CLAIMS

1. A method of preparing a sequencing library for determining the methylation status of a target nucleic acid comprising:

a. providing a plurality of solid support comprising immobilized transposome complexes immobilized thereon, wherein the transposome complexes comprise transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, the transferred strand comprising

(i) a first portion at the 3'-end comprising the transposase recognition sequence, and

(ii) a second portion located 5'to the first portion comprising a first adaptor sequence and first member of a binding pair, wherein the first member of the binding pair binds to a second member of the binding pair on the solid support, thereby immobilizes the transposon to the solid support, and wherein the first adaptor comprises a first primer binding sequence;

the non-transferred strand comprising

(i) a first portion at the 5'-end comprising the transposase recognition sequence

(ii) a second portion located 3'to the first portion comprising a second adaptor sequence, wherein the terminal nucleotide at the 3'-end is blocked, and wherein the second adaptor comprises a second primer binding sequence;

b. contacting a target nucleic acid with the plurality of solid support comprising immobilized transposome complexes;

c. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands to the 5' end of at least one strand of the fragments, thereby immobilizing the target nucleic acid fragments to the solid support;

d. extending the 3'-end of the fragmented target nucleic acid with a DNA polymerase;

e. ligating the non-transferred strand to the 3'-end of the fragmented target nucleic acid;

f. subjecting the immobilized target nucleic acid fragments to bisulfite treatment;

g. extending the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment by using a DNA polymerase such that the 3'-end of the immobilized target nucleic acid fragments comprises a homopolymeric tail;

h. incorporating a second adaptor sequence to the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment;

i. amplifying the bisulfite treated target nucleic acid fragments immobilized on solid support using a first and a second primer, thereby producing a sequencing library for determining the methylation status of a target nucleic acid,

wherein the first and second adaptors comprise first and second adaptors comprise a first and second barcodes.

2. A method of preparing a sequencing library for determining the methylation status of a target nucleic acid comprising:

a. contacting the target nucleic acid with transposome complexes, wherein the transposome complexes comprise transposons and transposases, wherein the transposons comprise transferred strands and non-transferred strands, the transferred strand comprising

(i) a first portion at the 3'-end comprising the transposase recognition sequence, and

(ii) a second portion located 5'to the first portion comprising a first adaptor sequence and first member of a binding pair, wherein the first member of the binding pair binds to a second member of the binding pair;

the non-transferred strand comprising

(i) a first portion at the 5'-end comprising the transposase recognition sequence

(ii) a second portion located 3'to the first portion comprising a second adaptor sequence, wherein the terminal nucleotide at the 3'-end is blocked, and wherein the second adaptor comprises a second primer binding sequence,

b. fragmenting the target nucleic acid into a plurality of fragments and inserting plurality of transferred strands to the 5' end of at least one strand of the fragments, thereby immobilizing the target nucleic acid fragments to the solid support;

c. contacting a target nucleic acid fragments comprising the transposon end with the plurality of solid support comprising second member of the binding pair, wherein binding of the first member of the binding pair to the second member of the binding pair immobilizes the target nucleic acid to the solid support;

d. extending the 3'-end of the fragmented target nucleic acid with a DNA polymerase;

e. ligating the non-transferred strand to the 3'-end of the fragmented target nucleic acid;

f. subjecting the immobilized target nucleic acid fragments to bisulfite treatment;

g. extending the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment by using a DNA polymerase such that the 3'-end of the immobilized target nucleic acid fragments comprises a homopolymeric tail;

h. incorporating a second adaptor sequence to the 3'-end of the immobilized target nucleic acid fragments damaged during the bisulfite treatment;

i. amplifying the bisulfite treated target nucleic acid fragments immobilized on solid support using a first and a second primer, thereby producing a sequencing library for determining the methylation status of a target nucleic acid,

wherein the first and second adaptors comprise first and second adaptors comprise a first and second barcodes.