

CLAIMS

1. A method of synthesizing a plurality of oligonucleotides and performing oligonucleotide-based assays in a single reaction vessel, the method comprising the steps of:

a) repeating in a reaction vessel cycles of (i) contacting under elongation conditions an initiator having a free 3'-hydroxyl or elongated fragments having free 3'-O-hydroxyls with a 3'-O-blocked nucleoside triphosphate and a template-independent DNA polymerase so that the initiator or elongated fragments are elongated by incorporation of a 3'-O-blocked nucleoside triphosphate to form 3'-O-blocked elongated fragments, and (ii) deblocking the elongated fragments to form elongated fragments having free 3'-hydroxyls, until elongated fragments are formed each containing a plurality of oligonucleotides separated from one another and from the initiator by cleavable nucleotides;

b) cleaving with the -application of an enzymatic activity the cleavable nucleotides to free at least one of the plurality of oligonucleotides;

c) adding reagents for the oligonucleotide-based assay; and

d) performing the oligonucleotide-based assay;

wherein after cleavage the enzymatic activity is deactivated.

2. The method of claim 1 wherein said oligonucleotide-based assay is a polymerase chain reaction (PCR) and wherein said step of adding further includes adding a polymerase, polymerase reaction buffer, nucleoside triphosphates, and one or more target polynucleotides at least one of which has complementary segments to at least two of said oligonucleotides so that sequences between the complementary segments are amplified in a PCR.

~~3. The method of claim 1 or 2, wherein said step of cleaving comprises the application of an enzymatic activity and wherein after cleavage the enzymatic activity is deactivated.~~

~~43.~~ The method of claim 1 wherein said oligonucleotide-based assay is a nucleic acid sequence-based amplification (NASBA) and wherein said step of adding further includes adding an RNA polymerase, an RNase H, a reverse transcriptase, NASBA reaction buffer, nucleoside triphosphates, and one or more single stranded target nucleic acids at least one of

which has complementary segment to at least one of said oligonucleotides so that sequences between the complementary segments are amplified in a NASBA reaction.

54. The method of anyone of claims 1 to 43, wherein said initiators and said elongation fragments are attached to a support and wherein said step of cleaving leaves one of said oligonucleotides of said plurality attached to the support.

65. The method of anyone of claims 1 to 54, wherein said template-independent DNA polymerase is a terminal deoxynucleotidyl transferase.

76. A method of synthesizing a plurality of oligonucleotides in a single reaction vessel, the method comprising the steps of:

a) providing one or more supports with two or more populations of initiators wherein the initiators of each population are terminated by a cleavable linkage or cleavable nucleotide having a population-specific 3'-O-blocking group removable by deblocking conditions orthogonal to the deblocking conditions of the 3'-O-blocking groups of every other population of initiators;

b) deblocking population-specific blocking groups of a population of initiators or elongated fragments to form initiators or elongated fragments having free 3'-hydroxyls;

c) contacting under elongation conditions the population of initiators or its elongated fragments having free 3'-hydroxyls with a 3'-O-blocked nucleoside triphosphate and a template-independent DNA polymerase so that the initiators or elongated fragments are elongated by incorporation of the 3'-O-blocked nucleoside triphosphate to form 3'-O-blocked elongated fragments;

d) repeating steps b) and c) for each population of initiators until elongated fragments are formed having nucleotide sequences of the plurality of oligonucleotides.

87. The method of 76 further including the steps of e) deblocking said elongated fragments, and f) cleaving said cleavable linkages or cleavable nucleotides to free said elongated fragments.

~~9. The method of claim 8 further including the steps of g) adding reagents for said oligonucleotide-based assay, and h) performing said oligonucleotide-based assay.~~

~~108.~~ The method of anyone of claims ~~7-6~~ to ~~97~~, wherein said steps b) through d) are implemented for each of said populations of initiators consecutively so that each of said oligonucleotides of said plurality are synthesized consecutively.

~~119.~~ The method of anyone of claims ~~7-6~~ to ~~98~~, wherein said steps b) through d) are implemented for each of said populations of initiators alternatively so that each of said oligonucleotides of said plurality are synthesized in parallel.

~~1210.~~ The method of anyone of claims ~~7-6~~ to ~~119~~, wherein said support is a solid support.

~~1311.~~ The method of anyone of claims 1 to ~~1210~~, wherein said template-independent DNA polymerase is a terminal deoxynucleotidyl transferase (TdT).

~~1412.~~ The method of claim ~~1311~~, wherein said TdT is a TdT variant having an amino acid sequence with at least 90 percent identity to one of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 with a substitution of methionine at position 63 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 12 and 14; or methionine at position 73 with respect to SEQ ID NO: 9; or methionine at position 64 with respect to SEQ ID NO: 10; or methionine at position 61 with respect to SEQ ID NO: 11; or methionine at position 66 with respect to SEQ ID NO: 15; and a substitution of a first arginine at position 207 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 9, 12 and 13; or a first arginine at position 206 with respect to SEQ ID NO: 5; or a first arginine at position 208 with respect to SEQ ID NOs: 8 or 10; or a first arginine at position 205 with respect to SEQ ID NO: 11; or a first arginine at position 216 with respect to SEQ ID NO: 14; or a first arginine at position 210 with respect to SEQ ID NO: 15.

~~1513.~~ The method of claim ~~1412~~, wherein said TdT variant further has one or more of the following substitutions: a cysteine at position 173 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 9, 12 and 13; or cysteine at position 172 with respect to SEQ ID NO: 5; or cysteine at position

174 with respect to SEQ ID NOs: 8 and 10; or cysteine at position 171 with respect to SEQ ID NO: 11; or cysteine at position 176 with respect to SEQ ID NO: 15; or cysteine at position 182 with respect to SEQ ID NO: 14; or a second arginine at position 325 with respect to SEQ ID NO: 2, 9 and 13; or a second arginine at position 324 with respect to SEQ ID NOs 3 and 4; or a second arginine at position 320 with respect to SEQ ID NO: 5; or a second arginine at position 331 with respect to SEQ ID NOs: 6 and 8; or a second arginine at position 323 with respect to SEQ ID NO: 11; or a second arginine at position 328 with respect to SEQ ID NOs: 12 and 15; or a second arginine at position 338 with respect to SEQ ID NO: 14; or a glutamic acid at position 328 with respect to SEQ ID NOs: 2, 7, 9 and 13; or glutamic acid at position 327 with respect to SEQ ID NOs: 3 and 4; or glutamic acid at position 334 with respect to SEQ ID NOs: 6 and 8; or glutamic acid at position 329 with respect to SEQ ID NO: 10; or glutamic acid at position 326 with respect to SEQ ID NO: 11; or glutamic acid at position 331 with respect to SEQ ID NOs: 12 and 15.

~~14~~14. The method of claim ~~13~~13, wherein said substitution of said methionine is R or Q; said substitution of said cysteine is G or R; said substitution of said first arginine is L or N; said substitution of said second arginine is P, N, A or V; and said substitution of said glutamic acid is N, L, T, S or K.

~~15~~15. A method for synthesizing a plurality of oligonucleotides in a single reaction vessel, the method comprising the steps of:

(a) providing a plurality of different initiators attached to one or more supports, wherein at least one initiator of the plurality has free 3'-hydroxyls and wherein at least one initiator of the plurality has 3'-O-blocked terminal nucleotides;

(b) synthesizing the plurality oligonucleotides by repeated cycles of template-free enzymatic nucleotide additions to each different initiator or its extension products of 3'-O-blocked nucleoside triphosphates, wherein the 3'-O-blocked nucleoside triphosphate has a blocking group that is removable under deblocking conditions orthogonal to deblocking conditions for removing blocking groups of other initiators of the plurality; and

(c) releasing oligonucleotides from the extension products and the one or more solid supports.

~~18~~16. The method of claim ~~17~~15, wherein said plurality of oligonucleotides is equal to or greater than said plurality of different initiators.

~~19~~17. The method of claim ~~17~~15 or ~~18~~16, further including the steps of (d) adding reagents for an oligonucleotide-based assay and (e) performing the oligonucleotide-based assay.