

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 target binding domain and a barcode domain;

wherein the target binding domain is at least 12 nucleotides in length;

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 that hybridizes to a complementary nucleic acid molecule, and wherein the barcode domain consists of synthetic backbone ~~comprises L-DNA~~,

wherein each of the at least three attachment positions have a different nucleic acid sequence, and

wherein each attachment position in the barcode domain is 6 to 20 nucleotides in length ~~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

2. The method of claim 1, wherein the probe comprises a single-stranded DNA synthetic backbone.

3. The method of claim 2, wherein the single-stranded DNA synthetic backbone is ~~about~~ 10 nucleotides to ~~about~~ 100 nucleotides in length.

4. The 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.

5. The method of any one of the preceding claims, wherein the barcode domain comprises at least four 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.

6. The method of any one of the preceding claims, wherein each attachment position in the barcode domain comprises one attachment region.

7. The method of any one of the preceding claims, wherein the at least one nucleic acid sequence of each attachment position in the barcode domain 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.

8. The method of any one of the preceding claims, wherein each nucleotide of the at least 12 nucleotides of the target binding domain is D-DNA.

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

10. The method of any one of the preceding claims, wherein steps (7) and (8) occur sequentially or concurrently.

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

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

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

14. The method of any one of the preceding claims, 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.

15. The method of claim 14, wherein the cleavable linker is photo-cleavable.
16. The method of any one of the preceding claims, wherein the first complementary nucleic acid molecule comprises a reporter probe comprising a primary nucleic acid, 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 each of the secondary nucleic acid molecules is hybridized to five tertiary nucleic molecules, wherein each of the tertiary nucleic acid molecules comprises a detectable label
17. The method of claim 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.
19. The method of 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 hybridizes to the five tertiary nucleic acid molecules.
20. The method of claim 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.
22. The method of any one of claims 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.