CLAIMS

Claim 1. A method of preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or single cells, the method comprising:

(a) providing a plurality of cells in a first plurality of compartments preferably comprising a well or a droplet;

(b) exposing the plurality of cells of each compartment to a predetermined condition;

(c) contacting nuclei isolated from the cells of each compartment or the cells of each compartment with a hashing oligo,

wherein at least one copy of the hashing oligo is associated with isolated nuclei or cells,

wherein the hashing oligo comprises a hashing index,

wherein the hashing index in each compartment comprises an index sequence that is different from index sequences in the other compartments to generate hashed nuclei or hashed cells;

wherein the association between isolated nuclei or cells and hashing oligo is non-specific and

(d) combining the hashed nuclei or hashed cells of different compartments to generate pooled hashed nuclei or pooled hashed cells.

Claim 2. The method of claim 1, further comprising exposing the cells or nuclei to a cross-linking compound to fix hashing oligos cells or to isolated nuclei optionally, wherein the cross-linking compound comprises paraformaldehyde, formalin, or methanol.

Claim 3. The method of claim 1, wherein the predetermined condition comprises exposure to an agent or an agent comprising a protein, a non-ribosomal protein, a polyketide, an organic molecule, an inorganic molecule, an RNA or RNAi molecule, a carbohydrate, a glycoprotein, a nucleic acid, a drug, or a combination thereof.

Claim 4. The method of claim 1, wherein the hashing oligo comprises or consists of

a) a single stranded nucleic acid;

b) a single stranded nucleic acid wherein the nucleic acid of the hashing oligo comprises DNA, RNA, or a combination thereof; or

c) a domain that mediates specific binding of the hashing oligo to the surface of cells or nuclei preferably, wherein the domain comprises a ligand, an antibody, or an aptamer.

Claim 5. The method of claim 1 wherein the non-specific association between the hashing oligo and the cells or isolated nuclei is by absorption.

Claim 6. The method of claim 1, further comprising processing the pooled hashed cells or pooled hashed nuclei using a single-cell combinatorial indexing method to result in a sequencing library comprising nucleic acids from the plurality of single nuclei, wherein the nucleic acids comprise a plurality of indexes, and optionally wherein the single-cell combinatorial indexing method is single-nuclei transcriptome sequencing, single-cell transcriptome sequencing, single-cell transcriptome and transposon-accessible chromatin sequencing, whole genome sequencing of single nuclei, single nuclei sequencing of transposon accessible chromatin, sci-HiC, DRUG-seq, sci-CAR, sci-MET, sci-Crop, sci-perturb, or sci-Crispr.

Claim 7. The method of claim 6, the method further comprising

(e) distributing subsets of the pooled hashed cells or hashed nuclei into a second plurality of compartments and contacting each subset with reverse transcriptase or DNA polymerase and a primer, wherein the primer in each compartment comprises a first index sequence that is different from first index sequences in the other compartments to generate indexed nuclei comprising indexed nucleic acid fragments;

(f) combining the indexed cells or indexed nuclei to generate pooled indexed cells or pooled indexed nuclei;

(g) distributing subsets of the pooled indexed cells or pooled indexed nuclei into a third plurality of compartments and introducing a second index sequence to indexed nucleic acid fragments to generate dual-indexed cells or dual-indexed nuclei comprising dual-indexed nucleic acid fragments, wherein the introducing comprises ligation, primer extension, amplification, or transposition, preferably comprising contacting each subset with a transposome complex, wherein the transposome complex in each compartment comprises a transposase and a second index sequence under conditions suitable for ligation of the second index sequence to the ends of indexed nucleic acid fragments comprising a first index sequence to generate dual-indexed nuclei comprising dual-indexed nucleic acid fragments, wherein the second index sequence is different from second index sequences in the other compartments;

(h) combining the dual-indexed cells or dual-indexed nuclei to generate pooled dual-indexed nuclei or cells;

(i) distributing subsets of dual-indexed cells or the pooled dual-indexed nuclei into a fourth plurality of compartments and introducing a third index sequence to dual-indexed nucleic acid fragments to generate triple-indexed cells or triple-indexed nuclei comprising triple-indexed nucleic acid fragments, wherein the introducing comprises ligation, primer extension, amplification, or transposition, preferably comprising contacting each subset with a primer comprising a third index sequence and a universal primer sequence, wherein the contacting comprises conditions suitable for amplification and incorporation of the third index sequence to the ends of the dual-indexed nucleic acid fragments, wherein the third index sequence is different from third index sequences in the other compartments;

(j) combining the triple-indexed fragments, thereby producing a sequencing library comprising transcriptome nucleic acids from the plurality of single nuclei.

Claim 8. The method of claim 7, further comprising:

providing a surface comprising a plurality of amplification sites,

wherein the amplification sites comprise at least two populations of attached single stranded capture oligonucleotides having a free 3’ end, and

contacting the surface comprising amplification sites with the triple-indexed fragments under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual fragment comprising a plurality of indexes.

Claim 9. A composition comprising the hashed cells or hashed nuclei of claim 1 or the pooled hashed cells or pooled hashed nuclei of claim 1.

Claim 10. A multi-well plate, wherein compartments of the multi-well plate comprise the composition of claim 9 preferably, wherein a compartment of the multi-well plate comprises from 50 to 100,000,000 cells or nuclei.

Claim 11. A droplet, wherein the droplet comprises the composition of claim 9 preferably, wherein the droplet comprises from 50 to 100,000,000 cells or nuclei.

Claim 12. A method of preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or single cells, the method comprising:

(a) providing a first plurality of compartments comprising isolated nuclei or cells and contacting the isolated nuclei or cells of each compartment with a hashing oligo,

wherein at least one copy of the hashing oligo is associated with isolated nuclei or cells and the association between isolated nuclei or cells and hashing oligo is non-specific,

wherein the hashing oligo comprises a nucleic acid and a hashing index,

wherein the hashing index in each compartment comprises an index sequence that is different from index sequences in the other compartments to generate hashed nuclei or hashed cells; and

(b) combining the hashed nuclei or hashed cells of different compartments to generate pooled hashed nuclei or pooled hashed cells.

Claim 13. A method of preparing a sequencing library comprising nucleic acids from a plurality of single nuclei or single cells, the method comprising:

(a) providing a first plurality of compartments comprising isolated nuclei or cells and contacting the isolated nuclei or cells of each compartment with a hashing oligo,

wherein at least one copy of the hashing oligo is associated with isolated nuclei or cells by absorption,

wherein the hashing oligo comprises a nucleic acid and a hashing index,

wherein the hashing index in each compartment comprises an index sequence that is different from index sequences in the other compartments to generate hashed nuclei or hashed cells; and

(b) combining the hashed nuclei or hashed cells of different compartments to generate pooled hashed nuclei or pooled hashed cells.

Claim 14. A method of preparing a sequencing library comprising nucleic acids from a plurality of nuclei or cells, the method comprising:

(a) providing a plurality of compartments comprising nuclei or cells, wherein the nuclei or cells comprise a hashing oligo that comprises a compartment specific index, wherein the association between nuclei or cells and hashing oligo is non-specific;

(b) combining the nuclei or cells from different compartments into a second compartment to generate pooled hashed nuclei or pooled hashed cells optionally, further comprising exposing the cells of each compartment to a predetermined condition or exposing the cells of each compartment to a predetermined condition and then isolating nuclei from a plurality of cells prior to step (a), wherein the predetermined condition comprises exposure to an agent preferably, wherein the agent comprises a protein, a non-ribosomal protein, a polyketide, an organic molecule, an inorganic molecule, an RNA or RNAi molecule, a carbohydrate, a glycoprotein, a nucleic acid, a drug, or a combination thereof.

Claim 15. A composition comprising multiple populations of normalization oligos, wherein the composition comprises a first population of normalization oligos comprising a first index sequence and other populations of normalization oligos each comprising a unique index sequence that is different from the index sequences of the other populations,

and wherein the concentration of each population is the same optionally, wherein the composition comprises from 2 to 100 populations of normalization oligos.

Claim 16. A composition comprising multiple populations of normalization oligos, wherein the composition comprises a first population of normalization oligos comprising a first index sequence and other populations of normalization oligos each comprising a unique index sequence that is different from the index sequences of the other populations, and wherein the concentrations of at least two of the populations are different optionally, wherein the composition comprises from 2 to 100 populations of normalization oligos.

Claim 17. A composition comprising multiple populations of normalization oligos, wherein the composition comprises a first population of normalization oligos comprising a set of first index sequences and other populations of normalization oligos each comprise a set of unique index sequences that is different from the sets of index sequences of the other populations, and wherein the concentration of each population is the same optionally, wherein the composition comprises from 2 to 100 populations of normalization oligos.

Claim 18. A composition comprising multiple populations of normalization oligos, wherein the composition comprises a first population of normalization oligos comprising a set of first index sequences and other populations of normalization oligos each comprise a set of unique index sequences that is different from the sets of index sequences of the other populations, and wherein the concentrations of at least two of the populations are different optionally, wherein the composition comprises from 2 to 100 populations of normalization oligos.

Claim 19. The composition of any one of claims 15-18, wherein the normalization oligos comprise:

a) single-stranded DNA;

b) a unique molecular identifier;

c) a universal sequence; or

d) a non-nucleic acid component optionally, wherein the non-nucleic acid component comprises protein.

Claim 20. The composition of any one of claims 16 or 18, wherein the first population is present in the composition at a lowest concentration of normalization oligos and one of the other populations is present in the composition at a highest concentration of normalization oligos, and wherein the lowest and highest concentrations differ by a factor of from 1 to 10,000.

Claim 21. A plurality of compartments, wherein each compartment comprises the composition of any one of claims 15-18 preferably, wherein the compartments comprise wells or droplets.

Claim 22. The plurality of compartments of claim 21, wherein each compartment further comprises nuclei or cells, and wherein the multiple populations of normalization oligos are associated with the nuclei or cells, and/or
wherein the concentration of normalization oligos of each of the populations is selected from at least 0.001 zeptomoles to no greater than 100 attomoles.

Claim 23. A population of nuclei or cells, wherein the nuclei or cells comprise the composition of any one of claims 15-18, and wherein members of each population of normalization oligos are associated with the nuclei or the cells, preferably, wherein the association between nuclei or cells and normalization oligos is non-specific.

Claim 24. A method for normalizing a sequencing library comprising nucleic acids from a plurality of single nuclei or single cells, the method comprising:

(a) providing a first plurality of compartments comprising isolated nuclei or cells;

(b) contacting the isolated nuclei or cells of each compartment with the composition of any one of claims 15-18, wherein members of each population of normalization oligos are associated with isolated nuclei or cells; and

(c) combining the labeled nuclei or labeled cells of different compartments to generate pooled labeled nuclei or pooled labeled cells.

Claim 25. The method of claim 24, further comprising exposing the cells of each compartment to a predetermined condition, or exposing the cells of each compartment to a predetermined condition and then isolating nuclei from a plurality of cells prior to step (a) optionally, wherein the predetermined condition comprises exposure to an agent

preferably, wherein the agent comprises a protein, a non-ribosomal protein, a polyketide, an organic molecule, an inorganic molecule, an RNA or RNAi molecule, a carbohydrate, a glycoprotein, a nucleic acid, a drug, or a combination thereof.

Claim 26. The method of claim 24, further comprising prior to step (b) contacting the isolated nuclei or cells of each compartment with a hashing oligo,

wherein at least one copy of the hashing oligo is associated with isolated nuclei or cells,

wherein the hashing oligo comprises a nucleic acid and a hashing index,

wherein the hashing index in each compartment comprises an index sequence that is different from index sequences in the other compartments and different from index sequences of normalization oligos present in the compartment to generate labeled hashed nuclei or labeled hashed cells; and

combining the labeled hashed nuclei or labeled hashed cells of different compartments to generate pooled labeled hashed nuclei or pooled labeled hashed cells.

Claim 27. The method of claim 24, further comprising exposing the cells or nuclei to a cross-linking compound to fix normalization oligos cells or to isolated nuclei preferably, wherein the cross-linking compound comprises paraformaldehyde, formalin, or methanol.

Claim 28. The method of claim 24, wherein the association between the normalization oligo and the cells or isolated nuclei is non-specific preferably, wherein the non-specific association between the normalization oligo and the cells or isolated nuclei is by absorption.

Claim 29. The method of claim 26, further comprising processing the pooled labeled hashed cells or pooled labeled hashed nuclei using a single-cell combinatorial indexing method to result in a sequencing library comprising nucleic acids from the plurality of single nuclei or single cells, wherein the nucleic acids comprise a plurality of indexes preferably, wherein the single-cell combinatorial indexing method is single-nuclei transcriptome sequencing, single-cell transcriptome sequencing, single-cell transcriptome and transposon-accessible chromatin sequencing, whole genome

sequencing of single nuclei, single nuclei sequencing of transposon accessible chromatin, sci-HiC, DRUG-seq, sci-CAR, sci-MET, sci-Crop, sci-perturb, or sci-Crispr.

Claim 30. A method for normalizing a sequencing library comprising nucleic acids from a plurality of single nuclei or single cells, the method comprising:

(a) providing isolated nuclei or cells;

(b) contacting the isolated nuclei or cells with the composition of any one of claims 15-18, wherein members of each population of normalization oligos are associated with isolated nuclei or cells; and

(c) distributing subsets of the labeled nuclei or labeled cells into a plurality of compartments.

Claim 31. The method of claim 30, further comprising exposing the cells to a predetermined condition, or exposing the cells to a predetermined condition and then isolating nuclei from a plurality of cells prior to step (a).

Claim 32. The method of claim 31, wherein the predetermined condition comprises exposure to an agent preferably, wherein the agent comprises a protein, a non-ribosomal protein, a polyketide, an organic molecule, an inorganic molecule, an RNA or RNAi molecule, a carbohydrate, a glycoprotein, a nucleic acid, a drug, or a combination thereof.

Claim 33. The method of claim 30, further comprising after step (c) contacting the isolated nuclei or cells of each compartment with a hashing oligo,

wherein at least one copy of the hashing oligo is associated with isolated nuclei or cells,

wherein the hashing oligo comprises a nucleic acid and a hashing index,

wherein the hashing index in each compartment comprises an index sequence that is different from index sequences in the other compartments and different from index sequences of normalization oligos present in the compartment to generate labeled hashed nuclei or labeled hashed cells; and

combining the labeled hashed nuclei or labeled hashed cells of different compartments to generate pooled labeled hashed nuclei or pooled labeled hashed cells.

Claim 34. The method of claim 30, further comprising exposing the cells or nuclei to a cross-linking compound to fix normalization oligos cells or to isolated nuclei preferably, wherein the cross-linking compound comprises paraformaldehyde, formalin, or methanol.

Claim 35. The method of claim 30, wherein the association between the normalization oligo and the cells or isolated nuclei is non-specific preferably, wherein the non-specific association between the normalization oligo and the cells or isolated nuclei is by absorption.

Claim 36. The method of claim 33, further comprising processing the pooled labeled hashed cells or pooled labeled hashed nuclei using a single-cell combinatorial indexing method to result in a sequencing library comprising nucleic acids from the plurality of single nuclei or single cells, wherein the nucleic acids comprise a plurality of indexes preferably, wherein the single-cell combinatorial indexing method is single-nuclei transcriptome sequencing, single-cell transcriptome sequencing, single-cell transcriptome and transposon-accessible chromatin sequencing, whole genome sequencing of single nuclei, single nuclei sequencing of transposon accessible chromatin, sci-HiC, DRUG-seq, sci-CAR, sci-MET, sci-Crop, sci-perturb, or sci-Crispr.