CLAIMS:

1. A method of preparing at least one polynucleotide library strand template, wherein the method comprises:

attaching a first adaptor to a first end of a double-stranded polynucleotide sequence, wherein the first end comprises the 3’ end of the forward strand and the 5’ end of the reverse strand of the double-stranded polynucleotide sequence; and

attaching a second adaptor to a second end of a double-stranded polynucleotide sequence, wherein the second end comprises the 5’ end of the forward strand and the 3’ end of the reverse strand of the double-stranded polynucleotide sequence;

wherein the first adaptor comprises a polynucleotide loop and the second adaptor comprises a base-paired stem and a fork, wherein the fork comprises at least one primer-binding sequence and at least one primer-binding complement sequence;

wherein the first adaptor comprises a first restriction site for a nicking endonuclease and wherein the second adaptor further comprises at least one cleavable site and/or a complement of a cleavable site.

2. The method according to claim 1, wherein the first adaptor comprises a base-paired stem and a loop, wherein the first restriction site is in the base-paired stem or wherein the first restriction site is in the loop.

3. The method according to any preceding claim, wherein the cleavable site and/or a complement of a cleavable site is in the base-paired stem.

4. The method according to any preceding claim, wherein the at least one cleavable site and/or a complement of a cleavable site is a restriction site for a nicking endonuclease, wherein preferably the restriction site is a second restriction site.

5. The method according to any preceding claim, wherein the first adaptor further comprises an affinity tag.

6. A polynucleotide library strand for sequencing comprising a first adaptor, a double-stranded polynucleotide sequence to be identified and a second adaptor;

wherein the first adaptor is attached to a first end of the double-stranded polynucleotide sequence, wherein the first end comprises the 3’ end of the forward strand and the 5’ end of the reverse strand of the double-stranded polynucleotide sequence; and

wherein the second adaptor is attached to a second end of the double-stranded polynucleotide sequence, wherein the second end comprises the 5’ end of the forward strand and the 3’ end of the reverse strand of the double-stranded polynucleotide sequence;

wherein the first adaptor comprises a base-paired stem and a loop; and

wherein the second adaptor comprises a base-paired stem, a primer-binding complement sequence and a primer-binding sequence; and

wherein the first adaptor comprises at least one restriction site for a nicking endonuclease and wherein the second adaptor further comprises at least one cleavable site and/or a complement of a cleavable site,

wherein preferably the cleavable site and/or a complement of a cleavable site is a restriction site for a nicking endonuclease.

7. A method of identifying at least a first region of a polynucleotide sequence, wherein the method comprises:

a. preparing at least one polynucleotide library strand template comprising: attaching a first adaptor to a first end of a double-stranded polynucleotide sequence, wherein the first end comprises the 3’ end of the forward strand and the 5’ end of the reverse strand of the double-stranded polynucleotide sequence; and attaching a second adaptor to a second end of a double-stranded polynucleotide sequence, wherein the second end comprises the 5’ end of the forward strand and the 3’ end of the reverse strand of the double-stranded polynucleotide sequence; wherein the first adaptor comprises a polynucleotide loop and the second adaptor comprises at least one primer-binding sequence and at least one primer-binding complement sequence; wherein the first adaptor comprises a first restriction site for a endonuclease and/or wherein the second adaptor further comprises at least one cleavable site and/or a complement of a cleavable site;

b. amplifying the polynucleotide library strand to generate a first and second library strand, wherein each library strand comprises a first and second region;

c. hybridising the first or second library strands to first and second immobilised primers respectively on a solid support and carrying out a first extension reaction to generate a first or second immobilised template strand;

d. hybridising the first or second immobilised template strands to a second or first immobilised primer respectively and carrying out a second extension reaction to generate a second and first immobilised template strand;

e. hybridising the first and second immobilised template strands;

f. applying a first endonuclease; and

g. sequencing the first and second immobilised template strands, wherein sequencing the first and second immobilised template strands identifies the first region.

8. The method of claim 7 wherein identifying comprises determining the sequences of a first region and/or identifying any epigenetic modification, wherein the epigenetic modification is preferably a modified cytosine.

9. The method of claim 7 or 8, wherein each first and second library strands comprise a primer-binding complement sequence, a first portion, a first adaptor sequence, a second portion and a primer-binding sequence
wherein preferably the primer-binding sequence and primer-binding complement sequence comprises at least one cleavable and/or a complement of a cleavable site; and
wherein the first adaptor comprises a first restriction site for an endonuclease, preferably a restriction site for a nicking endonuclease or a restriction endonuclease.

10. The method of claim 9, wherein the primer-binding sequence and primer-binding complement sequence comprises at least one cleavable and/or a complement of a cleavable site, wherein the cleavable site and/or a complement of a cleavable site is a second restriction site.

11. The method of any of claims 7 to 10, wherein following applying the first endonuclease and cleavage of the first restriction site, non-immobilised library strands are de-hybridised and the immobilised template strands are sequenced by single-stranded SBS (sequencing by synthesis).

12. The method of any of claims 7 to 10, wherein following applying the first endonuclease and cleavage of the first restriction site, the immobilised template strands are sequenced by double-stranded SBS (sequencing by synthesis).

13. The method of any of claims 7 to 12, wherein the method further comprises applying a second nicking endonuclease and sequencing the first and second immobilised template strands identifies the second region, wherein the second nicking endonuclease cleaves a different restriction site from the first nicking endonuclease.

14. An inverted-repeat tandem-insert polynucleotide library strand for sequencing, wherein the library strand comprises a primer-binding complement sequence, a first portion to be identified, a first adaptor sequence, a second portion to be identified and a primer-binding sequence, wherein the sequence of the second portion is inverted with respect to the first portion, wherein the first adaptor comprises a polynucleotide loop and the second adaptor comprises at least one primer-binding sequence and at least one primer-binding complement sequence and wherein the polynucleotide loop comprises at least one restriction site for a endonuclease and/or wherein the second adaptor further comprises at least one cleavable site and/or a complement of a cleavable site.

15. A library preparation kit comprising of a plurality of first adaptors and a plurality of second adaptors, wherein the first adaptors comprise a base-paired stem and a loop, and wherein the first adaptors comprise at least one restriction site for a nicking endonuclease, and wherein the second adaptors comprise a base-paired stem, a primer-binding sequence and a primer-binding complement sequence, wherein the second adaptors comprise at least one restriction site for an endonuclease.