

CLAIMS

1. A solid support for use in sequencing, having a patterned surface, comprising a plurality of library DNA binding regions separated by interstitial regions, and a plurality of library DNA complexes attached to the library DNA binding regions;

wherein each library DNA binding region comprises a number of capture moieties adapted for capturing a library DNA complex, and at least a portion of the interstitial regions comprise clustering oligonucleotides;

wherein each library DNA complex comprises a single library polynucleotide and a number of accessory binding sites capable of binding to the capture moieties in the library DNA binding region by noncovalent or covalent interactions;

wherein the number of accessory binding sites on each library DNA complex is more than the number of capture moieties in each library DNA binding region; and

wherein the plurality of library DNA complexes are attached to the library binding regions through the capture moieties, and wherein the capture moieties are orthogonal to the clustering oligonucleotides.

2. The solid support of claim 1, wherein at least 50% of the library DNA binding regions are each occupied with no more than three library DNA complexes.

3. The solid support of claim 1, wherein at least 50% of the library DNA binding regions are each occupied with only one library DNA complex or only one dominant library DNA complex.

4. The solid support of any one of claims 1 to 3, wherein each library DNA complex comprises a scaffold, wherein the number of accessory binding sites are on the scaffold, and the library polynucleotide is attached to the scaffold by non-covalent or covalent interaction with a single template binding site on the scaffold.

5. The solid support of claim 4, wherein the scaffold comprises a nanoparticle, a dendrimer, a polymer with bottlebrush structure, a single strand DNA scaffold, or a polypeptide scaffold.

6. The solid support of claim 4, wherein the scaffold comprises a DNA dendrimer, a single strand DNA with bottlebrush structure, or a single strand DNA produced by rolling circle amplification (RCA).

7. The solid support of any one of claims 4 to 6, wherein the library polynucleotide is attached to the scaffold by noncovalent hybridization with the single template binding site.

8. The solid support of any one of claims 4 to 6, wherein the library polynucleotide is attached to the scaffold by covalent bonding with the single template binding site.

9. The solid support of claim 8, wherein the covalent bonding is selected from the group consisting of amine-NHS ester bonding, amine-imidoester bonding, amine-pentofluorophenyl ester bonding, amine-hydroxymethyl phosphine bonding, carboxyl-carbodiimide bonding, thiol-maleimide bonding, thiol-haloacetyl bonding, thiol-pyridyl disulfide bonding, thiol-thiosulfonate bonding, thiol-vinyl sulfone bonding, aldehyde-hydrazide bonding, aldehyde-alkoxyamine bonding, hydroxy-isocyanate bonding, azide-alkyne bonding, azide-phosphine bonding, transcyclooctene-tetrazine bonding, norbornene-tetrazine bonding, azide-cyclooctyne bonding, azide-norbornene bonding, oxoamine-aldehyde bonding, SpyTag-SpyCatcher bonding, Snap-tag-O⁶-benzylguanine bonding, CLIP-tag-O²-benzylcytosine bonding, and sortase-coupling bonding.

10. The solid support of any one of claims 1 to 9, wherein at least a portion of the capture moieties are adapted to capture the library DNA complex by non-covalent interactions with one or more accessory binding sites on the library DNA complex.

11. The solid support of claim 10, wherein at least a portion of the capture moieties each comprises an oligonucleotide seeding sequence that is capable of hybridizing with one accessory binding site on the library DNA complex.

12. The solid support of claim 11, wherein the oligonucleotide seeding sequence comprises 10 to 40 nucleotides.

13. The solid support of claims 10, wherein at least a portion of the capture moieties are adapted to capture the library DNA complex by avidin biotin interaction.

14. The solid support of any one of claims 1 to 9, wherein at least a portion of the capture moieties are adapted to capture the library DNA complex by forming covalent bonding with accessory binding sites on the library DNA complex.

15. The solid support of any one of claims 1 to 14, wherein the solid support is a patterned flowcell, comprising a plurality of nanowells on the patterned surface of the flowcell.

16. The solid support of claim 15, wherein at least a portion of the nanowells each comprises a single library DNA binding region that is inside the nanowell.

17. The solid support of claim 15, wherein at least a portion of the library DNA binding regions are nanowells or capture pads, forming a patterned surface on the flowcell.

18. The solid support of any one of claims 1 to 17, wherein the size of each library binding regions is from 10 nm to 100 nm.

19. The solid support of any one of claims 1 to 18, wherein at least a portion of the library DNA binding regions each comprises from 1 to 200 capture moieties.

20. The solid support of any one of claims 1 to 19, wherein the ratio of the clustering oligonucleotides to the capture moieties is from 10 to 100,000.

21. The solid support of any one of claim 1 to 20, wherein the clustering oligonucleotides comprise P5 and P7 primers, or P15 and P17 primers.

22. A method of preparing a patterned surface of a solid support for sequencing, comprising:

contacting a buffer solution comprising a plurality of library DNA complexes with the patterned surface comprising a plurality of library DNA binding regions separated by interstitial regions;

wherein each library DNA binding region comprises a number of capture moieties adapted for capturing one library DNA complex, at least a portion of the interstitial regions comprise clustering oligonucleotides, and the capture moieties are orthogonal to the clustering oligonucleotides;

wherein each library DNA complex comprises a single library polynucleotide and a number of accessory binding sites, and the number of accessory binding sites on each library DNA complex is more than the number of capture moieties in each library DNA binding region; and

attaching the plurality of library DNA complexes to the library DNA binding regions of the patterned surface by noncovalent or covalent interactions between the accessory binding sites of the library DNA complexes and the capture moieties of library DNA binding regions.

23. The method of claim 22, wherein at least 50% of the library DNA binding regions are each occupied with no more than three library DNA complexes.

24. The method of claim 22 or 23, wherein at least 50% of the library DNA binding regions are each occupied with only one library DNA complex or only one dominant library DNA complex.

25. The method of any one of claims 22 to 24, further comprising amplifying the library polynucleotides.

26. The method of claim 25, wherein at least 50% of clusters generated from the amplification are monoclonal clusters.

27. The method of any one of claims 22 to 26, wherein the solid support comprises a flowcell.

28. The method of claim 27, wherein the flowcell has a patterned surface comprising a plurality of nanowells, and at least a portion of the nanowells each comprises a single library DNA binding region that is inside the nanowell.

29. The method of claim 27, wherein each of the library DNA binding region is a nanowell or a capture pad, forming a patterned surface on the flowcell.

30. The method of any one of claims 22 to 29, wherein the size of each library binding regions is from 10 nm to 100 nm, or 20 nm to 50 nm.

31. The method of any one of claims 22 to 30, wherein each library DNA complex comprises a scaffold, wherein the number of accessory binding sites are on the scaffold, and the single library polynucleotide is attached to the scaffold by non-covalent or covalent interaction with a single template binding site on the scaffold.

32. The method of claim 31, wherein the scaffold comprises a nanoparticle, a dendrimer, a polymer with bottlebrush structure, a single strand DNA scaffold, or a polypeptide scaffold.

33. The method of claim 31 or 32, wherein the library polynucleotide is attached to the scaffold by noncovalent hybridization with the single template binding site, or by covalent bonding with the single template binding site.

34. The method of any one of claims 22 to 33, wherein at least a portion of the capture moieties each comprises an oligonucleotide seeding sequence, and each library DNA complex is attached to the library DNA binding region by hybridization of one or more accessory binding sites on the library DNA complex with the oligonucleotide seeding sequences.

35. The method of claim 34, wherein the oligonucleotide seeding sequence comprises 10 to 40 nucleotides.

36. The method of any one of claims 22 to 33, wherein at least a portion of the capture moieties each comprises an avidin moiety, at least a portion of the accessory binding sites on the library DNA complex each comprises a biotin moiety, and the library DNA complex is attached to the library DNA binding region by avidin biotin interaction.

37. The method of any one of claims 22 to 33, wherein each library DNA complex is attached to the library DNA binding region by covalent bonding selected from the group consisting of amine-NHS ester bonding, amine-imidoester bonding, amine-pentofluorophenyl ester bonding, amine-hydroxymethyl phosphine bonding, carboxyl-carbodiimide bonding, thiol-maleimide bonding, thiol-haloacetyl bonding, thiol-pyridyl disulfide bonding, thiol-thiosulfonate bonding, thiol-vinyl sulfone bonding, aldehyde-hydrazide bonding, aldehyde-alkoxyamine bonding, hydroxy-isocyanate bonding, azide-alkyne bonding, azide-phosphine bonding, transcyclooctene-tetrazine bonding, norbornene-tetrazine bonding, azide-cyclooctyne bonding, azide-norbornene bonding, oxoamine-aldehyde bonding, SpyTag-SpyCatcher bonding, Snap-tag-O⁶-benzylguanine bonding, CLIP-tag-O²-benzylcytosine bonding, and sortase-coupling bonding.

38. The method of any one of claims 22 to 37, wherein at least a portion of the library DNA binding region each comprises from 1 to 200 capture moieties.

39. The method of any one of claims 22 to 38, wherein the ratio of the clustering oligonucleotides to the capture moieties is from 10 to 100,000.

40. The method of any one of claims 22 to 39, wherein the clustering oligonucleotides comprise P5 and P7 primers, or P15 and P17 primers.