

What is Claimed is:

1. A method of preparing an immobilized library of randomly fragmented, double-stranded nucleic acid fragments comprising:
 - (a) providing a solid support having CRISPR/Cas9 enzymes immobilized thereon; and
 - (b) applying a target double-stranded nucleic acid to the solid support under conditions whereby the target double-stranded nucleic acid is randomly fragmented by the CRISPR/Cas9 enzymes, and the CRISPR/Cas9 binds at least one strand of the double-stranded nucleic acid fragments; thereby producing an immobilized library of randomly fragmented, double-stranded nucleic acid fragments.
2. The method of claim 1, wherein the target double-stranded nucleic acid comprises double-stranded DNA (dsDNA), double-stranded RNA (dsRNA), or a double-stranded RNA/DNA hybrid.
3. The method of claim 1, wherein the target double-stranded nucleic acid is dsDNA.
4. The method of any one of claims 1 to 3, wherein CRISPR/Cas9 enzymes are bound to a first polynucleotide that directs the CRISPR/Cas9 enzymes to bind the target double-stranded nucleic acid in a non-sequence specific manner.
5. The method of claim 4, wherein the first polynucleotide is immobilized to the solid support.
6. The method of claim 4 or claim 5, wherein the first polynucleotide comprises a 3' portion comprising a CRISPR/Cas9 end sequence and a 5' portion comprising a single-stranded guide RNA (sgRNA) that directs the CRISPR/Cas9 enzymes to bind the target nucleic acid in a non-sequence specific manner.
7. The method of claim 6, wherein the sgRNA comprises (GC)_n or (AT)_n, wherein n is 5-20.
8. The method of claim 7, wherein n is 10.
9. The method of claim 6, wherein the sgRNA is 10 to 40 nucleotides.
10. The method of claim 9, wherein the sgRNA is 17 or 20 nucleotides.
11. The method of any one of claims 4 to 10, wherein the first polynucleotide is biotinylated and the solid support comprises one or more biotin binding proteins.
12. The method of claim 11, wherein the biotin binding proteins comprise avidin, streptavidin, neutravidin, an anti-biotin antibody, a biotin receptor, and/or a biotin-binding enzyme.
13. The method of claim 12, wherein the biotin-binding enzyme comprises biotinidase or

biotin holocarboxylase synthetase.

14. The method of any one of claims 1 to 13, comprising washing the solid support with the double-stranded nucleic acid fragments immobilized thereon to remove any unbound nucleic acids.

15. The method of any one of claims 4 to 14, comprising amplifying the double-stranded nucleic acid fragments immobilized on the solid surface.

16. The method of claim 15, wherein the amplifying comprises providing a polymerase and an amplification primer corresponding to a portion of the first polynucleotide.

17. The method of any one of claims 1 to 16, wherein applying the target double-stranded nucleic acid to the solid support comprises treating the CRISPR/Cas9 enzymes with one or more reagents that reduce the nucleic acid binding specificity of the CRISPR/Cas9 enzymes.

18. The method of claim 18, wherein the one or more reagents comprise betaine, dimethyl sulfoxide (DMSO), ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, and/or sulphalane.

19. The method of any one of claims 1 to 18, wherein the CRISPR/Cas9 enzymes are present on the solid support at a density of at least 10^3 , 10^4 , 10^5 , or 10^6 enzymes per mm^2 .

20. The method of any one of claims 1 to 19, wherein the lengths of the double-stranded nucleic acid fragments in the immobilized library are proportional to the density of CRISPR/Cas9 enzymes on the solid support.

21. The method of any one of claims 1 to 20, wherein the solid support comprises microparticles, a patterned surface, or wells.

22. The method of claim 21, wherein the microparticles are beads.

23. The method of any one of claims 1 to 22, comprising: (c) applying an intercalating dye to at least a portion of the immobilized library of double-stranded nucleic acid fragments to obtain a set of stained immobilized fragments; and obtaining an image of the stained immobilized fragments.

24. The method of any one of claims 1 to 23, wherein applying a target double-stranded nucleic acid comprises adding a biological sample to the solid support.

25. The method of claim 24, wherein the biological sample comprises a cell lysate.

26. The method of claim 24, wherein the biological sample comprises whole cells.

27. The method of claim 24, wherein the biological sample is selected from the group consisting of blood, plasma, serum, lymph, mucus, sputum, urine, semen, cerebrospinal fluid, bronchial aspirate, feces, and macerated tissue.

28. The method of any one of claims 1 to 27, comprising tagging the double-stranded

nucleic acid fragments.

29. The method of claim 28, wherein the double-stranded nucleic acid fragments are tagged with a first tag comprising a first tag domain.

30. The method of claim 29, wherein first tag domain comprises a region for cluster amplification.

31. The method of claim 29 or claim 30, wherein the first tag domain comprises a region for priming a sequencing reaction.

32. The method of any one of claims 1 to 31, comprising liberating the immobilized double-stranded nucleic acid fragments from the solid support.

33. The method of claim 32, wherein the liberating comprises cleavage of the CRISPR/Cas9 enzymes from the solid support.

34. The method of claim 32, wherein the liberating comprises performing polymerase chain reaction (PCR), strand displacement amplification (SDA), transcription mediated amplification (TMA) and nucleic acid sequence based amplification (NASBA), or other amplification process.

35. The method of claim 34, wherein the PCR comprises suppression PCR.

36. The method of claim 32, wherein the liberating comprises applying light or heat.

37. A method of preparing an immobilized library of randomly fragmented, double-stranded nucleic acid fragments comprising:

- (a) providing a solid support having CRISPR/Cas9 complexes immobilized thereon, wherein the CRISPR/Cas9 complexes comprise a CRISPR/Cas9 enzyme bound to a biotinylated first polynucleotide comprising a 3' portion comprising a CRISPR/Cas9 end sequence and a 5' portion comprising a single-stranded guide RNA (sgRNA) that directs the CRISPR/Cas9 enzymes to bind the target nucleic acid in a non-sequence specific manner, and wherein the biotinylated first polynucleotide is bound to a biotin binding protein on the solid support; and
- (b) applying a target double-stranded nucleic acid to the solid support under conditions whereby the target double-stranded nucleic acid is randomly fragmented by the CRISPR/Cas9 complexes, and the CRISPR/Cas9 complexes bind at least one strand of the double-stranded nucleic acid fragments; thereby producing an immobilized library of randomly fragmented, double-stranded nucleic acid fragments.

38. The method of claim 37, wherein the biotin binding protein comprises avidin, streptavidin, neutravidin, an anti-biotin antibody, a biotin receptor, and/or a biotin-binding enzyme.

39. The method of claim 38, wherein the biotin-binding enzyme comprises biotinidase or biotin holocarboxylase synthetase.

40. The method of claim 38 or claim 39, wherein applying the target double-stranded nucleic acid to the solid support comprises treating the CRISPR/Cas9 enzymes with one or more reagents that reduce the nucleic acid binding specificity of the CRISPR/Cas9 enzymes.

41. The method of claim 40, wherein the one or more reagents comprise betaine, dimethyl sulfoxide (DMSO), ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, and/or sulphalane.

42. The method of any one of claims 37 to 41, comprising tagging the double-stranded nucleic acid fragments.

43. The method of claim 42, wherein the double-stranded nucleic acid fragments are tagged with a first tag comprising a first tag domain.

44. The method of claim 43, wherein first tag domain comprises a region for cluster amplification.

45. The method of claim 43 or claim 44, wherein the first tag domain comprises a region for priming a sequencing reaction.

46. The method of any one of claims 37 to 45, comprising liberating the immobilized double-stranded nucleic acid fragments from the solid support.

47. The method of claim 46, wherein the liberating comprises cleavage of the CRISPR/Cas9 enzymes from the solid support.

48. The method of claim 47, wherein the liberating comprises performing PCR or other amplification process.

49. The method of claim 48, wherein the PCR comprises suppression PCR.

50. The method of claim 48, wherein the liberating comprises applying light or heat.

51. A solid support having a library of double-stranded nucleic acid fragments immobilized thereon prepared according to the method of any one of claims 1 to 45.

52. A solid support having CRISPR/Cas9 complexes immobilized thereon, wherein the CRISPR/Cas9 complexes comprise CRISPR/Cas9 enzymes that randomly fragment a target double-stranded nucleic acid.

53. The solid support of claim 52, wherein the target double-stranded nucleic acid comprises double-stranded DNA (dsDNA), double-stranded RNA (dsRNA), or a double-stranded RNA/DNA hybrid.

54. The solid support of claim 53, wherein the target double-stranded nucleic acid is dsDNA.

55. The solid support of any one of claims 51 to 54, wherein CRISPR/Cas9 enzymes are bound to a first polynucleotide that directs the CRISPR/Cas9 enzymes to bind the target double-stranded nucleic acid in a non-sequence specific manner.

56. The solid support of claim 55, wherein the first polynucleotide is immobilized to the solid support.

57. The solid support of claim 55 or claim 56, wherein the first polynucleotide comprises a 3' portion comprising a CRISPR/Cas9 end sequence and a 5' portion comprising a single-stranded guide RNA (sgRNA) that directs the CRISPR/Cas9 enzymes to bind the target nucleic acid in a non-sequence specific manner.

58. The solid support of claim 57, wherein the sgRNA comprises (GC)_n or (AT)_n, wherein n is 5-20.

59. The method of claim 58, wherein n is 10.

60. The method of claim 57, wherein the sgRNA is 10 to 40 nucleotides.

61. The method of claim 60, wherein the sgRNA is 17 or 20 nucleotides.

62. The solid support of any one of claims 55 to 51, wherein the first polynucleotide is biotinylated and the solid support comprises biotin binding proteins.

63. The solid support of claim 62, wherein the biotin binding proteins comprise avidin, streptavidin, neutravidin, an anti-biotin antibody, a biotin receptor, and/or a biotin-binding enzyme.

64. The solid support of claim 63, wherein the biotin-binding enzyme comprises biotinidase or biotin holocarboxylase synthetase.

65. The solid support of any one of claims 52 to 64, wherein the CRISPR/Cas9 enzymes are present on the solid support at a density of at least 10³, 10⁴, 10⁵, or 10⁶ enzymes per mm².

66. The solid support of any one of claims 52 to 65, wherein the solid support comprises microparticles, a patterned surface, or wells.

67. The solid support of claim 66, wherein the microparticles are beads.

68. A composition comprising the solid support of any one of claims 52 to 67 and one or more reagents that reduce the nucleic acid binding specificity of the CRISPR/Cas9 enzymes.

69. The composition of claim 68, wherein the one or more reagents comprise betaine, dimethyl sulfoxide (DMSO), ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, and/or sulphalane.

70. A solid support having CRISPR/Cas9 complexes immobilized thereon, wherein the CRISPR/Cas9 complexes comprise a CRISPR/Cas9 enzyme bound to a biotinylated first polynucleotide comprising a 3' portion comprising a CRISPR/Cas9 end sequence and a 5' portion comprising a single-stranded guide RNA (sgRNA) that directs the CRISPR/Cas9 enzymes to bind a target double-stranded nucleic acid in a non-sequence specific manner, and wherein the biotinylated first polynucleotide is bound to biotin binding protein on the solid support.

71. The solid support of claim 70, wherein the biotin binding protein comprises avidin, streptavidin, neutravidin, an anti-biotin antibody, a biotin receptor, and/or a biotin-binding enzyme.

72. The solid support of claim 71, wherein the biotin-binding enzyme comprises biotinidase or biotin holocarboxylase synthetase.

73. The solid support of any one of claims 70 to 72, wherein the CRISPR/Cas9 complexes are present on the solid support at a density of at least 10^3 , 10^4 , 10^5 , 10^6 complexes per mm^2 .

74. The solid support of any one of claims 70 to 73, wherein the solid support comprises microparticles, a patterned surface, or wells.

75. The solid support of claim 74, wherein the microparticles are beads.

76. A composition comprising the solid support of any one of claims 70 to 75 and one or more reagents that reduces the nucleic acid binding specificity of the CRISPR/Cas9 enzymes.

77. The composition of claim 76, wherein the one or more reagents comprise betaine, dimethyl sulfoxide (DMSO), ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, and/or sulphalane.