

**MODIFIED TRANSPOSASES FOR IMPROVED INSERTION SEQUENCE
BIAS AND INCREASED DNA INPUT TOLERANCE**

RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application Nos.:
61/979,871, filed on April 15, 2014; 62/062,006, filed on October 9, 2014; 62/080,882
filed on November 17, 2014 which are hereby incorporated by reference in its entirety.

BACKGROUND

10 Transposase enzymes are useful in *in vitro* transposition systems. They allow for
massive-scale fragmentation and tagging of genomic DNA and are useful for making
libraries of tagged DNA fragments from target DNA for use in nucleic acid analysis
methods such as next-generation sequencing and amplification methods. There remains
a need for modified transposases with improved properties and which generate tagged
15 DNA fragments that are qualitatively and quantitatively representative of the target
nucleic acids in the sample from which they are generated.

SEQUENCE LISTING

20 The present application is being filed along with a Sequence Listing in
electronic format. The Sequence Listing is provided as a file entitled IP-1198-
PCT_SequenceListing.txt, created April 13, 2015, which is 103 Kb in size. The
information in the electronic format of the Sequence Listing is incorporated herein by
reference in its entirety.

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BRIEF SUMMARY

Presented herein are transposase enzymes for improved fragmentation and
tagging of nucleic acid samples. The present inventors have surprisingly identified
certain altered transposases which exhibit improved insertion sequence bias and have a
30 number of other associated advantages.

Presented herein are mutant Tn5 transposases modified relative to a wild type Tn5 transposase. In some embodiments, the mutant transposase can comprise a mutation at position Asp248. In certain aspects, the mutation at position Asp248 is a substitution mutation. In certain aspects, the substitution mutation at position Asp248
5 can comprise a mutation to a residue selected from the group consisting of Tyr, Thr, Lys, Ser, Leu, Ala, Trp, Pro, Gln, Arg, Phe, and His.

In certain aspects, the mutation at position Asp248 is an insertion mutation after position Asp248. In certain aspects, the insertion mutation can comprise insertion of a hydrophobic residue after position Asp248. In certain aspects, the insertion mutation
10 can comprise insertion of a valine residue after position Asp248.

Also presented herein are mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Asp119. In certain aspects, the mutation at position Asp119 is a substitution mutation. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation
15 to a hydrophobic residue. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation to a hydrophilic residue. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation to a residue selected from the group consisting of Leu, Met, Ser, Ala, and Val.

Also presented herein are mutant Tn5 transposases modified relative to a wild
20 type Tn5 transposase, the mutant transposases comprising a mutation at position Trp125. In certain aspects, the mutation at position Trp125 is a substitution mutation. In certain aspects, the substitution mutation at position Trp125 can comprise a mutation to a methionine residue.

Also presented herein are mutant Tn5 transposases modified relative to a wild
25 type Tn5 transposase, the mutant transposases comprising a mutation at position Lys120. In certain aspects, the mutation at position Lys120 is a substitution mutation. In certain aspects, the substitution mutation at position Lys120 can comprise a mutation to a bulky aromatic residue. In certain aspects, the substitution mutation at position Lys120 can comprise a mutation to a residue selected from the group consisting of Tyr,
30 Phe, Trp, and Glu.

Also presented herein are mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Lys212 and/or Pro214 and/or Ala338. In certain aspects, the mutation or mutations at position Lys212 and/or Pro214 and/or Ala338 is a substitution mutation. In certain aspects, the substitution mutation at position Lys212 comprises a mutation to arginine. In certain aspects, the substitution mutation at position Pro214 comprises a mutation to arginine. In certain aspects, the substitution mutation at position Ala338 comprises a mutation to valine. In some embodiments the transposase can further comprise a substitution mutation at Gly251. In certain aspects, the substitution mutation at position Gly251 comprises a mutation to arginine.

Also presented herein are mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Glu146 and/or Glu190 and/or Gly251. In certain aspects, the mutation or mutations at position Glu146 and/or Glu190 and/or Gly251 is a substitution mutation. In certain aspects, the substitution mutation at position Glu146 can comprise a mutation to glutamine. In certain aspects, the substitution mutation at position Glu190 can comprise a mutation to glycine. In certain aspects, the substitution mutation at position Gly251 can comprise a mutation to arginine.

Also provided is an altered transposase comprising a substitution mutation to the semi-conserved domain comprising the amino acid sequence of SEQ ID NO: 21 wherein the substitution mutation comprises a mutation at position 2 to any residue other than Trp, Asn, Val, or Lys. In certain embodiments, the mutation comprises a substitution at position 2 to Met.

In any of the above-described embodiments, the mutant Tn5 transposase can further comprise substitution mutations at positions functionally equivalent to Glu54 and/or Met56 and/or Leu372 in the Tn5 transposase amino acid sequence. In certain embodiments, the transposase comprises substitution mutations homologous to Glu54Lys and/or Met56Ala and/or Leu372Pro in the Tn5 transposase amino acid sequence.

Also presented herein is a mutant Tn5 transposase comprising the amino acid sequence of any one of SEQ ID NOs: 2-10 and 12-20.

Also presented herein is a fusion protein comprising a mutant Tn5 transposase as defined in any the above embodiments fused to an additional polypeptide. In some
5 embodiments, the polypeptide domain fused to the transposase can comprise a purification tag, an expression tag, a solubility tag, or a combination thereof. In some embodiments, the polypeptide domain fused to the transposase can comprise, for example, Maltose Binding Protein (MBP). In some embodiments, the polypeptide domain fused to the transposase can comprise, for example, Elongation Factor Ts (Tsf).

10 Also presented herein is a nucleic acid molecule encoding mutant Tn5 transposase as defined in any the above embodiments. Also presented herein is an expression vector comprising the nucleic acid molecule described above. Also presented herein is a host cell comprising the vector described above.

Also presented herein are methods for *in vitro* transposition comprising:
15 allowing the following components to interact: (i) a transposome complex comprising a mutant Tn5 transposase according to any one of embodiments described hereinabove, and (ii) a target DNA.

Also presented herein are methods for sequencing a target DNA, utilizing the Tn5 transposases described hereinabove. In some embodiments, the methods can
20 comprise (a) incubating the target DNA with transposome complexes comprising (1) a mutant Tn5 transposase according to any one of embodiments described hereinabove; and (2) a first polynucleotide comprising (i) a 3' portion comprising a transposon end sequence, and (ii) a first tag comprising a first sequencing tag domain, under conditions whereby the target DNA is fragmented, and the 3' transposon end sequence of the first
25 polynucleotide is transferred to the 5' ends of the fragments, thereby producing double-stranded fragments wherein the 5' ends are tagged with the first tag, and there is a single-stranded gap at the 3' ends of the 5'-tagged strands; (b) incubating the fragments with a nucleic-acid-modifying enzyme under conditions whereby a second tag is attached to the 3' ends of the 5'-tagged strands, (c) optionally amplifying the fragments
30 by providing a polymerase and an amplification primer corresponding to a portion of

the first polynucleotide, thereby generating a representative library of di-tagged fragments having the first tag at the 5' ends and a second tag at the 3' ends; (d) providing first sequencing primers comprising a portion corresponding to the first sequencing tag domain; and (e) extending the first sequencing primers and detecting the identity of nucleotides adjacent to the first sequencing tag domains of the representative library of di-tagged fragments in parallel.

Also presented herein are kits for performing an *in vitro* transposition reactions. In some embodiments, the kits can comprise transposome complexes comprising (1) a mutant Tn5 transposase according to any one of embodiments described hereinabove; and (2) a polynucleotide comprising a 3' portion comprising a transposon end sequence.

The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a schematic showing structural alignment of the catalytic core domain of Tn5 transposase (1MUH), Hermes transposase (2BW3), HIV Integrase (1ITG), MuTransposase (1BCM), and Mos1 Transposase (3HOS). The numbering shown represents the numbering of amino acid residues in Tn5 transposase.

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Figure 1B is a schematic showing structural alignment catalytic core domain of Tn5 transposase (1MUH, pink), Hermes transposase (2BW3, black), HIV Integrase (1ITG, tan), MuTransposase (1BCM), and Mos1 Transposase (3HOS, yellow). The Tn5 transposase W125 position is shown in stick representation.

Figure 2 is an IVC plot showing altered sequence insertion bias for a D248Y mutant Tn5 transposase, compared to Tn5 control.

Figure 3 is an IVC plot showing altered sequence insertion bias for a D119L mutant Tn5 transposase, compared to Tn5 control.

Figure 4 is an IVC plot showing altered sequence insertion bias for a W125M mutant Tn5 transposase, compared to Tn5 control.

Figure 5 is an IVC plot showing altered sequence insertion bias for ia248V insertion mutant Tn5 transposase, compared to Tn5 control.

Figure 6 is an IVC plot showing altered sequence insertion bias for K120Y, K120F, and K120W Tn5 transposase insertion mutants, compared to Tn5 control.

5 Figure 7 is an IVC plot showing altered sequence insertion bias for three mutant Tn5 transposases, compared to Tn5 control.

Figure 8A is a graph showing AT/GC dropout in a *B. cereus* library created by three mutant Tn5 transposases, compared to Tn5 control. Figure 8B is a graph showing estimated library size for a *B. cereus* library created by three mutant Tn5 transposases,
10 compared to Tn5 control.

Figure 9A is a graph showing coverage uniformity in Rapid Capture Enrichment experiments in libraries created by two mutant Tn5 transposases, compared to Tn5 control. Figure 9B is a graph showing 10X and 20X target coverage and mean target coverage in Rapid Capture Enrichment experiments in libraries created by two mutant
15 Tn5 transposases, compared to Tn5 control.

Figure 10A is a graph showing percent passing filter of unique reads and hybrid selection library size in Rapid Capture Enrichment experiments in libraries created by two mutant Tn5 transposases, compared to Tn5 control. Figure 10B is a graph showing penalty scores to reach 10X, 20X and 30X coverage in Rapid Capture Enrichment
20 experiments in libraries created by two mutant Tn5 transposases, compared to Tn5 control.

Figure 11 shows a bar graph of the number of unique molecules in TS-Tn5059 and TS-Tn5 tagged DNA libraries prepared using different tagmentation buffers.

Figure 12 shows a bar graph of the percent GC dropout in TS-Tn5059 and TS-
25 Tn5 tagged DNA libraries prepared using different tagmentation buffers.

Figure 13 shows a bar graph of the percent AT dropout in TS-Tn5059 and TS-Tn5 tagged DNA libraries prepared using different tagmentation buffers.

Figure 14 shows a plot of Bioanalyzer traces of the fragment size distribution in TS-Tn5059 libraries prepared using the standard buffer (TD) and the cobalt buffer (Co)
30 formulations.

Figure 15 shows a plot of Bioanalyzer traces of the fragment size distribution in TS-Tn5059 libraries prepared using the cobalt-DMSO (Co-DMSO), NF2, and HMW buffer formulations.

Figure 16 shows a plot of Bioanalyzer traces of the fragment size distribution in
5 TS-Tn5 libraries prepared using the standard buffer formulation (TD) and the cobalt buffer (Co).

Figure 17 shows a plot of Bioanalyzer traces of the fragment size distribution in TS-Tn5 libraries prepared using the cobalt-DMSO (Co-DMSO), NF2, and HMW buffer formulations.

10 Figures 18A, 18B, 18C, and 18D show a bias graph of the sequence content in the TS-Tn5 library, a bias graph of the sequence content in the TS-TN5-Co library, a bias graph of the sequence content in the TS-Tn5-Co-DMSO library, and a bias graph of the sequence content in the TS-Tn5-NF2 library, respectively.

Figures 19A, 19B, 19C, and 19D show a bias graph of the sequence content in
15 the TS-Tn5059 library, a bias graph of the sequence content in the TS-TN5059-Co library, a bias graph of the sequence content in the TS-Tn5059-Co-DMSO library, and a bias graph of the sequence content in the TS-Tn5059-NF2 library, respectively.

Figure 20 shows a bar graph of the average total number of reads and average diversity in MBP-Mos1 tagmented libraries prepared using different tagmentation
20 buffers.

Figure 21 shows a bar graph of GC and AT dropout in the MBP-Mos1 tagmented libraries.

Figures 22A, 22B, 22C, and 22D show a bias graph of the sequence content in the Mos1-HEPES library, a bias graph of the sequence content in the Mos1-HEPES-DMSO library, a bias graph of the sequence content in the Mos1-HEPES-DMSO-Co
25 library, and a bias graph of the sequence content in the Mos1-HEPES-DMSO-Mn library, respectively.

Figure 23 illustrates a flow diagram of an example of a method of preparing and enriching a genomic DNA library for exome sequencing.

Figure 24A shows a plot of the coverage in tagmented *B. cereus* genomic DNA libraries prepared using TS-Tn5059 transposomes.

Figure 24B shows a plot of the coverage in tagmented *B. cereus* genomic DNA libraries prepared using NexteraV2 transposomes.

5 Figure 25A shows a plot of gap location and gap length in tagmented *B. cereus* genomic DNA libraries prepared using TS-Tn5059 transposomes.

Figure 25B shows a plot of gap location and gap length in tagmented *B. cereus* genomic tagmented DNA libraries prepared using NexteraV2 transposomes.

Figure 26 shows a panel of Bioanalyzer traces of fragment size distributions in
10 tagmented genomic DNA libraries prepared using TDE1 (Tn5 version-1) and TS-Tn5 normalized to TS-Tn5059 at 40nM (1X normalized concentration) to 25ng human gDNA.

Figure 27 shows an analysis of the size distributions in tagmented genomic DNA libraries prepared using TDE1 (Tn5 version-1) and TS-Tn5 normalized to TS-
15 Tn5059 at 40nM (1X normalized concentration) using 25ng human gDNA.

Figure 28 shows a panel of Bioanalyzer traces of fragment size distributions in tagmented genomic DNA libraries prepared using a range of DNA input.

Figure 29A shows a plot of Bioanalyzer traces of fragment size distributions in TS-Tn5059 tagmented libraries prepared by a first user; and using Coriel Human DNA.

20 Figure 29B shows a plot of Bioanalyzer traces of fragment size distributions in TS-Tn5059 tagmented libraries prepared by a second user, and using Coriel Human DNA.

Figure 30 shows a plot of Bioanalyzer traces of fragment size distributions in Tn5 version 1 (TDE1) tagmented libraries prepared by TDE1 at 6x “normalized”
25 concentration using 25ng-100ng gDNA.

Figure 31 shows a plot of Bioanalyzer traces of fragment size distributions in TS-Tn5 tagmented libraries prepared by TS-Tn5 at 6x “normalized” concentration using 25ng-100ng gDNA.

Figure 32 shows a plot of Bioanalyzer traces of fragment size distributions in TS-Tn5059 tagmented libraries prepared by TS-Tn5059 at 6x “normalized” concentration using 10ng-100ng of gDNA.

Figure 33 shows a plot of Bioanalyzer traces of fragment size distributions in
5 TS-Tn5059 tagmented libraries prepared by TS-Tn5059 at 6x “normalized” concentration using wider ranges of gDNA (5 ng-500 ng).

DETAILED DESCRIPTION

In some sample preparation methods for DNA sequencing, each template contains an adaptor at either end of the insert and often a number of steps are required
10 to both modify the DNA or RNA and to purify the desired products of the modification reactions. These steps are typically performed in solution prior to the addition of the adapted fragments to a flowcell where they are coupled to the surface by a primer extension reaction that copies the hybridized fragment onto the end of a primer covalently attached to the surface. These ‘seeding’ templates then give rise to
15 monoclonal clusters of copied templates through several cycles of amplification. However, as disclosed in U.S. 2010/0120098, the content of which is incorporated herein in its entirety, the number of steps required to transform DNA into adaptor-modified templates in solution ready for cluster formation and sequencing can be minimized by the use of transposase mediated fragmentation and tagging, referred to
20 herein as tagmentation. For example, tagmentation can be utilized for fragmenting DNA, for example as exemplified in the workflow for Nextera™ DNA sample preparation kits (Illumina, Inc.) wherein genomic DNA can be fragmented by an engineered transposome that simultaneously fragments and tags input DNA thereby creating a population of fragmented nucleic acid molecules which comprise unique
25 adapter sequences at the ends of the fragments. However, a need exists for transposase enzymes which exhibit improved insertion bias.

Accordingly, presented herein are transposase enzymes for improved fragmentation and tagging of nucleic acid samples. The present inventors have surprisingly identified certain altered transposases which exhibit improved insertion
30 sequence bias and have a number of other associated advantages. One embodiment of

the altered transposases presented herein are transposases which exhibit improved insertion bias.

As used herein, the term “normalized transposome activity” refers to the minimum concentration of transposome that on 25ng gDNA input yields a bioanalyzer
5 fragment size distribution of: the total area under the curve: 100-300bp = 20% - 30%; 301-600bp = 30% - 40%; 601-7,000 bp = 30 - 40%; 100-7,000bp $\geq$ 90% in a 50 μ l reaction. This minimum concentration is referred to as 1X.

As used throughout the application, the concentration of transposome is used interchangeably with the normalized activity. Additionally, as used throughout the
10 application, the concentration of transposome is used interchangeably with the concentration of the transposase.

As used herein, the term “insertion bias” refers to the sequence preference of a transposase for insertion sites. For example, if the background frequency of A/T/C/G in a polynucleotide sample is equally distributed (25% A, 25% T, 25% C, 25% G), then
15 any over-representation of one nucleotide over the other three at a transposase binding site or cleavage site reflects an insertion bias at that site. Insertion bias can be measured using any one of a number of methods known in the art. For example, the insertion sites can be sequenced and the relative abundance of any particular nucleotide at each position in an insertion site can be compared, as set forth generally in Example 1 below.

20 An “improvement in insertion bias” indicates that the frequency of a particular base at one or more positions of the binding site of an altered transposase is reduced or increased to be closer to the background frequency of that base in the polynucleotide sample. The improvement can be an increase in the frequency at that position, relative to the frequency in that position in an unaltered transposase. Alternatively, the
25 improvement can be a decrease in the frequency at that position, relative to the frequency in that position in an unaltered transposase. Thus, for example, if the background frequency of T nucleotide in a polynucleotide sample is 0.25, and an altered transposase reduces the frequency of T nucleotide at a specified position in a transposase binding site from a frequency higher than 0.25 to a frequency closer to 0.25,
30 the altered transposase has an improvement in insertion bias. Similarly, for example, if

the background frequency of T nucleotide in a polynucleotide sample is 0.25, and an altered transposase increases the frequency of T nucleotide at a specified position in a transposase binding site from a frequency lower than 0.25 to a frequency closer to 0.25, the altered transposase has an improvement in insertion bias.

5 One methodology of measuring insertion bias is by massive-scale sequencing of insertion sites and measuring the frequency of bases at each position in a binding site relative to the insertion site, as described for example in Green et al. *Mobile DNA* (2012) 3:3, which is incorporated herein by reference in its entirety. A typical tool to display abundance at each position is an intensity vs. cycle distribution plot, for
10 example as shown in Fig. 2. As described in Example 1 below, fragment ends generated by transposon-mediated tagging and fragmentation can be sequenced on a massive scale, and the frequency of distribution of bases at each position of an insertion site can be measured to detect bias at one or more positions of the insertion site. Thus, for instance, as indicated in Fig. 2, the base distribution at position (1) of frequencies of
15 0.55 for 'G' nucleotide and 0.16 for 'A' nucleotide reflect a sharp preference for G and a bias away from A at that position. As another example, and in contrast, as shown in Fig. 3, the base distribution at position (20) is essentially 0.25 for each of the four bases, reflecting little or no sequence bias at that position.

 In some embodiments presented herein, the altered transposase enzymes provide
20 a reduction in insertion bias at one or more sites located 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more than 20 bases upstream or downstream of the insertion site. In some embodiments, the altered transposase enzymes provide a reduction in insertion bias at one or more sites located from 1 to 15 bases downstream of the insertion site. In some embodiments, the altered transposase enzymes provide a
25 reduction in insertion bias at one or more sites located from 1 to 15 bases upstream of the insertion site.

 As described in greater detail hereinbelow, the inventors have surprisingly found that one or more mutations to residues at certain positions of a transposase amino acid sequence result in improved sequence insertion bias during transposition events.
30 These altered transposases give improved performance in tagmentation of high- and

low-diversity nucleic acid samples, resulting in greater coverage uniformity and less dropout of various regions being sequenced.

As used herein, the term “DNA input tolerance” refers to the ability of a transposase to generate uniform DNA fragment size across a range of input DNA
5 amounts.

As used herein, the notation for elongation factor: TS is used interchangeably with Tsf.

In some embodiments, the input DNA is genomic DNA. In some embodiments, the range of input DNA can be from 0.001 μ g to 1 mg, from 1ng to 1 mg, from 1ng to
10 900 ng, from 1 ng to 500 ng, from 1 ng to 300 ng, from 1 ng to 250 ng, from 1 ng to 100 ng, from 5 ng to 250 ng, or from 5 ng to 100 ng and the concentration of transposase is between 5nM and 500 nM. In some embodiments, the concentration of the transposase for the above mentioned range of input DNA is about 25 nM, 30 nM, 35 nM, 40 nM, 50 nM, 60 nM, 65 nM, 70 nM, 75 nM, 80 nM, 90 nM, 95 nM, 100 nM, 125 nM, 130 nM,
15 140 nM, 150 nM, 175 nM, 180 nM, 190 nM, 200 nM, 210 nM, 225 nM, 230nM, 240 nM, 250 nM, 260 nM, 275 nM, 280 nM, 290 nM, 300 nM, 325 nM, 350 nM, 360 nM, 375 nM, 380 nM, 390 nM, 400 nM, 425 nM, 450 nM, 475 nM, or 500 nM. In some embodiments, the concentration of the normalized concentration of the transposase or the normalized transposome for the above mentioned range of input DNA is selected
20 from the range of about 0.1X to 10X, 1X to 10X, 3X to 8X, 4X to 7X. In some embodiments, the normalized concentration of the transposase or the normalized transposome for the above mentioned range of input DNA is about 0.1X, 0.2X, 0.3X, 0.4X, 0.5X, 0.6X, 0.7X, 0.8X, 0.9X, 1X, 1.1X, 1.2X, 1.3X, 1.4X, 1.5X, 1.6X, 1.7X, 1.8X, 1.9X, 2X, 2.1X, 2.2X, 2.3X, 2.4X, 2.5X, 2.6X, 2.7X, 2.8X, 2.9X, 3X, 3.1X, 3.2X,
25 3.3X, 3.4X, 3.5X, 3.6X, 3.7X, 3.8X, 3.9X, 4X, 4.1X, 4.2X, 4.3X, 4.4X, 4.5X, 4.6X, 4.7X, 4.8X, 4.9X, 5X, 5.1X, 5.2X, 5.3X, 5.4X, 5.5X, 5.6X, 5.7X, 5.8X, 5.9X, 6X, 6.1X, 6.2X, 6.3X, 6.4X, 6.5X, 6.6X, 6.7X, 6.8X, 6.9X, 7X, or 7.5X, 8X, 8.5X, 9X, 9.5X, 10X.

In some embodiments, the amount of input DNA is 1ng, 2 ng, 3 ng, 4 ng, 5 ng, 6
30 ng, 7 ng, 8 ng, 9 ng, 10 ng, 11 ng, 12 ng, 13 ng, 15 ng, 20 ng, 25 ng, 30 ng, 35 ng, 40

ng, 45 ng, 50 ng, 55 ng, 60 ng, 65 ng, 70 ng, 75 ng, 80 ng, 85 ng, 90 ng, 95 ng, 100 ng, 110 ng, 115 ng, 120 ng, 125 ng, 130 ng, 135 ng, 140 ng, 150 ng, 155 ng, 160 ng, 165 ng, 170 ng, 180 ng, 185 ng, 190 ng, 195 ng, 200 ng, 210 ng, 220 ng, 225 ng, 230 ng, 235 ng, 240 ng, 245 ng, 250 ng, 260 ng, 270 ng, 280 ng, 290 ng, 300 ng, 325 ng, 350 ng, 375 ng, 400 ng, 425 ng, 450 ng, 475 ng, 500 ng, 525 ng, 550 ng, 600 ng, 650 ng, 700 ng, 750 ng, 800 ng, 850 ng, or 900 ng. In some embodiments, the concentration of the transposase for the above mentioned amount of input DNA is about 25 nM, 30 nM, 35 nM, 40 nM, 50 nM, 60 nM, 65 nM, 70 nM, 75 nM, 80 nM, 90 nM, 95 nM, 100 nM, 125 nM, 130 nM, 140 nM, 150 nM, 175 nM, 180 nM, 190 nM, 200 nM, 210 nM, 225 nM, 230 nM, 240 nM, 250 nM, 260 nM, 275 nM, 280 nM, 290 nM, 300 nM, 325 nM, 350 nM, 360 nM, 375 nM, 380 nM, 390 nM, 400 nM, 425 nM, 450 nM, 475 nM, or 500 nM. In some embodiments, the concentration of the normalized concentration of the transposase or the normalized transposome for the above mentioned amount of input DNA is selected from the range of about 0.1X to 10X, 1X to 10X, 3X to 8X, 4X to 7X.

In some embodiments, the normalized concentration of the transposase or the normalized transposome for the above mentioned amount of input DNA is about 0.1X, 0.2X, 0.3X, 0.4X, 0.5X, 0.6X, 0.7X, 0.8X, 0.9X, 1X, 1.1X, 1.2X, 1.3X, 1.4X, 1.5X, 1.6X, 1.7X, 1.8X, 1.9X, 2X, 2.1X, 2.2X, 2.3X, 2.4X, 2.5X, 2.6X, 2.7X, 2.8X, 2.9X, 3X, 3.1X, 3.2X, 3.3X, 3.4X, 3.5X, 3.6X, 3.7X, 3.8X, 3.9X, 4X, 4.1X, 4.2X, 4.3X, 4.4X, 4.5X, 4.6X, 4.7X, 4.8X, 4.9X, 5X, 5.1X, 5.2X, 5.3X, 5.4X, 5.5X, 5.6X, 5.7X, 5.8X, 5.9X, 6X, 6.1X, 6.2X, 6.3X, 6.4X, 6.5X, 6.6X, 6.7X, 6.8X, 6.9X, 7X, or 7.5X, 8X, 8.5X, 9X, 9.5X, 10X.

In some embodiments, the ratio of nM concentration of transposase to ng amount of input DNA is from about 0.5 to 5, from 1 to 5, from 2 to 5, from 2.1 to 3, or from 2.1 to 2.5.

As used herein, the term “genomic DNA” refers to the nucleic acid that is present in the cell which comprises one or more genes that encode various proteins of the cell. In some embodiments, genomic DNA is from a prokaryotic organism, for example, bacteria and archaea. In some embodiments, genomic DNA is from an eukaryotic organism, for example, human, plant, fungi, amoeba.

The term "Mutant", or "modified" as used herein refers to a gene or gene product which displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. "Mutant", or "modified" also refers to the sequence at a specific nucleotide position or positions, or the sequence at a particular codon position or positions, or the sequence at a particular amino acid position or positions which displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product.

"Including" as used herein has the same meaning as the term comprising.

"About" as used herein means in quantitative terms, plus or minus 10%.

As described in greater detail hereinbelow, the inventors have surprisingly found that one or more mutations to residues at certain positions of a transposase amino acid sequence result in increased DNA input tolerance, such that the mutant transposase generates uniform DNA fragment size across a range of input DNA amounts as compared to the wild-type transposase. In one embodiment, TS-Tn5059 transposase exhibits increased DNA input tolerance as compared to other transposases, for example, TS-Tn5 and Tn5 version 1 (TDE1).

In some embodiments, TS-Tn5059 exhibits increased DNA input tolerance as compared to other transposases where the range of input DNA is between 1 ng to 200 ng of genomic DNA and the concentration of TS-Tn5059 is between 100-300 nM. In some embodiments, in which TS-Tn5059 exhibits increased DNA input tolerance as compared to other transposases, the range of input DNA is between 5 ng to 200 ng of genomic DNA and the concentration of TS-Tn5059 is between 100-250 nM. In some embodiments, in which TS-Tn5059 exhibits increased DNA input tolerance as compared to other transposases, the range of input DNA is between 5 ng to 100 ng of genomic DNA and the concentration of TS-Tn5059 is between 240 nM and 250 nM.

Improved insertion bias together with increased DNA input tolerance provides faster and more flexible sample preparation and exome enrichment protocol than the current Nextera® Rapid Capture protocol (Illumina, Inc.).

As used herein, the term "tagmentation" refers to the modification of DNA by a transposome complex comprising transposase enzyme complexed with adaptors comprising transposon end sequence. Tagmentation results in the simultaneous fragmentation of the DNA and ligation of the adaptors to the 5' ends of both strands of duplex fragments.

As used herein, a "transposome complex" or "transposome" is comprised of at least a transposase enzyme and a transposase recognition site. In some such systems, the transposase can form a functional complex with a transposon recognition site that is capable of catalyzing a transposition reaction. The transposase may bind to the transposase recognition site and insert the transposase recognition site into a target nucleic acid in a process referred to herein as tagmentation. In some such insertion events, one strand of the transposase recognition site may be transferred into the target nucleic acid.

The altered transposase enzymes presented herein can form part of transposome complex. Exemplary transposition complexes include, but are not limited to, a hyperactive Tn5 transposase and a Tn5-type transposase recognition site. Hyperactive Tn5 transposases can include those described in U.S. Patent No. 5,925,545, U.S. Patent No. 5,965,443, U.S. Patent No. 7,083,980, and U.S. Patent No. 7,608,434, as well as in the disclosure of Goryshin and Reznikoff, *J. Biol. Chem.*, 273:7367 (1998), the content of each of which is incorporated herein by reference in its entirety. However, it will be appreciated that the altered transposase enzymes presented herein can be utilized in any transposition system that is capable of inserting a transposon end in a random or in an almost random manner with sufficient efficiency to tag target nucleic acids for its intended purpose can be used in the provided methods.

For example, the altered transposases presented can include comprise at least one amino acid substitution mutation at the position or positions functionally equivalent to sites in the Tn5 amino acid sequence. Regions of homology to Tn5 are set forth herein, as exemplified in Fig. 1 and allow for identification of functionally equivalent sites in other transposase enzymes, for example, Hermes transposase, HIV Integrase, Mu Transposase and Mos1 Transposase. Likewise, functionally equivalent sites in

other transposase or integrase enzymes will be readily apparent to one of ordinary skill in the art, for example by performing a sequence alignment of the Tn5 amino acid sequence and identifying conserved or semi-conserved residues or domains. Thus, it will be appreciated that transposition systems that can be used with certain
5 embodiments provided herein include any known transposase with sites that are functionally equivalent to those of Tn5. For example, such systems can include MuA transposase and a Mu transposase recognition site comprising R1 and R2 end sequences (Mizuuchi, K., Cell, 35: 785, 1983; Savilahti, H, et al., EMBO J., 14: 4893, 1995).

More examples of transposition systems included in certain embodiments
10 provided herein include Staphylococcus aureus Tn552 (Cologio et al., J. Bacteriol., 183: 2384-8, 2001; Kirby C et al., Mol. Microbiol., 43: 173-86, 2002), Ty1 (Devine & Boeke, Nucleic Acids Res., 22: 3765-72, 1994 and International Publication WO 95/23875), Transposon Tn7 (Craig, N L, Science. 271: 1512, 1996; Craig, N L, Review in: Curr Top Microbiol Immunol., 204:27-48, 1996), Tn/O and IS10 (Kleckner N, et al.,
15 Curr Top Microbiol Immunol., 204:49-82, 1996), Mariner transposase (Lampe D J, et al., EMBO J., 15: 5470-9, 1996), Tc1 (Plasterk R H, Curr. Topics Microbiol. Immunol., 204: 125-43, 1996), P Element (Gloor, G B, Methods Mol. Biol., 260: 97-114, 2004), Tn3 (Ichikawa & Ohtsubo, J Biol. Chem. 265:18829-32, 1990), bacterial insertion sequences (Ohtsubo & Sekine, Curr. Top. Microbiol. Immunol. 204: 1-26, 1996),
20 retroviruses (Brown, et al., Proc Natl Acad Sci USA, 86:2525-9, 1989), and retrotransposon of yeast (Boeke & Corces, Annu Rev Microbiol. 43:403-34, 1989). More examples include IS5, Tn10, Tn903, IS911, and engineered versions of transposase family enzymes (Zhang et al., (2009) PLoS Genet. 5:e1000689. Epub 2009 Oct. 16; Wilson C. et al (2007) J. Microbiol. Methods 71:332-5). The references cited
25 above are incorporated herein by reference in their entirety.

Briefly, a "transposition reaction" is a reaction wherein one or more transposons are inserted into target nucleic acids at random sites or almost random sites. Essential components in a transposition reaction are a transposase and DNA oligonucleotides that exhibit the nucleotide sequences of a transposon, including the transferred transposon
30 sequence and its complement (i.e., the non- transferred transposon end sequence) as

well as other components needed to form a functional transposition or transposome complex. The DNA oligonucleotides can further comprise additional sequences (e.g., adaptor or primer sequences) as needed or desired.

The adapters that are added to the 5' and/or 3' end of a nucleic acid can comprise
 5 a universal sequence. A universal sequence is a region of nucleotide sequence that is common to, i.e., shared by, two or more nucleic acid molecules. Optionally, the two or more nucleic acid molecules also have regions of sequence differences. Thus, for example, the 5' adapters can comprise identical or universal nucleic acid sequences and the 3' adapters can comprise identical or universal sequences. A universal sequence that
 10 may be present in different members of a plurality of nucleic acid molecules can allow the replication or amplification of multiple different sequences using a single universal primer that is complementary to the universal sequence.

Transposase mutants

15 Thus, presented herein are mutant transposases modified relative to a wild type transposase. The altered transposase can comprise at least one amino acid substitution mutation at the position or positions functionally equivalent to those residues set forth in Table 1 below. Table 1 sets forth substitution mutations at transposase residues that have been shown to result in improved insertion bias. As set forth in Table 1, the
 20 substitution mutations presented herein can be in any functional transposase backbone, such as wild type Tn5 transposase exemplified herein as SEQ ID NO: 1, or a transposase having further mutations to other sites, including those found in a transposase sequence known as hyperactive Tn5 transposase, such as, for example one or more mutations set forth in the incorporated materials of U.S. Patent No. 5,925,545,
 25 U.S. Patent No. 5,965,443, U.S. Patent No. 7,083,980, and U.S. Patent No. 7,608,434, and as exemplified herein as SEQ ID NO: 11.

Table 1 – Examples of mutations resulting in improved insertion bias

Backbone	Tn5 Mutant	SEQ ID NO:
Tn5 WT	D248Y D248T	2

	D248K D248S D248L D248A D248W D248P D248G D248R D248F D248H	
Tn5 WT	D119L D119M D119S D119A D119V	3
Tn5 WT	W125M	4
Tn5 WT	iaD248	5
Tn5 WT	K120F K120Y K120E K120W	6
Tn5 WT	D248 to Y, T, K, S, L, A, W, P, G, R, F or H D119 to L, M, S, A or V W125M K120F	7
Tn5 WT	K212R P214R A338V	8
Tn5 WT	K212R P214R G251R A338V	9
Tn5 WT	E146Q E190G G251R	10
Tn5 Hyperactive	D248Y D248T D248K D248S D248L D248A D248W D248P D248G D248R D248F	12

	D248H	
Tn5 Hyperactive	D119L D119M D119S D119A D119V	13
Tn5 Hyperactive	W125M	14
Tn5 Hyperactive	iaD248	15
Tn5 Hyperactive	K120F K120Y K120E K120W	16
Tn5 Hyperactive	D248 to Y, T, K, S, L, A, W, P, G, R, F or H D119 to L, M, S, A or V W125M K120F	17
Tn5 Hyperactive	K212R P214R A338V	18
Tn5 Hyperactive	K212R P214R G251R A338V	19
Tn5 Hyperactive	E146Q E190G G251R	20

As understood in the art, the reference numbers listed in the table above refer to the amino acid positions of the wild-type Tn5 sequence (SEQ ID NO: 1). One with ordinary skill in the art will understand that the numbering may change because of N-terminal truncation, insertion or fusion. The functional position of the amino acids listed above will remain the same even though the numbering of the position may have changed. For example, first 285 amino acid residues of the sequence set forth in SEQ ID NO: 25 comprises an N-terminal fusion of E. coli TS followed by amino acid residues 2-476 of SEQ ID NO: 11. Thus, for example, Pro 656 of SEQ ID NO: 25 corresponds functionally to Pro 372 of SEQ ID NO: 11.

Thus, in certain embodiments, an altered transposase presented herein comprises at least one amino acid substitution mutation relative to a wild type transposase at the

position or positions functionally equivalent to, for example, Asp248, Asp119, Trp125, Lys120, Lys212, Pro214, Gly251, Ala338, Glu146, and/or Glu190 in the Tn5 transposase amino acid sequence.

5 In some embodiments, the mutant transposase can comprise a mutation at position Asp248. The mutation at position Asp248 can be, for example, a substitution mutation or an insertion mutation. In certain embodiments, the mutation is a substitution mutation to any residue other than Asp. In certain embodiments, the substitution mutation at position Asp248 includes a mutation to a residue selected from the group consisting of Tyr, Thr, Lys, Ser, Leu, Ala, Trp, Pro, Gln, Arg, Phe, and His.

10 In certain embodiments, the mutation at position Asp248 is an insertion mutation after position Asp248. In certain aspects, the insertion mutation can comprise insertion of any residue after Asp248. In certain aspects, the insertion mutation can comprise insertion of a hydrophobic residue after position Asp248. Hydrophobic residues are known to those of skill in the art and include, for example, Val, Leu, Ile, Phe, Trp, Met, Ala, Tyr and Cys. In certain aspects, the insertion mutation can
15 comprise insertion of a valine residue after position Asp248.

Some embodiments presented herein include mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Asp119. In certain aspects, the mutation at position Asp119 is a substitution
20 mutation. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation to a hydrophobic residue. Hydrophobic residues are known to those of skill in the art and include, for example, Val, Leu, Ile, Phe, Trp, Met, Ala, Tyr and Cys. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation to a hydrophilic residue. Hydrophilic residues are known to those of skill in the art and
25 include, for example, Arg, Lys, Asn, His, Pro, Asp and Glu. In certain aspects, the substitution mutation at position Asp119 can comprise a mutation to a residue selected from the group consisting of Leu, Met, Ser, Ala, and Val.

Some embodiments presented herein include mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation
30 at position Trp125. In certain aspects, the mutation at position Trp125 is a substitution

mutation. In certain aspects, the substitution mutation at position Trp125 can comprise a mutation to a methionine residue.

Some embodiments presented herein include mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation
5 at position Lys120. In certain aspects, the mutation at position Lys120 is a substitution mutation. In certain aspects, the substitution mutation at position Lys120 can comprise a mutation to a bulky aromatic residue. Residues characterized as bulky aromatic residues are known to those of skill in the art and include, for example, Phe, Tyr and Trp. In certain aspects, the substitution mutation at position Lys120 can comprise a
10 mutation to a residue selected from the group consisting of Tyr, Phe, Trp, and Glu.

Some embodiments presented herein include mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Lys212 and/or Pro214 and/or Ala338. In certain aspects, the mutation or mutations at position Lys212 and/or Pro214 and/or Ala338 is a substitution mutation.
15 In certain aspects, the substitution mutation at position Lys212 comprises a mutation to arginine. In certain aspects, the substitution mutation at position Pro214 comprises a mutation to arginine. In certain aspects, the substitution mutation at position Ala338 comprises a mutation to valine. In some embodiments the transposase can further comprise a substitution mutation at Gly251. In certain aspects, the substitution
20 mutation at position Gly251 comprises a mutation to arginine.

Some embodiments presented herein include mutant Tn5 transposases modified relative to a wild type Tn5 transposase, the mutant transposases comprising a mutation at position Glu146 and/or Glu190 and/or Gly251. In certain aspects, the mutation or mutations at position Glu146 and/or Glu190 and/or Gly251 is a substitution mutation.
25 In certain aspects, the substitution mutation at position Glu146 can comprise a mutation to glutamine. In certain aspects, the substitution mutation at position Glu190 can comprise a mutation to glycine. In certain aspects, the substitution mutation at position Gly251 can comprise a mutation to arginine.

In any of the above-described embodiments, the mutant Tn5 transposase can
30 further comprise substitution mutations at positions functionally equivalent to Glu54

and/or Met56 and/or Leu372 in the Tn5 transposase amino acid sequence. In certain embodiments, the transposase comprises substitution mutations homologous to Glu54Lys and/or Met56Ala and/or Leu372Pro in the Tn5 transposase amino acid sequence.

5 Some embodiments presented herein include a mutant Tn5 transposase comprising the amino acid sequence of any one of SEQ ID NOs: 2-10 and 12-20.

Also presented herein is an altered transposase comprising a substitution mutation to a semi-conserved domain. As used herein, the term "semi-conserved domain" refers to a portion of transposase that is fully conserved, or at least partially
10 conserved among various transposases and/or among various species. The semi-conserved domain comprises amino acid residues that reside in the catalytic core domain of the transposase. It has been surprisingly discovered that mutation of one or more residues in the semi-conserved domain affects the transposase activity, resulting in improvement in insertion bias.

15 In some embodiments, the semi-conserved domain comprises amino acids having the sequence set forth in SEQ ID NO: 21. SEQ ID NO: 21 corresponds to residues 124-133 of the Tn5 transposase amino acid sequence, which is set forth herein as SEQ ID NO: 1. A structural alignment showing the conservation among various transposases in the semi-conserved domain is set forth in Figure 1. The transposase
20 sequences shown in Figure 1 include the catalytic core domain of Tn5 transposase (1MUH), Hermes transposase (2BW3), HIV Integrase (1ITG), MuTransposase (1BCM), and Mos1 Transposase (3HOS).

Mutations to one or more residues in the semi-conserved domain have been surprisingly found to result in improvement in insertion bias. For example, in some
25 embodiments of the altered transposase presented herein, the substitution mutation comprises a mutation at position 2 of SEQ ID NO: 21 to any residue other than Trp. In certain embodiments, the altered transposase comprises a mutation to Met at position 2 of SEQ ID NO: 21.

By "functionally equivalent" it is meant that the control transposase, in the case
30 of studies using a different transposase entirely, will contain the amino acid substitution

that is considered to occur at the amino acid position in the other transposase that has the same functional role in the enzyme. As an example, a mutation at position 288 from Lysine to Methionine (K288M) in the Mu transposase would be functionally equivalent to a substitution at position 125 from Tryptophan to Methionine (W125M) in the Tn5
5 transposase.

Generally functionally equivalent substitution mutations in two or more different transposase occur at homologous amino acid positions in the amino acid sequences of the transposases. Hence, use herein of the term “functionally equivalent” also encompasses mutations that are “positionally equivalent” or “homologous” to a
10 given mutation, regardless of whether or not the particular function of the mutated amino acid is known. It is possible to identify positionally equivalent or homologous amino acid residues in the amino acid sequences of two or more different transposases on the basis of sequence alignment and/or molecular modelling. An example of sequence alignment and molecular modeling to identify positionally equivalent and/or
15 functionally equivalent residues is set forth in Figure 1. Thus, for example, as shown in Figure 1, the residues in the semi-conserved domain are identified as positions 124-133 of the Tn5 transposase amino acid sequence. The corresponding residues in Hermes transposase, HIV Integrase, MuTransposase, and Mos1 Transposase transposases are identified in the Figure as vertically aligned and are considered positionally equivalent
20 as well as functionally equivalent to the corresponding residue in the Tn5 transposase amino acid sequence.

The altered transposases described hereinabove can comprise additional substitution mutations that are known to enhance one or more aspects of transposase activity. For example, in some embodiments, in addition to any of the above mutations,
25 the altered Tn5 transposase can further comprise substitution mutations at positions functionally equivalent to Glu54 and/or Met56 and/or Leu372 in the Tn5 transposase amino acid sequence. Any of a variety of substitution mutations at one or more of positions at positions functionally equivalent to Glu54 and/or Met56 and/or Leu372 in the Tn5 transposase amino acid sequence which results in improved activity can be
30 made, as is known in the art and exemplified by the disclosure of U.S. Patent No.

5,925,545, U.S. Patent No. 5,965,443, U.S. Patent No. 7,083,980, and U.S. Patent No. 7,608,434, as well as in the disclosure of Goryshin and Reznikoff, J. Biol. Chem., 273:7367 (1998), each of which is incorporated by reference in its entirety. embodiments, the transposase comprises substitution mutations homologous to
5 Glu54Lys and/or Met56Ala and/or Leu372Pro in the Tn5 transposase amino acid sequence. For example, the substitution mutations can comprise substitution mutations homologous to Glu54Lys and/or Met56Ala and/or Leu372Pro in the Tn5 transposase amino acid sequence.

10 Mutating Transposases

Various types of mutagenesis are optionally used in the present disclosure, e.g., to modify transposases to produce variants, e.g., in accordance with transposase models and model predictions as discussed above, or using random or semi-random mutational approaches. In general, any available mutagenesis procedure can be used for making
15 transposase mutants. Such mutagenesis procedures optionally include selection of mutant nucleic acids and polypeptides for one or more activity of interest (e.g., improved insertion bias). Procedures that can be used include, but are not limited to: site-directed point mutagenesis, random point mutagenesis, in vitro or in vivo homologous recombination (DNA shuffling and combinatorial overlap PCR),
20 mutagenesis using uracil containing templates, oligonucleotide-directed mutagenesis, phosphorothioate-modified DNA mutagenesis, mutagenesis using gapped duplex DNA, point mismatch repair, mutagenesis using repair-deficient host strains, restriction-selection and restriction-purification, deletion mutagenesis, mutagenesis by total gene synthesis, degenerate PCR, double-strand break repair, and many others known to
25 persons of skill. The starting transposase for mutation can be any of those noted herein, including available transposase mutants such as those identified e.g., in U.S. Patent No. 5,925,545, U.S. Patent No. 5,965,443, U.S. Patent No. 7,083,980, and U.S. Patent No. 7,608,434, as well as in the disclosure of Goryshin and Reznikoff, J. Biol. Chem., 273:7367 (1998), each of which is incorporated by reference in its entirety.

Optionally, mutagenesis can be guided by known information from a naturally occurring transposase molecule, or of a known altered or mutated transposase (e.g., using an existing mutant transposase as noted in the preceding references), e.g., sequence, sequence comparisons, physical properties, crystal structure and/or the like as discussed above. However, in another class of embodiments, modification can be essentially random (e.g., as in classical or "family" DNA shuffling, see, e.g., Cramer et al. (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" *Nature* 391:288-291).

Additional information on mutation formats is found in: Sambrook et al., *Molecular Cloning--A Laboratory Manual* (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2000 ("Sambrook"); *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2011) ("Ausubel")) and *PCR Protocols A Guide to Methods and Applications* (Innis et al. eds) Academic Press Inc. San Diego, Calif. (1990) ("Innis"). The following publications and references cited within provide additional detail on mutation formats: Arnold, Protein engineering for unusual environments, *Current Opinion in Biotechnology* 4:450-455 (1993); Bass et al., Mutant Trp repressors with new DNA-binding specificities, *Science* 242:240-245 (1988); Bordo and Argos (1991) Suggestions for "Safe" Residue Substitutions in Site-directed Mutagenesis 217:721-729; Botstein & Shortle, Strategies and applications of in vitro mutagenesis, *Science* 229:1193-1201 (1985); Carter et al., Improved oligonucleotide site-directed mutagenesis using M13 vectors, *Nucl. Acids Res.* 13: 4431-4443 (1985); Carter, Site-directed mutagenesis, *Biochem. J.* 237:1-7 (1986); Carter, Improved oligonucleotide-directed mutagenesis using M13 vectors, *Methods in Enzymol.* 154: 382-403 (1987); Dale et al., Oligonucleotide-directed random mutagenesis using the phosphorothioate method, *Methods Mol. Biol.* 57:369-374 (1996); Eghtedarzadeh & Henikoff, Use of oligonucleotides to generate large deletions, *Nucl. Acids Res.* 14: 5115 (1986); Fritz et al., Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro, *Nucl. Acids Res.* 16: 6987-6999 (1988);

Grundstrom et al., Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis, *Nucl. Acids Res.* 13: 3305-3316 (1985); Hayes (2002) Combining Computational and Experimental Screening for rapid Optimization of Protein Properties *PNAS* 99(25) 15926-15931; Kunkel, The efficiency of oligonucleotide directed mutagenesis, in *Nucleic Acids & Molecular Biology* (Eckstein, F. and Lilley, D. M. J. eds., Springer Verlag, Berlin)) (1987); Kunkel, Rapid and efficient site-specific mutagenesis without phenotypic selection, *Proc. Natl. Acad. Sci. USA* 82:488-492 (1985); Kunkel et al., Rapid and efficient site-specific mutagenesis without phenotypic selection, *Methods in Enzymol.* 154, 367-382 (1987); Kramer et al., The gapped duplex DNA approach to oligonucleotide-directed mutation construction, *Nucl. Acids Res.* 12: 9441-9456 (1984); Kramer & Fritz Oligonucleotide-directed construction of mutations via gapped duplex DNA, *Methods in Enzymol.* 154:350-367 (1987); Kramer et al., Point Mismatch Repair, *Cell* 38:879-887 (1984); Kramer et al., Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations, *Nucl. Acids Res.* 16: 7207 (1988); Ling et al., Approaches to DNA mutagenesis: an overview, *Anal Biochem.* 254(2): 157-178 (1997); Lorimer and Pastan *Nucleic Acids Res.* 23, 3067-8 (1995); Mandecki, Oligonucleotide-directed double-strand break repair in plasmids of *Escherichia coli*: a method for site-specific mutagenesis, *Proc. Natl. Acad. Sci. USA*, 83:7177-7181(1986); Nakamaye & Eckstein, Inhibition of restriction endonuclease *Nci I* cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis, *Nucl. Acids Res.* 14: 9679-9698 (1986); Nambiar et al., Total synthesis and cloning of a gene coding for the ribonuclease S protein, *Science* 223: 1299-1301(1984); Sakamar and Khorana, Total synthesis and expression of a gene for the α -subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin), *Nucl. Acids Res.* 14: 6361-6372 (1988); Sayers et al., Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis, *Nucl. Acids Res.* 16:791-802 (1988); Sayers et al., Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide, (1988) *Nucl. Acids Res.* 16: 803-814; Sieber, et al., *Nature Biotechnology*, 19:456-460 (2001); Smith, In vitro

mutagenesis, Ann. Rev. Genet. 19:423-462 (1985); Methods in Enzymol. 100: 468-500 (1983); Methods in Enzymol. 154: 329-350 (1987); Stemmer, Nature 370, 389-91(1994); Taylor et al., The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA, Nucl. Acids Res. 13: 8749-8764 (1985);

5 Taylor et al., The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA, Nucl. Acids Res. 13: 8765-8787 (1985); Wells et al., Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin, Phil. Trans. R. Soc. Lond. A 317: 415-423 (1986); Wells et al., Cassette mutagenesis: an efficient method for generation of multiple mutations at

10 defined sites, Gene 34:315-323 (1985); Zoller & Smith, Oligonucleotide-directed mutagenesis using M 13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment, Nucleic Acids Res. 10:6487-6500 (1982); Zoller & Smith, Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors, Methods in Enzymol. 100:468-500 (1983); Zoller & Smith,

15 Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template, Methods in Enzymol. 154:329-350 (1987); Clackson et al. (1991) "Making antibody fragments using phage display libraries" Nature 352:624-628; Gibbs et al. (2001) "Degenerate oligonucleotide gene shuffling (DOGS): a method for enhancing the frequency of recombination with family

20 shuffling" Gene 271:13-20; and Hiraga and Arnold (2003) "General method for sequence-independent site-directed chimeragenesis: J. Mol. Biol. 330:287-296. Additional details on many of the above methods can be found in Methods in Enzymology Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

25

Making and Isolating Recombinant Transposases

Generally, nucleic acids encoding a transposase as presented herein can be made by cloning, recombination, *in vitro* synthesis, *in vitro* amplification and/or other available methods. A variety of recombinant methods can be used for expressing an

30 expression vector that encodes a transposase as presented herein. Methods for making

recombinant nucleic acids, expression and isolation of expressed products are well known and described in the art. A number of exemplary mutations and combinations of mutations, as well as strategies for design of desirable mutations, are described herein. Methods for making and selecting mutations in catalytic domains of transposases, are
5 found herein and exemplified in U.S. Patent No. 5,925,545, U.S. Patent No. 5,965,443, U.S. Patent No. 7,083,980, and U.S. Patent No. 7,608,434, which are incorporated by reference in their entireties.

Additional useful references for mutation, recombinant and in vitro nucleic acid manipulation methods (including cloning, expression, PCR, and the like) include Berger
10 and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Kaufman et al. (2003) Handbook of Molecular and Cellular Methods in Biology and Medicine Second Edition Ceske (ed) CRC Press (Kaufman); and The Nucleic Acid Protocols Handbook Ralph Rapley (ed) (2000) Cold Spring Harbor, Humana Press Inc (Rapley); Chen et al. (ed) PCR Cloning
15 Protocols, Second Edition (Methods in Molecular Biology, volume 192) Humana Press; and in Viljoen et al. (2005) Molecular Diagnostic PCR Handbook Springer, ISBN 1402034032.

In addition, a plethora of kits are commercially available for the purification of plasmids or other relevant nucleic acids from cells, (see, e.g., EasyPrepTM, FlexiPrepTM
20 both from Pharmacia Biotech; StrataCleanTM, from Stratagene; and, QIAprepTM from Qiagen). Any isolated and/or purified nucleic acid can be further manipulated to produce other nucleic acids, used to transfect cells, incorporated into related vectors to infect organisms for expression, and/or the like. Typical cloning vectors contain transcription and translation terminators, transcription and translation initiation
25 sequences, and promoters useful for regulation of the expression of the particular target nucleic acid. The vectors optionally comprise generic expression cassettes containing at least one independent terminator sequence, sequences permitting replication of the cassette in eukaryotes, or prokaryotes, or both, (e.g., shuttle vectors) and selection markers for both prokaryotic and eukaryotic systems. Vectors are suitable for
30 replication and integration in prokaryotes, eukaryotes, or both.

In some embodiments, the transposase presented herein is expressed as a fusion protein. The fusion protein can enhance features such as, for example, solubility, expression, and/or purification of the transposase. As used herein, the term "fusion protein" refers to a single polypeptide chain having at least two polypeptide domains that are not normally present in a single, natural polypeptide. Thus, naturally occurring proteins and point mutants thereof are not "fusion proteins", as used herein. Preferably, a polypeptide of interest is fused with at least one polypeptide domain via a peptide bond and the fusion protein may also include the linking regions of amino acids between amino acid portions derived from separate proteins. The polypeptide domain fused to the polypeptide of interest may enhance solubility and/or expression of the polypeptide of interest and may also provide a purification tag to allow purification of the recombinant fusion protein from the host cell or culture supernatant, or both. Polypeptide domains which increase solubility during expression, purification and/or storage are well known in the art and include, for example, maltose binding protein (MBP), and elongation factor Ts (Tsf), as exemplified by Fox, J.D. and Waugh D.S., *E. coli* Gene Expression Protocols *Methods in Molecular Biology*, (2003) 205:99-117 and Han et al. *FEMS Microbiol. Lett.* (2007) 274:132-138, each of which is incorporated by reference herein in its entirety. The polypeptide domain fused to the polypeptide of interest may be fused at the N-terminus or at the C-terminus of the polypeptide of interest. The term "recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of amino acids or of nucleic acids by genetic engineering techniques.

In one embodiment, the invention provides transposase fusion proteins comprising a modified Tn5 transposase and elongation factor Ts (Tsf). The Tsf-Tn5 fusion protein may be assembled into a functional dimeric transposome complex comprising the fusion transposase and free transposon ends. The Tsf-Tn5 fusion protein has increased solubility and thermal stability compared to the unfused Tn5 protein.

In another embodiment, the invention provides transposase fusion proteins comprising a modified Tn5 transposase and a protein domain that recognizes 5-methyl

cytosine. The 5-methyl cytosine-Tn5 fusion protein may be assembled into a functional dimeric transposome complex comprising the fusion transposase and free transposon ends. The 5-methyl cytosine binding protein domain may, for example, be used to target the Tn5 transposome complex to the methylated regions of a genome.

5 In yet another embodiment, the invention provides transposase fusion proteins comprising a modified Tn5 transposase and a protein A antibody binding domain. The protein A-Tn5 fusion protein may be assembled into a functional dimeric transposome complex comprising the fusion transposase and free transposon ends. The antibody binding domain of protein A may, for example, be used to target the Tn5 transposome
10 complex to antibody bound regions of a genome.

The invention provides transposase fusion proteins comprising a modified Tn5 transposase and all or portions of elongation factor TS (Tsf). Tsf is a protein tag that may be used to enhance the solubility of heterologous proteins expressed in a bacterial expression system, e.g., an *E. coli* expression system. The ability of Tsf to increase the
15 solubility of heterologous proteins may be due to the intrinsic high folding efficiency of the Tsf protein. In a protein purification experiment (data not shown), Tsf was purified as a complex with the protein Tu. In order for Tu to bind in the complex, Tsf needs to be correctly folded. Purification of the Tsf-Tu complex suggests that Tsf was folded correctly. Exemplary nucleic acid and corresponding amino acid sequence of the
20 *Escherichia coli* elongation factor TS are shown as SEQ ID NOs: 22 and 23, respectively.

In one example, a TS-Tn5 fusion protein was constructed by fusing TS to the N-terminus of a hyperactive Tn5 transposase. Exemplary amino acid sequences of TS-fusion with mutant Tn5 transposase proteins are shown as SEQ ID NOs: 25 and 26,
25 respectively. SEQ ID NO: 24 corresponds to the nucleic acid sequence encoding TS-mutant protein fusion of SEQ ID NO: 25.

Although all or portion of the TS can be fused at the N- or at the C-terminus, it will be understood by the artisan skilled in the art that a linker sequence can be inserted between the TS sequence and N-terminus or C-terminus of the transposase. In some
30 embodiments, the linker sequence can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more amino acids in length. In some embodiments, one or more amino acids of the transposase portion of the fusion protein may be deleted or replaced with a linker sequence. In some embodiments, the first
5 methionine of the transposase portion of the fusion protein may be replaced with two amino acids, for example, Gly-Thr as indicated in SEQ ID NOs: 25 and 26.

The TS-Tn5 fusion construct was expressed in *E. coli* and evaluated for expression, solubility, and thermal stability. Fusion of TS to the N-terminus of Tn5 increased the solubility of Tn5. The increase in solubility may be associated with
10 increased robustness of the transposome complex and a decrease in protein aggregation. The thermal stability of the TS-Tn5 transposome is substantially improved compared to the thermal stability of the unfused Tn5 transposome. In one example, heat induced aggregation of Tn5 is substantially reduced in the Tsf-Tn5 fusion construct compared to an unfused Tn5 control.

15 In one application, the TS-Tn5 fusion protein is used in the construction of directional RNA-seq libraries (e.g., TotalScript™ RNA-Seq Kit, Illumina) for sequencing on next generation sequencing platforms (e.g., Illumina GA or HiSeq platforms).

In another application, the TS-Tn5 fusion protein may be used in a
20 normalization process. In another application, the TS solubilization tag may be used for expression and purification of other modified (mutant) Tn5 transposase enzymes.

An antibody specific for the TS fusion tag may be used in a pull-down process to capture transposome tagged sequences. In one example, the TS fusion tag antibody is a rabbit polyclonal.

25 In another application, a TS-Tn5 transposome and anti-TS antibody may be used in a mixed transposome process. For example, a transposome reaction is performed using a Tsf-Tn5 transposome and a Tn5 transposome (i.e., not tagged with TS). The anti-TS antibody is used to specifically pull-down the Tsf-Tn5 transposome tagged sequences.

The invention provides a transposase fusion protein comprising a modified Tn5 transposase and a protein domain that recognizes 5-methyl cytosine. The 5-methyl cytosine binding protein domain may, for example, be used to target the Tn5 transposome complex to the methylated regions of a genome. In one application, the 5-methyl cytosine binding domain – Tn5 transposome complex may be used to generate a methyl-enriched fragmented and tagged (tagmented) library for methylation analysis.

In some embodiments, the polypeptide domain fused to the transposase comprises an antibody binding domain of protein A. Protein A is a relatively small, compact molecule with robust folding characteristics. The antibody binding domain of protein A may, for example, be used to target the Tn5 transposome complex to antibody bound regions of a genome. For example, an antibody specific for 5-methyl cytosine may be used to bind to and identify methylated regions of a genome. The antibody-bound regions of the genome may subsequently be targeted for fragmenting and tagging (i.e., tagmentation) using the protein A – Tn5 fusion transposome complex.

In some embodiments, the polypeptide domain fused to the transposase comprises a purification tag. The term "purification tag" as used herein refers to any peptide sequence suitable for purification or identification of a polypeptide. The purification tag specifically binds to another moiety with affinity for the purification tag. Such moieties which specifically bind to a purification tag are usually attached to a matrix or a resin, such as agarose beads. Moieties which specifically bind to purification tags include antibodies, other proteins (e.g. Protein A or Streptavidin), nickel or cobalt ions or resins, biotin, amylose, maltose, and cyclodextrin. Exemplary purification tags include histidine (HIS) tags (such as a hexahistidine peptide), which will bind to metal ions such as nickel or cobalt ions. Other exemplary purification tags are the myc tag (EQKLISEEDL), the Strep tag (WSHPQFEK), the Flag tag (DYKDDDDK) and the V5 tag (GKPIPNPLLGLDST). The term "purification tag" also includes "epitope tags", i.e. peptide sequences which are specifically recognized by antibodies. Exemplary epitope tags include the FLAG tag, which is specifically recognized by a monoclonal anti-FLAG antibody. The peptide sequence recognized by the anti-FLAG antibody consists of the sequence DYKDDDDK or a substantially identical variant thereof. In some

embodiments, the polypeptide domain fused to the transposase comprises two or more tags, such as a SUMO tag and a STREP tag, as exemplified below in Example 1. The term "purification tag" also includes substantially identical variants of purification tags. "Substantially identical variant" as used herein refers to derivatives or fragments of
5 purification tags which are modified compared to the original purification tag (e.g. via amino acid substitutions, deletions or insertions), but which retain the property of the purification tag of specifically binding to a moiety which specifically recognizes the purification tag.

In some embodiments, the polypeptide domain fused to the transposase
10 comprises an expression tag. The term "expression tag" as used herein refers to any peptide or polypeptide that can be attached to a second polypeptide and is supposed to support the solubility, stability and/or the expression of a recombinant polypeptide of interest. Exemplary expression tags include Fc-tag and SUMO-tag. In principle, any peptide, polypeptide or protein can be used as an expression tag.

15 Other useful references, e.g. for cell isolation and culture (e.g., for subsequent nucleic acid isolation) include Freshney (1994) Culture of Animal Cells, a Manual of Basic Technique, third edition, Wiley-Liss, New York and the references cited therein; Payne et al. (1992) Plant Cell and Tissue Culture in Liquid Systems John Wiley & Sons, Inc. New York, N.Y.; Gamborg and Phillips (eds) (1995) Plant Cell, Tissue and
20 Organ Culture; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York) and Atlas and Parks (eds) The Handbook of Microbiological Media (1993) CRC Press, Boca Raton, Fla.

Nucleic acids encoding the recombinant transposases of disclosed herein are also a feature of embodiments presented herein. A particular amino acid can be encoded
25 by multiple codons, and certain translation systems (e.g., prokaryotic or eukaryotic cells) often exhibit codon bias, e.g., different organisms often prefer one of the several synonymous codons that encode the same amino acid. As such, nucleic acids presented herein are optionally "codon optimized," meaning that the nucleic acids are synthesized to include codons that are preferred by the particular translation system being employed
30 to express the transposase. For example, when it is desirable to express the transposase

in a bacterial cell (or even a particular strain of bacteria), the nucleic acid can be synthesized to include codons most frequently found in the genome of that bacterial cell, for efficient expression of the transposase. A similar strategy can be employed when it is desirable to express the transposase in a eukaryotic cell, e.g., the nucleic acid
5 can include codons preferred by that eukaryotic cell.

A variety of protein isolation and detection methods are known and can be used to isolate transposases, e.g., from recombinant cultures of cells expressing the recombinant transposases presented herein. A variety of protein isolation and detection methods are well known in the art, including, e.g., those set forth in R. Scopes, Protein
10 Purification, Springer-Verlag, N.Y. (1982); Deutscher, Methods in Enzymology Vol. 182: Guide to Protein Purification, Academic Press, Inc. N.Y. (1990); Sandana (1997) Bioseparation of Proteins, Academic Press, Inc.; Bollag et al. (1996) Protein Methods, 2.sup.nd Edition Wiley-Liss, NY; Walker (1996) The Protein Protocols Handbook Humana Press, NJ, Harris and Angal (1990) Protein Purification Applications: A
15 Practical Approach IRL Press at Oxford, Oxford, England; Harris and Angal Protein Purification Methods: A Practical Approach IRL Press at Oxford, Oxford, England; Scopes (1993) Protein Purification: Principles and Practice 3.sup.rd Edition Springer Verlag, NY; Janson and Ryden (1998) Protein Purification: Principles, High Resolution Methods and Applications, Second Edition Wiley-VCH, NY; and Walker (1998)
20 Protein Protocols on CD-ROM Humana Press, NJ; and the references cited therein. Additional details regarding protein purification and detection methods can be found in Satinder Ahuja ed., Handbook of Bioseparations, Academic Press (2000).

Methods of use

25 The altered transposases presented herein can be used in a sequencing procedure, such as an *in vitro* transposition technique. Briefly, *in vitro* transposition can be initiated by contacting a transposome complex and a target DNA. Exemplary transposition procedures and systems that can be readily adapted for use with the transposases of the present disclosure are described, for example, in WO 10/048605; US

2012/0301925; US 2013/0143774, each of which is incorporated herein by reference in its entirety.

For example, in some embodiments, the transposase enzymes presented herein can be used in a method for generating a library of tagged DNA fragments from target DNA comprising any dsDNA of interest (e.g., for use as next-generation sequencing or amplification templates), the method comprising: incubating the target DNA in an *in vitro* transposition reaction with at least one transposase and a transposon end composition with which the transposase forms a transposition complex, the transposon end composition comprising (i) a transferred strand that exhibits a transferred transposon end sequence and, optionally, an additional sequence 5'-of the transferred transposon end sequence, and (ii) a non-transferred strand that exhibits a sequence that is complementary to the transferred transposon end sequence, under conditions and for sufficient time wherein multiple insertions into the target DNA occur, each of which results in joining of a first tag comprising or consisting of the transferred strand to the 5' end of a nucleotide in the target DNA, thereby fragmenting the target DNA and generating a population of annealed 5'-tagged DNA fragments, each of which has the first tag on the 5'-end; and then joining the 3'-ends of the 5'-tagged DNA fragments to the first tag or to a second tag, thereby generating a library of tagged DNA fragments (e.g., comprising either tagged circular ssDNA fragments or 5'- and 3'-tagged DNA fragments (or "di-tagged DNA fragments")).

In some embodiments, the amount of the transposase and the transposon end composition or of the transposome composition used in the *in vitro* transposition reaction is between about 1 picomole and about 25 picomoles per 50 nanograms of target DNA per 50-microliter reaction. In some preferred embodiments of any of the methods of the invention, the amount of the transposase and the transposon end composition or of the transposome composition used in the *in vitro* transposition reaction is between about 5 picomoles and about 50 picomoles per 50 nanograms of target DNA per 50-microliter reaction. In some preferred embodiments of any of the methods of the invention wherein the transposase is the hyperactive Tn5 transposase and the transposon end composition comprises the MEDS transposon end composition

or wherein the transposome composition comprises said hyperactive Tn5 transposase and a transposon end composition that comprises the MEDS transposon end, the amounts of said transposase and transposon end composition or said transposome composition used in the in vitro transposition reaction is between about 5 picomoles and
5 about 25 picomoles per 50 nanograms of target DNA per 50-microliter reaction. In some preferred embodiments of any of the methods of the invention wherein the transposase is a hyperactive Tn5 transposase or MuA transposase, the final concentrations of the transposase and the transposon end composition or of the transposome composition used in the in vitro transposition reaction is at least 250 nM;
10 in some other embodiments, the final concentrations of hyperactive Tn5 transposase or MuA transposase and of their respective transposon end composition or transposome composition is at least 500 nM.

In some embodiments, the invention provides a method of preparing and enriching a genomic DNA library for exome sequencing. In various embodiments, the
15 method of the invention uses an altered Tn5 transposase, for example, TS-Tn5059, for preparation of the genomic library. In one embodiment, the method of the invention provides for preparation of a genomic library that has reduced bias driven by the reduced insertional sequence bias of altered transposase, for example, TS-TN5059. Tagmentation of genomic DNA using TS-Tn5059 provides more complete coverage of
20 a genome across a wide GC/AT range.

In another embodiment, the invention provides for a method of preparation of a genomic library using altered transposase that has increased DNA input tolerance. Tagmentation of genomic DNA using TS-Tn5059 provides uniform insert sizes across a range of DNA input amounts. In some embodiments, the invention provides for a
25 method of exome sequencing.

Tagmentation reaction conditions

Presented herein are reaction conditions and buffers for tagmentation reactions. In some embodiments, a divalent cation is included in the tagmentation reaction buffer. In particular embodiments, the divalent cation can be, for example, Co^{2+} , Mn^{2+} , Mg^{2+} ,
30 Cd^{2+} , or Ca^{2+} . The divalent cation can be included in the form of any suitable salt, such

as a chloride salt, for example, CoCl_2 , MnCl_2 , MgCl_2 , Mg acetate, CdCl_2 , or CaCl_2 . In particular embodiments, the tagmentation buffer comprises CoCl_2 , as exemplified in the examples hereinbelow. As demonstrated by the experimental evidence in Example 5, the addition of CoCl_2 in tagmentation buffer formulations surprisingly ameliorates sequence bias during tagmentation.

In certain embodiments, the tagmentation buffer may have a concentration of a divalent cation, that is, is about, or is more than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc. In some embodiments, the tagmentation buffer may have a concentration of a CoCl_2 , that is, is about, or is more than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc. In some embodiments, the tagmentation buffer may have a concentration of a MnCl_2 , that is, is about, or is more than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc. In some embodiments, the tagmentation buffer may have a concentration of a MgCl_2 , that is, is about, or is more than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc. In some embodiments, the tagmentation buffer may have a concentration of a CdCl_2 , that is, is about, or is more than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM,

40 mM, 50 mM, 60, mM, 70, mM, 80, mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc. In some embodiments, the tagmentation buffer may have a concentration of a CaCl_2 , that is, is about, or is more
5 than 0.01 mM, 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 12 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60, mM, 70, mM, 80, mM, 90 mM, 100 mM or a concentration of a divalent cation that is a range between any of these values, for example, 0.01 mM to 0.05 mM, 0.02 mM to 0.5 mM, 8 mM to 12 mM etc.

10 In some embodiments, the fragmentation of genomic DNA by transposases or the tagmentation reaction can be carried out at temperature range from 25°C to 70°C, from 37°C to 65°C, from 50°C to 65°C, or from 50°C to 60°C. In some embodiments, the fragmentation of genomic DNA by transposases or the tagmentation reaction can be carried out at 37°C, 40°C, 45°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C,
15 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, or 65°C.

Nucleic acids encoding altered transposases

Further presented herein are nucleic acid molecules encoding the altered transposase enzymes presented herein. For any given altered transposase which is a mutant version of a transposase for which the amino acid sequence and preferably also
20 the wild type nucleotide sequence encoding the transposase is known, it is possible to obtain a nucleotide sequence encoding the mutant according to the basic principles of molecular biology. For example, given that the wild type nucleotide sequence encoding Tn5 transposase is known, it is possible to deduce a nucleotide sequence encoding any given mutant version of Tn5 having one or more amino acid substitutions using the
25 standard genetic code. Similarly, nucleotide sequences can readily be derived for mutant versions other transposases. Nucleic acid molecules having the required nucleotide sequence may then be constructed using standard molecular biology techniques known in the art.

In accordance with the embodiments presented herein, a defined nucleic acid
30 includes not only the identical nucleic acid but also any minor base variations including,

in particular, substitutions in cases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code in conservative amino acid substitutions. The term “nucleic acid sequence” also includes the complementary sequence to any single stranded sequence given regarding base variations.

The nucleic acid molecules described herein may also, advantageously, be included in a suitable expression vector to express the transposase proteins encoded therefrom in a suitable host. Incorporation of cloned DNA into a suitable expression vector for subsequent transformation of said cell and subsequent selection of the transformed cells is well known to those skilled in the art as provided in Sambrook et al. (1989), *Molecular cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, which is incorporated by reference in its entirety.

Such an expression vector includes a vector having a nucleic acid according to the embodiments presented herein operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term “operably linked” refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for the expression of a protein according to the embodiments presented herein.

The nucleic acid molecule may encode a mature protein or a protein having a prosequence, including that encoding a leader sequence on the preprotein which is then cleaved by the host cell to form a mature protein. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, and optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable markers, such as, for example, an antibiotic resistance gene.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and to direct an appropriate level of transcription initiation and also translation initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation

initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained
5 commercially or be assembled from the sequences described by methods well known in the art.

Transcription of DNA encoding the transposase by higher eukaryotes may be optimised by including an enhancer sequence in the vector. Enhancers are cis-acting elements of DNA that act on a promoter to increase the level of transcription. Vectors
10 will also generally include origins of replication in addition to the selectable markers.

EXAMPLE 1

General Assay Methods and Conditions

The following paragraphs describe general assay conditions used in the
15 Examples presented below.

Tagmentation using TN5 for WGS on human gDNA

This section describes tagmentation assay used in the examples below for monitoring the insertion bias of a transposase. Briefly, the 50 ng of human genomic
20 DNA were incubated at 55°C for 5 min with 5 µL of TDE1 in 10 mM Tris-acetate, pH 7.6, 25 mM Mg-acetate. Then 1/5 of the reaction volume of 125 mM HEPES, pH 7.5, 1 M NaCl, 50 mM MgCl₂ was added followed by addition of Tn5 transposome to 100 nM and incubation at 30°C for 60 min. The reaction was then cleaned-up and amplified as described in Illumina's Nextera protocol and submitted for sequencing using a HiSeq
25 2000 instrument.

Tn5 transposome assembly

Tn5 was incubated with the annealed transposons to 20 µM at room temperature for 30 min in 25 mM HEPES, pH 7.6, 125 mM KCl, 18.75 mM NaCl, 0.375 mM
30 EDTA, 31.75 % glycerol.

Transposon assembly

Transposons were annealed separately to 40 μ M in 10 mM TrisHCl, pH 7.5, 50 mM NaCl, 1 mM EDTA by heating the reaction to 94°C and slowly cooling it to room temperature. For Tn5-ME-A, Tn5 Mosaic End Sequence A14 (Tn5MEA) was annealed to Tn5 Non-transferred sequence (NTS) and for Tn5-ME-B, Tn5 Mosaic End Sequence B15 (Tn5MEB) was annealed to Tn5 Non-transferred sequence (NTS). These sequences are indicated below:

Tn5MEA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Tn5MEB: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Tn5 NTS: 5'-CTGTCTCTTATACACATCT-3'

2. Cloning and expression of transposases

This section describes the approach used for cloning and expression of the various transposase mutants used in the Examples below.

Mutagenesis was performed on the gene encoding the backbone gene sequence for the transposase using standard site-directed mutagenesis methodology. For each mutation made, proper sequence of the mutated genes was confirmed by sequencing the cloned gene sequence.

The Tn5 transposase gene was cloned into a modified pET11a plasmid. The modified plasmid contained the purification tag StrepTag-II, derived from pASK5plus and SUMO, derived from pET-SUMO. Expression was performed using BL21(DE3)pLysY competent cells (New England Biolabs). Cells were grown at 25°C to an OD_{600nm} of 0.5 and then induced using 100 μ M IPTG. Expression was carried out at 18°C for 19h. Cell pellets were lysed using a microfluidizer in 100 mM TrisHCl, pH 8.0, 1 M NaCl, 1 mM EDTA in the presence of protease inhibitors. After lysis, cell lysates were incubated with deoxycholate to a final concentration of 0.1% for 30 min. Polyethyleneimine was added to 0.5% before centrifugation at 30,000xg for 20 min. Supernatants were collected and mixed with an equal volume of saturated ammonium sulphate solution followed by stirring on ice for at least 1h. The solutions were then

centrifuged for 20 min at 30,000xg and pellets were resuspended in 100 mM Tris, pH 8.0, 1 M NaCl, 1 mM EDTA and 1 mM DTT. The resuspended and filtered solutions were then applied to Streptactin columns using an AKTA purification system. Columns were washed using 100 mM Tris, pH 8.0, 1 mM EDTA, 1 mM DTT, 4 M NaCl, 5 followed by 100 mM Tris, pH 7.5, 1 mM EDTA, 1 mM DTT, 100 mM NaCl. Elution was performed using 100 mM Tris, pH 7.5, 1 mM EDTA, 1 mM DTT, 100 mM NaCl and 5 mM desthiobiotin. The eluate was loaded onto a heparin trap column using 100 mM Tris pH 7.5, 100 mM NaCl, 0.2 mM EDTA and 2 mM DTT. After washing using the same buffer, Tn5 variants were eluted with a salt gradient using 100 mM Tris pH 10 7.5, 1 M NaCl, 0.2 mM EDTA and 2 mM DTT. Fractions were collected, pooled, concentrated and glycerol was added to yield 50% final concentration before long-term storage at -20°C.

3. IVC Analysis of Insertion Bias in *E.coli* genomic DNA

15 Analysis of the insertion sequence bias was performed using IVC-plot data (intensity vs. cycle). This data was generated available after sequencing a DNA library that was created using the respective DNA transposase. Briefly, mutagenesis and expression were performed as described above. Transposases were incubated with transposons A and B described above, and incubated with *E. coli* genomic DNA to 20 generate a DNA library. Each library was sequenced for at least 35 cycles on an Illumina Genome Analyzer system running the MiSeq Fast chemistry (Illumina, Inc., San Diego, CA), according to manufacturer instructions. Illumina RTA Software was used to generate base calls and intensity values at each cycle. To generate IVC plots, sequencing reads were aligned to *E. coli* reference genome and intensity (occurrence) of 25 each of the four bases at each cycle were plotted as a fraction of all intensity values for all the aligned sequencing reads.

EXAMPLE 2

Identification of Tn5 transposase Mutants for Insertion Bias

This example describes analysis of the insertion sequence bias using IVC-plot data (intensity vs. cycle). This data was available after sequencing a DNA library that was created using the respective DNA transposase. This analysis required only a few sequencing reads (20k-40k) to give stable results and could be performed in *E.coli* cell
5 lysates that were used to express the respective Tn5 transposase variant, and was suitable for HTS (high-throughput screening) purposes

Representative results for various single-amino acid substitution Tn5 variants are set forth in Figs 2-7. The variants shown indicate, for example, loss of symmetry by substitution at position 248 (Fig. 2), flattened IVC plot by substitution at position 119
10 (Fig. 3), reduced IVC in 2nd half of IVC plot by substitution at position 125 or insertion after position 248 (Figs. 4-5), and increase of duplication from 9 bp to 10 bp by use of different aromatic amino acids at pos. K120 (Fig. 6, showing that symmetry changes from 1 and 9th to 1 and 10th bp). These results indicate that the specified mutations provide improved insertion bias compared to wt control.

15

EXAMPLE 3

Whole Genome Sequencing on Bacterial gDNA

These experiments were performed in order to compare various transposase mutants for a) estimated library size/diversity and b) AT/GC-dropout. These
20 experiments were done with purified and activity-normalized Tn5 transposase variants. These experiments require 500k – 1M sequencing reads/experiment.

Results were obtained by performing transposition on *B.cereus* gDNA using the indicated purified Tn5 transposase variants. The enzymes were normalized by activity and set to match the activity of the commercial TDE1 enzyme sold with the
25 Nextera™ kits.

The mutant indicated as Tn5001 has the same amino acid sequence as SEQ ID NO: 11 and serves as “wt” control. As shown in Table above, the mutants indicated as Tn5058, Tn5059 and Tn5061 have the same amino acid sequences as SEQ ID NO: 18, 19, and 20, respectively. Experiments were performed in triplicate, the data shows the

average and standard deviation of the collected data. The “Estimated Library Size” is calculated without the use of optical duplicates, providing reproducible results.

As shown in Fig. 8A, there is a marked reduction of AT-dropout for Tn5058 and Tn5059 compared to Tn5001, while keeping GC-dropout low. Similarly, as shown in Fig. 8B, there is a significant increase in library size by 1.7x (Tn5058) and 2.2x (Tn5059). These results indicate that mutants Tn5058 and Tn5059 greatly improve sequence insertion bias compared to wild type transposase, leading to further experiments described in Example 4 below.

10

EXAMPLE 4

Nextera Rapid Capture Enrichment Experiments on Human gDNA

The following experiments were performed with the same purified and activity-normalized Tn5 transposase variants described above in Example 3. These experiments typically require 40M – 100M sequencing reads/experiment, and sequencing data was analyzed to compare a) diversity, b) enrichment, c) coverage, d) coverage uniformity, e) penalty scores.

The capture was performed in triplicate using Nextera Rapid Capture Exome (IlluminaTM) CEX pool capture probes according to manufacturer instructions. As shown in Fig. 9A, the indicated mutants yielded marked improvement in coverage uniformity, compared to wt control Tn5001. Further, as indicated in Fig. 9B, statistically significant improvements were yielded by that tested mutants on the 10x and 20x on target coverage, despite the lower mean target coverage.

As shown in Fig. 10A, the indicated mutants yielded an increase in the number of unique reads and hybrid selection library size. Likewise, as shown in Fig. 10B, the mutants yielded lower penalty scores compared to Tn5001, which is the fold greater sequencing required to reach 10x, 20x or 30x depth of coverage. These results indicate that the tested mutants provide greater insertion bias and more uniform coverage, compared to control.

30

EXAMPLE 5

Effect of Tagmentation Buffer Composition on Tn5 activity

The following experiments were performed to characterize the effect of Tn5 tagmentation buffer composition and reaction conditions on library output and sequencing metrics.

To evaluate the effect of tagmentation buffer composition and reaction conditions on library output and sequencing metrics, Tn5 tagged DNA libraries were constructed using *Bacillus cereus* genomic DNA. Two different Tn5 transposases were used for the construction of the tagged libraries, i.e., a mutant Tn5 (“TS-Tn5059”), and a control hyperactive Tn5 (“TS-Tn5”). TS is a fusion tag that is used for purification of the Tn5 and Tn5059 proteins. Tn5059 has 4 additional mutations K212R, P214R, G251R, and A338V with respect to the hyperactive Tn5 amino acid sequence (SEQ ID NO: 11). TS-Tn5059 comprise a TS tag at the N-terminus of Tn5059. In some embodiments, the C-terminus of TS-tag may be fused to the N-terminus of Tn5059 by a linker, which substitutes the first methionine residue. In some embodiments, the linker is Gly-Thr.

TS-Tn5059 was used at final concentrations of 10, 40, and 80 nM. TS-Tn5 was used at final concentrations of 4, 15, and 30 nM. Enzyme concentrations for TS-Tn5059 and TS-Tn5 were normalized (using the standard buffer formulation) to provide about the same level of tagmentation activity, i.e., TS-Tn5059 at 10, 40, and 80 nM has about the same level of activity as Tn5 at 4, 15, and 30 nM, respectively. Each tagged library was prepared using 25 ng input of *B. cereus* genomic DNA. The genomic content of *B. cereus* is about 40% GC and about 60% AT.

Tagmentation buffers were prepared as 2x formulations. The 2x formulations were as follows: standard buffer (TD; 20 mM Tris Acetate, pH 7.6, 10 mM MgAcetate, and 20% dimethylformamide (DMF)); cobalt buffer (Co; 20 mM Tris Acetate, pH 7.6, , and 20 mM CoCl₂); cobalt + DMSO buffer (Co-DMSO; 20 mM Tris Acetate, pH 7.6, , 20 mM CoCl₂, and 20% dimethyl sulfoxide (DMSO)); high molecular weight buffer (HMW; 20 mM Tris Acetate, pH 7.6, and 10 mM MgAcetate); NF2 buffer (NF2; 20 mM Tris Acetate, pH 7.6, 20 mM CoCl₂, and 20% DMF). Tagmentation buffers that

include CoCl_2 were prepared fresh daily. For each library, a tagmentation reaction was performed by mixing 20 μL *B. cereus* genomic DNA (25 ng), 25 μL 2x tagmentation buffer, and 5 μL enzyme (10x TS-Tn5059 or 10x TS-Tn5) in a total reaction volume of 50 μL . Reactions were incubated at 55 °C for 5 minutes. Following the tagmentation reaction, the samples were processed according to the standard Nextera™ sample preparation protocol. Libraries were sequenced using Illumina's SBS (sequencing-by-synthesis) chemistry on a MiSeq device. Sequencing runs were 2x71 cycles using a V2 MiSeq kit. Fragment size distribution in each library was evaluated on a Bioanalyzer.

Figure 11 shows a bar graph 100 of the number of unique molecules in TS-Tn5059 and TS-Tn5 tagmented DNA libraries prepared using different tagmentation buffers. The number of unique molecules in a library is an indication of the diversity (complexity) of the library. Each bar on the graph represents a tagmented library. The experiment was repeated three times ($n = 3$). Control libraries (i.e., libraries that were prepared using the standard tagmentation buffer) are designated by “enzyme – enzyme concentration – DNA input”. For example, the first bar in bar graph 100 is labeled “TS-Tn5059-10nM-25ng” and designates a control library that was prepared using TS-Tn5059 at a final concentration of 10 nM in the standard buffer formulation and 25 ng of input DNA. Libraries that were prepared using a modified tagmentation buffer formulation are designated by “enzyme – enzyme concentration – buffer additive(s) – DNA input”. For example, the fourth bar in bar graph 100 is labeled “TS-Tn5059-10nM-Co-25ng” and designates a library that was prepared using TS-Tn5059 at a final concentration of 10 nM in a modified tagmentation buffer that included 10 mM CoCl_2 . The data show that TS-Tn5059 and TS-Tn5 tagmented libraries prepared using tagmentation buffers that include 10 mM CoCl_2 (i.e., Co, Co-DMSO, and NF2 buffers) have a higher average diversity compared to libraries prepared in buffers without the addition of CoCl_2 (i.e., standard buffer or HMW).

Figure 12 shows a bar graph 200 of the percent GC dropout in TS-Tn5059 and TS-Tn5 tagmented DNA libraries prepared using different tagmentation buffers. Control libraries and libraries prepared using a modified tagmentation buffer are designated as described in Figure 11. GC dropout may be defined as the percentage of

GC rich regions in the genome that are dropped (absent) from the tagmented library. The data show that for the control TS-Tn5059 and TS-Tn5 libraries that were prepared using the standard tagmentation buffer, the percentage of GC dropout is relatively low. The data also shows that TS-Tn5059 and TS-Tn5 tagmented libraries prepared using
5 tagmentation buffers that include 10 mM CoCl₂ (i.e., Co, Co-DMSO, and NF2 buffers) have a higher percentage GC dropout (i.e., up to about 6%) compared to libraries prepared in buffers without the addition of CoCl₂ (i.e., standard buffer or HMW). The increase in GC dropout in libraries prepared using Co-containing buffers is ameliorated by increase the concentration of TS-Tn5059 and TS-Tn5. For example, the percentage
10 GC dropout in the TS-Tn5059-10nm-Co-25ng library is relatively high compared to the TS-Tn5059-10nm-25ng control library. As the concentration of TS-Tn5059 is increased to 40 nM (i.e., TS-Tn5059-40nm-Co-25ng) and 80 nM (i.e., TS-Tn5059-80nm-Co-25ng), the percentage of GC dropout decreases.

Figure 13 shows a bar graph 300 of the percent AT dropout in TS-Tn5059 and
15 TS-Tn5 tagmented DNA libraries prepared using different tagmentation buffers. Control libraries and libraries prepared using a modified tagmentation buffer are designated as described in Figure 11. AT dropout may be defined as the percentage of AT rich regions in the genome that are dropped (absent) from the tagmented library. The data show that for the control TS-Tn5059 and TS-Tn5 libraries that were prepared
20 using the standard tagmentation buffer, a certain amount (i.e., from about 1% to about 3% and from about 7% to about 3%, respectively) of AT dropout is observed. The data also shows that TS-Tn5059 tagmented libraries prepared using the low enzyme concentration (i.e., 10 nM) and tagmentation buffers that include 10 mM CoCl₂ (i.e., Co, Co-DMSO, and NF2 buffers) have a lower percentage AT dropout compared to
25 libraries prepared in buffers without the addition of CoCl₂ (i.e., standard buffer or HMW). Similarly, TS-Tn5 libraries prepared using tagmentation buffers that include 10 mM CoCl₂ (i.e., Co, Co-DMSO, and NF2 buffers) have a lower percentage AT dropout compared to libraries prepared in buffers without the addition of CoCl₂ (i.e., standard buffer or HMW).

Now referring to Figures 12 and 13, the addition of CoCl_2 (10 nM) in the tagmentation buffers (i.e., Co, Co-DMSO, and NF2 buffers) may “flip” the percentage of GC and AT dropout in a tagmented library. For example, the percentage of GC dropout (Figure 12) in the TS-Tn5059-10nm-Co-25ng library is relatively high
5 compared to the TS-Tn5059-10nm-25ng control library; whereas the percentage of AT dropout (Figure 13) in the TS-Tn5059-10nm-Co-25ng library is relatively low (or none) compared to the TS-Tn5059-10nm-25ng control library.

Figure 14 shows a plot 400 of Bioanalyzer traces of the fragment size distribution in TS-Tn5059 libraries prepared using the standard buffer (TD) and the cobalt buffer (Co) formulations. Plot 400 shows a curve 410 which is a curve of the
10 fragment size distribution in the Ts-Tn5059-10nM-Co-25 ng library, a curve 415 which is a curve of the fragment size distribution in the Ts-Tn5059-40nM-Co-25 ng library, a curve 420 which is a curve of the fragment size distribution in the Ts-Tn5059-80nM-Co-25 ng library, a curve 425 which is a curve of the fragment size distribution in the
15 Ts-Tn5059-10nM-TD-25 ng library, a curve 430 which is a curve of the fragment size distribution in the Ts-Tn5059-10nM-TD-25 ng library, and a curve 435 which is a curve of the fragment size distribution in the Ts-Tn5059-80nM-TD-25 ng library. Plot 400 also shows a curve 440 which is a standard ladder of DNA fragment size in base pairs (bp). The fragment sizes in the ladder (from left to right) are shown in Table 2.

Table 2. Size ladder	
Ladder peak	Size (bp)
1	35
2	50
3	100
4	150
5	200
6	300
7	400
8	500
9	600
10	700
11	1,000
12	2,000
13	3,000
14	7,000
15	10,380

The data show that increasing the concentration of TS-Tn5059 used in the tagmentation reaction from 10 nM to 40 nM and 80 nM shifts the fragment size distribution to smaller fragment sizes. The shift in fragment size distribution is more pronounced in libraries prepared using the standard buffer (TD) formulation. For example, the fragment size distribution in libraries prepared using the cobalt buffer (Co) formulation is about 3,000 bp in libraries prepared using 10 nM TS-Tn5059 (curve 410) and from about 1,000 to about 2,000 bp in libraries prepared using 40 and 80 nM TS-Tn5059 (curves 415 and 420, respectively). For the library prepared using the standard buffer (TD) formulation and 80 nM TS-Tn5059 (curve 435), the fragments size distribution is from about 200 bp to about 1,000 bp.

Figure 15 shows a plot 500 of Bioanalyzer traces of the fragment size distribution in TS-Tn5059 libraries prepared using the cobalt-DMSO (Co-DMSO), NF2, and HMW buffer formulations. Plot 500 shows a curve 510 which is a curve of the fragment size distribution in the Ts-Tn5059-10nM-Co-DMSO-25 ng library, a curve 515 which is a curve of the fragment size distribution in the Ts-Tn5059-10nM-NF2-25 ng library, a curve 520 which is a curve of the fragment size distribution in the Ts-Tn5059-10nM-HMW-25 ng library, a curve 525 which is a curve of the fragment size distribution in the Ts-Tn5059-40nM-Co-DMSO-25 ng library, a curve 530 which is a

curve of the fragment size distribution in the Ts-Tn5059-40nM-NF2-25 ng library, a curve 535 which is a curve of the fragment size distribution in the Ts-Tn5059-40nM-HMW-25 ng library, a curve 540 which is a curve of the fragment size distribution in the Ts-Tn5059-80nM-Co-DMSO-25 ng library, a curve 545 which is a curve of the fragment size distribution in the Ts-Tn5059-80nM-NF2-25 ng library, and a curve 550 which is a curve of the fragment size distribution in the Ts-Tn5059-80nM-HMW-25 ng library. Plot 500 also shows curve 440 of plot 400 of Figure 14, which is the standard ladder of DNA fragment size in base pairs (bp).

The data show that in general, increasing the concentration of TS-Tn5059 used in the tagmentation reaction from 10 nM to 40 nM and 80 nM shifts the fragment size distribution to smaller fragment sizes. The shift in fragment size distribution is more pronounced in libraries prepared using HMW buffer (e.g., curves 520 and 535) which does not include CoCl_2 compared to libraries prepared using Co-DMSO (e.g., curves 510 and 525).

Figure 16 shows a plot 600 of Bioanalyzer traces of the fragment size distribution in TS-Tn5 libraries prepared using the standard buffer formulation (TD) and the cobalt buffer (Co). Plot 600 shows a curve 610 which is a curve of the fragment size distribution in the Ts-Tn5-4nM-Co-25 ng library, a curve 615 which is a curve of the fragment size distribution in the Ts-Tn5-15nM-Co-25 ng library, a curve 620 which is a curve of the fragment size distribution in the Ts-Tn5-30nM-Co-25 ng library, a curve 625 which is a curve of the fragment size distribution in the Ts-Tn5-4nM-TD-25 ng library, a curve 630 which is a curve of the fragment size distribution in the Ts-Tn5-15nM-TD-25 ng library, and a curve 635 which is a curve of the fragment size distribution in the Ts-Tn5-30nM-TD-25 ng library. Plot 600 also shows curve 440 of plot 400 of Figure 14, which is the standard ladder of DNA fragment size in base pairs (bp).

The data show that increasing the concentration of TS-Tn5 used in the tagmentation reaction from 4 nM to 15 nM and 30 nM shifts the fragment size distribution to smaller fragment sizes. The shift in fragment size distribution is more pronounced in libraries prepared using the standard buffer (TD) formulation. This

observation is similar to the fragment size distributions in TS-Tn5059 libraries of Figure 14.

Figure 17 shows a plot 700 of Bioanalyzer traces of the fragment size distribution in TS-Tn5 libraries prepared using the cobalt-DMSO (Co-DMSO), NF2, and HMW buffer formulations. Plot 700 shows a curve 710 which is a curve of the fragment size distribution in the Ts-Tn5-4nM-Co-DMSO-25 ng library, a curve 715 which is a curve of the fragment size distribution in the Ts-Tn5-4nM-NF2-25 ng library, a curve 720 which is a curve of the fragment size distribution in the Ts-Tn5-4nM-HMW-25 ng library, a curve 725 which is a curve of the fragment size distribution in the Ts-Tn5-15nM-Co-DMSO-25 ng library, a curve 730 which is a curve of the fragment size distribution in the Ts-Tn5-15nM-NF2-25 ng library, a curve 735 which is a curve of the fragment size distribution in the Ts-Tn5-15nM-HMW-25 ng library, a curve 740 which is a curve of the fragment size distribution in the Ts-Tn5-30nM-Co-DMSO-25 ng library, a curve 745 which is a curve of the fragment size distribution in the Ts-Tn5-30nM-NF2-25 ng library, and a curve 750 which is a curve of the fragment size distribution in the Ts-Tn5-30nM-HMW-25 ng library. Plot 700 also shows curve 440 of plot 400 of Figure 14, which is the standard ladder of DNA fragment size in base pairs (bp).

The data show that increasing the concentration of TS-Tn5 used in the tagmentation reaction from 4 nM to 15 nM and 30 nM shifts the fragment size distribution to smaller fragment sizes. The shift in fragment size distribution is more pronounced in libraries prepared using the standard buffer (TD) formulation. This observation is similar to the fragment size distributions in TS-Tn5059 libraries of Figure 15.

In general, now referring to Figures 14 through 17, the fragment size in TS-Tn5059 and TS-Tn5 libraries prepared using tagmentation buffers that include 10 nM CoCl_2 (e.g., Co, Co-DMSO, and NF2 buffers) are larger than in TS-Tn5059 and TS-Tn5 libraries prepared using tagmentation buffers without CoCl_2 (i.e., TD and HMW buffers).

Figures 18A, 18B, 18C, and 18D show a bias graph 800 of the sequence content in the TS-Tn5 library, a bias graph 830 of the sequence content in the TS-TN5-Co library, a bias graph 840 of the sequence content in the TS-Tn5-Co-DMSO library, and a bias graph 850 of the sequence content in the TS-Tn5-NF2 library, respectively. A bias graph (or intensity vs cycle number (IVC) graph) plots the ratio of the observed base (A, C, G, or T) as a function of SBS cycle number and shows the preferred sequence context that Tn5 has during tagmentation.

Bias graphs 800, 830, 840, and 850 each show a curve 810 which is a curve of A content by cycle number, a curve 815 which is a curve of C content by cycle number, a curve 820 which is a curve of G content by cycle number, and a curve 825 which is a curve of T content by cycle number. For example, in the TS-TN5 library of Figure 18A, curve 820, which represents the base G, shows that about 38% of bases observed at cycle 1 are G; curve 825, which represents the base T, shows that about 15% of bases observed at cycle 1 are T, etc.

Referring to Figure 18A, the data show that Tn5 sequence bias is observed for about the first 15 cycles of SBS in the TS-Tn5 library, which was prepared using the standard tagmentation buffer formulation. After about 15 cycles, the sequence bias is gradually reduced and the A, T, C, and G content reflects the expected genome composition. For *B. cereus*, the genome is about 40% GC and about 60% AT, which is represented in the bias graphs from about cycle 16 or 17 through cycle 35 where curve 810 (i.e., A) and curve 825 (i.e., T) converge at about 30% ($A + T \sim 60\%$); and curve 815 (i.e., C) and curve 820 (i.e., G) converge at about 20% ($C + G \sim 40\%$).

Referring to Figures 18B, 18C, and 18D, the data also shows that Tn5 sequence bias is observed for about the first 15 cycles of SBS in the TS-Tn5-Co, TS-Tn5-Co-DMSO, and Ts-Tn5-NF2 libraries, which are libraries that were prepared using tagmentation buffers that included CoCl_2 . Again, after about 15 cycles, the sequence bias is gradually reduced and the A, T, C, and G content reflects the expected genome composition as described with reference to Figure 18A. However, in the TS-Tn5-Co, TS-Tn5-Co-DMSO, and Ts-Tn5-NF2 libraries, curve 810 (i.e., A) and curve 825 (i.e., T) begin to shift toward the expected genome composition at about cycle 10 to cycle 15;

and curve 815 (i.e., C) and curve 820 (i.e., G) begin to shift toward the expected genome composition at about cycle 10 to cycle 15. In addition, the bias between cycles 2-8 is reduced when compared to Figure 18A. The data show that the addition of CoCl_2 in tagmentation buffer formulations ameliorates Tn5 sequence bias during tagmentation.

5 Figures 19A, 19B, 19C, and 19D show a bias graph 900 of the sequence content in the TS-Tn5059 library, a bias graph 930 of the sequence content in the TS-TN5059-Co library, a bias graph 940 of the sequence content in the TS-Tn5059-Co-DMSO library, and a bias graph 950 of the sequence content in the TS-Tn5059-NF2 library, respectively. Bias graphs 900, 930, 940, and 950 each show a curve 910 which is a
10 curve of A content by cycle number, a curve 915 which is a curve of C content by cycle number, a curve 920 which is a curve of G content by cycle number, and a curve 925 which is a curve of T content by cycle number.

Referring to Figure 19A, the data show that Tn5059 sequence bias is observed for about the first 15 cycles of SBS in the TS-Tn5059 tagmented library. After about 15
15 cycles, the sequence bias is reduced and the A, T, C, and G content reflects the expected genome composition as described with reference to Figure 18A. However, the mutant Tn5059 shows reduced sequence bias compared to Tn5 sequence bias shown in Figure 18A. In the TS-Tn5059 library, curve 910 (i.e., A) and curve 925 (i.e., T) begin to shift toward the expected genome composition at about cycle 10 to cycle 15; and curve 915
20 (i.e., C) and curve 920 (i.e., G) begin to shift toward the expected genome composition at about cycle 10 to cycle 15.

Referring to Figures 19B, 19C, and 19D, the data also shows that Tn5059 sequence bias is observed for about the first 15 cycles of SBS in the TS-Tn5059-Co, TS-Tn5059-Co-DMSO, and Ts-Tn5059-NF2 libraries, which are libraries that were
25 prepared using tagmentation buffers that included CoCl_2 . Again, after about 15 cycles, the sequence bias is gradually reduced and the A, T, C, and G content reflects the expected genome composition as described with reference to Figure 18A. However, in the TS-Tn5059-Co library, curve 910 (i.e., A) and curve 925 (i.e., T) begin to shift toward the expected genome composition at about cycle 5; and curve 915 (i.e., C) and
30 curve 920 (i.e., G) begin to shift toward the expected genome composition at about

cycle 5. In the TS-Tn5059-Co-DMSO, and Ts-Tn5059-NF2 libraries, curve 910 (i.e., A) and curve 925 (i.e., T) begin to shift toward the expected genome composition before cycle 5; and curve 915 (i.e., C) and curve 920 (i.e., G) begin to shift toward the expected genome composition before cycle 5.

5

EXAMPLE 6

Effect of Tagmentation Buffer Composition on Mos1 activity

The following experiments were performed to characterize the effect of Tn5 tagmentation buffer composition and reaction conditions on library output and sequencing metrics.

Mos1 tagmented DNA libraries were constructed using *B. cereus* genomic DNA. The Mos1 transposase used for construction of the tagmented libraries was an MBP-Mos1 fusion protein. Maltose binding protein (MBP) is a fusion tag that is used for purification of the Mos1 protein. MBP-Mos1 was used at a final concentration of 100 μ M. Each tagmented library was prepared using 50 ng input of *B. cereus* genomic DNA.

Tagmentation buffers were prepared as 2x formulations. The 2x formulations were as follows: standard buffer (TD; 20 mM Tris Acetate, pH 7.6, 10 mM MgAcetate, and 20% dimethylformamide (DMF); TD + NaCl (TD-NaCl; 20 mM Tris Acetate, pH 7.6, 10 mM MgAcetate, 20% DMF, and 200 mM NaCl); high molecular weight buffer (HMW; 20 mM Tris Acetate, pH 7.6, and 10mM MgAcetate); HEPES (50 mM HEPES, pH 7.6, 10mM MgAcetate, 20% DMF); HEPES-DMSO (50 mM HEPES pH 7.6, 10mM MgAcetate, and 20% DMSO); HEPES-DMSO-Co (50 mM HEPES, pH 7.6, 20% DMSO, and 20 mM CoCl_2), and HEPES-DMSO-Mn (50 mM HEPES, pH 7.6, 20% DMSO, and 20 mM manganese (Mn)). Tagmentation buffers that include CoCl_2 were prepared fresh daily.

For each library, a tagmentation reaction was performed by mixing 20 μ L *B. cereus* genomic DNA (50 ng), 25 μ L 2x tagmentation buffer, and 5 μ L enzyme (10x MBP-Mos1) in a total reaction volume of 50 μ L. Reactions were incubated at 30 $^{\circ}$ C for 60 minutes. Following the tagmentation reaction, the samples were processed

according to the standard Nextera™ sample preparation protocol. Libraries were sequenced using Illumina's SBS (sequencing-by-synthesis) chemistry on a MiSeq device. Sequencing runs were 2x71 cycles.

Figure 20 shows a bar graph 1000 of the average total number of reads and average diversity in MBP-Mos1 tagmented libraries prepared using different
5 tagmentation buffers. The total number of reads is the total number of reads from the flow cell. The diversity is the number of unique molecules in the library and is used as an indication of library complexity. Each pair of bars on the graph represents a tagmented library. The experiment was repeated three times ($n = 3$). The first two
10 graph bars, EZTn5-std-bcereus and NexteraV2-30C, are comparative libraries that were prepared using Tn5 and the standard buffer formulation at 55 °C and 30 °C, respectively. Libraries that were prepared using MBP-Mos1 for the tagmentation reaction are designated by "enzyme – enzyme concentration – buffer". For example, the third pair of graph bars are labeled "MBPMos1-100μM-TD" and designate a library
15 that was prepared using MBP-Mos1 at a final concentration of 100 μM in the standard tagmentation buffer (TD). The data show. The effect of different buffers on the diversity of the library prepared by Mos1 tagmentation under relatively same number or sequencing reads. In particular, HEPES-DMSO-Mn buffer helps increasing the diversity of the library.

Figure 21 shows a bar graph 1100 of GC and AT dropout in the MBP-Mos1
20 tagmented libraries. GC and AT dropout may be defined as the percentage of GC rich regions and AT rich regions, respectively, in the genome that are dropped (absent) from the tagmented library. Libraries are designated as described in Figure 20. The data show that libraries prepared using EZTn5 and NexteraV2 (i.e., Tn5 transposase) have
25 essentially no GC dropout, but about 7% and about 5%, respectively, of AT rich regions are dropped from the tagmented library. The library prepared using MBP-Mos1 and the standard tagmentation buffer (MBPMos1-100μM-TD) has essentially no AT dropout, but about 2% or less of the GC rich regions are dropped from the tagmented library. The percent GC dropout in a MBP-Mos1 tagmented library is effected by the
30 composition of the tagmentation buffer. The percent GC dropout is increased in MBP-

Mos1 tagmented libraries prepared using HMW, HEPES, HEPES-DMSO, HEPES-DMSO-Co, and HEPES-DMSO-Mn buffers.

Figures 22A, 22B, 22C, and 22D show a bias graph 1200 of the sequence content in the Mos1-HEPES library, a bias graph 1230 of the sequence content in the Mos1-HEPES-DMSO library, a bias graph 1240 of the sequence content in the Mos1-HEPES-DMSO-Co library, and a bias graph 1250 of the sequence content in the Mos1-HEPES-DMSO-Mn library, respectively. Bias graphs 1200, 1230, 1240, and 1250 each show a curve 1210 which is a curve of A content by cycle number, a curve 1215 which is a curve of C content by cycle number, a curve 1220 which is a curve of G content by cycle number, and a curve 1225 which is a curve of T content by cycle number.

Referring to Figures 22A and 22B, the data show that Mos1 sequence bias is observed for the first few cycles of SBS in the Mos1-HEPES and the Mos1-HEPES-DMSO tagmented libraries. In the first SBS cycle, detection of T is about 100% throughout the flow cell. In the second SBS cycle, detection of A is about 100% throughout the flow cell. After about 4 cycles, the sequence bias is reduced and the A, T, C, and G content reflects the expected genome composition.

Referring to Figures 22C and 22D, the data also shows that Mos1 sequence bias is observed for the first few cycles of SBS in the Mos1-HEPES-DMSO-Co and the Mos1-HEPES-DMSO-Mn tagmented libraries, which are libraries that were prepared using tagmentation buffers that replaced magnesium (Mg) with cobalt (Co) or manganese (Mn), respectively. Again, after about 4 cycles, the sequence bias is reduced and the A, T, C, and G content reflects the expected genome composition. However, in the Mos1-HEPES-DMSO-Co and the Mos1-HEPES-DMSO-Mn libraries, curve 1210 (i.e., A) and curve 1225 (i.e., T) a shift toward the expected genome composition is observed at cycle 1 and cycle 2. The shift toward the expected genome composition is more pronounced in the Mos1-HEPES-DMSO-Mn library.

EXAMPLE 7

TS-Tn5059 Library Preparation and Exome Enrichment Protocol

In one embodiment, the method of the invention provides a streamlined workflow for preparation and enrichment of a Tn5 transposome-based exome library.

Figure 23 illustrates a flow diagram of an example of a method 1260 of preparing and enriching a genomic DNA library for exome sequencing. Method 1260 uses TS-Tn5059 transposomes and modifications to certain process steps of the current Nextera® Rapid Capture protocol to provide improved library yields across a range of DNA input amounts and sequencing metrics. For example, method 1260 uses a “double-sided” solid phase reversible immobilization (SPRI) protocol (Agencourt AMPure XP beads; Beckman Coulter, Inc.) to purify the tagmented DNA and prior to PCR amplification provides a first DNA fragment size selection step and a second DNA fragment size selection step. In another example, a pre-concentration process is used to concentrate tagmented DNA libraries prior to exome enrichment. Method 1260 includes, but is not limited to, the following steps.

At a step 1270, genomic DNA is tagmented (tagged and fragmented) by the transposome. The transposome simultaneously fragments the genomic DNA and adds adapter sequences to the ends, allowing subsequent amplification by PCR. In one example, the transposome is TS-Tn5059. At the completion of the tagmentation reaction, a tagmentation stop buffer is added to the reaction. The tagmentation stop buffer may be modified to ensure sufficient denaturation of TS-Tn5059 transposome complexes from the tagmented DNA (e.g., the concentration of SDS in the stop buffer is increased from 0.1% to 1.0% SDS in combination with high heat).

At a step 1275, a first clean-up is performed to purify the tagmented DNA from the transposomes and provide a first DNA fragment size selection step. DNA fragment size may be selected by varying the volume-to-volume ratio of SPRI beads to DNA (e.g., 1X SPRI = 1:1 vol SPRI: DNA). For example, in the first size selection the volume ratio of SRPI beads to DNA is selected to bind DNA fragments greater than a certain size (i.e., remove larger DNA fragments from the sample) while DNA fragments smaller than a certain size remain in the supernatant. The supernatant with size-selected DNA fragments therein is transferred to a clean reaction vessel for subsequent processing. The SPRI beads with larger DNA fragments thereon may be discarded. In one embodiment, the concentration of SPRI beads can vary from 0.8X to 1.5X. In one embodiment, the concentration of SPRI beads is 0.8X.

At a step 1280, a second clean-up is performed to further select DNA fragments in a certain size range. For example, the volume ratio of SPRI beads to DNA is selected to bind DNA fragments greater than a certain size (i.e., DNA fragments in the desired size range are bound to the SPRI beads). Smaller DNA fragments remain in the supernatant and are discarded. The bound DNA fragments are then eluted from the SPRI beads for subsequent processing.

At an optional step 1285, the DNA fragment size distribution is determined. The DNA fragment size distribution is, for example, determined using a Bioanalyzer.

At a step 1290, the purified tagged DNA is amplified via a limited-cycle PCR program. The PCR step also adds index 1 (i7) and index 2 (i5) and sequencing, as well as common adapters (P5 and P7) required for subsequent cluster generation and sequencing. Because a double-side SPRI process (i.e., steps 1275 and 1280) was used to select a desired DNA fragment size range, only tagged DNA fragments in the desired size range are available for PCR amplification. Consequently, the library yield is significantly increased and subsequent sequencing metrics (e.g., percent read enrichment) are improved.

At step 1295, the amplified tagged DNA library is purified using a bead-based purification process.

At an optional step 1300, the DNA fragment size distribution post-PCR is determined. The DNA fragment size distribution is, for example, determined using a Bioanalyzer.

At a step 1310, the tagged DNA library is pre-concentrated prior to subsequent hybridization for exome enrichment. For example, the tagged DNA library is pre-concentrated from about 50 μ L to about 10 μ L. Because the tagged DNA library is pre-concentrated, the hybridization kinetics are faster and the hybridization times are reduced.

At a step 1320, a first hybridization for exome enrichment is performed. For example, The DNA library is mixed with biotinylated capture probes targeted to regions of interest. The DNA library is denatured at about 95 °C for about 10 minutes and

hybridized to the probes at about 58 °C for about 30 minutes for a total reaction time of about 40 minutes.

At a step 1325, streptavidin beads are used to capture biotinylated probes hybridized to the targeted regions of interest. Two heated wash procedures are used to
5 remove non-specifically bound DNA from the beads. The enriched library is then eluted from the beads and prepared for a second round of hybridization.

At a step 1330, a second hybridization for exome enrichment is performed using the same probes and blockers as the first hybridization. For example, the eluted DNA library from step 155 is denatured at about 95 °C for about 10 minutes and hybridized at
10 about 58 °C for about 30 minutes for a total reaction time of about 40 minutes. The second hybridization is used to ensure high specificity of the captured regions.

At a step 1335, streptavidin beads are used to capture biotinylated probes hybridized to the targeted regions of interest. Two heated wash procedures are used to remove non-specifically bound DNA from the beads. The exome enriched library is
15 then eluted from the beads and amplified by ten cycles of PCR in preparation for sequencing.

At a step 1340, the exome enriched capture sample (i.e., exome enriched DNA library) is purified using a bead-based purification protocol.

At a step 1345, the exome enriched DNA library is PCR amplified for
20 sequencing.

At a step 1350, the amplified enriched DNA library is optionally purified using a bead-based purification protocol. For example, a 1X SPRI bead protocol is used to remove unwanted products (e.g., excess primers) that may interfere with subsequent cluster amplification and sequencing.

25 Method 100 provides for library preparation and exome enrichment in about 11 hours. If optional steps 1285 and 1300 are omitted, method 1260 provides for library preparation and exome enrichment in about 9 hours.

EXAMPLE 8

TS-Tn5059 Insertional Bias

A transposase may have a certain insertion site (DNA sequence) bias in a tagmentation reaction. The DNA sequence bias may cause certain regions (e.g., GC-rich or AT-rich) of a genome to be dropped from a tagmented library. For example, Tn5 transposase has a certain bias for GC-rich regions of the genome; consequently, AT regions of the genome may be dropped in a Tn5 tagmented library. To provide a more complete coverage of a genome, minimal sequence bias is desired.

To evaluate the effect of TS-Tn5059 transposome on library output and sequencing metrics, TS-Tn5059 tagmented DNA libraries were prepared using a standard Nextera™ DNA library preparation kit for whole genome sequencing and *Bacillus cereus* genomic DNA. TS-Tn5059 was used at a final concentration of 40 nM. A reference control library was prepared using standard reaction conditions of 25 nM NexteraV2 transposomes. Libraries were evaluated by sequencing-by-synthesis (SBS).

Figure 24A shows a plot 1400 of the coverage in tagmented *B. cereus* genomic DNA libraries prepared using TS-Tn5059 transposomes. The TS-Tn5059 transposome becomes resistant to increasing levels of bias as the GC content increases. Figure 24B shows a plot 1450 of the coverage in tagmented *B. cereus* genomic DNA libraries prepared using NexteraV2 transposomes. Figure 24B demonstrates that as GC content increases, the Nextera V2 coverage of GC rich regions becomes skewed, with an increasing bias. The data show that tagmented DNA libraries prepared using TS-Tn5059 have improved and more even coverage across a wide GC/AT range with lower insertional bias compared to tagmented libraries prepared using NexteraV2.

Figure 25A shows a plot 1500 of gap location and gap length in tagmented *B. cereus* genomic DNA libraries prepared using TS-Tn5059 transposomes. Figure 25B shows a plot of gap location and gap length in tagmented *B. cereus* genomic tagmented DNA libraries prepared using NexteraV2 transposomes. The number of gaps in the TS-Tn5059 tagmented library is 27. The number of gaps in the NexteraV2 tagmented library is 208. The data show that tagmented DNA libraries prepared using TS-Tn5059

transposomes have more even coverage with fewer gaps compared to tagmented libraries prepared using NexteraV2 transposomes.

EXAMPLE 8

TS-Tn5059 DNA Input Tolerance

5 Preparation of a tagmented DNA library uses an enzymatic DNA fragmentation step (e.g., transposome mediated tagmentation) and therefore may be more sensitive to DNA input compared to, for example, mechanical fragmentation methods. In one example, the current Nextera® Rapid Capture Enrichment protocol has been optimized for input of 50 ng of total genomic DNA. A higher mass input of genomic DNA can
10 result in incomplete tagmentation and larger insert sizes, which may affect subsequent enrichment performance. A lower mass input of genomic DNA or low quality genomic DNA in the tagmentation reaction may generate smaller than expected insert sizes. Smaller inserts may be lost during subsequent clean-up steps and result in lower library diversity.

15 To evaluate the effect of different DNA input amounts on fragment (insert) size distributions, TS-Tn5059 tagmented DNA libraries were prepared using various amount of input genomic DNA at various enzyme concentrations and the fragment sizes were compared with the fragment sizes obtained for other transposases, whose activities are normalized to the activity of 40 nM TS-Tn5059 and 25 ng of genomic DNA input.

20 The size distribution of the fragments generated by 40nM TS-Tn5059, normalized TDE1 (Tn5 version-1) and normalized TS-Tn5 and using 25 ng of human genomic DNA were similar as shown in Figure 26 and 27.

 However, TS-Tn5059 showed increased DNA input tolerance at higher enzyme concentration and over a wide range of input DNA amounts. Figure 28 shows a panel
25 1600 of Bioanalyzer traces of fragment size distributions in tagmented genomic DNA libraries prepared using a range of DNA input. 240 nM of TS-Tn5059 tagmented libraries were prepared by tagmentation, 1.8X SPRI clean up, followed by Bioanalyzer trace. Reference control libraries were prepared using the current Nextera® Rapid Capture kit (“Nextera”) and the Agilent QXT kit (“Agilent QXT”). Tagmented libraries
30 were prepared using 25, 50, 75, and 100 ng of *B. cereus* genomic DNA. The data show

that tagmented DNA libraries prepared using TS-Tn5059 transposomes have a more consistent fragment size distribution across a 25 to 100 ng DNA input range compared to libraries prepared using Nextera or Agilent QXT transposomes. As the amount of DNA input is increased from 25 to 100 ng, the yield of tagmented DNA in TS-Tn5059 tagmented libraries is increased, while the fragment size distribution remains substantially the same. In contrast, at 75 ng and 100 ng of DNA input, the Nextera and Agilent QXT tagmented libraries show a substantial shift in the DNA fragment size distribution to larger fragment sizes.

Figure 29A shows a plot 1700 of Bioanalyzer traces of fragment size distributions in TS-Tn5059 tagmented libraries prepared by a first user using varying input of human Coriel DNA from 5 ng to 100 ng. Figure 29B shows a plot 1750 of Bioanalyzer traces of fragment size distributions in TS-Tn5059 tagmented libraries prepared by a second user. Tagmented libraries were prepared using 5, 10, 25, 50, 75, and 100 ng of *B. cereus* genomic DNA. Both plot 1700 of Figure 29A and plot 1750 of Figure 29B show a line 1710 of the fragment size distribution in a tagmented library prepared using 5 ng of DNA input, a line 1715 of the fragment size distribution in a tagmented library prepared using 10 ng of DNA input, a line 1720 of the fragment size distribution in a tagmented library prepared using 25 ng of DNA input, a line 1725 of the fragment size distribution in a tagmented library prepared using 50 ng of DNA input, a line 1730 of the fragment size distribution in a tagmented library prepared using 75 ng of DNA input, and a line 1735 of the fragment size distribution in a tagmented library prepared using 100 ng of DNA input. The data show that the fragment size distributions in TS-Tn5059 tagmented DNA libraries are consistent in a DNA input range from 5 to 100 ng. The consistency in fragment size distribution is observed for different users.

In another example, Table 3 shows the median library insert size in TS-Tn5059 tagmented DNA libraries across a DNA input range from 5 to 200 ng.

Table 3. Median insert size with 5 ng to 200 ng DNA input									
Input									Av
DNA (ng)		0	5	0	5	00	50	00	e. $\pm$ SD

Median									16
insert (bp)	64	69	58	44	71	78	79	75	7 ± 11

In yet another example, Table 4 shows the library insert size and exome enrichment sequencing metrics for TS-Tn5059 tagmented DNA libraries prepared using 25, 50, 75, and 100 ng of DNA input. The data show that the percent (%) read enrichment is about 80%. The percent read enrichment for tagmented libraries prepared using the current Nextera® Rapid Capture Enrichment protocol is about 60% (data not shown). The data also shows a consistent insert size across the DNA input range from 15 ng to 100 ng.

Table 4. Insert size and exome enrichment metrics with 25 to 100 ng DNA input					
Exome (Picard) Metrics	5 ng	0 ng	5 ng	00 ng	A
% Read Enrichment	8	9	0	7	78 ± 1
% Duplicates	.6	.7	.9	.4	2. 9 ± 0.3
% Zero Coverage	.6	.9	.2	.2	2. 2 ± 0.2
% Exome Coverage at 10x	1.6	2.4	2	2.4	82 $.1 \pm 0.4$
Insert size 150 ± 25 bp	67	69	68	76	17 0 ± 4
Pre-enrichment library quant (ng/ μ L)	23	40	65	52	14 5 ± 15

In another example, Table 5 shows the pre-enrichment library yield across a range of DNA input from 25 ng to 100 ng in TS-Tn5059 tagmented libraries.

Table 5. Pre-enrichment library yields with 25 to 100 ng DNA input

Experiment #	Yield for 15 μ L (25 ng input)	Yield for 15 μ L (50 ng input)	Yield for 15 μ L (75 ng input)	Yield for 15 μ L (100 ng input)
Exp. #1	1098	1468	2420	2240
Exp. #2	1332	2200	2480	1974
Exp. #3	1845	2100	2475	2295
Exp. #4	1860	2295	2895	2760
Exp. #5	1830	2895	1515	1665
Exp. #6	1785	2760	1725	1920

In yet another example, Table 6 shows the exome enrichment sequencing metrics for TS-Tn5059 tagged DNA libraries. Starting with an input DNA of 50 ng, Libraries were prepared using 500 ng, 625 ng, and 750 ng input of library DNA for
 5 exome enrichment. The data show that exome enrichment metrics are consistent across a range of pre-enrichment library input amounts (i.e., 500 ng to 750 ng).

Table 6. Exome enrichment metrics for 500 to 750 ng pre-enrichment library input amount				
Marketing Metrics	00 ng	25 ng	50 ng	Av e. $\pm$ SD
% Read Enrichment	2.2	1.7	2.3	82. 1 $\pm$ 0.32
% Duplicates	.1	.3	.9	4.1 $\pm$ 0.2
% Zero Coverage	.7	.6	.7	1.6 7 $\pm$ 0.06
% Exome Coverage at 10x	4.3	5.4	5.6	85. 5 $\pm$ 0.7
HS library size	9M	0M	1M	50 M $\pm$ 1M

Mean coverage	5.1	8.2	8.6	47. 3 ± 1.92
HS 20x penalty	.5	.3	.4	6.4 ± 0.1

In yet another example, Table 7 shows the exome enrichment sequencing metrics for tagmented DNA libraries prepared using the current Nextera® Rapid Capture Enrichment hybridization protocol (“NRC”) and enrichment steps 1310 through 1350 of method 1260 of Figure 23. The data show that the exome enrichment metrics are improved and/or maintained in TS-Tn5059 tagmented libraries prepared using method 1260 of Figure 23 compared to libraries prepared using the hybridization protocol in the current Nextera® Rapid Capture Enrichment protocol (“NRC”).

Table 7. Exome enrichment sequencing metrics for tagmented DNA libraries prepared using “NRC” and method 100 hybridization protocols		
Key Exome Metrics	NRC hybridization	Method 100 hybridization
Read enrichment (not padded)	54.3%	77.7%
Read enrichment (padded)	64.3%	85%
Mean coverage	50.3X	52.1X
Zero target drop out	1.6%	1.9%
% Duplicates (10 mil. Reads)	3.1%	4%
Coverage at 10X	83.3	84.3%
HS library size	62.3 M	61 M
% Selected on target	76.5%	85.2%
HS 20 x penalty	7.4	6.8

In separate experiments, TS-Tn5059 demonstrated increased DNA input tolerance at higher concentration (normalized to 6X concentration) as compared to Tn5 version-1 and TS-Tn5 transposases normalized to the same concentration. The results are shown in Figures 30-33. Both Tn5 version 1 (Figure 30) and TS-Tn5 (Figure 31) at 6X “normalized” concentration show a fragment size distribution shift with gDNA input varied between 25-100ng. In contrast, TS-Tn5059 at a 6X normalized concentration shows no significant size shift with DNA input between 10-100ng (Figure 32). Fragment size distribution begins to shift when increasing the gDNA input to 200-500ng (Figure 33). The result of the increased DNA input tolerance of TS-Tn5059 is summarized in Table 8 below.

Table 8: Ratio of TS-Tn5059 (nM) : gDNA (ng) in final 50uL reaction

TS-Tn5059 Vol (uL)	Stock Vol (nM)	Final Rxn Volume (uL)	Final Conc in rxn (nM)	gDNA input (ng)	ratio of TS-Tn5059 (nM):gDNA(ng) in 50uL rxn	Comment
5	400	50	40	25	1.6	WGS ratios
5	400	50	40	50	0.8	
15	800	50	240 (6X)	500	0.48	
15	800	50	240 (6X)	400	0.6	
15	800	50	240 (6X)	300	0.8	
15	800	50	240 (6X)	200	1.2	
15	800	50	240 (6X)	100	2.4	input tolerance seen
15	800	50	240 (6X)	75	3.2	
15	800	50	240	50	4.8	

TS-Tn5059 Vol (uL)	Stock Vol (nM)	Final Rxn Volume (uL)	Final Conc in rxn (nM)	gDNA input (ng)	ratio of TS-Tn5059 (nM):gDNA(ng) in 50uL rxn	Comment
			(6X)			
15	800	50	240 (6X)	25	9.6	
15	800	50	240 (6X)	10	24	
15	800	50	240 (6X)	5	48	

Thus, for TS-Tn5059, at a ratio ≥ 2.4 (nM TS-Tn5059: ng input DNA) there was no size shift indicating an increased DNA input tolerance.

Throughout this application various publications, patents and/or patent
5 applications have been referenced. The disclosure of these publications in their entireties is hereby incorporated by reference in this application.

The term comprising is intended herein to be open-ended, including not only the recited elements, but further encompassing any additional elements.

A number of embodiments have been described. Nevertheless, it will be
10 understood that various modifications may be made. Accordingly, other embodiments are within the scope of the following claims.

CLAIMS

1. A mutant Tn5 transposase having transposase activity, wherein said mutant Tn5 transposase comprises all of SEQ ID NO:1 or SEQ ID NO:11 except for:
 - (a) mutations at one or more positions corresponding to positions 214 and 338 of SEQ ID NO:1 or SEQ ID NO:11, and optionally
 - (b) mutations at one or more positions corresponding to positions 54, 56, 212, 251, and 372 of SEQ ID NO:1 or SEQ ID NO:11.
2. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises all of SEQ ID NO:1 or SEQ ID NO:11 except for:
 - (a) mutations at one or more positions corresponding to positions 214 and 338 of SEQ ID NO:1 or SEQ ID NO:11,
 - (b) mutations at one or more positions corresponding to positions 212 and 251 of SEQ ID NO:1 or SEQ ID NO:11, and optionally
 - (c) mutations at one or more positions corresponding to positions 54, 56, and 372 of SEQ ID NO:1 or SEQ ID NO:11.
3. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises all of SEQ ID NO:1 or SEQ ID NO:11 except for:
 - (a) mutations at one or more positions corresponding to positions 212, 214, and 338 of SEQ ID NO:1 or SEQ ID NO:11, and optionally
 - (b) mutations at one or more positions corresponding to positions 54, 56, 251, and 372 of SEQ ID NO:1 or SEQ ID NO:11.
4. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises arginine at the position corresponding to position 214 of SEQ ID NO:1 or SEQ ID NO:11.
5. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises valine at the position corresponding to position 338 of SEQ ID NO:1 or SEQ ID NO:11.

6. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises arginine at the position corresponding to position 251 of SEQ ID NO:1 or SEQ ID NO:11.
7. A fusion protein comprising the mutant Tn5 transposase of claim 1 and a polypeptide fusion domain.
8. The fusion protein of claim 7, wherein the polypeptide fusion domain is fused to the N-terminus of the mutant Tn5 transposase.
9. The fusion protein of claim 7, wherein the polypeptide fusion domain is fused to the C-terminus of the mutant Tn5 transposase.
10. The fusion protein of claim 7, wherein the polypeptide fusion domain comprises a tag to increase solubility.
11. The fusion protein of claim 7, wherein the polypeptide fusion domain comprises a domain selected from the group consisting of maltose binding protein (MBP), elongation factor Ts (Tsf), 5-methyl cytosine binding domain, and protein A.
12. A kit for performing an in vitro transposition reaction, wherein the kit comprises a transposome complex, wherein the transposome complex comprises the mutant Tn5 transposase of claim 1 and a polynucleotide, wherein the 3' portion of the polynucleotide comprises a transposon end sequence.
13. The mutant Tn5 transposase of claim 3, wherein the mutant Tn5 transposase comprises a substitution at the position corresponding to position 212 of SEQ ID NO:1 or SEQ ID NO:11.
14. The mutant Tn5 transposase of claim 13, wherein the mutant Tn5 transposase comprises arginine at the position corresponding to position 212 of SEQ ID NO:1 or SEQ ID NO:11.

15. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises all of SEQ ID NO:1 or SEQ ID NO:11 except for:
- (a) mutations at one or more positions corresponding to positions 212, 214, 251, and 338 of SEQ ID NO:1 or SEQ ID NO:11, and
 - (b) mutations at one or more positions corresponding to positions 54, 56, and 372 of SEQ ID NO:1 or SEQ ID NO:11.
16. The mutant Tn5 transposase of claim 15, wherein the mutant Tn5 transposase comprises arginine, arginine, arginine, and valine, respectively, at the positions corresponding to positions 212, 214, 251, and 338 of SEQ ID NO:1 or SEQ ID NO:11.
17. The fusion protein of claim 11, wherein the polypeptide fusion domain comprises elongation factor Ts (Tsf) and wherein the mutant Tn5 transposase comprises arginine, arginine, arginine, and valine, respectively, at the positions corresponding to positions 212, 214, 251, and 338 of SEQ ID NO:1 or SEQ ID NO:11.
18. The fusion protein of claim 7, wherein said fusion protein comprises SEQ ID NO:26.
19. The mutant Tn5 transposase of claim 1, wherein said mutant Tn5 transposase comprises all of SEQ ID NO:1 or SEQ ID NO:11 except for mutations at positions corresponding to positions 54, 56, 212, 214, 251, 338, and 372 of SEQ ID NO:1 or SEQ ID NO:11.
20. The mutant Tn5 transposase of claim 1, wherein the mutant Tn5 transposase comprises SEQ ID NO:26.