

CLAIMS

[Claim 1]. A method for producing a sequencing library comprising:

providing a plurality of symmetric modified target nucleic acids comprising a first adapter sequence at each end, wherein the first adapter sequence comprises a DNA lesion,
the DNA lesion optionally comprising at least one of an abasic site, a modified base, a mismatch, a single-stranded break, or cross-linked nucleotides or at least one uracil;
optionally wherein the plurality of symmetric modified target nucleic acids are double stranded and each strand comprises, from 5' to 3', the first adapter sequence comprising the DNA lesion, the target nucleic acid, a gap comprising at least one nucleotide, and the complement of a portion of the first adapter sequence without the DNA lesion;
optionally wherein the cytosine residues of the first adapter are replaced with 5-methylcytosine; and

extending the modified target nucleic acids with a lesion-intolerant polymerase to generate a plurality of asymmetric modified target nucleic acids comprising at the 5' end of each strand the first adapter sequence and at the 3' end of each strand the complement of a portion of the first adapter wherein the extending optionally initiates at the gap and/or preferably wherein the symmetric or asymmetric target nucleic acids are subjected to chemical or enzymatic methylation conversion.

~~2. The method of claim 1, wherein the plurality of symmetric modified target nucleic acids are double stranded and each strand comprises, from 5' to 3', the first adapter sequence comprising the DNA lesion, the target nucleic acid, a gap comprising at least one nucleotide, and the complement of a portion of the first adapter sequence without the DNA lesion.~~

~~3. The method of claim 1, wherein the extending initiates at the gap.~~

[Claim 42]. The method of claim 21, further comprising:

annealing a primer to the plurality of asymmetric modified target nucleic acids, the primer comprising from 5' to 3' a second adapter sequence and an annealing domain, the annealing domain comprising a nucleotide sequence that anneals to the complement of the portion of the first adapter of the plurality of asymmetric modified target nucleic acids,
optionally wherein the annealing domain of the primer comprises (a) at least one altered

nucleotide that increases the melting temperature compared to the corresponding native DNA nucleotide, and/or (b) the at least one altered nucleotide preferably comprising a locked nucleic acid, PNA, or RNA; or
optionally wherein the cytosine residues of the second adapter are replaced with 5-methylcytosine;

extending the 3' end of the annealed asymmetric modified target nucleic acids with a lesion-intolerant polymerase, preferably repeated at least 3 times, and/or preferably wherein the symmetric or asymmetric target nucleic acids are subjected to chemical or enzymatic methylation conversion,

wherein the extending results in a plurality of asymmetric modified target nucleic acids comprising from 5' to 3' (i) the first adapter, (ii) the target nucleic acid, (iii) the complement of the portion of the first adapter, and (iv) the complement of the second adapter.

~~5. The method of claim 4, wherein the extending the 3' end of the annealed asymmetric modified target nucleic acids is repeated at least 3 times.~~

~~6. The method of claim 1, wherein the DNA lesion comprises at least one of an abasic site, a modified base, a mismatch, a single stranded break, or cross-linked nucleotides.~~

~~7. The method of claim 1, wherein the DNA lesion comprises at least one uracil.~~

~~8. The method of claim 4, wherein the annealing domain of the primer comprises at least one altered nucleotide that increases the melting temperature compared to the corresponding native DNA nucleotide.~~

~~9. The method of claim 8, wherein the altered nucleotide comprises a locked nucleic acid, PNA, or RNA.~~

[Claim 403]. The method of claim 42, wherein the 3' end of the primer is blocked.

[Claim 4]. The method of claim 2, 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 plurality of asymmetric modified target nucleic acids under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual asymmetric modified target nucleic acid.

[Claim 445]. The method according to any one of claims 1-3, wherein the first adapter comprises one or more universal sequences, one or more index sequences, one or more universal molecular identifiers, or a combination thereof.

~~12. The method of claim 11, optionally~~ wherein at least one of the one or more universal sequences, one or more index sequences, and one or more universal molecular identifiers is located in the adapter between the DNA lesion and the end of the adapter distal to the target nucleic acid, preferably wherein the one or more index sequences of the first adapter are compartment specific.

| [Claim 43]. The method of claim 42, wherein the second adapter comprises one or more
| universal sequences, one or more index sequences, one or more universal molecular
| identifiers, or a combination thereof.

~~14. The method of claim 13, optionally wherein the one or more universal sequences, one or more index sequences, and one or more universal molecular identifiers of the first adapter are unique compared to the one or more universal sequences, one or more index sequences, and one or more universal molecular identifiers of the second adapter.~~

~~15. The method of claim 11, wherein the one or more index sequences of the first adapter are compartment specific.~~

~~16. The method of claim 13, preferably wherein the one or more index sequences of the second adapter are compartment specific.~~

~~[Claim 177]. The method according to any one of claims 1-6, wherein the first adapter comprises a transposase recognition site.~~

~~[Claim 188]. The method according to any one of claims 1-7, wherein the target nucleic acids:~~

~~(i) are from nucleic acids originating from a single cell; or~~

~~19. The method of claim 1, wherein the target nucleic acids (ii) are from nucleic acids originating from a plurality of cells; or~~

~~(iii) the target nucleic acids are from nucleic acids originating from cell free DNA.~~

~~20. The method of claim 18 or 19, wherein the target nucleic acids originating from a single cell or a plurality of cells comprise RNA.~~

~~21. The method of claim 20, wherein the RNA comprises, optionally mRNA, or~~

~~22. The method of claim 18 or 19, wherein the target nucleic acids originating from a single cell or a plurality of cells comprise DNA.~~

~~23. The method of claim 22, wherein the DNA comprises, optionally whole cell genomic DNA wherein the method preferably further comprises fragmentation of the whole cell genomic DNA optionally by digestion of the whole cell genomic DNA with a restriction endonuclease and/or wherein the fragmented DNA is subjected to proximity ligation to join chimeric target nucleic acids.~~

~~24. — The method of claim 23, preferably~~ wherein the whole cell genomic DNA comprises nucleosomes.

~~25. — The method of claim 1, wherein the target nucleic acids are from nucleic acids originating from cell free DNA.~~

[Claim 269]. The method of any one of claims ~~114-168~~, wherein the method comprises combinatorial indexing.

[Claim 2710]. The method of any one of claims ~~1-9~~, further comprising amplifying the asymmetric modified target nucleic acids,

wherein the amplifying comprises a second primer and a lesion-tolerant polymerase,

wherein the second primer comprises a nucleotide sequence that anneals to the first adapter sequence or the complement thereof and/or.

~~28. — The method of claim 27, wherein the second primer further comprises one or more universal sequences, one or more index sequences, one or more universal molecular identifiers, or a combination thereof.~~

~~29. — The method of claim 28, optionally~~ wherein the one or more universal sequences, one or more index sequences, and one or more universal molecular identifiers of the second primer are unique compared to the one or more universal sequences, one or more index sequences, and one or more universal molecular identifiers of the first adapter and the second adapter.

[Claim 3011]. The method of claim 1, wherein subsets of the plurality of asymmetric modified target nucleic acids are present in a plurality of compartments, preferably wherein the compartments comprise a well or a droplet, and wherein ~~either~~ (i) the first adapter comprises a first compartment specific index, or (ii) the second adapter comprises a second compartment specific index, or (iii) both (i) and (ii)

~~31. The method of claim 30, optionally~~ further comprising combining the asymmetric modified target nucleic acids from different compartments to generate pooled indexed asymmetric modified target nucleic acids and/or:

~~32. The method of claim 31, further comprising~~

distributing subsets of the pooled indexed asymmetric modified target nucleic acids into a second plurality of compartments and modifying the indexed asymmetric modified target nucleic acids,

wherein the modifying comprises adding to the indexed asymmetric modified target nucleic acids present in each subset an additional compartment specific index sequence to result in indexed DNA nucleic acids,

wherein the modifying comprises ligation or extension.

~~33. The method of any one of claims 30-32, wherein the compartments comprise a well or a droplet.~~

[Claim 3412]. The method of claim 1, wherein the providing comprises contacting a plurality of DNA fragments with the first adapter under conditions to ligate the first adapter to both ends of the DNA fragments, optionally wherein one 3' end of the first adapter is blocked, optionally wherein the adapter is a forked adapter, preferably wherein (i):

~~35. The method of claim 34, wherein~~ the DNA fragments are double stranded and blunt-ended; or:

~~36. The method of claim 34 or 35, wherein (ii)~~ the first adapter is a double stranded DNA oligonucleotide, and optionally the double stranded DNA oligonucleotide comprised a single stranded region at one end, wherein the single stranded region can anneal to the single stranded region present on the DNA fragments, or:

~~37. The method of claim 34 or 35, wherein one 3' end of the first adapter is blocked.~~

~~38. The method of claim 34, wherein (iii)~~ the DNA fragments are double stranded and comprise a single stranded region at one or both 3' ends.

~~39.—The method of claim 34 or 38, wherein the first adapter is a double stranded DNA oligonucleotide comprising a single stranded region at one end, wherein the single stranded region can anneal to the single stranded region present on the DNA fragments.~~

~~40.—The method of any one of claims 34, 35, or 38, wherein the adapter is a forked adapter.~~

[Claim 413]. The method of claim 1, wherein the providing comprises contacting DNA with a transposome complex, wherein the transposome complex comprises a transposase and the first adapter, wherein the contacting occurs under conditions suitable for ligation of the first adapter into the DNA to generate the symmetric modified target nucleic acids.

~~42.—The method of claim 41, optionally wherein the generated symmetric modified target nucleic acids comprise a gap of at least one nucleotide in one strand the between the ligated first adapter and the target nucleic acid and/or.~~

~~43.—The method of claim 41 or 42, optionally wherein the DNA is present in a plurality of compartments, and wherein the first adapter in each compartment comprises a compartment specific index.~~

~~44.—The method of claim 42, optionally further comprising combining the single stranded modified target nucleic acids from different compartments to generate pooled symmetric modified target nucleic acids, and distributing the symmetric modified target nucleic acids into a second plurality of compartments.~~

~~45.—The method of claim 23, wherein the method further comprises fragmentation of the whole cell genomic DNA.~~

~~46.—The method of claim 45, wherein the fragmentation comprises digestion of the whole cell genomic DNA with a restriction endonuclease.~~

~~47.—The method of claim 45 or 46, wherein the fragmented DNA is subjected to proximity ligation to join chimeric target nucleic acids.~~

~~48.—The method of claim 1 or 4, wherein the cytosine residues of an adapter are replaced with 5-methylcytosine.~~

~~49.—The method of claim 48, wherein the symmetric or asymmetric target nucleic acids are subjected to chemical or enzymatic methylation conversion.~~

[Claim 5014]. The method of claim 1, wherein the providing comprises fixing isolated nuclei, subjecting the isolated nuclei to conditions to dissociate nucleosomes from genomic DNA, fragmenting the genomic DNA, subjecting the fragments to proximity ligation to join chimeric target nucleic acids, and contacting the ligated fragments to a transposome complex, wherein the transposome complex comprises a transposase and the first adapter, wherein the contacting occurs under conditions suitable for ligation of the first adapter into the DNA to generate the symmetric modified target nucleic acids.

~~51.—The method of claim 50, optionally wherein the fragmenting comprises digestion with a restriction endonuclease.~~

~~52.—The method of claim 4, 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 plurality of asymmetric modified target nucleic acids under conditions suitable to produce a plurality of amplification sites that each comprise a clonal population of amplicons from an individual asymmetric modified target nucleic acid.~~

[Claim 153]. A composition comprising a transposome complex and a DNA polymerase, wherein the transposome comprises a transposase bound to a transposon sequence,

wherein the transposon sequence comprises an adapter and a DNA lesion, and
wherein the DNA polymerase is a lesion-intolerant polymerase;

~~54. — The composition of claim 53, preferably~~ wherein the adapter comprises one or more universal sequence, one or more index sequence, one or more UMI, or a combination thereof;

~~55. — The composition of claim 53 or 54, and/or optionally~~ further comprising a lesion-tolerant DNA polymerase.

~~[Claim 516].~~ A composition comprising

a plurality of modified target nucleic acids comprising 5' to 3' a first adapter comprising a DNA lesion, a target nucleic acid, and the complement of the first adapter, ~~optionally wherein the first adapter comprises a transposase recognition site;~~

a primer comprising from 5' to 3' a second adapter, ~~optionally wherein the 3' end of the primer is blocked or wherein the primer comprises at least one altered nucleotide that increases the melting temperature compared to the corresponding native DNA nucleotide;~~ and an annealing domain, the annealing domain comprising a nucleotide sequence that anneals to the complement of the first adapter, ~~preferably wherein the primer is annealed to target nucleic acids;~~

a lesion-intolerant DNA polymerase.

~~57. — The composition of claim 56, wherein the primer comprises at least one altered nucleotide that increases the melting temperature compared to the corresponding native DNA nucleotide.~~

~~58. — The composition of claim 56 or 57, wherein the primer is annealed to target nucleic acids.~~

~~59. — The composition of claim 56, wherein the 3' end of the primer is blocked.~~

~~60. — The composition of claim 56, wherein the first adapter comprises a transposase recognition site.~~

[Claim 61]7]. A kit comprising in separate containers a transposome complex and a DNA polymerase,

wherein the transposome comprises a transposase bound to a transposon sequence,

wherein the transposon sequence comprises a first adapter and a DNA lesion,

optionally wherein the first adapter comprises one or more of a universal sequence, one or more of an index sequence, one or more of a UMI, or a combination thereof and

wherein the DNA polymerase is a lesion-intolerant polymerase; and

instructions for use.

~~62. The kit of claim 61,~~ optionally further comprising in a separate container a second DNA polymerase,

wherein the second DNA polymerase is a lesion-tolerant polymerase.

~~63~~[Claim 18]. The kit of claim ~~61 or 62~~17, further comprising a primer,

wherein the primer comprises from 5' to 3' a second adapter and an annealing domain, the annealing domain comprising a nucleotide sequence that anneals to the complement of the first adapter.

~~64. The kit of claim 63,~~ optionally wherein the 3' end of the primer is blocked.

~~65. The kit of claim 61, wherein the first adapter comprises one or more of a universal sequence, one or more of an index sequence, one or more of a UMI, or a combination thereof.~~

~~66. The kit of claim 63,~~ and/or preferably wherein the second adapter primer further comprises one or more of a universal sequence, one or more of an index sequence, one or more of a UMI, or a combination thereof.

[Claim 67]19]. A transposome complex comprising:

a transposase; and

a transposon comprising a nucleic acid comprising an adapter comprising on a first strand, from 5' to 3', at least one universal sequence, at least one index sequence, at least one UMI, or a combination thereof, a DNA lesion, and a transposase recognition sequence,

and on a second strand nucleotides complementary to at least a portion of the transposase recognition sequence.

[Claim 6820]. The transposome complex of claim 6719, wherein the first strand further comprises

a capture agent at the 5' end of the first strand; or

~~69. — The transposome complex of claim 68, wherein the first strand further comprises a cleavable linker located between capture agent and the 5' end; and/or .~~

~~70. — The transposome complex of claim 67, wherein the second strand further comprises a capture agent at the 3' end of the second strand.~~

~~71. — The transposome complex of claim 70, wherein the second strand further comprises or a cleavable linker located between capture agent and the 3' end.~~