sequences, which are compatible for downstream next generation sequencing technologies. Provided herein are compositions, methods and kits addressing this need.

SUMMARY

[0004a] In a first aspect, the present disclosure provides 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).

[0004b] In a second aspect, the present disclosure provides 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).

[0005] The disclosure provides 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 (long splint strand (300)) and a second splint strand (short 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 a first region (320), an internal region (310), and 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 library molecule, and wherein a second region of the first splint strand (330) is hybridized to the at least first right universal sequence (130) of the library molecule, thereby circularizing the library molecule to generate a library-splint complex (500).

[0006] In some examples, the nucleic acid library molecule (100) further comprises: a second left universal adaptor sequence (140). In some examples, the nucleic acid library molecule (100) further comprises: a second right universal adaptor sequence (150). In some examples, the nucleic acid library molecule (100) further comprises: a first left index sequence (160). In some examples, the nucleic acid library molecule (100) further comprises: a first right index sequence (170). In some examples, the nucleic acid library molecule (100) further comprises: a first left unique identification sequence (180). In some examples, the nucleic acid library molecule (100) further comprises: a first right unique identification sequence (190).

[0007] In some examples, the nucleic acid library molecule (100) further comprises any one or any combination of two or more: (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).

[0008] In some examples, 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0009] In some examples, 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0010] In some examples, the second splint strand (400) includes at least two sub-regions, the first sub-region comprising a universal binding sequence for a third surface primer, and the second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and second sub-regions do not hybridize to (or at least exhibit very little hybridization to) the first and second surface primers.

[0011] In some examples, the second splint strand (400) further comprises an optional 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 (e.g., NN).

[0012] In some examples, the first splint strand (300) includes an internal region (310) comprising at least two sub-regions, the fourth sub-region comprising a universal binding sequence for a third surface primer and the fourth sub-region hybridizes to the first sub-region of the second splint strand (400), and the fifth sub-region comprising a universal binding sequence for a fourth surface primer and the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), 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. In some examples, the first splint strand (300) includes an internal region (310) which further comprises a sixth sub-region which comprises a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases (e.g., NN), wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

[0013] The disclosure provides a library-splint complex (500) comprising: **a)** a single-stranded nucleic acid library molecule (100) comprising components arranged in a 5’ to 3’

order: (i) a first left universal adaptor sequence (120) having a binding sequence for a first surface primer (120); (ii) a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer; (iii) a sequence of interest (110); (iv) a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; and (v) a first right universal adaptor sequence (130) having a binding sequence for a second surface primer (130); **b)** a first splint strand (300) comprising components arranged in a 5' to 3' order: a first region (320); an internal region (310); and a second region (330); and **c)** a second splint strand (400) comprising sub-regions arranged in a 3' to 5' order: a first sub-region having a universal binding sequence for a third surface primer; and a second sub-region having a universal binding sequence for a fourth surface primer; wherein the first splint strand (300) is hybridized to portions of the library molecule (100) thereby circularizing the library molecule to generate a library-splint complex (500), such that the first region (320) of the first splint strand is hybridized to the binding sequence for the first surface primer (120), and the third region (330) of the first splint strand is hybridized to the binding sequence for the second surface primer (130), wherein the second splint strand (400) is hybridized to the internal region (310) of the first splint strand (300), wherein the library-splint complex (500) comprises a first nick between the 5' end of the library molecule and the 3' end of the second splint strand, wherein the library-splint complex (500) comprises a second nick between the 5' end of the second splint strand and the 3' end of the library molecule, and wherein the first and second nicks are enzymatically ligatable.

[0014] In some examples, the library-splint complex (500) further comprising a plurality of library-splint complexes (500), wherein the sequence of interest (110) of individual library-splint complexes in the plurality comprise the same sequence of interest or different sequences of interest.

[0015] The disclosure provides a reaction mixture comprising the plurality of library-splint complexes (500) of the disclosure and T4 polynucleotide kinase. The disclosure provides a reaction mixture comprising the plurality of library-splint complexes (500) of the disclosure and a ligase enzyme. The disclosure provides a reaction mixture comprising the plurality of library-splint complexes (500) of the disclosure, T4 polynucleotide kinase, and a ligase enzyme. In some examples, the ligase enzyme comprises T7 DNA ligase, T3 ligase, T4 ligase, or Taq ligase.

[0016] The disclosure provides a covalently closed circular library molecule (600) comprising: a sequence of interest (110), at least a first left universal adaptor sequence (120),

at least a first right universal adaptor sequence (130), and a second splint strand sequence (400).

[0017] In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a second left universal adaptor sequence (140). In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a second right universal adaptor sequence (150). In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a first left index sequence (160). In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a first right index sequence (170). In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a first left unique identification sequence (180). In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: a first right unique identification sequence (190).

[0018] In some examples, the covalently closed circular library molecule (600) of the disclosure further comprises: any one or any combination of two or more: (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).

[0019] In some examples, 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0020] In some examples, 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0021] In some examples, the closed circular library molecule (600) of the disclosure is hybridized to a first splint strand (long splint strand (300)) which comprises a first region

(320), an internal region (310), and a second region (330), wherein the second splint strand sequence (400) of the covalently closed circular library molecule is hybridized to the internal region (310) of the first splint strand, wherein the first region of the first splint strand (320) is hybridized to the at least first left universal adaptor sequence (120) of the library molecule, and wherein a second region of the first splint strand (330) is hybridized to the at least first right universal sequence (130) of the library molecule.

[0022] In some examples, the second splint strand (400) includes at least two sub-regions, the first sub-region comprising a universal binding sequence for a third surface primer, and the second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and second sub-regions do not hybridize to (or at least exhibit very little hybridization to) the first and second surface primers.

[0023] In some examples, the second splint strand (400) further comprises an optional 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 (e.g., NN).

[0024] In some examples, the first splint strand (300) includes an internal region (310) comprises at least two sub-regions, the fourth sub-region comprising a universal binding sequence for a third surface primer and the fourth sub-region hybridizes to the first sub-region of the second splint strand (400), and the fifth sub-region comprising a universal binding sequence for a fourth surface primer and the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), wherein the fourth and fifth sub-regions do not hybridize (or at least exhibit very little hybridization to) the first and second surface primers. In some examples, the first splint strand (300) includes an internal region (310) which further comprises a sixth sub-region which comprises a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases (e.g., NN), wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

[0025] The disclosure provides a single-stranded covalently closed circular molecule (600) comprising: **(i)** a first left universal adaptor sequence (120) having a binding sequence for a first surface primer (120); **(ii)** a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer; **(iii)** a sequence of interest (110); **(iv)** a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; **(v)** a first right universal adaptor sequence (130) having a binding sequence for a second surface primer (130); and (vi) a second splint strand (400), wherein the covalently closed circular molecule (600) is optionally hybridized to the first splint strand (300).

[0026] In some examples, the covalently closed circular library molecule (600) further comprises a plurality of covalently closed circular library molecules (600), wherein the sequence of interest (110) of individual covalently closed circular library molecules (600) in the plurality comprise the same sequence of interest or different sequences of interest.

[0027] The disclosure provides a reaction mixture comprising the plurality of plurality of covalently closed circular library molecules (600) of the disclosure, wherein individual covalently closed circular library molecules (600) are hybridized to a first splint strand (300). In some examples, plurality of covalently closed circular library molecules (600) and at least one exonuclease enzyme. In some examples, the exonuclease enzyme comprises exonuclease I, thermolabile exonuclease I, or T7 exonuclease.

[0028] The disclosure provides a kit, comprising: a double-stranded splint adaptor (200) which includes **(i)** a first splint strand (long splint strand (300)) and **(ii)** a second splint strand (short splint strand (400)), wherein the first splint strand (300) can hybridize to the second splint strand (400) to form a double-stranded region and two flanking single-stranded regions, wherein the first splint strand (300) comprises a first region (320), an internal region (310), and a second region (330), and wherein the internal region of the first splint strand (310) can hybridize to the second splint strand (400). In some examples, the first splint strand (300) and the second splint strand (400) are hybridized to each other to form a double-stranded splint adaptor (200) having a double-stranded region and two flanking single-stranded regions, and wherein the internal region of the first splint strand (310) can hybridize to the second splint strand (400).

[0029] In some examples, the kit of the disclosure is for the use in circularizing a single-stranded nucleic acid library molecule (100) having 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). In some examples, the first region of the first splint strand (320) can hybridize to at least first left universal adaptor sequence (120) of the library molecule (100), and wherein a second region of the first splint strand (330) can hybridize to the at least first right universal sequence (130) of the library molecule. In some examples, the at least first left universal adaptor sequence (120) of the library molecule (100) 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0030] In some examples, the at least first right universal adaptor sequence (130) of the library molecule (100) 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0031] In some examples, the second splint strand (400) includes at least two sub-regions, the first sub-region comprising a universal binding sequence for a third surface primer, and the second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and second sub-regions do not hybridize (or at least exhibit very little hybridization to) the first and second surface primers. In some examples, the second splint strand (400) further comprises an optional 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 (e.g., NN).

[0032] In some examples, the first splint strand (300) includes an internal region (310) comprising at least two sub-regions, the fourth sub-region comprising a universal binding sequence for a third surface primer and the fourth sub-region hybridizes to the first sub-region of the second splint strand (400), and the fifth sub-region comprising a universal binding sequence for a fourth surface primer and the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), wherein the fourth and fifth sub-regions do not hybridize (or at least exhibit very little hybridization to) the first and second surface primers. In some examples, the first splint strand (300) includes an internal region (310) which further comprises a sixth sub-region which comprises a sample index sequence having 5-20 bases and/or a unique identification sequence having 2-10 or more bases (e.g., NN), wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

[0033] In some examples, the kit of the disclosure further comprises a T4 polynucleotide kinase. In some examples, the kit of the disclosure further comprises a ligase enzyme. In some examples, the kit of the disclosure further comprises a T4 polynucleotide kinase and a ligase enzyme. In some examples, the ligase enzyme comprises T7 DNA ligase, T3 ligase, T4 ligase, or Taq ligase.

[0034] The disclosure provides 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 the double-stranded splint adaptor includes a double-stranded region and two flanking single-stranded regions, wherein the first splint strand comprises a first region (320), an internal region (310), and 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 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 library molecule, and the condition is suitable to hybridize the second region of the first splint strand (330) to the at least first right universal sequence (130) of the library molecule, thereby circularizing the plurality of library molecules to form a plurality of library-splint complexes (500).

[0035] In some examples, the 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 wherein individual library-splint complexes (500) in the plurality comprise a second nick between the 5’ end of the second splint strand and the 3’ end of the library molecule. In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a second left universal adaptor sequence (140). In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a second right universal adaptor sequence (150). In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a first left index sequence (160). In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a first right index sequence (170). In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a first left unique identification sequence (180). In some examples, the individual nucleic acid library molecules (100) in the plurality further comprise: a first right unique identification sequence (190).

[0036] In some examples, the individual 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). In some examples, the first and/or the second left 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0037] In some examples, 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 an first surface primer; (iv) a universal binding sequence for an 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.

[0038] In some examples, the second splint strand (400) includes at least two sub-regions, the first sub-region comprising a universal binding sequence for a third surface primer, and the second sub-region comprising a universal binding sequence for a fourth surface primer, wherein the first and second sub-regions do not hybridize to (or at least exhibit very little hybridization to) the first and second surface primers. In some examples, the second splint strand (400) further comprises an optional 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 (e.g., NN).

[0039] In some examples, the first splint strand (300) includes an internal region (310) comprising at least two sub-regions, the fourth sub-region comprising a universal binding sequence for a third surface primer and the fourth sub-region hybridizes to the first sub-region of the second splint strand (400), and the fifth sub-region comprising a universal binding sequence for a fourth surface primer and the fifth sub-region hybridizes to the second sub-region of the second splint strand (400), wherein the fourth and fifth sub-regions do not hybridize (or at least exhibit very little hybridization to) the first and second surface primers. In some examples, the first splint strand (300) includes an internal region (310) which further comprises a sixth sub-region which comprises a sample index sequence having 5-20

bases and/or a unique identification sequence having 2-10 or more bases (e.g., NN), wherein the sixth sub-region hybridizes to the third sub-region of the second splint strand (400).

[0040] The disclosure provides a method for forming a plurality of library-splint complexes (500) comprising: **a)** providing a plurality of double-stranded splint adaptors (200) wherein individual double-stranded splint adaptors (200) comprise a first splint strand (300) hybridized to a second splint strand (400), wherein the first splint strand (300) comprises regions arranged in a 5' to 3' order a first region (320), an internal region (310), and a second region (330), and wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400), wherein the second splint strand comprises regions arranged in a 5' to 3' order **(i)** a second sub-region having a universal binding sequence for a fourth surface primer, and **(ii)** a first sub-region having a universal binding sequence for a third surface primer; and **b)** hybridizing the plurality of double-stranded splint adaptors with a plurality of single-stranded nucleic acid library molecules (100) wherein individual library molecules comprise regions arranged in a 5' to 3' order: **(i)** a first left universal adaptor sequence (120) having a binding sequence for a first surface primer (120); **(ii)** a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer; **(iii)** a sequence of interest (110); **(iv)** a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; and **(v)** a first right universal adaptor sequence (130) having a binding sequence for a second surface primer (130), wherein the hybridizing is conducted under a condition suitable to hybridize portions of the first splint strand (300) to portions of the library molecule (100) thereby circularizing the library molecule to generate a library-splint complex (500), such that the first region (320) of the first splint strand is hybridized to the binding sequence for the first surface primer (120), and the third region (330) of the first splint strand is hybridized to the binding sequence for the second surface primer (130), wherein the library-splint complex (500) comprises a first nick between the 5' end of the library molecule and the 3' end of the second splint strand (300), wherein the library-splint complex (500) comprises a second nick between the 5' end of the second splint strand (300) and the 3' end of the library molecule (100), and wherein the first and second nicks are enzymatically ligatable.

[0041] In some examples, the plurality of single-stranded nucleic acid library molecules (100) further comprises a first left index sequence (160) and/or a first right index sequence (170). In some examples, the plurality of single-stranded nucleic acid library molecules (100) further comprises a first left unique identification sequence (180) and/or a first right unique identification sequence (190).

[0042] In some examples, the method further comprises: contacting the plurality of the double-stranded splint adaptors (200) and the plurality of the single-stranded nucleic acid library molecules (100) with a T4 polynucleotide kinase enzyme under a condition suitable to phosphorylate the 5’ ends of the plurality of double-stranded splint adaptors (200) and/or the plurality of single-stranded nucleic acid library molecules (100).

[0043] In some examples, the method further comprises: contacting the plurality of the double-stranded splint adaptors (200) and the plurality of the single-stranded nucleic acid library molecules (100) with a ligase, under a condition suitable to enzymatically ligate the first and second nicks, thereby generating a plurality of covalently closed circular library molecules (600) each hybridized to the first splint strand (300).

[0044] In some examples, the method further comprising conducting the sequential steps of: **a)** contacting the plurality of the double-stranded splint adaptors (200) and the plurality of the single-stranded nucleic acid library molecules (100) with a T4 polynucleotide kinase enzyme under a condition suitable to phosphorylate the 5’ ends of the plurality of double-stranded splint adaptors (200) and the plurality of single-stranded nucleic acid library molecules (100); and **b)** contacting the phosphorylated double-stranded splint adaptors (200) and the phosphorylated single-stranded nucleic acid library molecules (100) with a ligase under a condition suitable to enzymatically ligate the first and second nicks, thereby generating a plurality of covalently closed circular library molecules (600) each hybridized to the first splint strand (300).

[0045] In some examples, the method further comprises conducting the essentially simultaneous steps of contacting the plurality of the double-stranded splint adaptors (200) and the plurality of the single-stranded nucleic acid library molecules (100) with a (i) T4 polynucleotide kinase enzyme and (ii) a ligase enzyme, under a condition suitable to phosphorylate the 5’ ends of the plurality of double-stranded splint adaptors (200) and the plurality of single-stranded nucleic acid library molecules (100), and the conditions are suitable to enzymatically ligate the first and second nicks, thereby generating a plurality of covalently closed circular library molecules (600) each hybridized to the first splint strand (300).

[0046] In some examples, the method further comprises contacting the plurality of covalently closed circular library molecules (600) with at least one exonuclease enzyme to remove the plurality of first splint strands (300) and retaining the plurality of covalently closed circular library molecules (600). In some examples, the at least one exonuclease enzyme comprises exonuclease I, thermolabile exonuclease I and/or T7 exonuclease.

[0047] In some examples, the individual covalently closed circular library molecules (600) in the plurality comprise a second splint strand region (400) which includes a universal binding sequence for a third surface primer, and wherein the method further comprises: distributing the retained 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).

[0048] In some examples, the method further comprises: 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. In some examples, the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0049] In some examples, the first (120) or the second (140) left universal adaptor sequence of individual library molecules in the plurality comprise a universal binding sequence for a forward amplification primer, and the method further comprises: **a)** hybridizing a plurality of soluble forward amplification primers to the first or second left universal adaptor sequence which comprise the universal binding sequence for a forward amplification primer; and **b)** conducting a rolling circle amplification reaction thereby generating a plurality of nucleic acid concatemer molecules.

[0050] In some examples, the first (130) or the second (150) right universal adaptor sequence of individual library molecules in the plurality comprise a universal binding sequence for a forward amplification primer, and the method further comprises: **a)** hybridizing a plurality of soluble forward amplification primers to the first or second right universal adaptor sequence which comprise the universal binding sequence for a forward amplification primer; and **b)** conducting a rolling circle amplification reaction thereby generating a plurality of nucleic acid concatemer molecules each hybridized to a covalently closed circular library molecule (600).

[0051] In some examples, the individual concatemer molecules in the plurality comprise a second splint strand region (400) having a universal binding sequence for third surface primer, wherein individual concatemer molecules are hybridized to a covalently

closed circular library molecule (600), and the method further comprises: distributing the plurality of concatemer molecules wherein onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules.

[0052] In some examples, the method further comprises: contacting the immobilized plurality of concatemer molecules (which are hybridized to a covalently closed circular library molecule (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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules. In some examples, the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0053] In some examples, individual covalently closed circular library molecules (600) in the plurality are hybridized to a first splint strand (300), and the method further comprises: contacting the plurality of covalently closed circular library molecules (600) with a plurality of strand-displacing polymerases and a plurality of nucleotides under a condition suitable for conducting a rolling circle amplification reaction using the first splint strand (300) as an amplification primer thereby generating a plurality of concatemer molecules.

[0054] In some examples, individual concatemer molecules in the plurality comprise a second splint strand region (400) which includes a universal binding sequence for third surface primer, and the method further comprises: distributing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600) onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600).

[0055] In some examples, the method further comprises: contacting the plurality of immobilized concatemer molecules 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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules. In some examples, the plurality of nucleotides comprises dATP,

dGTP, dCTP, dTTP and/or dUTP. In some examples, the support further comprises a plurality of the fourth surface primers immobilized on the support.

[0056] In some examples, the plurality of immobilized nucleic acid concatemer molecules comprise at least two second splint strand regions (400) each having a universal binding sequence for the third and fourth surface primer, and the method further comprises: incubating the plurality of immobilized nucleic acid concatemer molecules under a condition suitable for hybridizing at least one second splint strand region (400) of at least one concatemer molecule to an immobilized fourth surface primer thereby pinning down at least one portion of the immobilized concatemer molecule to the support.

[0057] In some examples, the method further comprises: sequencing the plurality of immobilized concatemers, the method comprising: **a)** contacting the plurality of immobilized concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of the soluble sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of complexed polymerases each comprising a sequencing polymerase bound to a nucleic acid duplex wherein the nucleic acid duplex comprises a 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 the sugar 3’ position; **c)** incorporating at least one nucleotide into the 3’ end of the hybridized 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.

[0058] In some examples, the method further comprises: sequencing the plurality of immobilized concatemers, the method comprising: **a)** contacting the plurality of immobilized concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of the soluble sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of first complexed polymerases each comprising a sequencing polymerase bound to a nucleic acid duplex wherein the nucleic acid duplex comprises a 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 each nucleotide arm is attached to a nucleotide unit; **c)** detecting the plurality of multivalent-complexed polymerases; and **d)** identifying the nucleo-base 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.

[0059] In some examples, the method further comprises: **e)** dissociating the plurality of multivalent-complexed polymerases and removing the plurality of first sequencing polymerases and their bound multivalent molecules, and retaining the plurality of nucleic acid duplexes; **f)** contacting the plurality of the retained 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 the retained nucleic acid duplexes, thereby forming a plurality of second complexed polymerases each comprising a second sequencing polymerase bound to a retained nucleic acid duplex; **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. In some examples, 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.

[0060] In some examples, the method further comprises: **h)** detecting the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases. In some examples, the method further comprises: **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.

[0061] The disclosure provides a method for forming at least one avidity complex, comprising: **a)** binding a first universal nucleic acid primer, a first DNA polymerase, and a

first multivalent molecule to a first portion of any of the concatemer molecules of any one of claims 80, 85, 89 or 92, thereby forming a first binding complex, wherein a first nucleotide unit of the first multivalent molecule binds to the first DNA polymerase; and **b)** binding a second universal nucleic acid primer, a second DNA polymerase, and the first multivalent molecule to a second portion of the same concatemer template molecule thereby forming a second binding complex, wherein a second nucleotide unit of the first multivalent molecule binds to the second DNA polymerase, wherein the first and second binding complexes which include the same multivalent molecule forms an avidity complex, wherein the first multivalent molecule comprises a core attached to multiple nucleotide arms and each nucleotide arm is attached to a nucleotide unit, and wherein the concatemer molecule comprises two or more tandem repeat sequences of a sequence of interest (110) and a universal primer binding site that binds the first and second universal nucleic acid primers.

[0062] The disclosure provides a method for sequencing by forming at least one avidity complex, comprising: **a)** binding a first universal nucleic acid primer, a first DNA polymerase, and a first multivalent molecule to a first portion of any of the concatemer molecules of the disclosure, thereby forming a first binding complex, wherein a first nucleotide unit of the first multivalent molecule binds to the first DNA polymerase; **b)** binding a second universal nucleic acid primer, a second DNA polymerase, and the first multivalent molecule to a second portion of the same concatemer template molecule thereby forming a second binding complex, wherein a second nucleotide unit of the first multivalent molecule binds to the second DNA polymerase, wherein the first and second binding complexes which include the same multivalent molecule forms an avidity complex, wherein the first multivalent molecule comprises a core attached to multiple nucleotide arms and each nucleotide arm is attached to a nucleotide unit, wherein the concatemer molecule comprises two or more tandem repeat sequences of a sequence of interest (110) and a universal primer binding site that binds the first and second universal nucleic acid primers, and wherein the contacting is conducted under a condition suitable to inhibit polymerase-catalyzed incorporation of the bound first and second nucleotide units in the first and second binding complexes; **c)** detecting the first and second binding complexes on the same concatemer template molecule, and **d)** identifying the first nucleotide unit in the first binding complex thereby determining the sequence of the first portion of the concatemer template molecule, and identifying the second nucleotide unit in the second binding complex thereby determining the sequence of the second portion of the concatemer template molecule.

[0063] The disclosure provides a method for preparing a multiplex mixture of sequences-of-interest isolated from a plurality of sample sources, comprising: **a)** providing two or more populations of single-stranded nucleic acid library molecules (100), each population of library molecules (100) contained in a separate compartment, wherein the nucleic acid library molecules in a given population comprise **(i)** a first left universal adaptor sequence (120) having a binding sequence for a first surface primer, **(ii)** a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer; **(iii)** a sequence of interest (110) isolated from a sample source, **(iv)** a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; **(v)** a first right universal adaptor sequence (130) having a binding sequence for a second surface primer, and **(vii)** a first left index sequence (160) and/or a first right index sequence (170), wherein the sequences of the left (160) and/or right (170) indexes separately or in combination identify the sample source from which the sequences of interest (110) have been isolated; **b)** providing a plurality of double-stranded splint adaptors (200) wherein individual double-stranded splint adaptors (200) comprise a first splint strand (300) hybridized to a second splint strand (400), wherein the first splint strand (300) comprises regions arranged in a 5' to 3' order a first region (320), an internal region (310), and a second region (330), and wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400), wherein the second splint strand comprises regions arranged in a 5' to 3' order **(i)** a second sub-region having a universal binding sequence for a fourth surface primer, and **(ii)** a first sub-region having a universal binding sequence for a third surface primer; **c)** contacting in the separate compartments the population of single-stranded nucleic acid library molecules (100) with a plurality of double-stranded splint adaptors (200), wherein the contacting is conducted under a condition suitable to hybridize portions of the first splint strand (300) to portions of the library molecules (100) thereby circularizing the library molecules to generate a population of library-splint complexes (500), such that the first region (320) of an individual first splint strand is hybridized to the binding sequence for the first surface primer (120) of an individual library molecule (100), and the third region (330) of the individual first splint strand is hybridized to the binding sequence for the second surface primer (130) of the individual library molecule (100), wherein each of the library-splint complexes (500) comprise a first nick between the 5' end of the library molecule and the 3' end of the second splint strand (300), wherein each of the library-splint complexes (500) comprises a second nick between the 5' end of the second splint strand (300) and the 3' end of the library molecule (100), and wherein the first and second nicks are enzymatically ligatable; **d)**

contacting in the separate compartments the populations of library-splint complexes (500) with a ligase, under a condition suitable to enzymatically ligate the first and second nicks, thereby generating two or more populations of covalently closed circular library molecules (600) each hybridized to the first splint strand (300); and **e)** pooling together the two or more populations of covalently closed circular library molecules (600) from the separate compartments to generate a multiplex mixture of covalently closed circular library molecules (600) which comprise the multiplex mixture of sequences-of-interest isolated from a plurality of sample sources.

[0064] In some examples, the individual covalently closed circular library molecules (600) in the multiplex mixture of step (e) comprise a second splint strand region (400) which includes a universal binding sequence for a third surface primer, and the method further comprises: **a)** distributing the multiplex mixture 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); and **b)** contacting the multiplex mixture 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, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0065] In some examples, the method further comprises: conducting a rolling circle amplification reaction by: **a)** contacting the two or more populations of covalently closed circular library molecules (600) that are hybridized to the first splint strand (300) of step (d) or (e) with at least one exonuclease enzyme under a condition suitable to remove the plurality of first splint strands (300) and retaining the population of covalently closed circular library molecules (600); and **b)** contacting the retained two or more populations of covalently closed circular library molecules (600) with a plurality of soluble amplification primers, a plurality of strand-displacing polymerases, and a plurality of nucleotides, under a condition suitable to conduct a rolling circle amplification reaction thereby generating a plurality of nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0066] In some examples, the method further comprises: **a)** distributing the plurality of concatemer molecules onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules; and **b)** contacting the immobilized plurality of concatemer molecules (which are hybridized to a covalently closed circular library molecule (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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0067] In some examples, the method further comprises: conducting a rolling circle amplification reaction by contacting the two or more populations of covalently closed circular library molecules (600) each hybridized to the first splint strand (300) of step **(d)** or **(e)** with a plurality of strand-displacing polymerases and a plurality of nucleotides, under a condition suitable to conduct a rolling circle amplification reaction thereby generating a plurality of concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0068] In some examples, the method further comprises: **a)** distributing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600) onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600); and **b)** contacting the plurality of immobilized concatemer molecules 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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0069] In some examples, the method further comprises: sequencing the insert region (110) and the first left index sequence (160) and/or the first right index sequence (170) of the plurality of immobilized nucleic acid concatemer molecules, wherein the sequences of the

insert region (110) along with the first left index sequence (160) and/or first right index sequence (170) can be used to identify the sample source of the sequence of interest.

[0070] In some examples, the sequencing reaction comprises: **a)** binding the plurality of immobilized nucleic acid concatemer molecules with a plurality of sequencing primers, a plurality of sequencing polymerases, and a plurality of detectably labeled nucleotide analogs each comprising a 2' or 3' chain terminating moiety, under a condition suitable for incorporating the detectably labeled nucleotide analogs into the 3' end of the sequencing primers; **b)** detecting the bound detectably labeled nucleotide analog; and **c)** identifying the nucleo-base of the detectably labeled nucleotide analog. In some examples, the sequencing reaction employs a two-stage sequencing reaction, wherein the first stage comprises: **a)** binding the plurality of immobilized nucleic acid concatemer molecules with a plurality of sequencing primers, a plurality of a first sequencing polymerase, and a plurality of detectably labeled multivalent molecules, under a condition suitable to bind individual concatemer molecules to a sequencing primer, a first sequencing polymerase, and a detectably labeled multivalent molecule (e.g., forming a plurality of multivalent-complexed polymerases); **b)** detecting the bound detectably labeled multivalent molecule; and **c)** identifying the nucleo-base of the detectably labeled multivalent molecule. In some examples, the binding of the detectably labeled multivalent molecules to the plurality of immobilized nucleic acid concatemer molecules forms at least one avidity complex.

[0071] In some examples, the method further comprises conducting a second stage which comprises: **d)** removing the plurality of first sequencing polymerases and the plurality of bound multivalent molecules from the plurality of immobilized nucleic acid concatemer molecules and retaining the immobilized concatemer molecules each hybridized to a sequencing primer (nucleic acid duplex); and **e)** contacting the retained immobilized concatemer molecules and hybridized sequencing primers with a plurality of second polymerases and a plurality of nucleotides under a condition suitable for incorporating the nucleotides into the 3' end of the sequencing primers.

[0072] The disclosure provides a method for preparing a multiplex mixture of sequences-of-interest isolated from a plurality of sample sources, comprising: **a)** providing two or more populations of single-stranded nucleic acid library molecules (100), each population of library molecules (100), each population of single-stranded nucleic acid library molecules is contained in a separate compartment, wherein the nucleic acid library molecules in a given population comprise **(i)** a first left universal adaptor sequence (120) having a binding sequence for a first surface primer, **(ii)** a second left universal adaptor sequence (140) having

a binding sequence for a first sequencing primer; **(iii)** a sequence of interest (110) isolated from a sample source, **(iv)** a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; **(v)** a first right universal adaptor sequence (130) having a binding sequence for a second surface primer, and **(vi)** a first left index sequence (160) and/or a first right index sequence (170), wherein the sequences of the left (160) and/or right (170) indexes separately or in combination identify the sample source from which the sequences of interest (110) are isolated; **b)** pooling together the two or more populations of single-stranded nucleic acid library molecules (100) into the same compartment; **c)** adding to the compartment of step (b) a plurality of double-stranded splint adaptors (200) wherein individual double-stranded splint adaptors (200) comprise a first splint strand (300) hybridized to a second splint strand (400), wherein the first splint strand (300) comprises regions arranged in a 5' to 3' order a first region (320), an internal region (310), and a second region (330), and wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400), wherein the second splint strand comprises regions arranged in a 5' to 3' order of **(i)** a second sub-region having a universal binding sequence for a fourth surface primer, and **(ii)** a first sub-region having a universal binding sequence for a third surface primer; **d)** incubating the population of single-stranded nucleic acid library molecules (100) and the plurality of double-stranded splint adaptors (200) which are in the same compartment of step (c) under a condition suitable to hybridize portions of the first splint strand (300) to portions of the library molecules (100) thereby circularizing the library molecules to generate a population of library-splint complexes (500), such that the first region (320) of an individual first splint strand is hybridized to the binding sequence for the first surface primer (120) of an individual library molecule (100), and the third region (330) of the individual first splint strand is hybridized to the binding sequence for the second surface primer (130) of the individual library molecule (100), wherein each of the library-splint complexes (500) comprise a first nick between the 5' end of the library molecule and the 3' end of the second splint strand (300), wherein each of the library-splint complexes (500) comprises a second nick between the 5' end of the second splint strand (300) and the 3' end of the library molecule (100), and wherein the first and second nicks are enzymatically ligatable; and **e)** contacting the populations of library-splint complexes (500) in the compartment of step (d) with a ligase, under a condition suitable to enzymatically ligate the first and second nicks, thereby generating a population of covalently closed circular library molecules (600) each hybridized to the first splint strand (300), thereby generating a

multiplex mixture of covalently closed circular library molecules (600) which comprise the multiplex mixture of sequences-of-interest isolated from a plurality of sample sources.

[0073] In some examples, the individual covalently closed circular library molecules (600) in the multiplex mixture of step (e) comprise a second splint strand region (400) which includes a universal binding sequence for a third surface primer, and the method further comprises: **a)** 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); and **b)** 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, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0074] In some eombidment,s the method further comprises: conducting a rolling circle amplification reaction by: **a)** contacting the population of covalently closed circular library molecules (600) that are hybridized to the first splint strand (300) of step (d) or (e) with at least one exonuclease enzyme under a condition suitable to remove the plurality of first splint strands (300) and retaining the population of covalently closed circular library molecules (600); **b)** contacting the retained population of covalently closed circular library molecules (600) with a plurality of soluble amplification primers, a plurality of strand-displacing polymerases, and a plurality of nucleotides, under a condition suitable to conduct a rolling circle amplification reaction thereby generating a plurality of nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0075] In some examples, the method further comprises: **c)** distributing the plurality of concatemer molecules onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules; and **d)** contacting the immobilized plurality of concatemer molecules (which are hybridized to a covalently closed

circular library molecule (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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0076] In some examples, the method further comprises: conducting a rolling circle amplification reaction by contacting the population of covalently closed circular library molecules (600) each hybridized to the first splint strand (300) of step (d) or (e) with a plurality of strand-displacing polymerases and a plurality of nucleotides, under a condition suitable to conduct a rolling circle amplification reaction thereby generating a plurality of concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0077] In some examples, the method further comprises: **c)** distributing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600) onto a support having a plurality of the third surface primers immobilized thereon, under a condition suitable for hybridizing at least a portion of the concatemers to at least a portion of the plurality of the immobilized third surface primers thereby immobilizing the plurality of concatemer molecules which are hybridized to a covalently closed circular library molecule (600); and **d)** contacting the plurality of immobilized concatemer molecules 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 covalently closed circular library molecules (600) as template molecules, thereby extending the plurality of immobilized nucleic acid concatemer molecules, wherein the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0078] In some examples, the method further comprises sequencing the insert region (110) and the first left index sequence (160) and/or the first right index sequence (170) of the plurality of immobilized nucleic acid concatemer molecules, wherein the sequences of the insert region (110) along with the first left index sequence (160) and/or first right index sequence (170) can be used to identify the sample source of the sequence of interest.

[0079] In some examples, the sequencing reaction comprises: **a)** binding the plurality of immobilized nucleic acid concatemer molecules with a plurality of sequencing primers, a plurality of sequencing polymerases, and a plurality of detectably labeled nucleotide analogs each comprising a 2’ or 3’ chain terminating moiety, under a condition suitable for

incorporating the detectably labeled nucleotide analogs into the 3’ end of the sequencing primers; **b)** detecting the bound detectably labeled nucleotide analog; and **c)** identifying the nucleo-base of the detectably labeled nucleotide analog.

[0080] In some examples, the sequencing reaction employs a two-stage sequencing reaction, wherein the first stage comprises: **a)** binding the plurality of immobilized nucleic acid concatemer molecules with a plurality of sequencing primers, a plurality of a first sequencing polymerase, and a plurality of detectably labeled multivalent molecules, under a condition suitable to bind individual concatemer molecules to a sequencing primer, a first sequencing polymerase, and a detectably labeled multivalent molecule (e.g., forming a plurality of multivalent-complexed polymerases); **b)** detecting the bound detectably labeled multivalent molecule; and **c)** identifying the nucleo-base of the detectably labeled multivalent molecule. In some examples, the detectably labeled multivalent molecules to the plurality of immobilized nucleic acid concatemer molecules forms at least one avidity complex.

[0081] In some examples, the method further comprises conducting a second stage which comprises: **d)** removing the plurality of first sequencing polymerases and the plurality of bound multivalent molecules from the plurality of immobilized nucleic acid concatemer molecules and retaining the immobilized concatemer molecules each hybridized to a sequencing primer (nucleic acid duplex); and **e)** contacting the retained immobilized concatemer molecules and hybridized sequencing primers with a plurality of second polymerases and a plurality of nucleotides under a condition suitable for incorporating the nucleotides into the 3’ end of the sequencing primers.

[0082] The disclosure provides a method for appending additional universal adaptor sequencing to nucleic acid library molecules, comprising: **a)** providing a plurality of nucleic acid library molecules (100) each comprising sequences arranged in a 5’ to 3’ order: (i) a first left universal adaptor sequence (120), (ii) a second left universal adaptor sequence (140), (iii) a sequence of interest (110), (iv) a second right universal adaptor sequence (150), and (v) a first right universal adaptor sequence (130); **b)** hybridizing individual library molecules of step (a) with a first tailed primer and conducting a first primer extension reaction (or first PCR), wherein the first tailed primers comprise a 5’ region carrying a third left universal adaptor sequence and a 3’ region that is complementary to at least a portion of the first left universal adaptor sequence (120) of the library molecule (100), wherein the first primer extension reaction (or first PCR) generates a plurality of first modified library molecules each appended at one end with the third left universal adaptor sequence, wherein the third left universal adaptor sequence of the first tailed primers comprise a universal binding sequence

for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide; and **c)** hybridizing individual first modified library molecules of step (a) with a second tailed primer and conducting a second primer extension reaction (or second PCR), wherein the second tailed primers comprise a 5' region carrying a third right universal adaptor sequence and a 3' region that is complementary to at least a portion of the first right universal adaptor sequence (130) of the first modified library molecule, wherein the second primer extension reaction (or second PCR) generates a plurality of second modified library molecules each appended at one end with the third right universal adaptor sequence, wherein the third right universal adaptor sequence of the second tailed primers comprise a universal binding sequence for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide.

[0083] In some examples, the nucleic acid library molecules each comprise sequences arranged in a 5' to 3' order: (i) a first left universal adaptor sequence (120) having a binding sequence for a first surface primer, (ii) a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer, (iii) a sequence of interest (110), (iv) a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer, and (v) a first right universal adaptor sequence (130) having a binding sequence for a second surface primer.

[0084] In some examples, the method further comprises: **d)** hybridizing individual second modified library molecules of step (c) with a third tailed primer and conducting a third primer extension reaction (or third PCR), wherein the third tailed primers comprise a 5' region carrying a fourth left universal adaptor sequence and a 3' region that is complementary to at least a portion of the third left universal adaptor sequence of the second modified library molecule, wherein the third primer extension reaction (or third PCR) generates a plurality of third modified library molecules each appended at one end with the fourth left universal adaptor sequence, wherein the fourth left universal adaptor sequence of the third tailed primers comprise a universal binding sequence for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide; and **e)** hybridizing individual third modified library molecules of step (d) with a fourth tailed primer and conducting a fourth primer extension reaction (or

fourth PCR), wherein the fourth tailed primers comprise a 5' region carrying a fourth right universal adaptor sequence and a 3' region that is complementary to at least a portion of the third right universal adaptor sequence of the third modified library molecule, wherein the fourth primer extension reaction (or fourth PCR) generates a plurality of fourth modified library molecules each appended at one end with the fourth right universal adaptor sequence, wherein the fourth right universal adaptor sequence of the fourth tailed primers comprise a universal binding sequence for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide.

[0085] In some examples, the method further comprises: **f)** hybridizing individual fourth modified library molecules of step (e) with a fifth tailed primer and conducting a fifth primer extension reaction (or fifth PCR), wherein the fifth tailed primers comprise a 5' region carrying a fifth left universal adaptor sequence and a 3' region that is complementary to at least a portion of the fourth left universal adaptor sequence of the fourth modified library molecule, wherein the fifth primer extension reaction (or fifth PCR) generates a plurality of fifth modified library molecules each appended at one end with the fifth left universal adaptor sequence, wherein the fifth left universal adaptor sequence of the fifth tailed primers comprise a universal binding sequence for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide; and **g)** hybridizing individual fifth modified library molecules of step (f) with a sixth tailed primer and conducting a sixth primer extension reaction (or sixth PCR), wherein the sixth tailed primers comprise a 5' region carrying a sixth right universal adaptor sequence and a 3' region that is complementary to at least a portion of the fourth right universal adaptor sequence of the fifth modified library molecule, wherein the sixth primer extension reaction (or sixth PCR) generates a plurality of sixth modified library molecules each appended at one end with the sixth right universal adaptor sequence, wherein the sixth right universal adaptor sequence of the sixth tailed primers comprise a universal binding sequence for a forward or reverse sequencing primer, a universal binding sequence for a first or second surface primer, a universal binding sequence for a forward or reverse amplification primer and/or a universal binding sequence for a compaction oligonucleotide.

[0086] In some examples, the first tailed primer, the third tailed primer and/or the fifth tailed primer further comprises a left index sequence. In some examples, the first tailed

primer, the third tailed primer and/or the fifth tailed primer further comprises a left unique identification sequence. In some examples, the second tailed primer, the fourth tailed primer and/or the sixth tailed primer further comprises a right index sequence. In some examples, the second tailed primer, the fourth tailed primer and/or the sixth tailed primer further comprises a right unique identification sequence. In some examples, the appended third left universal adaptor sequence of step (b) can bind a first region (320) of a first splint strand. In some examples, the appended third right universal adaptor sequence of step (c) can bind a second region (330) of a first splint strand. In some examples, the appended fourth left universal adaptor sequence of step (d) can bind a first region (320) of a first splint strand. In some examples, the appended fourth right universal adaptor sequence of step (e) can bind a second region (330) of a first splint strand.

[0087] In some examples, the appended fifth left universal adaptor sequence of step (f) can bind a first region (320) of a first splint strand. In some examples, the appended fifth right universal adaptor sequence of step (g) can bind a second region (330) of a first splint strand.

[0088] The disclosure provides a method for forming a plurality of library-splint complexes (500) comprising: **a)** providing a plurality of double-stranded splint adaptors (200) wherein individual double-stranded splint adaptors (200) comprise a first splint strand (300) hybridized to a second splint strand (400), wherein the first splint strand (300) comprises regions arranged in a 5' to 3' order a first region (320), an internal region (310), and a second region (330), and wherein the internal region of the first splint strand (310) is hybridized to the second splint strand (400), wherein the second splint strand comprises regions arranged in a 5' to 3' order **(i)** a second sub-region having a universal binding sequence for a fourth surface primer, and **(ii)** a first sub-region having a universal binding sequence for a third surface primer; and **b)** hybridizing the plurality of double-stranded splint adaptors with a plurality of single-stranded nucleic acid library molecules (100) wherein individual library molecules comprise regions arranged in a 5' to 3' order: **(i)** a first left universal adaptor sequence (120) having a binding sequence for a third surface primer; **(ii)** a second left universal adaptor sequence (140) having a binding sequence for a first sequencing primer; **(iii)** a sequence of interest (110); **(iv)** a second right universal adaptor sequence (150) having a binding sequence for a second sequencing primer; and **(v)** a first right universal adaptor sequence (130) having a binding sequence for a fourth surface primer, wherein the hybridizing is conducted under a condition suitable to hybridize portions of the first splint strand (300) to portions of the library molecule (100) thereby circularizing the library

molecule to generate a library-splint complex (500), such that the first region (320) of the first splint strand is hybridized to the binding sequence for the third surface primer (120), and the third region (330) of the first splint strand is hybridized to the binding sequence for the fourth surface primer (130), wherein the library-splint complex (500) comprises a first nick between the 5’ end of the library molecule and the 3’ end of the second splint strand (400), wherein the library-splint complex (500) comprises a second nick between the 5’ end of the second splint strand (400) and the 3’ end of the library molecule (100), and wherein the first and second nicks are enzymatically ligatable.

[0089] In some examples, the plurality of single-stranded nucleic acid library molecules (100) further comprise a first left index sequence (160) and/or a first right index sequence (170). In some examples, the plurality of single-stranded nucleic acid library molecules (100) further comprise a first left unique identification sequence (180) and/or a first right unique identification sequence (190).

[0090] In some examples, the method further comprises: contacting the plurality of the double-stranded splint adaptors (200) and the plurality of the single-stranded nucleic acid library molecules (100) with a ligase, under a condition suitable to enzymatically ligate the first and second nicks, thereby generating a plurality of covalently closed circular library molecules (600) each hybridized to the first splint strand (300). In some examples, the method further comprises: contacting the plurality of covalently closed circular library molecules (600) with at least one exonuclease enzyme to remove the plurality of first splint strands (300) and retaining the plurality of covalently closed circular library molecules (600).

[0091] In some examples, the individual covalently closed circular library molecules (600) in the plurality comprise a second splint strand region (400) which includes the universal binding sequence for the second surface primer, and the method further comprises: distributing the retained plurality of covalently closed circular library molecules (600) onto a support having a plurality of the second surface primers immobilized on the support, under a condition suitable for hybridizing individual covalently closed circular library molecules (600) to individual immobilized second surface primers thereby immobilizing the plurality of covalently closed circular library molecules (600).

[0092] In some examples, the method further comprises: 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 second 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. In some examples, the plurality of nucleotides comprises dATP, dGTP, dCTP, dTTP and/or dUTP.

[0093] In some examples, the plurality of immobilized second surface capture primers on the support are located at pre-determined or random locations on the support. In some examples, the plurality of immobilized second surface capture primers on the support are in fluid communication with each to permit flowing a solution of reagents onto the support so that the plurality of immobilized second surface primers can be essentially simultaneously reacted with the reagents in a massively parallel manner, optionally wherein the solution of reagents comprises enzymes, nucleotides and divalent cations. In some examples, the density of the plurality of immobilized nucleic acid concatemer molecules on the support is $10^4 – 10^8$ per mm^2.

[0094] In some examples, the method further comprises: sequencing the plurality of immobilized concatemers, wherein the sequencing comprises: **a)** contacting the plurality of immobilized concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of the soluble sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of complexed polymerases each comprising a sequencing polymerase bound to a nucleic acid duplex wherein the nucleic acid duplex comprises a 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 the sugar 3’ position; **c)** incorporating at least one nucleotide into the 3’ end of the hybridized 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.

[0095] In some examples, the plurality of nucleotides comprise a removable chain terminating moiety at the 3’ sugar group, wherein the removable chain terminating moiety comprises an alkyl group, alkenyl group, alkynyl group, allyl group, aryl group, benzyl group, azide group, azido group, O-azidomethyl group, amine group, amide group, keto group, isocyanate group, phosphate group, thio group, disulfide group, carbonate group, urea group, or silyl group, and wherein the removable chain terminating moiety is cleavable with a chemical compound to generate an extendible 3’OH moiety on the sugar group. In some examples, the plurality of nucleotides comprise one type of nucleotide selected from a

group consisting of dATP, dGTP, dCTP, dTTP and dUTP. In some examples, the plurality of nucleotides comprise a mixture of any combination of two or more types of nucleotides selected from a group consisting of dATP, dGTP, dCTP, dTTP and/or dUTP.

[0096] In some examples, the method further comprises: sequencing the plurality of immobilized concatemers, wherein the sequencing comprises: **a)** contacting the plurality of immobilized concatemer molecules with (i) a plurality of sequencing polymerases and (ii) a plurality of the soluble universal sequencing primers, wherein the contacting is conducted under a condition suitable to form a plurality of first complexed polymerases each comprising a sequencing polymerase bound to a nucleic acid duplex wherein the nucleic acid duplex comprises a 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 each nucleotide arm is attached to a nucleotide unit; and **c)** detecting the plurality of multivalent-complexed polymerases.

[0097] In some examples, the method further comprises: **d)** dissociating the plurality of multivalent-complexed polymerases and removing the plurality of first sequencing polymerases and their bound multivalent molecules, and retaining the plurality of nucleic acid duplexes; **e)** contacting the plurality of the retained nucleic acid duplexes of step d) 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 the retained nucleic acid duplexes, thereby forming a plurality of second complexed polymerases each comprising a second sequencing polymerase bound to a retained nucleic acid duplex; **f)** contacting the plurality of second complexed polymerases with a plurality of nucleotides comprising at least one nucleotide analog having a removable chain terminating moiety at the sugar 3’ position, 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 e) 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.

[0098] In some examples, the method further comprises: **g)** detecting the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases. In some examples, the method further comprises: **g)** detecting the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases; and **h)** identifying the nucleo-bases of the complementary nucleotides which are incorporated into the sequencing primers of the nucleotide-complexed polymerases.

[0099] The disclosure provides a method for sequencing by forming at least one avidity complex, comprising: **a)** binding a first universal sequencing primer, a first sequencing polymerase, and a first detectably labeled multivalent molecule to a first portion of a concatemer molecule of claim 16, thereby forming a first binding complex, wherein a first nucleotide unit of the first multivalent molecule binds to the first sequencing polymerase; **b)** binding a second universal sequencing primer, a second sequencing polymerase, and the first detectably labeled multivalent molecule to a second portion of the same concatemer molecule thereby forming a second binding complex, wherein a second nucleotide unit of the first multivalent molecule binds to the second sequencing polymerase, wherein the first and second binding complexes which include the same multivalent molecule forms an avidity complex, wherein the first detectably labeled multivalent molecule comprises a core attached to multiple nucleotide arms and each nucleotide arm is attached to a nucleotide unit, wherein the concatemer molecule comprises two or more tandem repeat sequences of a sequence of interest (110) and a universal primer binding site that binds the first and second universal sequencing primers, and wherein the contacting is conducted under a condition suitable to inhibit polymerase-catalyzed incorporation of the bound first and second nucleotide units in the first and second binding complexes; **c)** detecting the first and second binding complexes on the same concatemer molecule, and **d)** identifying the first nucleotide unit in the first binding complex thereby determining the sequence of the first portion of the concatemer template molecule, and identifying the second nucleotide unit in the second binding complex thereby determining the sequence of the second portion of the concatemer template molecule.

[0100] In some examples, the plurality of nucleotide arms attached to the core of the individual multivalent molecules have the same type of a nucleotide unit, and wherein the types of nucleotide unit is selected from a group consisting of dATP, dGTP, dCTP, dTTP and dUTP. In some examples, the plurality of multivalent molecules comprise a mixture of

any combination of two or more types of multivalent molecules each type having nucleotide units selected from a group consisting of dATP, dGTP, dCTP, dTTP and/or dUTP.

[0101] In some examples, the plurality of nucleotides comprise a removable chain terminating moiety at the 3' sugar group, wherein the removable chain terminating moiety comprises an alkyl group, alkenyl group, alkynyl group, allyl group, aryl group, benzyl group, azide group, azido group, O-azidomethyl group, amine group, amide group, keto group, isocyanate group, phosphate group, thio group, disulfide group, carbonate group, urea group, or silyl group, and wherein the removable chain terminating moiety is cleavable with a chemical compound to generate an extendible 3'OH moiety on the sugar group.

[0102] In some examples, the plurality of nucleotides comprise one type of nucleotide selected from a group consisting of dATP, dGTP, dCTP, dTTP and dUTP. In some examples, the plurality of nucleotides comprise a mixture of any combination of two or more types of nucleotides selected from a group consisting of dATP, dGTP, dCTP, dTTP and/or dUTP.

[0103] In some examples, the support comprises a glass or plastic substrate. In some examples, the support is passivated with at least one hydrophilic polymer coating having a water contact angle of no more than 45 degrees. In some examples, the at least one hydrophilic polymer coating comprises a molecule selected from a group consisting of polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(vinyl pyridine), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly(methyl methacrylate) (PMA), poly(2-hydroxylethyl methacrylate) (PHEMA), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), polyglutamic acid (PGA), poly-lysine, poly-glucoside, streptavidin, and dextran.

DESCRIPTION OF THE DRAWINGS

[0104] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0105] **FIG. 1** is a schematic showing an exemplary linear single stranded library molecule (100) hybridizing with a double-stranded splint molecule (200) thereby circularizing the library molecule to form a library-splint complex (500) with two nicks. The library molecule (100) comprises a sequence of interest (110) flanked on one side by a first left universal