

[0004] Solid tumors contain hundreds to thousands of mutant alleles that are spatially segregated throughout the three-dimensional tumor mass. Traditional methods for sequence capture utilize extremely small amounts of input DNA (about 5 to about 200 nanograms) isolated from formalin fixed, paraffin embedded tissue sections (e.g. from biopsy specimens), such as depicted in FIG. 2. Typical sequence capture methods have evolved to fit the input DNA requirements in today's clinical pathology labs. Due to the small amounts of input DNA, and loss of DNA at several steps in the sequence capture workflow, the DNA fragments must be amplified or too little will remain at the end of the capture workflow for sequencing to be performed. This amplification generally is performed twice, a first time prior to the specific probe capture, and a second time following the specific probe capture of the selected targets (see FIGs. 1 and 2). While this amplification is useful for increasing the absolute mass of the DNA available for subsequent protocol steps, it does not increase the amount of information present. Rather, and without wishing to be bound by any particular theory, when a population of different DNA fragments is amplified in the same reaction (i.e. multiplex PCR), the process of amplification can alter the information that was contained within the original sample. For example, if two different DNA fragments, A and B, are initially present in a sample at one copy each (a 1:1 numerical ratio), PCR may result in an amplified sample that contains 1,000 copies of DNA fragment A and 2,000 copies of DNA fragment B (a 1:2 numerical ratio). It is believed that the risk of introducing bias to the original information is increased when smaller numbers of individual molecules are used as input into the amplification process and when the amount of amplification is increased (i.e. a greater number of PCR cycles are applied).

BRIEF SUMMARY OF THE DISCLOSURE

[0005] The present disclosure provides a targeted sequencing workflow where an input sample comprising a sufficient quantity of genomic material is provided such that minimal or no amplification processes are required prior to sequencing. In some embodiments, the input sample is derived from an intact tumor or from lymph nodes. In some embodiments, the input sample is obtained through homogenization of an intact tumor sample (whole or partial) and/or one or more lymph nodes obtained from a patient or mammalian subject, as discussed further herein. In some

[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. 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

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. In another aspect of 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 at most 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

patient diagnosed with cancer. In some embodiments, the sample is derived from a patient at risk of developing cancer. In some embodiments, the sample is derived from healthy tissue samples. In some embodiments, 0.5 micrograms of DNA is isolated from the sample. In some embodiments, at least 1 microgram of genomic material is isolated from the sample. In some embodiments, at least 5 micrograms of genomic material is isolated from the sample. In some embodiments, at least 10 micrograms of genomic material is isolated from the sample.

[0016] The present disclosure provides a sequencing method where PCR-introduced mutations are reduced, the sequencing method comprising capturing at least 0.05 micrograms of genomic material, and performing between 0 and 2 amplification cycles prior to sequencing. In some embodiments, 0 amplification cycles are conducted. In other embodiments, 1 amplification cycle is conducted. In yet other embodiments, 2 amplification cycles are conducted.

[0017] The present disclosure provides a sequence capture method where PCR-introduced biases in the proportional representation of genome content are reduced, the sequencing method comprising providing an input sample comprising at least 0.5 micrograms of genomic material, and where the sequence capture method comprises performing between 0 and 2 amplification cycles prior to sequencing. In some embodiments, 0 amplification cycles are conducted. In other embodiments, 1 amplification cycle is conducted. In yet other embodiments, 2 amplification cycles are conducted. 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.

[0018] The present disclosure provides a sequence capture method where PCR-introduced mutations are eliminated, the sequence capture method comprising preparing 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.

[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.

[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