

effective in identifying regulatory elements across a variety of cell types and quantifying changes that lead to both activation or repression of gene expression.

[0008] One such method is the Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq). The ATAC-seq method probes DNA accessibility with an artificial transposon, which inserts specific sequences into accessible regions of chromatin. Because the transposase can only insert sequences into accessible regions of chromatin not bound by transcription factors and/or nucleosomes, sequencing reads can be used to infer regions of increased chromatin accessibility.

[0009] Traditional approaches to the ATAC-seq methodology requires large pools of cells, processes cells in bulk, and result in data representative of an entire cell population, but lack information about cell-to-cell variation inherently present in a cell population (see, *e.g.*, Buenrostro, *et al.*, Curr. Protoc. Mol. Biol., 2015 Jan 5; 21.29.1–21.29.9). While single cell ATAC-seq (scATAC-seq) methods have been developed, they suffer from several limitations. For example, scATAC-seq methods that utilize sample pooling, cell indexing, and cell sorting (see, *e.g.*, Cusanovich, *et al.*, Science, 2015 May 22; 348(6237):910-14) result in high variability and a low number of reads associated with any single cell. Other scATAC-seq methods that utilize a programmable microfluidic device to isolate single cells and perform scATAC-seq in nanoliter reaction chambers (see, *e.g.*, Buenrostro, *et al.*, Nature, 2015 Jul 23; 523(7561):486-90) are limited by the throughput of the assay and may not generate personal epigenomic profiles on a timescale compatible with clinical decision-making.

[0010] In an example, the present disclosure provides a method for nucleic acid processing, comprising: (a) generating a partition comprising: (i) a biological particle comprising chromatin comprising a template nucleic acid molecule; (ii) a plurality of nucleic acid barcode molecules comprising a common barcode sequence; (iii) a plurality of transposon end nucleic acid molecules comprising a transposon end sequence; and (iv) a plurality of transposase molecules; (b) generating a plurality of template nucleic acid fragments with the aid of a transposase-nucleic acid complex comprising a transposase molecule of the plurality of transposase molecules and a transposon end oligonucleotide molecule of the plurality of transposon end oligonucleotide molecules; and (c) generating a barcoded nucleic acid fragment using a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules and a template nucleic acid fragment of the plurality of template nucleic acid fragments.

[0011] In some examples, the barcoded nucleic acid fragment is generated by ligation of the nucleic acid barcode molecule with the template nucleic acid fragment, or a derivative thereof.

- [0012]** In some examples, the barcoded nucleic acid fragment is generated by amplification of the template nucleic acid fragment, or a derivative thereof, using the nucleic acid barcode molecule as a primer.
- [0013]** In some examples, the transposase-nucleic acid complex is generated in the partition using transposase molecules of the plurality of transposase molecules and transposon end nucleic acid molecules of the plurality of transposon end nucleic acid molecules.
- [0014]** In some examples, the transposase-nucleic acid complex is partitioned into the partition.
- [0015]** In some examples, the plurality of nucleic acid barcode molecules are attached to a bead and wherein the partition further comprises the bead. In some examples, the nucleic acid barcode molecules are releasably attached to the bead. In some examples, the method further comprises releasing the plurality of nucleic acid barcode molecules from the bead. In some examples, the bead is a gel bead. In some examples, the gel bead is a degradable gel bead. In some examples, the degradable gel bead is degradable upon application of a stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the stimulus is a reducing agent. In some examples, the partition further comprises the chemical stimulus.
- [0016]** In some examples, prior to (b), the template nucleic acid molecule is released from the biological particle. In some examples, subsequent to (b), the plurality of template nucleic acid fragments is released from the biological particle.
- [0017]** In some examples, the biological particle is a cell. In some examples, the biological particle is a nucleus.
- [0018]** In some examples, the partition is an aqueous droplet in an emulsion. In some examples, the partition is a well.
- [0019]** In some examples, the method further comprises releasing or removing the barcoded nucleic acid fragment or a derivative thereof from the partition.
- [0020]** In some examples, the method further comprises sequencing the barcoded nucleic acid fragment, or a derivative thereof.
- [0021]** In some examples, the plurality of transposon end nucleic acid molecules further comprise a sequence complementary to a sequence of the plurality of nucleic acid barcode molecules. In some examples, the partition further comprises a plurality of second nucleic acid molecules comprising a transposon end sequence and a primer sequence, and wherein the transposase-nucleic acid complex comprises: (i) transposase molecules of the plurality of transposase molecules; (ii) a transposon end oligonucleotide molecule of the plurality of transposon end oligonucleotide molecules; and (iii) a second nucleic acid molecule of the

plurality of second nucleic acid molecules. In some examples, the plurality of nucleic acid barcode molecules are partially double-stranded and comprise (i) a first strand comprising the common barcode sequence and a sequence complementary to a sequence of the plurality of first nucleic acid molecules; and (ii) a second strand comprising a sequence complementary to the common barcode sequence. In some examples, the plurality of nucleic acid barcode molecules are single-stranded and comprise the common barcode sequence and a sequence complementary to a sequence in the plurality of transposon end nucleic acid molecules.

[0022] In some examples, the biological particle further comprises a template ribonucleic acid (RNA) molecule, and wherein the method further comprises generating a barcoded complementary deoxyribonucleic acid (cDNA) molecule from the template RNA molecule or a derivative thereof. In some examples, the barcoded cDNA molecule is generated in the partition. In some examples, the partition comprises a second plurality of nucleic acid barcode molecules comprising the common barcode sequence and a capture sequence. In some examples, the capture sequence is a poly-T sequence. In some examples, the capture sequence is a template switching oligo sequence and wherein the barcoded cDNA molecule is generated using a template switching reaction. In some examples, the plurality of nucleic acid barcode molecules are attached to a first bead, the second plurality of nucleic acid barcode molecules are attached to a second bead, and wherein the partition comprises the first bead and the second bead. In some examples, the first bead or the second bead is a magnetic bead. In some examples, the first bead is a gel bead and the second bead is a magnetic bead. In some examples, the gel bead comprises the magnetic bead. In some examples, the method further comprises degrading the gel bead. In some examples, the first bead is a magnetic bead and the second bead is a gel bead. In some examples, the gel bead comprises the magnetic bead. In some examples, the method further comprises degrading the gel bead. In some examples, the plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules are attached to a bead, and wherein the partition further comprises the bead. In some examples, the barcoded nucleic acid fragment and the barcoded cDNA molecule is released from the partition. In some examples, the method further comprises sequencing the barcoded nucleic acid fragment or a derivative thereof and the barcoded cDNA molecule or a derivative thereof.

[0023] In some examples, the method further comprises cleaving one or more mitochondrial nucleic acid fragments, or derivatives thereof, using (i) one or more guide ribonucleic acid molecules (gRNAs) targeted to the one or more mitochondrial nucleic acid fragments; and (ii) a clustered regularly interspaced short palindromic (CRISPR) associated

(Cas) nuclease. In some examples, the partition further comprises the one or more gRNAs and the Cas nuclease. In some examples, the Cas nuclease is Cas9.

[0024] In another example, the present disclosure provides a method of generating barcoded nucleic acid fragments, comprising: (a) providing: (i) a plurality of biological particles, an individual biological particle of the plurality of biological particles comprising chromatin comprising a template nucleic acid; (ii) a plurality of transposon end nucleic acid molecules comprising a transposon end sequence; and (iii) a plurality of transposase molecules; (b) generating a plurality of template nucleic acid fragments in a biological particle of the plurality of biological particles with the aid of a transposase-nucleic acid complex comprising a transposase molecule of the plurality of transposase molecules and a transposon end nucleic acid molecule of the plurality of transposon end nucleic acid molecules; (c) generating a partition comprising the biological particle comprising the plurality of template nucleic acid fragments and a plurality of nucleic acid barcode molecules comprising a common barcode sequence; and (d) generating a barcoded nucleic acid fragment using a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules and a template nucleic acid fragment of the plurality of template nucleic acid fragments.

[0025] In some examples, the barcoded nucleic acid fragment is generated by ligation of the nucleic acid barcode molecule with the template nucleic acid fragment, or a derivative thereof.

[0026] In some examples, the barcoded nucleic acid fragment is generated by nucleic acid amplification of the template nucleic acid fragment, or a derivative thereof, using the nucleic acid barcode molecule as a primer.

[0027] In some examples, the plurality of nucleic acid barcode molecules are attached to a bead and wherein the partition further comprises the bead. In some examples, the nucleic acid barcode molecules are releasably attached to the bead. In some examples, the method further comprises releasing the plurality of nucleic acid barcode molecules from the bead. In some examples, the bead is a gel bead. In some examples, the gel bead is a degradable gel bead. In some examples, the degradable gel bead is degradable upon application of a stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the stimulus is a reducing agent. In some examples, the partition further comprises the chemical stimulus.

[0028] In some examples, subsequent to (c), the template nucleic acid fragments are released from the biological particle.

[0029] In some examples, the biological particle is a cell. In some examples, the cell is a permeabilized cell.

[0030] In some examples, the biological particle is a nucleus. In some examples, the nucleus is a permeabilized nucleus.

[0031] In some examples, the partition is an aqueous droplet in an emulsion. In some examples, the partition is a well.

[0032] In some examples, the method further comprises releasing or removing the barcoded nucleic acid fragment or a derivative thereof from the partition.

[0033] In some examples, the method further comprises sequencing the barcoded nucleic acid fragment, or a derivative thereof.

[0034] In some examples, the plurality of transposon end nucleic acid molecules comprise a plurality of first nucleic acid molecules comprising a transposon end sequence and a sequence complementary to a sequence of the plurality of nucleic acid barcode molecules. In some examples, the plurality of transposon end nucleic acid molecules further comprise a plurality of second nucleic acid molecules comprising a transposon end sequence and a primer sequence. In some examples, the plurality of nucleic acid barcode molecules are partially double-stranded and comprise a first strand comprising the common barcode sequence and a sequence complementary to a sequence of the plurality of first nucleic acid molecules; and a second strand comprising a sequence complementary to the common barcode sequence. In some examples, the plurality of nucleic acid barcode molecules are single-stranded and comprise the common barcode sequence and a sequence complementary to a sequence of the plurality of first nucleic acid molecules.

In some examples, the biological particle further comprises a template RNA molecule, and wherein the method further comprises generating a barcoded cDNA molecule from the template RNA molecule or a derivative thereof. In some examples, the barcoded cDNA molecule is generated in the partition. In some examples, the partition comprises a second plurality of nucleic acid barcode molecules comprising the common barcode sequence and a capture sequence. In some examples, the capture sequence is a poly-T sequence. In some examples, the capture sequence is a template switching oligo sequence and wherein the barcoded cDNA molecule is generated using a template switching reaction. In some examples, the plurality of nucleic acid barcode molecules are attached to a first bead, the second plurality of nucleic acid barcode molecules are attached to a second bead, and wherein the partition comprises the first bead and the second bead. In some examples, the first bead or the second bead is a magnetic bead. In some examples, the first bead is a gel bead and the second bead is a magnetic bead. In some examples, the gel bead comprises the magnetic bead. In some examples, the method further comprises degrading the gel bead. In some examples, the first bead is a magnetic bead and the second bead is a gel bead. In some

examples, the gel bead comprises the magnetic bead. In some examples, the method further comprises degrading the gel bead. In some examples, the plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules are attached to a bead, and wherein the partition further comprises the bead. In some examples, the barcoded nucleic acid fragment and the barcoded cDNA molecule is released from the partition. In some examples, the method further comprises sequencing the barcoded nucleic acid fragment or a derivative thereof and the barcoded cDNA molecule or a derivative thereof.

[0035] In some examples, the method further comprises (a) lysing a plurality of cells in the presence of a cell lysis agent and a blocking agent; and (b) separating a plurality of nuclei from the plurality of lysed cells to generate the plurality of biological particles, wherein the blocking agent reduces the fraction of sequencing reads corresponding to a mitochondrial genome compared to sequencing reads obtained in the absence of the blocking agent. In some examples, the blocking agent is bovine serum albumin (BSA).

[0036] In some examples, the method further comprises cleaving one or more mitochondrial nucleic acid molecules or derivatives thereof, using (i) one or more guide ribonucleic acid molecules (gRNAs) targeted to the one or more mitochondrial nucleic acid fragments; and (ii) a clustered regularly interspaced short palindromic (CRISPR) associated (Cas) nuclease. In some examples, the Cas nuclease is Cas9. In some examples, the mitochondrial nucleic acid molecules are cleaved prior to (c). In some examples, the mitochondrial nucleic acid molecules are cleaved in the partition. In some examples, the partition comprises the Cas nuclease. In some examples, the partition further comprises the one or more gRNAs. In some examples, the mitochondrial nucleic acid molecules are cleaved subsequent to (d).

[0037a] In one aspect, the present disclosure provides a method of generating barcoded nucleic acid fragments, comprising:

(a) providing: (i) a plurality of cells or cell nuclei comprising chromatin comprising a template nucleic acid molecule; (ii) a plurality of oligonucleotide molecules comprising a transposon end sequence; and (iii) a plurality of transposase molecules;

(b) in said plurality of cells or cell nuclei, generating a plurality of template nucleic acid fragments with the aid of a plurality of transposase-nucleic acid complexes comprising a transposase molecule of said plurality of transposase molecules and an oligonucleotide molecule of said plurality of oligonucleotide molecules;

(c) partitioning said plurality of cells or cell nuclei into a plurality of partitions, each comprising (i) a cell or a cell nucleus of said plurality of cells or cell nuclei, said cell or cell nucleus comprising a template nucleic acid fragment of said plurality of template nucleic acid fragments, and (ii) a plurality of nucleic acid barcode molecules comprising a common barcode sequence; and

(d) in a partition of said plurality of partitions, generating a barcoded nucleic acid fragment using the nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules

and a template nucleic acid fragment of said plurality of template nucleic acid fragments.

[0037] 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 respects, 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

[0038] 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

WHAT IS CLAIMED IS:

1. A method of generating barcoded nucleic acid fragments, comprising:
 - (a) providing: (i) a plurality of cells or cell nuclei comprising chromatin comprising a template nucleic acid molecule; (ii) a plurality of oligonucleotide molecules comprising a transposon end sequence; and (iii) a plurality of transposase molecules;
 - (b) in said plurality of cells or cell nuclei, generating a plurality of template nucleic acid fragments with the aid of a plurality of transposase-nucleic acid complexes comprising a transposase molecule of said plurality of transposase molecules and an oligonucleotide molecule of said plurality of oligonucleotide molecules;
 - (c) partitioning said plurality of cells or cell nuclei into a plurality of partitions, each comprising (i) a cell or a cell nucleus of said plurality of cells or cell nuclei, said cell or cell nucleus comprising a template nucleic acid fragment of said plurality of template nucleic acid fragments, and (ii) a plurality of nucleic acid barcode molecules comprising a common barcode sequence; and
 - (d) in a partition of said plurality of partitions, generating a barcoded nucleic acid fragment using a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and the template nucleic acid fragment of said plurality of template nucleic acid fragments.
2. The method of claim 1, wherein said barcoded nucleic acid fragment is generated by ligation of said nucleic acid barcode molecule with said template nucleic acid fragment, or a derivative thereof, or wherein said barcoded nucleic acid fragment is generated by nucleic acid amplification of said template nucleic acid fragment, or a derivative thereof, using said nucleic acid barcode molecule as a primer.
3. The method of claim 1 or claim 2, wherein said plurality of nucleic acid barcode molecules are attached to a bead and wherein said partition further comprises said bead.
4. The method of claim 3, wherein said nucleic acid barcode molecules are releasably attached to said bead.

5. The method of claim 4, further comprising releasing said plurality of nucleic acid barcode molecules from said bead.
6. The method of any one of claims 3 to 6, wherein said bead is a degradable gel bead, wherein said degradable gel bead is degradable upon application of a stimulus.
7. The method of any one of claims 1 to 6, wherein subsequent to (c), said template nucleic acid fragments are released from said cells or cell nuclei.
8. The method of any one of claims 1 to 7, wherein said partition is an aqueous droplet in an emulsion, or a well.
9. The method of any one of claims 1 to 8, further comprising: (i) releasing or removing said barcoded nucleic acid fragment or a derivative thereof from said partition, or (ii) sequencing said barcoded nucleic acid fragment, or a derivative thereof.
10. The method of any one of claims 1 to 9, wherein said plurality of oligonucleotide molecules comprise a plurality of first nucleic acid molecules comprising a transposon end sequence and a sequence complementary to a sequence of said plurality of nucleic acid barcode molecules, or wherein said cells or cell nuclei further comprises a template RNA molecule, and wherein the method further comprises generating a barcoded cDNA molecule from said template RNA molecule or a derivative thereof.
11. The method of claim 10, wherein said plurality of oligonucleotide molecules further comprise a plurality of second nucleic acid molecules comprising a transposon end sequence and a primer sequence, or wherein said plurality of nucleic acid barcode molecules are (A) partially double-stranded and comprise (i) a first strand comprising said common barcode sequence and a sequence complementary to a sequence of said plurality of first nucleic acid molecules; and (ii) a second strand comprising a sequence complementary to said common barcode sequence or (B) single-stranded and comprise said common barcode sequence and a sequence complementary to a sequence of said plurality of first nucleic acid molecules.

12. The method of claim 10, wherein said barcoded cDNA molecule is generated in said partition, or wherein said partition comprises a second plurality of nucleic acid barcode molecules comprising said common barcode sequence and a capture sequence.
13. The method of any one of claims 1 to 12, further comprising (a) lysing a plurality of cells in the presence of a cell lysis agent and a blocking agent; and (b) separating a plurality of nuclei from said plurality of lysed cells to generate said plurality of cells or cell nuclei, wherein said blocking agent reduces the fraction of sequencing reads corresponding to a mitochondrial genome compared to sequencing reads obtained in the absence of said blocking agent.
14. The method of any one of claims 1 to 13, further comprising cleaving one or more mitochondrial nucleic acid molecules or derivatives thereof, using (i) one or more guide ribonucleic acid molecules (gRNAs) targeted to said one or more mitochondrial nucleic acid fragments; and (ii) a clustered regularly interspaced short palindromic (CRISPR) associated (Cas) nuclease.