

allow the user to make use of the capacity typically lost to the processing of the control genome. This may provide users the ability to run multiple assays in parallel, thus improving sequencing efficiency and/or throughput, thereby saving on overall sequencing cost and time, while still maintaining the sample complexity required for a successful sequencing run.

[006] Additionally, the availability of commercially available sequencers may place constraints on what assays may be economically run on those sequencing instruments. For example, a model designed for higher sequencing output may be too costly to run for biased sequencing applications without multiplexing a large number of specimens in a single run, yet can meaningfully decrease the cost per base for unbiased sequencing runs. The ability to combine both biased and unbiased specimens into a single sequencing run may make the use of higher output instruments more versatile, as they can then be used across a broader spectrum of applications with a reduced run cost per specimen.

[007] An example of the present disclosure provides a method for increasing complexity of a sample for sequencing, the method comprising: providing a first nucleic acid sample having a first degree of complexity that differs from a desired degree of complexity; providing a second nucleic acid sample having a second degree of complexity that differs from the first degree of complexity and that differs from the desired degree of complexity; pooling at least a portion of the first nucleic acid sample and at least a portion of the second nucleic acid sample, thereby generating a pooled nucleic acid sample having the desired degree of complexity; and sequencing at least a portion of the pooled nucleic acid sample.

[008] In some examples, the sequencing comprises whole genome sequencing (WGS). In some examples, the sequencing comprises massively parallel sequencing. In some examples, the sequencing comprises sequencing on a sequencing platform that comprises an output of at least about 1 billion reads per flow cell. In some examples, the sequencing comprises sequencing on a sequencing platform that comprises an output of at least about 1.5 billion reads per flow cell. In some examples, the sequencing comprises sequencing on a sequencing platform that comprises an output of at least about 2 billion reads per flow cell.

[009] Another example of the present disclosure provides a method for sequencing nucleic acid molecules, comprising: processing a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing; processing a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing; pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; and using a single flow cell of a sequencing platform, sequencing the pooled plurality of libraries to generate a first plurality of sequencing reads corresponding to

the first plurality of nucleic acid molecules and a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules. In one aspect, the present disclosure refers to a method for sequencing nucleic acid molecules, comprising: processing a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing; processing a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci; pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; and using a single flow cell of a sequencing platform, sequencing the pooled plurality of libraries to generate a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules and a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0010] In some examples, the unbiased sequencing comprises whole genome sequencing (WGS). In some examples, the unbiased sequencing is performed at a depth of no more than about 0.1X, no more than about 0.5X, no more than about 1X, no more than about 2X, no more than about 3X, no more than about 4X, no more than about 5X, no more than about 6X, no more than about 7X, no more than about 8X, no more than about 9X, no more than about 10X, no more than about 12X, no more than about 14X, no more than about 16X, no more than about 18X, no more than about 20X, no more than about 22X, no more than about 24X, no more than about 26X, no more than about 28X, or no more than about 30X. In some examples, the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci. In some examples, the targeted sequencing comprises targeted methyl-seq. In some examples, the unbiased sequencing comprises methylation sequencing. In some examples, the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq). In some examples, generating the second plurality of sequencing reads comprises using at least a portion of the first plurality of libraries as control libraries. In some examples, the method further comprises pooling a third plurality of libraries to generate the pooled plurality of libraries, wherein the third plurality of libraries comprises control libraries for generating the first plurality of sequencing reads or the second plurality of sequencing reads. In some examples, the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules comprise DNA molecules. In some examples, the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules comprise RNA molecules. In some examples, the sequencing platform is an Illumina™ sequencer.

[0011] Another example of the present disclosure provides a method for sequencing nucleic acid molecules, comprising: processing a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first biased sequencing; processing a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second biased sequencing; pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; and using a single flow cell of a sequencing platform, sequencing the pooled plurality of libraries to generate a first plurality of sequencing reads corresponding to

the first plurality of nucleic acid molecules and a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0012] In some examples, the first biased sequencing comprises targeted sequencing of a first target capture panel comprising a first plurality of genetic loci, and wherein the second biased sequencing comprises targeted sequencing of a second target capture panel comprising a second plurality of genetic loci.

[0013] Another example of the present disclosure provides a method for sequencing nucleic acid molecules, comprising: processing a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first unbiased sequencing; processing a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second unbiased sequencing; pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; and using a single flow cell of a sequencing platform, sequencing the pooled plurality of libraries to generate a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules and a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0014] In some examples, the first unbiased sequencing comprises whole genome sequencing (WGS), and the second unbiased sequencing comprises methylation sequencing. In some examples, the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq). In some examples, the unbiased sequencing is performed at a depth of no more than about 0.1X, no more than about 0.5X, no more than about 1X, no more than about 2X, no more than about 3X, no more than about 4X, no more than about 5X, no more than about 6X, no more than about 7X, no more than about 8X, no more than about 9X, no more than about 10X, no more than about 12X, no more than about 14X, no more than about 16X, no more than about 18X, no more than about 20X, no more than about 22X, no more than about 24X, no more than about 26X, no more than about 28X, or no more than about 30X.

[0015] In some examples, the nucleic acid molecules are extracted from a sample. In some examples, the sample is a biological sample.

[0016] In some examples, the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules are generated from a same initial biological sample.

[0017] Another example of the present disclosure provides a system for sequencing nucleic acid molecules, comprising: a controller comprising one or more computer processors; and a support operatively coupled to the controller; wherein the one or more computer processors are individually or collectively programmed to: direct the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing; direct the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, direct the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generate, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generate, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules. In one aspect, the present disclosure refers to a system for sequencing nucleic acid molecules, comprising: a controller comprising one or more computer processors; and a support operatively coupled to the controller; wherein the one or more computer processors are individually or collectively programmed to: direct the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing; direct the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci; direct the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generate, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generate, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0018] In some examples, the unbiased sequencing comprises whole genome sequencing (WGS) or methylation sequencing. In some examples, the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq). In some examples, the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci. In some examples, the targeted sequencing comprises targeted methyl-seq.

[0019] Another example of the present disclosure provides a system for sequencing nucleic acid molecules, comprising: a controller comprising one or more computer processors; and a support operatively coupled to the controller; wherein the one or more computer processors are individually or collectively programmed to: direct the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first biased sequencing; direct the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second biased sequencing, direct the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generate, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generate, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0020] In some examples, the first biased sequencing comprises targeted sequencing of a first target capture panel comprising a first plurality of genetic loci, and the second biased sequencing comprises targeted sequencing of a second target capture panel comprising a second plurality of genetic loci.

[0021] Another example of the present disclosure provides a system for sequencing nucleic acid molecules, comprising: a controller comprising one or more computer processors; and a support operatively coupled to the controller; wherein the one or more computer processors are individually or collectively programmed to: direct the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first unbiased sequencing; direct the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second unbiased sequencing, direct the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generate, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generate, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0022] In some examples, the first unbiased sequencing or the second unbiased sequencing comprises whole genome sequencing (WGS) or methylation sequencing. In some examples, the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq).

[0023] Another example of the present disclosure provides a non-transitory computer-readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method for sequencing nucleic acid molecules, the method comprising: directing the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing; directing the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing; directing the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generating, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generating, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules. In one aspect, the present disclosure refers to a non-transitory computer-readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method for sequencing nucleic acid molecules, the method comprising: directing the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing, directing the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci; directing the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generating, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generating, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0024] Another example of the present disclosure provides a non-transitory computer-readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method for sequencing nucleic acid molecules, the method comprising: directing the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first biased sequencing; directing the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second biased sequencing; directing the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generating, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generating, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0025] Another example of the present disclosure provides a non-transitory computer-readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method for sequencing nucleic acid molecules, the method comprising: directing the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing a first unbiased sequencing; directing the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a second unbiased sequencing; directing the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; generating, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and generating, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

[0026] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious examples, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0027] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications, patents and patent applications incorporated by reference contradict

CLAIMS

WHAT IS CLAIMED IS:

1. A method for sequencing nucleic acid molecules, comprising:
processing a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing;
processing a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci;
pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries; and
using a single flow cell of a sequencing platform, sequencing the pooled plurality of libraries to generate a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules and a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.
2. The method of claim 1, wherein the unbiased sequencing comprises whole genome sequencing (WGS).
3. The method of claim 2, wherein the unbiased sequencing is performed at a depth of no more than about 10X.
4. The method of claim 1, wherein the targeted sequencing comprises hybridization capture of the target capture panel followed by sequencing.
5. The method of claim 1 or claim 4, wherein the targeted sequencing comprises targeted methyl-seq.
6. The method of claim 1, wherein the unbiased sequencing comprises methylation sequencing.
7. The method of claim 6, wherein the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite

sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq).

8. The method of claim 1, wherein generating the second plurality of sequencing reads comprises using at least a portion of the first plurality of libraries as control libraries.

9. The method of claim 1, further comprising pooling a third plurality of libraries to generate the pooled plurality of libraries, wherein the third plurality of libraries comprises control libraries for generating the first plurality of sequencing reads or the second plurality of sequencing reads.

10. The method of claim 1, wherein the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules comprise DNA molecules.

11. The method of claim 1, wherein the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules comprise RNA molecules.

12. The method of claim 1, wherein the sequencing platform is a massively parallel array sequencer.

13. The method of any one of claims 1 to 12, wherein the nucleic acid molecules are extracted from a sample.

14. The method of claim 13, wherein the sample is a biological sample.

15. The method of any one of claims 1-14, wherein the first plurality of nucleic acid molecules and the second plurality of nucleic acid molecules are generated from a same initial biological sample.

16. A system for sequencing nucleic acid molecules, comprising:
a controller comprising one or more computer processors; and
a support operatively coupled to the controller;
wherein the one or more computer processors are individually or collectively programmed to:
direct the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing;

direct the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci;

direct the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries;

generate, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and

generate, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.

17. The system of claim 16, wherein the unbiased sequencing comprises whole genome sequencing (WGS) or methylation sequencing.

18. The system of claim 17, wherein the methylation sequencing comprises bisulfite sequencing, whole genome bisulfite sequencing (WGBS), APOBEC-seq, methyl-CpG-binding domain (MBD) protein capture, methyl-DNA immunoprecipitation (MeDIP), methylation sensitive restriction enzyme sequencing (MSRE/MRE-Seq or Methyl-Seq), oxidative bisulfite sequencing (oxBS-Seq), reduced representative bisulfite sequencing (RRBS), or Tet-assisted bisulfite sequencing (TAB-Seq).

19. The system of claim 16, wherein the targeted sequencing comprises targeted methyl-seq.

20. The system of claim 16 or claim 19, wherein the targeted sequencing comprises hybridization capture of the target capture panel followed by sequencing.

21. A non-transitory computer-readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method for sequencing nucleic acid molecules, the method comprising:

directing the processing of a first plurality of nucleic acid molecules to generate a first plurality of libraries for performing an unbiased sequencing,

directing the processing of a second plurality of nucleic acid molecules to generate a second plurality of libraries for performing a biased sequencing, wherein the

biased sequencing comprises targeted sequencing of a target capture panel comprising a plurality of genetic loci;

directing the pooling the first plurality of libraries and the second plurality of libraries to generate a pooled plurality of libraries;

generating, from the pooled plurality of libraries, a first plurality of sequencing reads corresponding to the first plurality of nucleic acid molecules; and

generating, from the pooled plurality of libraries, a second plurality of sequencing reads corresponding to the second plurality of nucleic acid molecules.