CLAIMS

I/We claim:

1. A method of sequencing a double-stranded target nucleic acid molecule, the method comprising:

(a) amplifying a physically linked~~physically-linked~~ nucleic acid complex on a surface to produce physically linked~~physically-linked~~ nucleic acid complex amplicons bound to the surface in both a forward orientation and a reverse orientation, wherein the physically linked~~physically-linked~~ nucleic acid complex comprises (i) the double-stranded target nucleic acid molecule, (ii) a first adapter comprising a linker domain on a first end of the double-stranded target nucleic acid molecule, and (iii) a second adapter having a double-stranded portion and a single-stranded portion on a second end of the double-stranded target nucleic acid molecule;

(b) removing either (i) the physically linked~~physically-linked~~ nucleic acid complex amplicons bound to the surface in the reverse orientation or (ii) the physically linked~~physically-linked~~ nucleic acid complex amplicons bound to the surface in the forward orientation;

(c) cleaving a portion of the remaining bound physically linked~~physically-linked~~ nucleic acid complex amplicons to provide a subset of single-stranded amplicons comprising information from one strand and a subset of physically linked~~physically-linked~~ nucleic acid complex amplicons;

(d) sequencing the subset of single-stranded amplicons to provide a sequencing read derived from an original strand of the double-stranded target nucleic acid molecule;

(e) amplifying the subset of physically linked~~physically-linked~~ nucleic acid complex amplicons on the surface;

(f) removing the physically linked~~physically-linked~~ nucleic acid complex amplicons that are in the other orientation;

(g) cleaving the remaining bound physically linked~~physically-linked~~ nucleic acid complex amplicons to provide single-stranded amplicons comprising information from the other strand; and

(h) sequencing the single-stranded amplicons to provide sequencing reads derived from the other original strand of the double-stranded target nucleic acid molecule.

2. A method of sequencing a population of double-stranded target nucleic acid molecule, the method comprising:

(a) amplifying a plurality of physically linked~~physically-linked~~ nucleic acid complexes on a surface to produce a plurality of clonal clusters, each clonal cluster comprising a plurality of physically linked~~physically-linked~~ nucleic acid complex amplicons bound to the surface, wherein each physically linked~~the physically-linked~~ nucleic acid complex comprises (i) a~~the~~ double-stranded target nucleic acid molecule from the population, (ii) a first adapter comprising a linker domain on one end of the double-stranded target nucleic acid molecule, and (iii) a second adapter having a double-stranded portion and a single-stranded portion on the other end of the double-stranded target nucleic acid molecule;

(b) removing either the physically linked~~physically-linked~~ nucleic acid complex amplicons from each clonal cluster bound to the surface at (i) a 5' end of the physically linked~~physically-linked~~ nucleic acid complex amplicons or (ii) a 3' end of the physically linked~~physically-linked~~ nucleic acid complex amplicons;

(c) cleaving at least a portion of the remaining bound physically linked~~physically-linked~~ nucleic acid complex amplicons from each clonal cluster at a cleavage site to provide single-stranded amplicons comprising sequence information derived from one original strand of the double-stranded target nucleic acid molecule for each clonal cluster on the surface; and

(d) sequencing the single-stranded amplicons to provide a sequencing read derived from the one original strand of the double-stranded target nucleic acid molecule for each clonal cluster on the surface.

3. The method of claim 2, wherein cleaving at least a portion of the remaining bound physically linked~~physically-linked~~ nucleic acid complex amplicons from each clonal cluster comprises preserving at least one physically linked~~physically-linked~~ nucleic acid complex amplicon from each clonal cluster bound to the surface.

4. The method of claim 3, further comprising:

(e) amplifying the at least one physically linked~~physically-linked~~ nucleic acid complex amplicon from each clonal cluster on the surface to repopulate each clonal~~the~~ cluster~~ of physically-linked nucleic acid complex amplicons bound to the surface~~;

(f) removing the physically linked~~physically-linked~~ nucleic acid complex amplicons that are in the other orientation relative to the ampliconsnot removed in (b);

(g) cleaving the remaining bound physically linked~~physically-linked~~ nucleic acid complex amplicons from each clonal cluster to provide single-stranded amplicons comprising information derived from the other original strand of the double-stranded target nucleic acid molecule for each clonal cluster on the surface; and

(h) sequencing the single-stranded amplicons to provide a sequencing read derived from the other original strand of the double-stranded target nucleic acid molecule for each clonal cluster on the surface.

5. The method of any of the proceeding claims, further comprising comparing the sequence read from the one original strand to the sequence read from the other original strand to generate a consensus sequence for the double-stranded target nucleic acid molecule.

~~6. The method of any of claims 1-4, further comprising:~~

~~identifying sequence variations in the sequence read from the one original strand and the sequence read from the other original strand, wherein the sequence variations from the one original strand and the other original strand are consistent sequence variations; or~~

~~eliminating or discounting sequence variations that occur in the one original strand and not the other original strand.~~

6~~7~~. The method of any of claims 1-4, further comprising:

comparing the sequence read from the one original strand to the sequence read from the other original strand;

identifying a nucleotide position that does not agree between the sequence read from the one original strand and~~to~~ the sequence read from the other original strand; and

generating an error-corrected sequence of the double-stranded target nucleic acid molecule by discounting. eliminating, or correcting the nucleotide position identified that does not agree.

~~8. A method of sequencing a population of double-stranded target nucleic acid molecules, each comprising a first strand and a second strand, the method comprising:~~

~~(a) amplifying a plurality of physically-linked nucleic acid complexes on a surface to produce a plurality of clonal clusters, each clonal cluster comprising a plurality of physically-linked nucleic acid complex amplicons each comprising a first strand amplicon and a second strand amplicon, wherein each physically-linked nucleic acid complex comprises (i) a double-stranded target nucleic acid molecule from the population, (ii) a first adapter comprising a linker domain attached to a first end of the double-stranded target nucleic acid molecule, and (iii) a second adapter having a double-stranded portion and a single-stranded portion attached to a second end of the double-stranded target nucleic acid molecule;~~

~~(b) removing either the physically-linked nucleic acid complex amplicons from each clonal cluster bound to the surface in the (i) reverse orientation or (ii) in the forward orientation;~~

~~(c) cleaving a portion of the remaining surface bound physically-linked nucleic acid complex amplicons remaining after (b) and thereby physically separating the first strand amplicons and the second strand amplicons;~~

~~(d) removing the unbound physically separated first or second strand amplicons; and~~

~~(e) sequencing the remaining physically separated first or second strand amplicons bound to the surface to produce a nucleic acid sequence read of the first strand or the second strand for each clonal cluster on the surface.~~

~~9. The method of claim 8, wherein cleaving at least a portion of the remaining bound physically-linked nucleic acid complex amplicons comprises preserving at least one physically-linked nucleic acid complex amplicon in at least some of the clonal clusters bound to the surface.~~

~~10. The method of claim 9, further comprising:~~

~~(f) in at least some of the clonal clusters, amplifying the at least one physically-linked nucleic acid complex amplicon on the surface to repopulate the clonal clusters of physically-linked nucleic acid complex amplicons bound to the surface;~~

~~(g) removing the physically-linked nucleic acid complex amplicons that are in the other orientation from step (b);~~

~~(h) removing the unbound physically separated first or second strand amplicons;~~

~~(i) cleaving the remaining bound physically-linked nucleic acid complex amplicons remaining after (h) and thereby physically separating the first strand amplicons and the second strand amplicons; and~~

~~(j) sequencing the remaining physically separated first or second strand amplicons bound to the surface to produce a nucleic acid sequence read of the first strand or the second strand for each clonal cluster on the surface.~~

7~~11~~. A method of sequencing a population of double-stranded target nucleic acid molecules, each comprising a first strand and a second strand, the method comprising:

(a) amplifying a plurality of physically linked~~physically-linked~~ nucleic acid complexes bound on a surface to produce a plurality of clusters, each cluster comprising a plurality of physically linked~~physically-linked~~ nucleic acid complex amplicons representing an original double-stranded target nucleic acid molecule, wherein each physically linked~~physically-linked~~ nucleic acid complex amplicon comprises a first strand amplicon and a second strand amplicon, and wherein each physically linked~~physically-linked~~ nucleic acid complex comprises a double-stranded target nucleic acid molecule from the population attached to (i) a first adapter comprising a linker domain between the first strand and the second strand at one end and (ii) a second adapter having a double-stranded portion and a single-stranded portion at the other end;

(b) cleaving the surface bound physically linked~~physically-linked~~ nucleic acid complex amplicons and thereby physically separating the first strand amplicons from~~and~~ the second strand amplicons;

(c) removing the unbound physically separated first strand amplicons from the surface-bound second strand amplicons, and~~/or~~ the unbound physically separated second strand amplicons from the surface-bound first strand amplicons, such that~~wherein~~ the remaining amplicons bound to the surface comprise (i) the surface-bound physically separated first strand amplicons and (ii) the surface-bound physically separated second strand amplicons;

(d) sequencing the surface-bound physically separated first strand amplicons ~~bound to the surface~~ to produce a nucleic acid sequence read of the first strand for each cluster on the surface; and

(e) sequencing the surface-bound physically separated second strand amplicons ~~bound to the surface~~ to produce a nucleic acid sequence read of the second strand for each cluster on the surface.

~~12. The method of claim 10 or claim 11, further comprising: for at least some of the clusters on the surface, comparing the nucleic acid sequence read of the first strand to the nucleic acid sequence read of the second strand to generate an error-corrected sequence read of an original double-stranded target nucleic acid molecule.~~

8~~13~~. The method of claim 7~~any one of claims 10-12~~, further comprising relating the nucleic acid sequence read of the first strand of an original double-stranded target nucleic acid molecule from the population to the nucleic acid sequence read of the second strand of the same original double-stranded target nucleic acid molecule using a unique molecular identifier (UMI).

~~14. The method of claim 13, wherein the UMI comprises a physical location on the surface.~~

9~~15~~. The method of claim 8~~14~~, wherein the UMI comprises a tag sequence, a molecule-specific feature, cluster location on the surface or a combination thereof.

~~16. The method of claim 15, wherein the molecule-specific feature comprises nucleic acid mapping information against a reference sequence, sequence information at or near the ends of the double-stranded target nucleic acid molecule, a length of the double-stranded target nucleic acid molecule, or a combination thereof.~~

10~~17~~. The method of any one of claims 7-9~~10-16~~, further comprising differentiating the nucleic acid sequence read of the first strand of an original double-stranded target nucleic acid molecule from the nucleic acid sequence read of the second strand from the same original double-stranded target nucleic acid molecule using a strand defining element (SDE).

11~~18~~. The method of claim 10~~17~~, wherein the SDE is the association of sequence read information with ~~step (e) and step (j) of claim 10, or with~~ steps (d) and (e) of claim 7~~11~~.

~~19. The method of claim 17, wherein the SDE comprises a portion of an adapter sequence.~~

~~20. The method of any one of claims 8-19, wherein sequencing the physically separated first strand amplicons or the second strand amplicons comprises sequencing by synthesis.~~

~~21. The method of any one of claims 8-20, further comprising:~~

~~preparing the physically-linked nucleic acid complexes by ligating the first adapter and the second adapter to each of a plurality of double-stranded target nucleic acid molecules in the population; and~~

~~presenting the physically-linked nucleic acid complexes to the surface, the surface having a plurality of bound oligonucleotides at least partially complimentary to the single-stranded portion of the second adapters such that a plurality of physically-linked nucleic acid complexes are captured on the surface via hybridization to the plurality of bound oligonucleotides.~~

~~22. The method of any one of claims 8-21, wherein the amplification step in (a) comprises bridge amplification.~~

1223. The method of any one of claims 78-1122, further comprising:

for at least some of the double-stranded target nucleic acid molecules in the population:

(i) comparing the sequence read from the first strand to the sequence read from the second strand;

(ii) identifying a nucleotide position that does not agree between the sequence read from the first strand and the sequence read from the second strand; and

(iii) generating an error-corrected sequence read of the double-stranded target nucleic acid molecule by discounting, eliminating, or correcting the identified nucleotide position that does not agree.

1324. The method of any one of claims 1-1223, wherein the first adapter comprises a cleavable site, domain, or motif.

~~25. The method of any one of claims 1-24, wherein the first adapter comprises a cleavable domain.~~

1426. The method of any one of claims 1-1325, wherein the first adapter comprises a hairpin loop structure comprising a self-complementary stem portion and a single-stranded nucleotide loop portion.

~~27. The method of claim 26, wherein the cleavable domain is in the single-stranded nucleotide loop portion or the stem portion.~~

~~28. The method of claim 33, wherein the cleavable domain comprises an enzyme recognition site.~~

~~29. The method of claim 28, wherein the enzyme recognition site is targeted by a restriction enzyme or a targeted endonuclease.~~

1530. The method of any of claims 1-1429, wherein the single-stranded portion of the second adapter comprises a first arm having a first primer binding site and a second arm having a second primer binding site.

~~31. The method of claim 30, wherein, when denatured, the physically-linked double-stranded nucleic acid complex comprises from 5' to 3' or from 3' to 5': the first primer binding site, the first strand, the first adapter comprising the linker domain, the second strand, and the second primer binding site.~~

1632. The method of any of the previous claims, wherein the surface is a sequencing surface, and wherein the method further comprises flowing the plurality of physically linked double-stranded nucleic acid complexes over the surface prior to the amplification in (a).

~~33. The method of any of one of claims 8-32, further comprising flowing the plurality of physically-linked double stranded nucleic acid complexes over the surface prior to the amplification in (a).~~

~~34. The method of any of the previous claims, wherein the surface comprises a plurality of one or more bound oligonucleotides at least partially complimentary to one or more regions of the second adapter.~~

~~35. The method of claim 34, wherein the plurality of one or more bound oligonucleotides is at least partially complimentary to the single-stranded portion of the second adapter.~~

~~36. The method of any one of claims 1-35, wherein a first strand and a second strand of the physically-linked nucleic acid complex are amplified via multiple amplification reactions in step (a) to generate a cluster of the physically-linked nucleic acid complex amplicons on the surface.~~

~~37. The method of any of claim 8-36, wherein the first strand and the second strand of each of the plurality of physically-linked nucleic acid complexes are amplified in step (a) to generate the plurality of clusters on the surface simultaneously.~~

1738. The method of any one of claims 1-68 and 812-1637, wherein cleaving a portion of the bound physically linked~~physically-linked~~ nucleic acid complex amplicons comprises inefficiently cleaving at a cleavable site in the first adapter resulting in both cleaved nucleic acid complexes and uncleaved nucleic acid complexes within each cluster on the surface.

~~39. The method of claim 38, wherein the ratio of uncleaved nucleic acid complexes of all nucleic acid complexes within each cluster on the flow cell is 1%, 5%, 10%, 20%, 30%, 40%, 45%, or 50%.~~

1840. The method of claim 1738 ~~or 39~~, wherein the cleaved nucleic acid complexes are cleaved at a cleavable site in the linker domain of the first adapter by a cleavage facilitator.

~~41. The method of claim 40, wherein the cleavage is a site-directed enzymatic reaction.~~

1942. The method of claim 1840 ~~or claim 41~~, wherein the cleavage facilitator is an endonuclease, a CRISPR-associated enzyme, a nickase, a nickase variant, or a chemical process.

~~43. The method of claim 40 or claim 41, wherein the cleavage facilitator comprises a CRISPR-associated enzyme.~~

~~44. The method of claim 40 or claim 41, wherein the cleavage facilitator comprises a nickase or nickase variant.~~

~~45. The method of claim 40, wherein the cleavage facilitator comprises a chemical process.~~

2046. The method of any one of claims 1738-1945, wherein the amount of uncleaved nucleic acid complexes remaining on the surface can be scaled by controlling the amount or concentration of the cleavage facilitator being introduced for site-directed cleavage or by controlling the amount of time the cleavage facilitator is being introduced for site-directed cleavage.

2147. The method of any one of claims 1738-1945, wherein the uncleaved nucleic acid complexes are protected by addition of an anti-cleavage facilitator before or during the cleavage step.

~~48. The method of claim 47, wherein cleaving a portion of the bound physically-linked nucleic acid complex amplicons further comprises:~~

~~(i) introducing the anti-cleavage facilitator; and~~

~~(ii) either following or simultaneously with (i), introducing the cleavage facilitator,~~

~~wherein interaction with the anti-cleavage facilitator protects a physically-linked nucleic acid complex amplicon from cleavage.~~

2249. The method of any one of claims 1738-1944, wherein the cleavable site is created by hybridization of an oligonucleotide comprising an at least partially complementary sequence to the linker domain of the first adapter and wherein physically linked~~physically-linked~~ nucleic acid complex amplicons not hybridized with the oligonucleotide, are not cleaved.

2350. The method of any one of claims 1738-1944, wherein the cleavable site is created by hybridization of a first oligonucleotide comprising an at least partially complementary sequence to the linker domain of the adapter and an anti-cleavage motif is created by hybridization of a second oligonucleotide comprising an at least partially complementary sequence to the linker domain of the adapter, and wherein cleaving a portion of the bound physically linked~~physically-linked~~ nucleic acid complex amplicons further comprises:

(i) introducing a mixture of the first and second oligonucleotides; and

(ii) introducing the cleavage facilitator.

~~51. The method of claims 38-44, wherein the cleaved nucleic acid complexes are cleaved at a cleavable site in the first adapter by a catalytically active enzyme and the uncleaved nucleic acid complexes are protected from cleavage in the first adapter by a catalytically inactive enzyme.~~

~~52. The method of any one of claims 38-44, wherein the cleavage site is in a self-complementary portion of the first adapter or a single-stranded portion of the first adapter.~~

2453. The method of claim 1,52 wherein the cleavage site is available when the physically linked~~physically-linked~~ nucleic acid complex amplicons are in a self-hybridized configuration on the surface.

~~54. The method of any one of claims 38-44, wherein the cleavage site is available when the physically-linked nucleic acid complex amplicons are in a double-stranded bridge amplified configuration.~~