WHAT IS CLAIMED:

1. A library-splint complex (500) comprising:

(i) a single-stranded nucleic acid library molecule (100) which includes a sequence of interest (110) flanked on one side by at least a first left universal adaptor sequence (120) and flanked on the other side by at least a first right universal adaptor sequence (130); and

(ii) a double-stranded splint adaptor (200) which includes a first splint strand (300) and a second splint strand (400), wherein the double-stranded splint adaptor (200) includes a double-stranded region and two flanking single-stranded regions, wherein the first splint strand comprises regions arranged in a 5' to 3' order: (i) a first region (320), (ii) an internal region (310), and (iii) a second region (330);

wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400), wherein the first region of the first splint strand (320) is hybridized to the at least first left universal adaptor sequence (120) of the single-stranded nucleic acid library molecule, and wherein the second region of the first splint strand (330) is hybridized to the at least first right universal adaptor sequence (130) of the single-stranded nucleic acid library molecule, thereby circularizing the single-stranded nucleic acid library molecule to generate a library-splint complex (500).

2. The library-splint complex (500) of claim 1, wherein the single-stranded nucleic acid library molecule (100) further comprises any one or any combination of two or more of:

(i) a second left universal adaptor sequence (140);

(ii) a second right universal adaptor sequence (150);

(iii) a first left index sequence (160);

(iv) a first right index sequence (170);

(v) a first left unique identification sequence (180); and/or

(vi) a first right unique identification sequence (190).

3. The library-splint complex (500) of claim 1 or 2, wherein the first left universal adaptor sequence (120) and/or the second left universal adaptor sequence (140) comprises:

(i) a universal binding sequence for a forward sequencing primer;

(ii) a universal binding sequence for a reverse sequencing primer;

(iii) a universal binding sequence for a first surface primer;

(iv) a universal binding sequence for a second surface primer;

(v) a universal binding sequence for a forward amplification primer;

(vi) a universal binding sequence for a reverse amplification primer; and/or

(vii) a universal binding sequence for a compaction oligonucleotide.

4. The library-splint complex (500) of any one of claims 1-3, wherein the first right universal adaptor sequence (130) and/or the second right universal adaptor sequence (150) comprises:

(i) a universal binding sequence for a forward sequencing primer;

(ii) a universal binding sequence for a reverse sequencing primer;

(iii) a universal binding sequence for a first surface primer;

(iv) a universal binding sequence for a second surface primer;

(v) a universal binding sequence for a forward amplification primer;

(vi) a universal binding sequence for a reverse amplification primer; and/or

(vii) a universal binding sequence for a compaction oligonucleotide.

5. The library-splint complex (500) of claim 3 or 4, wherein:

(i) the second splint strand (400) includes at least two sub-regions: a first sub-region comprising a universal binding sequence for a third surface primer, and a second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and the second sub-regions do not hybridize to or at least exhibit very little hybridization to the first and the second surface primers; or

(ii) the first splint strand (300) comprises an internal region (310) comprising at least two sub-regions, a fourth sub-region comprising a universal binding sequence for a third surface primer, wherein the fourth sub-region hybridizes to the first sub-region of the second splint strand (400), and a fifth sub-region comprising a universal binding sequence for a fourth surface primer, wherein the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), and wherein the fourth and fifth sub-regions do not hybridize to or at least exhibit very little hybridization to the first and second surface primers.

6. The library-splint complex of claim 5, wherein the second splint strand (400) further comprises a third sub-region which comprises a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases.

7. The library-splint complex (500) of claim 5 or 6, wherein the internal region (310) of the first splint strand comprises a sixth sub-region comprising a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases, wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

8. The library-splint complex (500) of any one of claims 1-7, further comprising

a) a first nick between the 5’ end of the single-stranded nucleic acid library molecule and the 3’ end of the second splint strand, and/or

b) a second nick between the 5’ end of the second splint strand and the 3’ end of the single-stranded nucleic acid library molecule,

wherein the first nick and/or the second nicks are enzymatically ligatable.

9. A method for forming a plurality of library-splint complexes (500) comprising:

a) providing a plurality of double-stranded splint adaptors, wherein individual double-stranded splint adaptors (200) comprise a first splint strand (300) hybridized to a second splint strand (400), wherein individual double-stranded splint adaptors include a double-stranded region and two flanking single-stranded regions, wherein the first splint strand comprises regions arranged in a 5’ to 3’ order: (i) a first region

(320), (ii) an internal region (310), and (iii) a second region (330), and wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400); and

b) hybridizing the plurality of double-stranded splint adaptors with a plurality of single-stranded nucleic acid library molecules (100), wherein individual single-stranded nucleic acid library molecules include a sequence of interest (110) flanked on one side by at least a first left universal adaptor sequence (120) and flanked on the other side by at least a first right universal adaptor sequence (130), wherein the hybridizing is conducted under a condition suitable to hybridize the first region of the first splint strand (320) to the at least first left universal adaptor sequence (120) of the single-stranded nucleic acid library molecule, and the condition is suitable to hybridize the second region of the first splint strand (330) to the at least a first right universal adaptor sequence (130) of the single-stranded nucleic acid library molecule, thereby circularizing the plurality of single-stranded nucleic acid library molecules to form a plurality of library-splint complexes (500).

10. The method of claim 9, wherein:

(a) individual library-splint complexes (500) in the plurality comprise a first nick between the 5’ end of the library molecule and the 3’ end of the second splint strand, and a second nick between the 5’ end of the second splint strand and the 3’ end of the library molecule; or

(b) individual single-stranded nucleic acid library molecules (100) in the plurality further comprise any one or any combination of two or more of:

(i) a second left universal adaptor sequence (140);

(ii) a second right universal adaptor sequence (150);

(iii) a first left index sequence (160);

(iv) a first right index sequence (170);

(v) a first left unique identification sequence (180); and/or

(vi) a first right unique identification sequence (190).

11. The method of claim 10, wherein the first and/or the second left universal adaptor sequence comprises:

(a)

(i) a universal binding sequence for a forward sequencing primer;

(ii) a universal binding sequence for a reverse sequencing primer;

(iii) a universal binding sequence for a first surface primer;

(iv) a universal binding sequence for a second surface primer;

(v) a universal binding sequence for a forward amplification primer;

(vi) a universal binding sequence for a reverse amplification primer; and/or

(vii) a universal binding sequence for a compaction oligonucleotide; or

(b) wherein the first and/or the second right universal adaptor sequence comprises:

(i) a universal binding sequence for a forward sequencing primer;

(ii) a universal binding sequence for a reverse sequencing primer;

(iii) a universal binding sequence for a first surface primer;

(iv) a universal binding sequence for a second surface primer;

(v) a universal binding sequence for a forward amplification primer;

(vi) a universal binding sequence for a reverse amplification primer; and/or

(vii) a universal binding sequence for a compaction oligonucleotide.

12. The method of claim 11, wherein the second splint strand (400) comprises at least two sub-regions, a first sub-region comprising a universal binding sequence for a third surface primer, and a second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and the second sub-regions do not hybridize to or at least exhibit very little hybridization to the first and the second surface primers.

13. The method of claim 12, wherein the second splint strand (400) further comprises a third sub-region which comprises a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases.

14. The method of claim 12 or 13, wherein the internal region (310) of the first splint strand comprises at least two sub-regions, a fourth sub-region comprising a universal binding sequence for the third surface primer, wherein the fourth sub-region hybridizes to the first sub-region of the second splint strand (400); and a fifth sub-region comprising a universal binding sequence for the fourth surface primer, wherein the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), wherein the fourth and the fifth sub-regions do not hybridize to or at least exhibit very little hybridization to the first and second surface primers.

15. The method of claim 14, wherein the internal region (310) of the first splint strand further comprises a sixth sub-region comprising a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases, wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

16. The method of any one of claims 9-15, further comprising: contacting the plurality of library-splint complexes (500) with a ligase, thereby generating a plurality of covalently closed circular library molecules (600) hybridized to the first splint strand (300).

17. The method of claim 16, further comprising distributing the plurality of covalently closed circular library molecules (600) onto a support, wherein the support comprises a plurality of surface capture primers immobilized thereon, and converting the plurality of covalently closed circular library molecules (600) into immobilized nucleic acid concatemer template molecules by conducting a rolling circle amplification reaction on the support.

18. The method of claim 16 or 17, further comprising: contacting the plurality of covalently closed circular library molecules (600) with at least one exonuclease enzyme to remove the first splint strands (300) and retaining the plurality of covalently closed circular library molecules (600).

19. The method of any one of claims 16-18, wherein the second splint strand (400) comprises a universal binding sequence for a third surface primer, and wherein the method further comprises: c) distributing the plurality of covalently closed circular library molecules (600) onto a support having a plurality of the third surface primers immobilized on the support, under a condition suitable for hybridizing individual covalently closed circular library molecules (600) to individual immobilized third surface primers, thereby immobilizing the plurality of covalently closed circular library molecules (600).

20. The method of claim 19, further comprising: contacting the plurality of immobilized covalently closed circular library molecules (600) with a plurality of strand-displacing polymerases and a plurality of nucleotides, under a condition suitable to conduct a rolling circle amplification reaction on the support using the plurality of third surface primers as immobilized amplification primers and the plurality of covalently closed circular library molecules (600) as template molecules, thereby generating a plurality of immobilized nucleic acid concatemer molecules as template molecules, thereby generating a plurality of immobilized nucleic acid concatemer template molecules.

21. The method of claim 20, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

22. The method of claim 20 or 21, further comprising: sequencing the plurality of immobilized nucleic acid concatemer molecules, the method comprising:

    a) contacting the plurality of immobilized nucleic acid concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of soluble sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of complexed polymerases comprising a sequencing polymerase bound to a nucleic acid duplex, wherein the nucleic acid duplex comprises a nucleic acid concatemer molecule hybridized to a soluble sequencing primer;

    b) contacting the plurality of complexed sequencing polymerases with a plurality of nucleotides under a condition suitable for binding at least one nucleotide to a

complexed sequencing polymerase, wherein the plurality of nucleotides comprises at least one nucleotide analog labeled with a fluorophore and having a removable chain terminating moiety at a sugar 3' position;

c) incorporating the at least one nucleotide into a 3' end of the soluble sequencing primers, thereby generating a plurality of nascent extended sequencing primers; and

d) detecting the incorporated nucleotide and identifying the nucleo-base of the incorporated nucleotide.

23. The method of any one of claims 20 or 21, further comprising: sequencing the plurality of immobilized nucleic acid concatemer molecules, the method comprising:

a) contacting the plurality of immobilized nucleic acid concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of soluble sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of first complexed polymerases comprising a sequencing polymerase bound to a nucleic acid duplex, wherein the nucleic acid duplex comprises a nucleic acid concatemer molecule hybridized to a soluble sequencing primer;

b) contacting the plurality of complexed sequencing polymerases with a plurality of detectably labeled multivalent molecules to form a plurality of multivalent-complexed polymerases, under a condition suitable for binding complementary nucleotide units of the multivalent molecules to at least two of the plurality of first complexed polymerases thereby forming a plurality of multivalent-complexed polymerases, and the condition inhibits incorporation of the complementary nucleotide units into the sequencing primers of the plurality of multivalent-complexed polymerases, wherein individual multivalent molecules in the plurality of multivalent molecules comprise a core attached to multiple nucleotide arms and individual nucleotide arms are attached to a nucleotide unit;

c) detecting the plurality of multivalent-complexed polymerases; and

d) identifying the nucleo-bases of the complementary nucleotide units that are bound to the plurality of first complexed polymerases in the plurality of multivalent-complexed polymerases, thereby determining the sequence of the nucleic acid template.

24. The method of claim 23, further comprising:

e) dissociating the plurality of multivalent-complexed polymerases and removing the plurality of first sequencing polymerases and bound multivalent molecules, and retaining the plurality of nucleic acid duplexes;

f) contacting the plurality of nucleic acid duplexes of step (e) with a plurality of second sequencing polymerases, wherein the contacting is conducted under a condition suitable for binding the plurality of second sequencing polymerases to the plurality of nucleic acid duplexes, thereby forming a plurality of second complexed polymerases comprising a second sequencing polymerase bound to a nucleic acid duplex; and

g) contacting the plurality of second complexed polymerases with a plurality of nucleotides, wherein the contacting is conducted under a condition suitable for binding complementary nucleotides from the plurality of nucleotides to at least two of the second complexed polymerases of step (f) thereby forming a plurality of nucleotide-complexed polymerases and the condition is suitable for promoting incorporation of the bound complementary nucleotides into the sequencing primers of the nucleotide-complexed polymerases.

25. The method of claim 24, wherein the plurality of nucleotides includes at least one nucleotide analog labeled with a fluorophore and having a removable chain terminating moiety at the sugar 3' position.

26. The method of claim 24, further comprising:

h) detecting the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases; and

i) identifying the nucleo-bases of the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases.