CLAIMS

I/We claim:

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

(a) amplifying a physically linked nucleic acid complex on a surface to produce physically linked nucleic acid complex amplicons bound to the surface in both a forward orientation and a reverse orientation, wherein the 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 nucleic acid complex amplicons bound to the surface in the reverse orientation or (ii) the physically linked nucleic acid complex amplicons bound to the surface in the forward orientation;

(c) cleaving a portion of the remaining bound 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 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 nucleic acid complex amplicons on the surface;

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

(g) cleaving the remaining bound 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 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 bound to the surface, 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 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 nucleic acid complex amplicons from each clonal cluster bound to the surface at (i) a 5' end of the physically linked nucleic acid complex amplicons or (ii) a 3' end of the physically linked nucleic acid complex amplicons;

(c) cleaving at least a portion of the remaining bound 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 nucleic acid complex amplicons from each clonal cluster comprises preserving

at least one 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 nucleic acid complex amplicon from each clonal cluster on the surface to repopulate each clonal cluster;

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

(g) cleaving the remaining bound 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:

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 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.

7. 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 bound on a surface to produce a plurality of clusters, each cluster comprising a plurality of physically linked nucleic acid complex amplicons representing an original double-stranded target nucleic acid molecule, wherein each physically linked nucleic acid complex amplicon comprises a first strand amplicon and a second strand amplicon, and wherein each 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 nucleic acid complex amplicons and thereby physically separating the first strand amplicons from the second strand amplicons;

(c) removing the unbound physically separated first strand amplicons from the surface-bound second strand amplicons, and the unbound physically separated second strand amplicons from the surface-bound first strand amplicons, such that 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 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 to produce a nucleic acid sequence read of the second strand for each cluster on the surface.

8. The method of claim 7, 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).

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

10. The method of any one of claims 7-9, 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. The method of claim 10, wherein the SDE is the association of sequence read information with steps (d) and (e) of claim 7.

12. The method of any one of claims 7-11, 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.

13. The method of any one of claims 1-12, wherein the first adapter comprises a cleavable site, domain, or motif.

14. The method of any one of claims 1-13, wherein the first adapter comprises a hairpin loop structure comprising a self-complementary stem portion and a single-stranded nucleotide loop portion.

15. The method of any of claims 1-14, 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.

16. 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).

17. The method of any one of claims 1-6 and 8-16, wherein cleaving a portion of the bound 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.

18. The method of claim 17, wherein the cleaved nucleic acid complexes are cleaved at a cleavable site in the linker domain of the first adapter by a cleavage facilitator.

19. The method of claim 18, wherein the cleavage facilitator is an endonuclease, a CRISPR-associated enzyme, a nickase, a nickase variant, or a chemical process.

20. The method of any one of claims 17-19, 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.

21. The method of any one of claims 17-19, wherein the uncleaved nucleic acid complexes are protected by addition of an anti-cleavage facilitator before or during the cleavage step.

22. The method of any one of claims 17-19, 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 nucleic acid complex amplicons not hybridized with the oligonucleotide, are not cleaved.

23. The method of any one of claims 17-19, 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 nucleic acid complex amplicons further comprises:

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

(ii) introducing the cleavage facilitator.

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