 micrograms of isolated DNA for sequencing; and sequencing the prepared DNA, wherein the preparing of the at least 0.5 micrograms of isolated DNA for sequencing comprises hybridizing the at least 0.5 micrograms of isolated genomic to capture probes and capturing the hybridized genomic material, wherein the method comprising 1 to 4 amplification cycles prior to sequencing.
12. The method of claim 11, wherein an amount of captured genomic material ranges from 90ng to 900ng.
13. The method of claim 11, wherein 1 or 2 amplification cycles are performed on the captured genomic material.
14. The method of claim 11, wherein at least 1 microgram of DNA is isolated from the blood sample.
15. A method of targeted representational sequencing comprising: (i) homogenizing at least a portion of a tumor, one or more whole or partial lymph nodes, or any combination thereof to provide a homogenized sample; (ii) extracting genomic material from the homogenized sample; (iii) capturing the extracted genomic material onto beads; and (iv) sequencing the captured genomic material; wherein the targeted representational sequencing comprises performing 1 to 4 amplification cycles prior to sequencing of the captured genomic material.
16. The method of claim 15, wherein the at most 4 amplification cycles may be conducted prior to capture of the extracted genomic material or after capture of the extracted genomic material, or any combination thereof.
17. The method of claim 15, wherein an amount of captured genomic material ranges from 90ng to 900ng.
18. The method of claim 15, wherein from 1 to 3 amplification cycles are performed following capture of the extracted genomic material, but prior to sequencing.
19. The method of claim 15, wherein at least 1 microgram of genomic material is extracted from the homogenized sample.
20. A method of reducing PCR-introduced mutations during sequencing comprising isolating DNA from a sample comprising a sufficient amount of

- genomic material; preparing the isolated DNA for sequencing; and sequencing the prepared DNA, wherein the method comprises 1 to 3 amplification cycles prior to sequencing.
21. The method of claim 20, wherein the method comprises 1 or 2 amplification cycles prior to sequencing.
 22. The method of claim 20, wherein the sufficient amount of input genomic material is an amount such that no pre-capture amplification cycles are utilized.
 23. The method of claim 20, wherein the sample is derived from a patient suspected of having cancer.
 24. The method of claim 20, wherein the sample is derived from a patient diagnosed with cancer.
 25. The method of claim 20, wherein the sample is derived from a patient at risk of developing cancer.
 26. The method of claim 20, wherein the sample is derived from healthy tissue samples.
 27. The method of claim 20, wherein about 0.5 micrograms of DNA is isolated from the sample.
 28. A method of identifying cancer subtypes responsive to a particular treatment or active pharmaceutical ingredient, wherein the cancer subtype is identified by sequencing an input sample comprising a representative sampling of a tumor, lymph node, or blood, the input sample comprising a sufficient quantity of genomic material, and wherein 1 to 4 amplification cycles are conducted prior to sequencing.
 29. The method of claim 42, wherein 3 amplification cycles are conducted prior to sequencing.
 30. The method of claim 42, wherein 2 amplification cycles are conducted prior to sequencing.
 31. The method of claim 42, wherein 1 amplification cycle is conducted prior to sequencing.

32. The method of claim 42, wherein the quantity of genomic material is at least 0.5 micrograms.
33. The method of claim 42, wherein the quantity of genomic material is at least 1 microgram.
34. The method of claim 42, wherein the quantity of genomic material is at least 5 micrograms.
35. The method of claim 42, wherein the quantity of genomic material is at least 10 micrograms.