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(54) **HIGH THROUGHPUT NUCLEIC ACID SEQUENCING BY EXPANSION**

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(51) **Int. Cl.**
C07H 21/02 (2006.01)
C12Q 1/68 (2006.01)

(52) **U.S. Cl.** **536/23.1; 435/6**

(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

Nucleic acid sequencing methods and related products are disclosed. Methods for sequencing a target nucleic acid comprise providing a daughter strand produced by a template-directed synthesis, the daughter strand comprising a plurality of subunits coupled in a sequence corresponding to a contiguous nucleotide sequence of all or a portion of the target nucleic acid, wherein the individual subunits comprise a tether, at least one probe or nucleobase residue, and at least one selectively cleavable bond. The selectively cleavable bond(s) is/are cleaved to yield an Xpandomer of a length longer than the plurality of the subunits of the daughter strand, the Xpandomer comprising the tethers and reporter elements for parsing genetic information in a sequence corresponding to the contiguous nucleotide sequence of all or a portion of the target nucleic acid. Reporter elements of the Xpandomer are then detected. Corresponding products, including Xpandomers and oligomeric and monomeric substrate constructs are also disclosed.

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Fig. 1A

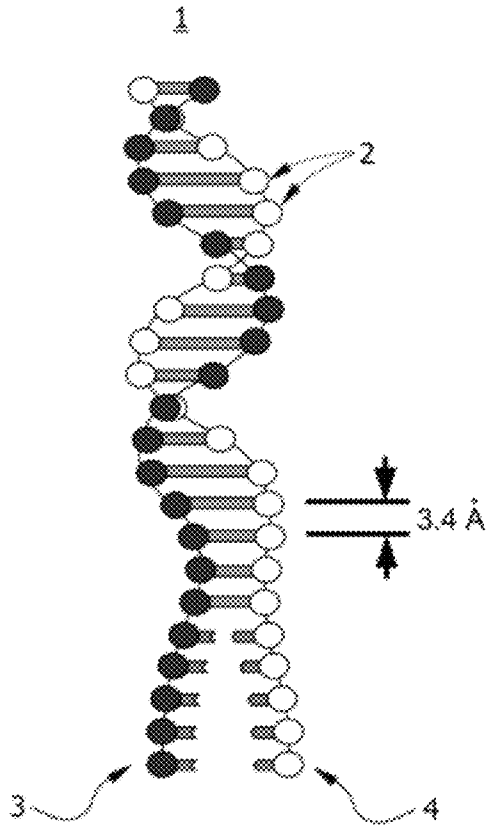
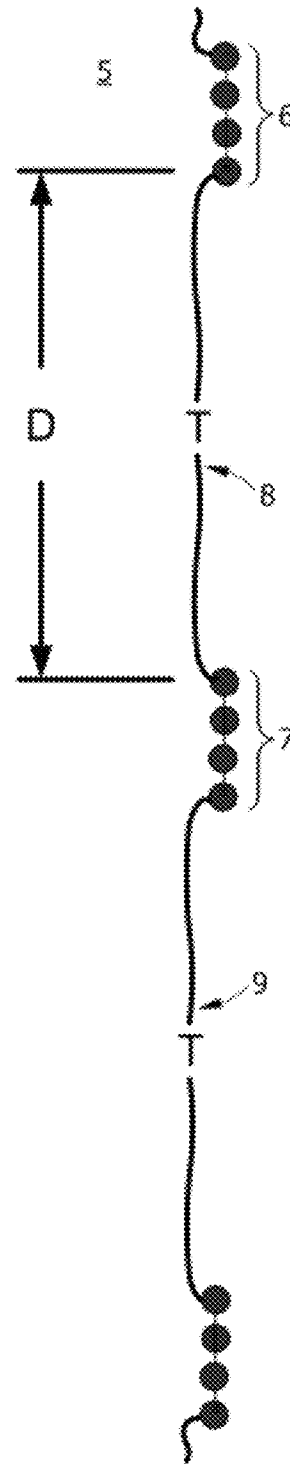
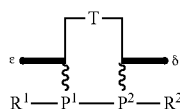


Fig. 1B



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6. The oligomer substrate construct of claim 1 having the following structure:



wherein

T represents a tether;

P¹ represents a first probe moiety;

P² represents a second probe moiety;

~ represents the at least one selectively cleavable bond;

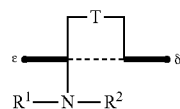
P¹ and R² represent the same or different end groups for template directed synthesis;

ε represents a first linker group; and

δ represents a second linker group.

7. An monomer substrate construct for use in a template directed synthesis for sequencing a target nucleic acid, comprising a nucleobase residue with end groups suitable for the template directed synthesis, and a tether having a first end and a second end with at least the first end of the tether joined to the nucleobase residue, wherein the monomer substrate construct when used in the template directed synthesis is capable of forming a daughter strand comprising a constrained Xpandomer, the constrained Xpandomer having a plurality of subunits coupled in a sequence corresponding to the contiguous nucleotide sequence of all or a portion of the target nucleic acid, wherein the individual subunits comprise a tether, the nucleobase residue and at least one selectively cleavable bond that, upon cleavage, yields an Xpandomer of a length longer than the plurality of subunits of the daughter strand.

8. The monomer substrate construct of claim 7 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

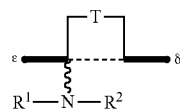
R¹ and R² represent the same or different end groups for the template directed synthesis of the daughter strand;

ε represents a first linker group;

δ represents a second linker group; and

“- - -” represents a cleavable intra-tether crosslink.

9. The monomer substrate construct of claim 7 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

R¹ and R² represent the same or different end groups for the template directed synthesis of the daughter strand;

~ represents the at least one selectively cleavable bond;

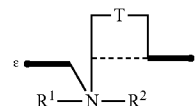
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ε represents a first linker group;

δ represents a second linker group; and

“- - -” represents a cleavable intra-tether crosslink.

10. The monomer substrate construct of claim 7 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

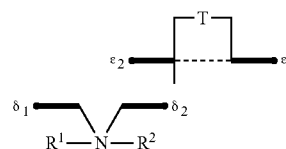
P¹ and R² represent the same or different end groups for the template directed synthesis of the daughter strand;

ε represents a first linker group;

δ represents a second linker group; and

“- - -” represents a cleavable intra-tether crosslink.

11. A monomer substrate construct having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

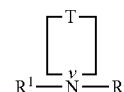
R¹ and R² represent the same or different end groups for the template directed synthesis of the daughter strand;

ε₁ and ε₂ represent the same or different first linker groups;

δ₁ and δ₂ represent the same or different second linker groups; and

“- - -” represents a cleavable intra-tether crosslink.

12. The monomer substrate construct of claim 7 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

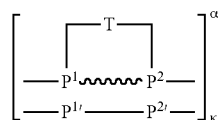
V represents an internal cleavage site of the nucleobase residue; and

R¹ and R² represent the same or different end groups for the template directed synthesis of the daughter strand.

13. A duplex daughter strand for use in a template directed synthesis for sequencing a target nucleic acid, comprising a daughter strand duplexed with a template strand, the daughter strand comprising a constrained Xpandomer and having a plurality of subunits coupled in a sequence corresponding to a contiguous nucleotide sequence of all or a portion of the target nucleic acid, wherein the individual subunits comprise a tether, at least one probe or nucleobase residue, and at least one selectively cleavable bond that, upon cleavage, yields an Xpandomer of a length longer than the plurality of subunits of the daughter strand.

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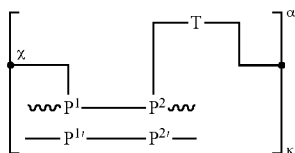
14. The duplex daughter strand of claim 13 having the following structure:



wherein

- T represents the tether;
- P¹ represents a first probe moiety;
- P² represents a second probe moiety;
- ~ represents the at least one selectively cleavable bond;
- P^{1'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P¹ is complementary;
- P^{2'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P² is complementary;
- κ represents the κth subunit in a chain of m subunits, where m is an integer greater than three; and
- α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid.

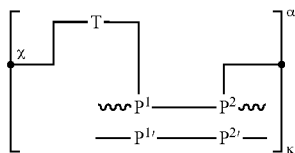
15. The duplex daughter strand of claim 13 having the following structure:



wherein

- T represents the tether;
- P¹ represents a first probe moiety;
- P² represents a second probe moiety;
- ~ represents the at least one selectively cleavable bond;
- P^{1'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P¹ is complementary;
- P^{2'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P² is complementary;
- κ represents the κth subunit in a chain of m subunits, where m is an integer greater than three;
- α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and
- χ represents a bond with the tether of an adjacent subunit.

16. The duplex daughter strand of claim 13 having the following structure:



wherein

- T represents the tether;
- P¹ represents a first probe moiety;
- P² represents a second probe moiety;
- ~ represents the at least one selectively cleavable bond;

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P^{1'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P¹ is complementary;

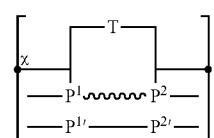
P^{2'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P² is complementary;

κ represents the κth subunit in a chain of m subunits, where m is an integer greater than three;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

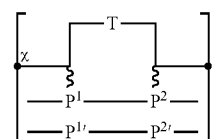
17. The duplex daughter strand of claim 13 having the following structure:



wherein

- T represents the tether;
- P¹ represents a first probe moiety;
- P² represents a second probe moiety;
- ~ represents the at least one selectively cleavable bond;
- P^{1'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P¹ is complementary;
- P^{2'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P² is complementary;
- κ represents the κth subunit in a chain of m subunits, where m is an integer greater than three;
- α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and
- χ represents a bond with the tether of an adjacent subunit.

18. The duplex daughter strand of claim 13 having the following structure:



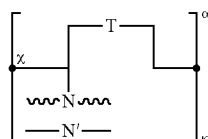
wherein

- T represents the tether;
- P¹ represents a first probe moiety;
- P² represents a second probe moiety;
- ~ represents the at least one selectively cleavable bond;
- P^{1'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P¹ is complementary;
- P^{2'} represents a contiguous nucleotide sequence of at least one nucleotide residue of the template strand to which P² is complementary;
- κ represents the κth subunit in a chain of m subunits, where m is an integer greater than three;
- α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and
- χ represents a bond with the tether of an adjacent subunit.

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is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and χ represents a bond with the tether of an adjacent subunit.

19. The duplex daughter strand of claim 13 having the following structure:



wherein

T represents the tether;

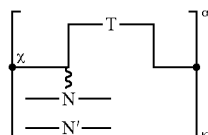
N represents a nucleobase residue;

N' represents a nucleotide residue of the template strand to which N is complementary;

~ represents the at least one selectively cleavable bond; κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and χ represents a bond with the tether of an adjacent subunit.

20. The duplex daughter strand of claim 13 having the following structure:



wherein

T represents the tether;

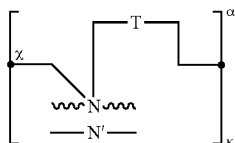
N represents a nucleobase residue;

N' represents a nucleotide residue of the template strand to which N is complementary;

~ represents the at least one selectively cleavable bond; κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and χ represents a bond with the tether of an adjacent subunit.

21. The duplex daughter strand of claim 13 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

N' represents a nucleotide residue of the template strand to which N is complementary;

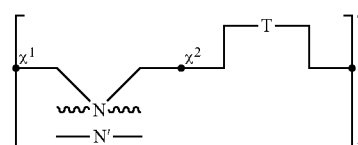
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~ represents the at least one selectively cleavable bond; κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

22. The duplex daughter strand of claim 13 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

N' represents a nucleotide residue of the template strand to which N is complementary;

~ represents the at least one selectively cleavable bond;

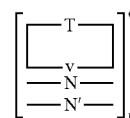
κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid;

χ^1 represents a bond with the tether of an adjacent subunit; and

χ^2 represents an inter-tether bond.

23. The duplex daughter strand of claim 13 having the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

N' represents a nucleotide residue of the template strand to which N is complementary;

V represents an internal cleavage site of the nucleobase residue;

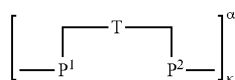
κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten; and

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species is complementary to the contiguous nucleotide sequence of a portion of the target nucleic acid.

24. The oligomer substrate construct of claim 2 or the duplex daughter strand of claim 14, wherein the Xpandomer

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of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

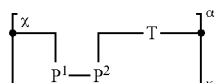
P¹ represents a first probe moiety;

P² represents a second probe moiety;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than three; and

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid.

25. The oligomer substrate construct of claim 3 or the duplex daughter strand of claim 15, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

P¹ represents a first probe moiety;

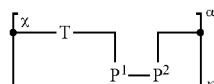
P² represents a second probe moiety;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than three;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

26. The oligomer substrate construct of claim 4 or the duplex daughter strand of claim 16, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

P¹ represents a first probe moiety;

P² represents a second probe moiety;

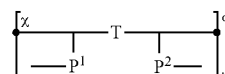
κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than three;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

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χ represents a bond with the tether of an adjacent subunit.

27. The oligomer substrate construct of claim 5 or the duplex daughter strand of claim 17, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

P¹ represents a first probe moiety;

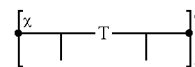
P² represents a second probe moiety;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than three;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

28. The oligomer substrate construct of claim 6 or the duplex daughter strand of claim 18, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

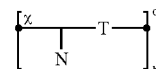
T represents the tether;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than three;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

29. The monomer substrate construct of claim 8 or the duplex daughter strand of claim 19, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

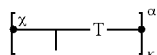
κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

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χ represents a bond with the tether of an adjacent subunit.

30. The monomer substrate construct of claim 9 or the duplex daughter strand of claim 20, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

31. The monomer substrate construct of claim 10 or the duplex daughter strand of claim 21, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

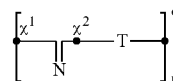
α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid; and

χ represents a bond with the tether of an adjacent subunit.

32. The monomer substrate construct of claim 11 or the duplex daughter strand of claim 22, wherein the Xpandomer

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of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

N represents a nucleobase residue;

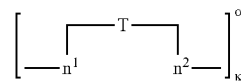
κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten;

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid;

χ^1 represents a bond with the tether of an adjacent subunit; and

χ^2 represents an inter-tether bond.

33. The monomer substrate construct of claim 12 or the duplex daughter strand of claim 23, wherein the Xpandomer of a length longer than the plurality of subunits of the daughter strand has the following structure:



wherein

T represents the tether;

n^1 and n^2 represent a first portion and a second portion, respectively, of a nucleobase residue;

κ represents the κ^{th} subunit in a chain of m subunits, where m is an integer greater than ten; and

α represents a species of a subunit motif selected from a library of subunit motifs, wherein each of the species comprises sequence information of the contiguous nucleotide sequence of a portion of the target nucleic acid.

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