CHEMICAL COMPOSITIONS AND METHODS OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 62/424,887, filed November 21, 2016; U.S. Provisional Application No. 62/457,237, filed February 10, 2017; and U.S. Provisional Application No. 62/536,147, filed July 24, 2017. The contents of each of the aforementioned patent applications are incorporated herein by reference in their entireties.

SEQUENCE LISTING

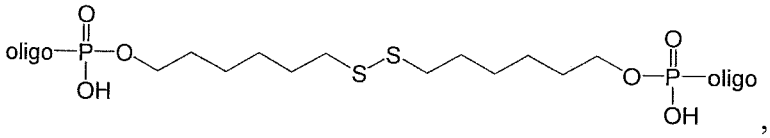
[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 20, 2017, is named NATE033-001WO_SeqList_ST25.txt and is 19,351 bytes in size.

BACKGROUND OF THE INVENTION

[0003] There are currently a variety of methods for nucleic acid sequencing, *i.e.,* the process of determining the precise order of nucleotides within a nucleic acid molecule. Current methods require amplifying a nucleic acid enzymatically, *e.g.,* PCR, and/or by cloning. Further enzymatic polymerizations are required to produce a detectable signal by a light detection means. Such amplification and polymerization steps are costly and/or time-consuming. Thus, there is a need in the art for a method of nucleic acid sequencing that is rapid and amplification- and enzyme-free. The present disclosure addresses these needs.

SUMMARY

[0004] The present disclosure provides sequencing probes, methods, kits, and apparatuses that provide rapid enzyme-free, amplification-free, and library-free nucleic acid sequencing that has long-read-lengths and with low error rate. The sequencing probes described herein include barcode domains in which each position in the barcode domain corresponds to at least two nucleotides in the target binding domain. Moreover, the methods, kits, and apparatuses have rapid sample-to-answer capability. These features are particularly useful for sequencing in a



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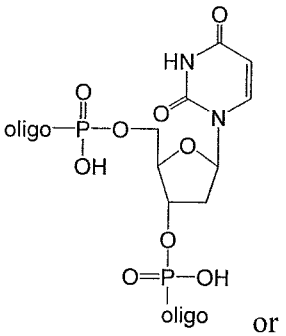

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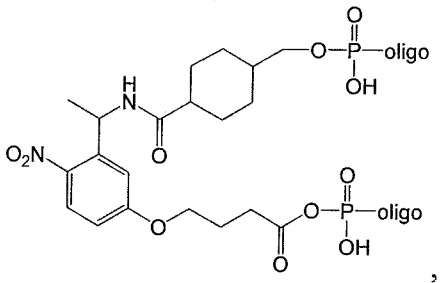


, and wherein the linker modification is located between the first and second domains.

[0005a] In one aspect, there is provided a complex comprising

a) a composition comprising a target binding domain and a barcode domain,

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, and wherein each of the at least three attachment positions have a different nucleic acid sequence; and

a first complementary primary nucleic acid molecule hybridized to a first attachment position of the at least three attachment positions,

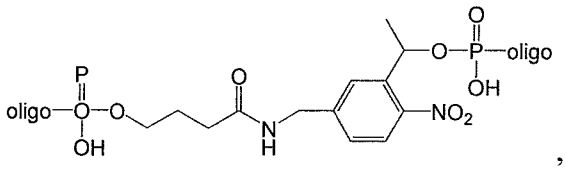
wherein the first complementary primary nucleic acid molecule comprises at least two domains and a cleavable linker,

wherein the first domain is hybridized to the first attachment position of the barcode domain and the second domain is capable of hybridizing to at least one complementary secondary nucleic acid molecule, and

wherein the cleavable linker is located between the first and second domains.

[0006] The present disclosure provides a complex comprising a) a composition comprising a target binding domain and a barcode domain, wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 at least two nucleotides of the at least six nucleotides in the target binding domain are modified nucleotides or nucleotide analogues; 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, 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 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; and a first complementary primary nucleic acid molecule hybridized to a first attachment position of the at least three attachment positions, wherein the first primary complementary nucleic acid molecule comprises at least two domains and a linker modification, wherein the first domain is hybridized to the first attachment position of the barcode domain and the second domain capable of hybridizing to at least one complementary secondary nucleic acid molecule, and wherein the linker modification is



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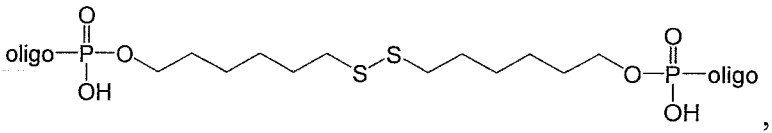

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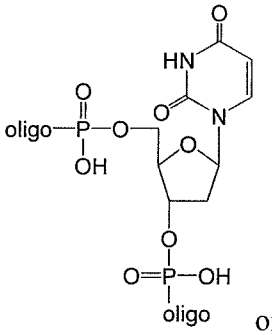
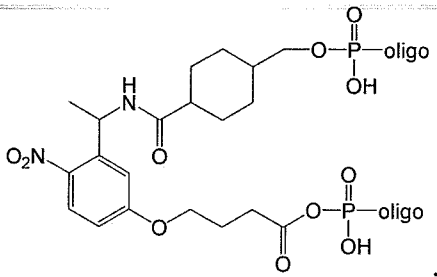


or



, and wherein the linker modification is located between the first and second domains.

[0006a] In another aspect, there is provided a complex comprising

a) a composition comprising a target binding domain and a barcode domain,

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain,

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 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; and

a first complementary primary nucleic acid molecule hybridized to a first attachment position of the at least three attachment positions,

wherein the first complementary primary nucleic acid molecule comprises at least two domains and a cleavable linker,

wherein the first domain is hybridized to the first attachment position of the barcode domain and the second domain is capable of hybridizing to at least one complementary secondary nucleic acid molecule, and

wherein the cleavable linker is located between the first and second domains.

[0007] The present disclosure provides a sequencing probe comprising a target binding domain and a barcode domain; wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 at least two nucleotides of the at least six nucleotides in the target binding domain are modified nucleotides or nucleotide analogues; 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, and wherein each of the at least three attachment positions have a different nucleic acid sequence.

[0007a] In another aspect, there is provided a probe comprising a target binding domain and a barcode domain;

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, and wherein each of the at least three attachment positions have a different nucleic acid sequence.

[0008] The present disclosure provides a sequencing probe comprising a target binding domain and a barcode domain; wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 at least two nucleotides of the at least six nucleotides in the target binding domain are modified nucleotides or nucleotide analogues; 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, 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 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.

[0008a] In another aspect, there is provided a probe comprising a target binding domain and a barcode domain;

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule,

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 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.

[0008b] In another aspect, there is provided a population of probes comprising a plurality of the probe as described herein.

[0009] The present disclosure provides a sequencing probe comprising a target binding domain and a barcode domain; wherein the target binding domain comprises at least ten nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying a corresponding nucleotide in the target nucleic acid molecule and wherein at least four 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the

the first and the second detectable label hybridized to the first attachment position; (5) hybridizing a second complementary nucleic acid molecule comprising a third detectable label and a fourth detectable label to a second attachment position of the at least three attachment positions of the barcode domain; (6) identifying the third and the fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position; (7) removing the third and fourth detectable label hybridized to the second attachment position; (8) hybridizing a third complementary nucleic acid molecule comprising a fifth detectable label and a sixth detectable label to a third attachment position of the at least three attachment positions of the barcode domain; (9) identifying the fifth and the sixth detectable label of the third complementary nucleic acid molecule hybridized to the third attachment position; and (10) identifying the linear order of at least six nucleotides of the optionally immobilized target nucleic acid hybridized to the target binding domain of the sequencing probe based on the identity of the first detectable label, second detectable label, third detectable label, fourth detectable label, fifth detectable label and sixth detectable label.

[0047a] In another aspect, there is provided a method for determining a nucleotide sequence of a nucleic acid comprising

(1) hybridizing a first probe of as described herein 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 a first detectable label and a second detectable label to a first attachment position of the at least three attachment positions of the barcode domain;

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

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

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

(6) identifying the third and the fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position;

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

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

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

(10) identifying the linear order of at least six nucleotides in the first region of the optionally immobilized target nucleic acid hybridized to the target binding domain of the first probe based on the identity of the first detectable label, second detectable label, third detectable label, fourth detectable label, fifth detectable label and sixth detectable label.

[0048] The method can further comprise (11) removing the at least first sequencing probe from the first region of the optionally immobilized target nucleic acid; (12) hybridizing at least a second sequencing probe as disclosed herein to a second region of the target nucleic acid that is optionally immobilized to a substrate at one or more positions, and wherein the target binding domain of the first sequencing probe and the at least second sequencing probe are different; (13) hybridizing a first complementary nucleic acid molecule comprising a first detectable label and a second detectable label to a first attachment position of the at least three attachment positions of the barcode domain; (14) detecting the first and the second detectable label of the first complementary nucleic acid molecule hybridized to the first attachment position; (15) hybridizing a second complementary nucleic acid molecule comprising a third detectable label and a fourth detectable label to a second attachment position of the at least three attachment positions of the barcode domain; (16) detecting the third and the fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position; (17) removing the third and fourth detectable label hybridized to the second attachment position; (18) hybridizing a third complementary nucleic acid molecule comprising a fifth detectable label and a sixth detectable label to a third attachment position of the at least three attachment positions of the barcode domain; (19) identifying the fifth and the sixth detectable label of the third

complementary nucleic acid molecule hybridized to the third attachment position; and (20) identifying the linear order of at least six nucleotides in the second region of the optionally immobilized target nucleic acid hybridized to the target binding domain of the at least second sequencing probe based on the identity of the first detectable label, second detectable label, third detectable label, fourth detectable label, fifth detectable label and sixth detectable label.

[0054] The method can further comprise assembling each identified linear order of nucleotides in the at least first region and at least second region of the immobilized target nucleic acid, thereby identifying a sequence for the immobilized target nucleic acid.

[0055] Steps (4) and (5) can occur sequentially or concurrently. The first and at least second detectable labels can have the same emission spectrum or can have different emission spectra. The third and at least fourth detectable labels can have the same emission spectrum or can have different emission spectra. The fifth and at least sixth detectable labels can have the same emission spectrum or can have different emission spectra.

[0056] The first complementary nucleic acid molecule can comprise a cleavable linker. The second complementary nucleic acid molecule can comprise a cleavable linker. The third complementary nucleic acid molecule can comprise a cleavable linker. The first complementary nucleic acid molecule, the second complementary nucleic acid molecule and the at least third complementary nucleic molecule can each comprise a cleavable linker. Preferably, the cleavable linker is photo-cleavable. A method of removing any one of the first complementary nucleic acid molecule, the second complementary nucleic acid molecule and the at least third complementary nucleic molecule can comprise contact with light. Preferably, UV light. The light can be provided by a light source selected from the group consisting of an arc-lamp, a laser, a focused UV light source, and light emitting diode.

[0057] The present disclosure also provides an apparatus for performing any of the methods disclosed herein.

[0057a] In another aspect, there is provided an apparatus for performing the method as described herein.

[0058] The present disclosure also provides one or more kits comprising a substrate, a population of sequencing probes disclosed herein, at least three complementary nucleic acid molecules comprising a first detectable label and at least two second detectable labels, and instructions for use. The one or more kits can further comprise at least one capture probe. The one or more kits can further comprise at least two capture probes.

[0058a] In another aspect, there is provided a kit comprising a substrate, a population of probes as described herein, at least one capture probe, at least three complementary nucleic acid molecules comprising a first detectable label and at least two second detectable labels, and instructions for use.

[0059] Any of the above aspects can be combined with any other aspect.

[0060] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms "a," "an," and "the" are understood to be singular or plural and the term "or" is understood to be inclusive. By way of example, "an element" means one or more element. Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

[0061] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed disclosure. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claim.

BRIEF DESCRIPTION OF THE DRAWINGS

[0062] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0063] The above and further features will be more clearly appreciated from the following detailed description when taken in conjunction with the accompanying drawings.

[0064] FIG. 1 is an illustration of one exemplary sequencing probe of the present disclosure.

[0065] FIG. 2 shows the design of standard and three-part sequencing probes of the present disclosure.

What is claimed is:

1. A complex comprising

a) a composition comprising a target binding domain and a barcode domain,

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, and wherein each of the at least three attachment positions have a different nucleic acid sequence; and

a first complementary primary nucleic acid molecule hybridized to a first attachment position of the at least three attachment positions,

wherein the first complementary primary nucleic acid molecule comprises at least two domains and a cleavable linker,

wherein the first domain is hybridized to the first attachment position of the barcode domain and the second domain is capable of hybridizing to at least one complementary secondary nucleic acid molecule, and

wherein the cleavable linker is located between the first and second domains.

2. A complex comprising

a) a composition comprising a target binding domain and a barcode domain,

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain,

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 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; and

a first complementary primary nucleic acid molecule hybridized to a first attachment position of the at least three attachment positions,

wherein the first complementary primary nucleic acid molecule comprises at least two domains and a cleavable linker,

wherein the first domain is hybridized to the first attachment position of the barcode domain and the second domain is capable of hybridizing to at least one complementary secondary nucleic acid molecule, and

wherein the cleavable linker is located between the first and second domains.

3. A probe comprising a target binding domain and a barcode domain;

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule, wherein the nucleic acid sequence of the at least three attachment positions determines the position and identity of the at least six nucleotides in the target nucleic acid that is bound by the target binding domain, and wherein each of the at least three attachment positions have a different nucleic acid sequence.

4. A probe comprising a target binding domain and a barcode domain;

wherein the target binding domain comprises at least eight nucleotides and is capable of binding a target nucleic acid, wherein at least six nucleotides in the target binding domain are capable of identifying 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 region comprising at least one nucleic acid sequence capable of being bound by a complementary nucleic acid molecule,

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 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.

5. The probe of claim 3, wherein said synthetic backbone comprises a polysaccharide, a polynucleotide, a peptide, a peptide nucleic acid, or a polypeptide.

6. The probe of claim 3, wherein said synthetic backbone comprises a single-stranded DNA.

7. The probe of claim 3, wherein said probe comprises a single-stranded DNA synthetic backbone and a double-stranded DNA spacer between the target binding domain and the barcode domain.

8. The probe of claim 7, wherein said double-stranded DNA spacer is from about 1 nucleotide to about 100 nucleotides in length.

9. The probe of claim 7, wherein said double-stranded DNA spacer is about 25 nucleotides in length.

10. The probe of claim 3, wherein the number of nucleotides in the target binding domain is greater than the number of attachment positions in the barcode domain.

11. The probe of claim 3, wherein the number of nucleotides in the target binding domain is at least five more than the number of attachment positions in the barcode domain.

12. The probe of claim 3, wherein the target binding domain comprises eight nucleotides and the barcode domain comprises three attachment positions.

13. The probe of claim 3, wherein at least four nucleotides of the at least six nucleotides in the target binding domain are modified nucleotides or nucleotide analogues.

14. The probe of claim 3, wherein at least six nucleotides of the at least six nucleotides in the target binding domain are modified nucleotides or nucleotide analogues.

15. The probe of claim 3, wherein the modified nucleotide or nucleic acid analogue is a locked nucleic acid (LNA).

16. The probe of claim 3, wherein at least one of the at least two 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 at least two 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.

17. The probe of claim 3, wherein each attachment position in the barcode domain comprises the same number of attachment regions.

18. The probe of claim 3, wherein at least one of the at least three attachment positions in the barcode domain comprises a different number of attachment regions than the other two.

19. The probe of claim 3, wherein each attachment position in the barcode domain comprises one attachment region.

20. The probe of claim 3, wherein each attachment position in the barcode domain comprises more than one attachment region.

21. The probe of claim 3, wherein when the attachment position in the barcode domain comprises more than one attachment region, the attachment regions are the same.

22. The probe of claim 3, wherein when the attachment position in the barcode domain comprises more than one attachment region, the attachment regions are different.

23. The probe of claim 3, wherein each nucleic acid sequence comprising each attachment region in the barcode domain is from about 8 nucleotides to about 20 nucleotides in length.

24. The probe of claim 3, wherein each nucleic acid sequence comprising each attachment region in the barcode domain is about 12 nucleotides in length.

25. The probe of claim 3, wherein each nucleic acid sequence comprising each attachment region in the barcode domain is about 14 nucleotides in length.

26. The probe of claim 3, wherein at least one of the at least three attachment positions in the barcode domain is adjacent to at least one flanking single-stranded polynucleotide.

27. The probe of claim 3, wherein each of the at least three attachment positions in the barcode domain is adjacent to at least one flanking single stranded polynucleotide.

28. The probe of claim 3, wherein at least one attachment region in at least one attachment position is integral to the synthetic backbone.

29. The probe of claim 3, wherein each attachment region in each of the at least three attachment positions is integral to the synthetic backbone.

30. The probe of claim 3, wherein the complementary nucleic acid molecule is RNA, DNA or PNA.

31. The probe of claim 30, wherein the complementary nucleic acid molecule is DNA.

32. The probe of claim 3, 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.

33. The probe of claim 32, 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.

34. The probe of claim 33, wherein the primary nucleic acid molecule comprises a cleavable linker.

35. The probe of claim 34, wherein the cleavable linker is located between the first domain and the second domain.

36. The probe of claim 34, wherein the linker is photo-cleavable.

37. The probe of claim 32, wherein the primary nucleic acid molecule is hybridized to at least one attachment region within at least one attachment position of the barcode domain and comprises a first detectable label and an at least second detectable label.

38. The probe of claim 33, wherein the primary nucleic acid 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.

39. The probe of claim 38, wherein the primary nucleic acid molecule is hybridized to at least one, two, three, four, five, or more secondary nucleic acid molecules.

40. The probe of claim 38, wherein the primary nucleic acid molecule is hybridized to four secondary nucleic acid molecules.

41. The probe of claim 37, wherein the first and at least second detectable labels have the same emission spectrum or have different emission spectra.

42. The probe of claim 33, 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.

43. The probe of claim 42, wherein the secondary nucleic acid molecule comprises a cleavable linker.

44. The probe of claim 43, wherein the cleavable linker is located between the first domain and the second domain.

45. The probe of claim 43, wherein the linker is photo-cleavable.

46. The probe of claim 42, wherein the secondary nucleic acid molecule is hybridized to at least one primary nucleic acid molecule and comprises a first detectable label and an at least second detectable label.

47. The probe of claim 42, wherein the secondary nucleic acid molecule is hybridized to at least one primary nucleic acid molecule and is hybridized to at least one tertiary nucleic acid molecule.

48. The probe of claim 42, wherein the secondary nucleic acid 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.

49. The probe of claim 47, wherein the secondary nucleic acid molecule is hybridized to at least one, two, three, four, five, six, seven, or more tertiary nucleic acid molecules.

50. The probe of claim 47, wherein the secondary nucleic acid molecule is hybridized to one tertiary nucleic acid molecule.

51. The probe of claim 46, wherein the first and at least second detectable labels have the same emission spectrum or have different emission spectra.

52. The probe of claim 42, wherein the tertiary nucleic acid molecule comprises at least two domains,

a first domain capable of binding to 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.

53. The probe of claim 52, wherein the tertiary nucleic acid molecule comprises a cleavable linker.

54. The probe of claim 53, wherein the cleavable linker is located between the first domain and the second domain.

55. The probe of claim 53, wherein the linker is photo-cleavable.

56. The probe of claim 52, wherein the tertiary nucleic acid molecule is hybridized to at least one secondary nucleic acid molecule and comprises a first detectable label and an at least second detectable label.

57. The probe of claim 52, wherein the first and at least second detectable labels have the same emission spectrum or have different emission spectra.

58. The probe of claim 55, 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.

59. A population of probes comprising a plurality of the probe of claim 3.

60. A method for determining a nucleotide sequence of a nucleic acid comprising

(1) hybridizing a first probe of claim 3 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 a first detectable label and a second detectable label to a first attachment position of the at least three attachment positions of the barcode domain;

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

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

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

(6) identifying the third and the fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position;

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

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

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

(10) identifying the linear order of at least six nucleotides in the first region of the optionally immobilized target nucleic acid hybridized to the target binding domain of the first probe based on the identity of the first detectable label, second detectable label, third detectable label, fourth detectable label, fifth detectable label and sixth detectable label.

61. The method of claim 60, wherein the target nucleic acid is obtained from a predetermined gene.

62. The method of claim 60, wherein steps (4) and (5) occur sequentially or concurrently.

63. The method of claim 60, wherein the first and second detectable labels have the same emission spectrum or have different emission spectra.

64. The method of claim 60, wherein the third and fourth detectable labels have the same emission spectrum or have different emission spectra.

65. The method of claim 60, wherein the fifth and sixth detectable labels have the same emission spectrum or have different emission spectra.

66. The method of claim 60, wherein the first complementary nucleic acid molecule comprises a cleavable linker.

67. The method of claim 60, wherein the second complementary nucleic acid molecule comprises a cleavable linker.

68. The method of claim 60, wherein the third complementary nucleic acid molecule comprises a cleavable linker.

69. The method of claim 60, 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.

70. The method of claim 66, wherein the cleavable linker is photo-cleavable.

71. The method of claim 70, wherein removing the first complementary nucleic acid molecule comprises contacting the first complementary nucleic acid molecule with light.

72. The method of claim 71, wherein the light is provided by a light source selected from the group consisting of an arc-lamp, a laser, a focused UV light source, and light emitting diode.

73. The method of claim 60, further comprising

(11) removing the first probe from the first region of the optionally immobilized target nucleic acid;

(12) hybridizing at least a second probe of claim 3 to a second region of the target nucleic acid that is optionally immobilized to a substrate at one or more positions, and wherein the target binding domain of the first probe and the at least second probe are different;

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

(14) detecting the first and the second detectable label of the first complementary nucleic acid molecule hybridized to the first attachment position;

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

(16) detecting the third and the fourth detectable label of the second complementary nucleic acid molecule hybridized to the second attachment position;

(17) removing the third and fourth detectable label hybridized to the second attachment position;

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

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

(20) identifying the linear order of at least six nucleotides in the second region of the optionally immobilized target nucleic acid hybridized to the target binding domain of the at least second probe based on the identity of the first detectable label, second detectable label, third detectable label, fourth detectable label, fifth detectable label and sixth detectable label.

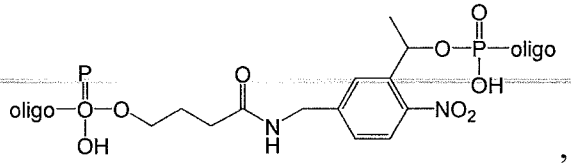
74. The method of claim 73, further comprising assembling each identified linear order of nucleotides in the at least first region and at least second region of the optionally immobilized target nucleic acid, thereby identifying a sequence for the optionally immobilized target nucleic acid.

75. An apparatus for performing the method of claim 60.

76. A kit comprising a substrate, a population of probes of claim 59, at least one capture probe, at least three complementary nucleic acid molecules comprising a first detectable label and at least two second detectable labels, and instructions for use.

77. The complex of claim 1 or claim 2, wherein the cleavable linker is a photo-cleavable linker.

78. The complex of claim 1 or claim 2, wherein the cleavable linker is



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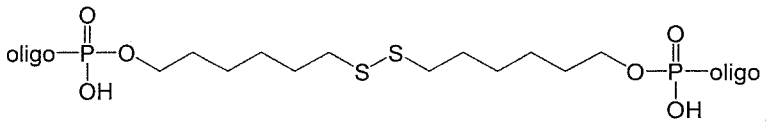

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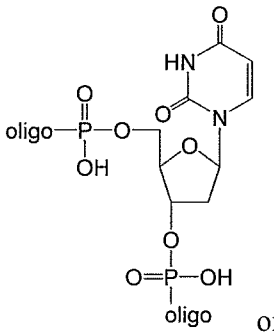

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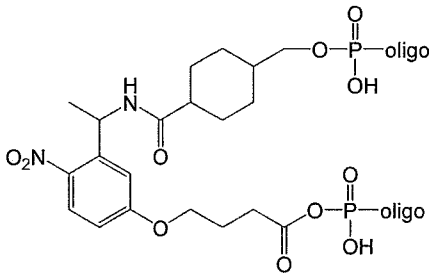

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