CLAIMS

1. A method of tagmentation comprising:

(a) providing a first transposome, the first transposome comprising a first transposon and a first transposase enzyme having a first tagmentation profile;

(b) combining a target nucleic acid with the first transposase enzyme under conditions suitable for tagmentation, thereby generating a first tagmented nucleic acid;

(c) combining the first tagmented nucleic acid with a second transposome under conditions suitable for tagmentation, the transposome comprising a second transposon and a second transposase enzyme having a second tagmentation profile, thereby generating a second tagmented nucleic acid,

wherein the first tagmentation profile and the second tagmentation profile are different, optionally wherein the tagmentation is sequential.

2. The method of claim 1, comprising a wash step between steps (b) and (c) to substantially separate the tagmented nucleic from reaction buffer used in step (b), optionally wherein the wash step comprises binding the tagmented nucleic acid to a solid support.

3. The method of claim 2, wherein the wash step comprises binding the tagmented nucleic acid to a solid support wherein (a) the solid support comprises beads or (b) the solid support comprises a spin column.

4. The method of claim 1, wherein the first transposase and the second transposase have different insertion biases.

5. The method of any one of claims 1-4, wherein the two tagmentation profiles have different percent of GC dropout, or wherein the two tagmentation profiles have different percent of AT dropout.

6. The method of any one of claims 1-5,

wherein the first transposon comprises a first adaptor, or

wherein the second transposon comprises a second adaptor, or

wherein the first transposon comprises a first adaptor and the second transposon comprises a second adaptor, wherein the first and the second adaptors are different.

7. The method of any one of claims 1-6,

wherein the first transposase enzyme is selected from the group consisting of Mos-1, HyperMu™, Tn5, Ts-Tn5, Ts-Tn5059, Hermes, and Tn7, or

wherein the second transposase enzyme is selected from the group consisting of Mos-1, HyperMu™, Tn5, Ts-Tn5, Ts-Tn5059, Hermes, and Tn7, and optionally

wherein when the first or second transposon comprises an adaptor, the adaptor comprises a sequence selected from the group consisting of barcodes, primer binding sequences, restriction endonuclease sites, and unique molecular indices.

8. The method of claim 1, wherein reaction buffer used in step (b) is diluted to permit tagmentation reaction with the second transposome.

9. The method of any of claims 1-8, wherein the methods are used for metagenomics for microbial samples.

10. A method of preparing a sequencing library, comprising:

(a) providing a first transposome, the first transposome comprising a first transposon and a first transposase enzyme having a first tagmentation profile;

(b) combining a target nucleic acid with the first transposase enzyme under conditions suitable for tagmentation, thereby generating a first tagmented nucleic acid;

(c) combining the first tagmented nucleic acid with a second transposome under conditions suitable for tagmentation, the transposome comprising a second transposon and a second transposase enzyme having a second tagmentation profile, thereby generating a second tagmented nucleic acid and creating a sequencing

library, wherein at least the first or the second transposome is immobilized on a first or second support.

11. The method of claim 10, wherein the first, second, or both transposomes are immobilized on solid supports, optionally wherein the first and the second solid supports are beads.

12. The method of claim 10:
optionally comprising substantially separating the first tagmented nucleic acid from reaction buffer used in step (b); and wherein the method further comprises
amplifying the second tagmented nucleic acid, thereby generating a sequencing library.

13. The method of any one of claims 1-11, further comprising amplifying the first tagmented nucleic acid, or further comprising amplifying the first tagmented nucleic acid and the second tagmented nucleic acid.

14. The method of claim 12, wherein the first, second, or both transposomes are immobilized on solid supports, optionally wherein the solid support is bead.

15. The method of any one of claims 12-14, optionally wherein
a) the first transposon comprises a first adaptor,
b) the second transposon comprises a second adaptor, or
c) the first transposon comprises a first adaptor and the second transposon comprises a second adaptor, wherein the first and the second adaptors are different, optionally
wherein the first and second adaptors comprise a sequence selected from the group consisting of barcodes, primer binding sequences, restriction endonuclease sites, and unique molecular indices,
optionally wherein the first transposase enzyme is selected from the group consisting of Mos-1, HyperMu™, Tn5, Ts-Tn5, Ts-Tn5059, Hermes, and Tn7,

optionally wherein the second transposase enzyme is selected from the group consisting of Mos-1, HyperMu™, Tn5, Ts-Tn5, Ts-Tn5059, Hermes, and Tn7, optionally

wherein the methods are used for meta-genomics for microbial samples,

optionally

i) wherein the two tagmentation profiles have different percent of GC dropout, or

ii) wherein the two tagmentation profiles have different percent of AT dropout.