

What is claimed is:

1. A method for identifying the presence of a predetermined nucleotide sequence in a target nucleic acid comprising:

(1) hybridizing a target binding domain of a first probe to a first region of a target nucleic acid, wherein the probe comprises

~~A probe comprising~~ a target binding domain and a barcode domain;

wherein the target binding domain is at least 12 nucleotides in length~~comprises at least eight nucleotides and hybridizes to a target nucleic acid, wherein at least six nucleotides in the target binding domain identify a corresponding nucleotide in the target nucleic acid molecule and wherein at least two nucleotides in the target binding domain do not identify a corresponding nucleotide in the target nucleic acid molecule;~~

wherein the barcode domain comprises a synthetic backbone, the barcode domain comprising at least three attachment positions, each attachment position comprising at least one attachment ~~position~~ region comprising at least one nucleic acid sequence that hybridizes to a complementary nucleic acid molecule, and wherein the synthetic backbone comprises L-DNA,

wherein ~~each attachment position of the at least three attachment positions corresponds to two nucleotides of the at least six nucleotides in the target binding domain and~~ each of the at least three attachment positions have a different nucleic acid sequence, and

wherein each nucleotide of the at least one nucleic acid sequence of each attachment region is L-DNA;

(2) hybridizing a first complementary nucleic acid molecule comprising at least one first detectable label and at least one second detectable label to a first attachment position of the at least three attachment positions of the barcode domain;

(3) identifying the at least one first and the at least one second detectable label of the first complementary nucleic acid molecule hybridized to the first attachment position;

(4) removing the at least one first and the at least one second detectable label hybridized to the first attachment position;

(5) hybridizing a second complementary nucleic acid molecule comprising at least one third detectable label and at least one fourth detectable label to a second attachment position of the at least three attachment positions of the barcode domain;

(6) identifying the at least one third and the at least one fourth detectable label of the

second complementary nucleic acid molecule hybridized to the second attachment position;

(7) removing the at least one third and the at least one fourth detectable label
hybridized to the second attachment position;

(8) hybridizing a third complementary nucleic acid molecule comprising at least one
fifth detectable label and at least one sixth detectable label to a third attachment position of
the at least three attachment positions of the barcode domain;

(9) identifying the at least one fifth and the at least one sixth detectable label of the
third complementary nucleic acid molecule hybridized to the third attachment position; and

(10) determining the presence of the predetermined nucleotide sequence based on at
least the identity of the at least one first detectable label, the at least one second detectable
label, the at least one third detectable label, the at least one fourth detectable label, the at least
one fifth detectable label and the at least one sixth detectable label

~~wherein the nucleic acid sequence of each position of the at least three~~
~~attachment positions determines the position and identity of the corresponding two~~
~~nucleotides of the at least six nucleotides in the target nucleic acid that is bound by the~~
~~target binding domain~~

2. ~~The probe of claim 1, wherein the probe comprises about 60 nucleotides.~~

32. ~~The probe of claim 1 or method of claim 21, wherein the probe comprises a single-~~
~~stranded DNA synthetic backbone and a double stranded DNA spacer between the target~~
~~binding domain and the barcode domain.~~

43. ~~The probe of any of claims 1-3 method of claim 1 or claim 2, wherein the single-~~
~~stranded DNA synthetic backbone comprises about 27 nucleotides is about 10 nucleotides to~~
~~about 100 nucleotides in length.~~

5. ~~The probe of claim 3 or claim 4, wherein the double stranded DNA spacer comprises L-~~
~~DNA.~~

6. ~~The probe of any of claims 3-5, wherein the double stranded DNA spacer comprises~~
~~about 25 nucleotides in length.~~

~~74.~~ The ~~probe of any of claims 1-6~~method of any one of the preceding claims, wherein the number of nucleotides in the target binding domain is greater than the number of attachment positions in the barcode domain.

~~85.~~ The ~~probe of any of claims 1-7~~method of any one of the preceding claims, wherein ~~the target binding domain comprises eight nucleotides and~~ the barcode domain comprises at least four~~three~~ attachment positions, wherein the method further comprises, after step (9) and prior to step (10):

i) removing the at least one fifth and the at least one sixth detectable label hybridized to the third attachment position;

ii) hybridizing a fourth complementary nucleic acid molecule comprising at least one seventh detectable label and at least one eighth detectable label to a fourth attachment position of the at least four attachment positions of the barcode domain; and

iii) identifying the at least one seventh and the at least one eighth detectable label of the fourth complementary nucleic acid molecule hybridized to the fourth attachment position, and

wherein step (10) further comprises determining the presence of the predetermined nucleotide sequence based on at least the identity of the at least one first detectable label, the at least one second detectable label, the at least one third detectable label, the at least one fourth detectable label, the at least one fifth detectable label, the at least one sixth detectable label, the at least one seventh detectable label and the at least one eighth detectable label.

~~9. The probe of any of claims 1-8, wherein at least one of the nucleotides in the target binding domain that does not identify a corresponding nucleotide in the target nucleic acid molecule precedes the at least six nucleotides in the target binding domain and wherein at least one of the nucleotides in the target binding domain that does not identify a corresponding nucleotide in the target nucleic acid molecule follows the at least six nucleotides in the target binding domain.~~

~~106.~~ The ~~probe of any of claims 1-9~~method of any one of the preceding claims, wherein each attachment position in the barcode domain comprises one attachment region.

~~117.~~ The ~~probe of any of claims 1-10~~method of any one of the preceding claims, wherein the at least one nucleic acid sequence of each attachment position in the barcode domain comprises is:

- a) about 6 to about 20 nucleotides in length;
- b) about 9 nucleotides in length;
- c) about 12 nucleotides in length;
- d) about 14 nucleotides in length; or
- e) about 16 nucleotides in length.

~~12. The probe of any of claims 1-11, wherein the at least one nucleic acid sequence of each attachment position comprises a 3' terminal guanosine nucleotide.~~

~~13. The probe of any of claims 1-12, wherein the at least one nucleic acid sequence of each attachment position comprises at least one adenine nucleotide, at least one thymine nucleotide, at least one cytosine nucleotide or any combination thereof and a 3' terminal guanosine nucleotide.~~

~~14. The probe of any of claims 1-13, wherein each nucleotide of the at least one nucleic acid sequence of each attachment position is L-DNA.~~

~~15. The probe of any of claims 1-14 method of any one of the preceding claims, wherein each nucleotide of the at least eight 12 nucleotides of the target binding domain is D-DNA.~~

~~16. The probe of any of claims 1-15, wherein the complementary nucleic acid molecule is a primary nucleic acid molecule, wherein the primary nucleic acid molecule directly binds to at least one attachment region within at least one attachment position of the barcode domain.~~

~~17. The probe of any of claims 1-16, wherein the primary nucleic acid molecule comprises at least two domains;~~

~~a first domain capable of binding to at least one attachment region within at least one attachment position of the barcode domain and~~

~~a second domain capable of binding to at least one complementary secondary nucleic acid molecule.~~

~~18. The sequence probe of claim 17, wherein the first domain of the primary nucleic acid molecule comprises L-DNA.~~

19. ~~The probe of claim 17 or 18, wherein the second domain of the primary nucleic acid molecule comprises D-DNA.~~

20. ~~The probe of any of claims 17-19, wherein the first domain of the primary nucleic acid molecule comprises a 5' terminal cytosine nucleotide.~~

21. ~~The probe of any of claims 17-20, wherein the first domain of the primary nucleic acid molecule comprises at least one adenine nucleotide, at least one thymine nucleotide, at least one guanine nucleotide or any combination thereof and a 5' terminal cytosine nucleotide.~~

22. ~~The probe of any of claims 17-21, further comprising a cleavable linker located between the first domain of the primary nucleic acid molecule and the second domain of the primary nucleic acid molecule.~~

23. ~~The probe of claim 22, wherein the cleavable linker comprises at least one cleavable moiety.~~

24. ~~The probe of claim 23, wherein the cleavable moiety is a photocleavable moiety.~~

25. ~~The probe of any of claims 17-24, wherein the primary nucleic molecule is hybridized to at least one attachment region within at least one attachment position of the barcode domain and is hybridized to at least one secondary nucleic acid molecule.~~

26. ~~The probe of any of claims 17-25, wherein the primary nucleic molecule is hybridized to four secondary nucleic acid molecules.~~

27. ~~The probe of any of claims 17-26, wherein the secondary nucleic acid molecule comprises at least two domains,~~

~~a first domain capable of binding to a complementary sequence in at least one primary nucleic acid molecule; and~~

~~a second domain capable of binding to (a) a first detectable label and an at least second detectable label, (b) to at least one complementary tertiary nucleic acid molecule, or (c) a combination thereof.~~

~~28. The probe of claim 27, wherein the secondary nucleic acid molecule comprises a cleavable linker.~~

~~29. The probe of claim 28, wherein the cleavable linker is located between the first domain and the second domain.~~

~~30. The probe of claim 28 or claim 29, wherein the linker is photo-cleavable.~~

~~31. The probe of any of claims 27-30, wherein the secondary nucleic molecule is hybridized to at least one primary nucleic acid molecule and is hybridized to at least one tertiary nucleic acid molecule.~~

~~32. The probe of any of claims 27-31, wherein the secondary nucleic molecule is hybridized to (a) at least one primary nucleic acid molecule, (b) at least one tertiary nucleic acid molecule, and (c) a first detectable label and an at least second detectable label.~~

~~33. The probe of claim 32, wherein each secondary nucleic molecule is hybridized to one tertiary nucleic acid molecule.~~

~~34. The probe of claim 32 or claim 33, wherein the first and at least second detectable labels have the same emission spectrum or have different emission spectra.~~

~~35. The probe of any of claims 27-34, wherein the tertiary nucleic acid molecule comprises at least two domains,~~

~~a first domain capable of binding to a complementary sequence in a secondary nucleic acid molecule; and~~

~~a second domain capable of binding to a first detectable label and an at least second detectable label.~~

~~36. The probe of claim 35, wherein the tertiary nucleic acid molecule comprises a cleavable linker.~~

37. ~~The probe of claim 36, wherein the cleavable linker is located between the first domain and the second domain.~~
38. ~~The probe of claim 36 or claim 37, wherein the linker is photo-cleavable.~~
39. ~~The probe of any of claims 27-38, wherein the tertiary nucleic molecule is hybridized to at least one secondary nucleic acid molecule and comprises a first detectable label and an at least second detectable label.~~
40. ~~The probe of claim 39, wherein the first and at least second detectable labels have the same emission spectrum or have different emission spectra.~~
41. ~~The probe of claim 39 or claim 40, wherein the at least first and second detectable labels located on the secondary nucleic acid molecule have the same emission spectra and the at least first and second detectable labels located on the tertiary nucleic acid molecule have the same emission spectra, and wherein the emission spectra of the detectable labels on the secondary nucleic acid molecule are different than the emission spectra of the detectable labels on the tertiary nucleic acid molecule.~~
42. ~~The probe of any of claims 25-41, wherein the primary nucleic acid molecule is hybridized to four secondary nucleic acid molecules;~~
~~— wherein each of the four secondary nucleic acid molecules comprises four first detectable labels, and~~
~~— wherein each of the four secondary nucleic acid molecules is hybridized to one tertiary nucleic acid molecule;~~
~~— wherein the tertiary nucleic acid molecule comprises five detectable labels.~~
43. ~~The probe of claim 42, wherein emission spectra of the first detectable labels of the secondary nucleic acid molecules are different than the emission spectra of the second detectable labels on the tertiary nucleic acid molecules.~~
44. ~~A method for determining a nucleotide sequence of a nucleic acid comprising~~

~~—— (1) hybridizing the target binding domain of at least one first probe of any of claims 1-43 to a first region of a target nucleic acid that is optionally immobilized to a substrate at one or more positions;~~
~~—— (2) hybridizing a first complementary nucleic acid molecule comprising at least one first detectable label and at least one second detectable label to a first attachment position of the at least three attachment positions of the barcode domain;~~
~~—— (3) identifying the at least one first and the at least one second detectable label of the first complementary nucleic acid molecule hybridized to the first attachment position;~~
~~—— (4) removing the at least one first and the at least one second detectable label hybridized to the first attachment position;~~
~~—— (5) hybridizing a second complementary nucleic acid molecule comprising at least one third detectable label and at least one fourth detectable label to a second attachment position of the at least three attachment positions of the barcode domain;~~
~~—— (6) identifying the at least one third and the at least one fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position;~~
~~—— (7) removing the at least one third and the at least one fourth detectable label hybridized to the second attachment position;~~
~~—— (8) hybridizing a third complementary nucleic acid molecule comprising at least one fifth detectable label and at least one sixth detectable label to a third attachment position of the at least three attachment positions of the barcode domain;~~
~~—— (9) identifying the at least one fifth and the at least one sixth detectable label of the third complementary nucleic acid molecule hybridized to the third attachment position; and~~
~~—— (10) determining the nucleotide sequence of at least six nucleotides of the optionally immobilized target nucleic acid hybridized to the at least six nucleotides of the target binding domain of the at least one first probe based on the identity of the at least one first detectable label, the at least one second detectable label, the at least one third detectable label, the at least one fourth detectable label, the at least one fifth detectable label and the at least one sixth detectable label.~~

459. The method of ~~claim 44~~any one of the preceding claims, wherein steps (4) and (5) occur sequentially or concurrently.

4610. The method of any one of the preceding claims~~claim 44 or claim 45~~, wherein steps (7) and (8) occur sequentially or concurrently.

~~47~~11. The method of any one of the preceding claims~~of claims 44-46~~, wherein the first and second detectable labels have the same emission spectrum or have different emission spectra.

~~48~~12. The method of any one of the preceding claims~~of claims 44-47~~, wherein the third and fourth detectable labels have the same emission spectrum or have different emission spectra.

~~49~~13. The method of any one of the preceding claims~~of claims 44-48~~, wherein the fifth and sixth detectable labels have the same emission spectrum or have different emission spectra.

~~50~~14. The method of any one of the preceding claims~~of claims 44-49~~, wherein the first complementary nucleic acid molecule, the second complementary nucleic acid molecule and the third complementary nucleic acid molecule each comprise a cleavable linker.

~~51~~15. The method of claim ~~50~~14, wherein the cleavable linker is photo-cleavable.

~~52~~16. The method of any one of the preceding claims~~of claims 44-51~~, wherein the first complementary nucleic acid molecule comprises a reporter probe comprising a primary nucleic acid, four secondary nucleic acid molecules and four tertiary nucleic acid molecules,
wherein the primary nucleic acid molecule comprises at least two domains,
a first domain that hybridizes to the first attachment position of the barcode domain
and
a second domain that is hybridized to six secondary nucleic acid molecules,
~~wherein the primary nucleic acid is hybridized to four secondary nucleic acid~~
~~molecules,~~
~~wherein each of the four secondary nucleic acid molecules comprises four first~~
~~detectable labels, and~~
~~wherein each of the four secondary nucleic acid molecules is hybridized to one five~~
~~tertiary nucleic molecules,~~ wherein each of the tertiary nucleic acid molecules comprises a
detectable label,
~~wherein each of the four tertiary nucleic acid molecules comprises five second~~
~~detectable labels~~

~~53. The method of claim 52, wherein the primary nucleic acid molecule comprises at least two domains,~~

~~a first domain that hybridizes to the first attachment position of the barcode domain and~~

~~a second domain that hybridizes to the four secondary nucleic acid molecules.~~

~~54~~17. The method of claim ~~53~~16, wherein the primary nucleic acid molecule comprises a cleavable linker located between the first domain and the second domain.

18. The method of claim 17, wherein the cleavable linker is a photocleavable linker.

~~55~~19. The method of ~~claim 53 or claim 54~~any one of claims 16-18, wherein each of the secondary nucleic acid molecules comprises at least two domains,

a first domain that hybridizes to the second domain of the primary nucleic acid molecule; and

a second domain ~~that comprises four first detectable labels and that hybridizes to one~~the five tertiary nucleic acid molecules.

~~56~~20. The method of claim ~~55~~19, wherein each of the secondary nucleic acid molecules comprises a cleavable linker located between the first domain and the second domain.

21. The method of claim 20, wherein the cleavable linker is a photocleavable linker.

~~57~~22. The method of any one of claims ~~44-56~~16-21, wherein removing the at least one first and the at least one second detectable label hybridized to the first attachment position comprises cleaving the cleavable linker between the first domain and the second domain of the primary nucleic acid, cleaving the cleavable linker between the first domain and the second domain of each secondary nucleic acid or any combination thereof. ~~58. The method of any of claims 44-57, further comprising:~~

~~— (11) removing the at least one first probe from the first region of the optionally immobilized target nucleic acid;~~

~~(12) hybridizing the target binding domain of a least one second probe of claim 1 to a second region of the optionally immobilized target nucleic acid and wherein the target binding domain of the first probe and the at least second probe are different;~~

(13) hybridizing a fourth complementary nucleic acid molecule comprising at least one seventh detectable label and at least one eighth detectable label to a first attachment position of the at least three attachment positions of the barcode domain of the at least one second probe;

—— (14) identifying the at least one seventh and the at least one eighth detectable label of the fourth complementary nucleic acid molecule hybridized to the first attachment position;

(15) removing the at least one seventh and the at least one eighth detectable label hybridized to the first attachment position;

—— (16) hybridizing a fifth complementary nucleic acid molecule comprising at least one ninth detectable label and at least one tenth detectable label to a second attachment position of the at least three attachment positions of the barcode domain of the at least second probe;

—— (17) identifying the at least one ninth and the at least one tenth detectable label of the fifth complementary nucleic acid molecule hybridized to the second attachment position;

(18) removing the at least one ninth and the at least one tenth detectable label hybridized to the second attachment position;

—— (19) hybridizing a sixth complementary nucleic acid molecule comprising at least one eleventh detectable label and at least one twelfth detectable label to a third attachment position of the at least three attachment positions of the barcode domain of the at least second probe;

—— (20) identifying the at least one eleventh and the at least one twelfth detectable label of the sixth complementary nucleic acid molecule hybridized to the third attachment position;

and

—— (21) determining the nucleotide sequence of at least six nucleotides of the optionally immobilized target nucleic acid hybridized to the at least six nucleotides of the target binding domain of the at least one second probe based on the identity of the at least one seventh detectable label, the at least one eighth detectable label, the at least one ninth detectable label, the at least one tenth detectable label, the at least one eleventh detectable label and the at least one twelfth detectable label.

59. — The method of claim 58, further comprising assembling each identified linear order of nucleotides in the at least first region and at least second region of the optionally immobilized target nucleic acid, thereby identifying a sequence for the optionally immobilized target nucleic acid