

Claims

1. A method of synthesizing a polynucleotide having a predetermined sequence, the
5 method comprising the steps of:
a) providing an initiator having a 3'-terminal nucleotide having a free 3'-hydroxyl; and
b) repeating, until the polynucleotide is formed, cycles of (i) contacting under
elongation conditions the initiator or elongated fragments having free 3'-hydroxyls with a 3'-
O-blocked-nucleoside triphosphate and a template-free polymerase so that the initiator or
10 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;
wherein the template-free polymerase is a poly(A) polymerase (PAP) or a poly(U)
polymerase.
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2. The method of claim 1, wherein said initiator is attached to a support by a 5' end.
3. The method of claim 2, wherein said support is a solid support.
- 20 4. The method of any of claims 1 to 3, further including a step of cleaving said
polynucleotide from the initiator.
5. The method of any of claims 1 to 4, wherein said polynucleotide is a poly-2'-
deoxyribonucleotide and said 3'- O-blocked-nucleoside triphosphate is a 3'-O-blocked-2'-
25 deoxyribonucleoside triphosphate.
6. The method of claim 5, wherein said 3'- O-blocked-2'-deoxyribonucleotide
triphosphate is a 3'-O-azidomethyl-2'-deoxyribonucleoside triphosphate or a 3'-O-amino-2'-
deoxyribonucleoside triphosphate.

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7. The method of any of claims 1 to 4, wherein said polynucleotide is a polyribonucleotide and said 3'-O-blocked-nucleoside triphosphate is a 3'-O-blocked-ribonucleoside triphosphate.

- 5 8. The method of any of claims 1 to 7, wherein said poly(A) polymerase is a poly(A) polymerase variant comprising an amino acid sequence that is at least 90 percent identical to an amino acid sequence selected from SEQ ID NO: 1, 2, 3, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 with a substitution of methionine at position 310 with respect to SEQ ID NO: 1,
- 10 at position 318 with respect to SEQ ID NO: 2,
at position 318 with respect to SEQ ID NO: 3,
at position 316 with respect to SEQ ID NO: 8,
at position 309 with respect to SEQ ID NO: 9,
at position 316 with respect to SEQ ID NO: 10,
- 15 at position 272 with respect to SEQ ID NO: 11,
at position 316 with respect to SEQ ID NO: 12,
at position 307 with respect to SEQ ID NO: 13,
at position 313 with respect to SEQ ID NO: 14,
at position 312 with respect to SEQ ID NO: 15,
- 20 at position 317 with respect to SEQ ID NO: 16,
at position 316 with respect to SEQ ID NO: 17,
at position 316 with respect to SEQ ID NO: 18,
at position 312 with respect to SEQ ID NO: 19,
at position 310 with respect to SEQ ID NO: 20,
- 25 at position 309 with respect to SEQ ID NO: 21,
at position 317 with respect to SEQ ID NO: 22,
at position 314 with respect to SEQ ID NO: 23,
at position 307 with respect to SEQ ID NO: 24,
at position 315 with respect to SEQ ID NO: 25,
- 30 at position 316 with respect to SEQ ID NO: 26, and
at position 311 with respect to SEQ ID NO: 27.

9. The method of claim 8, wherein said substitution of said methionine at said position is selected from F, Y, V, E or T.

- 5 10. The method of claim 8 or 9, wherein said amino acid sequence selected from said SEQ ID NO: 1, 2, 3, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 further comprises a substitution of valine
- at position 234 with respect to SEQ ID NO: 1,
at position 240 with respect to SEQ ID NO: 2,
10 at position 240 with respect to SEQ ID NO: 3,
at position 232 with respect to SEQ ID NO: 9,
at position 240 with respect to SEQ ID NO: 10,
at position 196 with respect to SEQ ID NO: 11,
at position 240 with respect to SEQ ID NO: 12,
15 at position 229 with respect to SEQ ID NO: 13,
at position 236 with respect to SEQ ID NO: 14,
at position 236 with respect to SEQ ID NO: 15,
at position 241 with respect to SEQ ID NO: 16,
at position 233 with respect to SEQ ID NO: 17,
20 at position 240 with respect to SEQ ID NO: 18,
at position 240 with respect to SEQ ID NO: 19,
at position 234 with respect to SEQ ID NO: 20,
at position 233 with respect to SEQ ID NO: 21,
at position 237 with respect to SEQ ID NO: 22,
25 at position 238 with respect to SEQ ID NO: 23,
at position 231 with respect to SEQ ID NO: 24,
at position 239 with respect to SEQ ID NO: 25,
at position 240 with respect to SEQ ID NO: 26, and
at position 235 with respect to SEQ ID NO: 27.

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11. The method of claim 10, wherein said substitution of said valine at said position is alanine or glycine.

12. The method of any of claims 7 to 11, wherein said 3'-blocked-O-ribonucleoside
5 triphosphate is 3'-azidomethyl-O-ribonucleoside triphosphate.

13. The method of claim 12, wherein said 3'-azidomethyl-O-ribonucleoside triphosphate is selected from the group consisting of 3'-azidomethyl-O-adenosine triphosphate, 3'-azidomethyl-O-guanosine triphosphate, and 3'-azidomethyl-O-cytidine triphosphate and 3'-
10 azidomethyl-O-uridine triphosphate.

14. A kit for performing template-free synthesis of a polyribonucleotide having a predetermined sequence, the kit comprising a poly(A) polymerase, an initiator attached to a solid support, and 3'-O-blocked ribonucleoside triphosphate monomers.

15. The kit of claim 14, wherein said 3'-O-protected ribonucleoside triphosphate monomers comprise one or more of 3'-O-azidomethyl-riboadenosine triphosphate, 3'-O-azidomethyl-riboguanosine triphosphate, 3'-O-azidomethyl-ribocytidine triphosphate, 3'-O-azidomethyl-ribothymidine triphosphate and 3'-O-amino-ribouridine triphosphate.

20 16. The kit of claim 14 or 15, wherein said poly(A) polymerase comprises a poly(A) polymerase variant having an amino acid sequence that is at least 90 percent identical to SEQ ID NO: 1 and having a substitution at M310, or at least 90 percent identical to SEQ ID NO: 3 and having a substitution at M318; wherein the poly(A) polymerase variant is capable of (a)
25 synthesizing a ribonucleic acid fragment without a template and (b) incorporating said 3'-O-azidomethyl-ribonucleoside triphosphate onto a ribonucleic acid fragment.

17. A poly(A) polymerase (PAP) variant comprising an amino acid sequence that is at least 90 percent identical to an amino acid sequence selected from SEQ ID NO: 1, 2, 3, 8, 9, 10, 11,
30 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 with a substitution of methionine at position 310 with respect to SEQ ID NO: 1,

- at position 318 with respect to SEQ ID NO: 2,
at position 318 with respect to SEQ ID NO: 3,
at position 316 with respect to SEQ ID NO: 8,
at position 309 with respect to SEQ ID NO: 9,
5 at position 316 with respect to SEQ ID NO: 10,
at position 272 with respect to SEQ ID NO: 11,
at position 316 with respect to SEQ ID NO: 12,
at position 307 with respect to SEQ ID NO: 13,
at position 313 with respect to SEQ ID NO: 14,
10 at position 312 with respect to SEQ ID NO: 15,
at position 317 with respect to SEQ ID NO: 16,
at position 316 with respect to SEQ ID NO: 17,
at position 316 with respect to SEQ ID NO: 18,
at position 312 with respect to SEQ ID NO: 19,
15 at position 310 with respect to SEQ ID NO: 20,
at position 309 with respect to SEQ ID NO: 21,
at position 317 with respect to SEQ ID NO: 22,
at position 314 with respect to SEQ ID NO: 23,
at position 307 with respect to SEQ ID NO: 24,
20 at position 315 with respect to SEQ ID NO: 25,
at position 316 with respect to SEQ ID NO: 26, and
at position 311 with respect to SEQ ID NO: 27,
wherein the PAP variant is capable of (a) synthesizing a ribonucleic acid fragment without a
template and (b) incorporating a 3'-O-azidomethyl-ribonucleoside triphosphate onto a
25 ribonucleic acid fragment or a 3'-O-azidomethyl-2'-deoxyribonucleoside triphosphate into a
deoxyribonucleic acid fragment.

18. The PAP variant of claim 17, wherein said substitution of said methionine at said
position is selected from F, Y, V, E or T.

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19. The PAP variant of claim 17 or 18, further comprising a substitution of valine

- at position 234 with respect to SEQ ID NO: 1,
at position 240 with respect to SEQ ID NO: 2,
at position 240 with respect to SEQ ID NO: 3,
at position 232 with respect to SEQ ID NO: 9,
5 at position 240 with respect to SEQ ID NO: 10,
at position 196 with respect to SEQ ID NO: 11,
at position 240 with respect to SEQ ID NO: 12,
at position 229 with respect to SEQ ID NO: 13,
at position 236 with respect to SEQ ID NO: 14,
10 at position 236 with respect to SEQ ID NO: 15,
at position 241 with respect to SEQ ID NO: 16,
at position 233 with respect to SEQ ID NO: 17,
at position 240 with respect to SEQ ID NO: 18,
at position 240 with respect to SEQ ID NO: 19,
15 at position 234 with respect to SEQ ID NO: 20,
at position 233 with respect to SEQ ID NO: 21,
at position 237 with respect to SEQ ID NO: 22,
at position 238 with respect to SEQ ID NO: 23,
at position 231 with respect to SEQ ID NO: 24,
20 at position 239 with respect to SEQ ID NO: 25,
at position 240 with respect to SEQ ID NO: 26, and
at position 235 with respect to SEQ ID NO: 27.

20. The PAP variant of claim 19 wherein said substitution of valine at said position is
25 alanine or glycine.

21. A poly(U) polymerase (PUP) variant comprising an amino acid sequence that is at least
90 percent identical to an amino acid sequence selected from SEQ ID NO: 4, 28, 29, 30, 31,
32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 with a substitution of tyrosine
30 at position 212 with respect to SEQ ID NO: 4,
at position 189 with respect to SEQ ID NO: 28,

- at position 184 with respect to SEQ ID NO: 29,
at position 227 with respect to SEQ ID NO: 30,
at position 478 with respect to SEQ ID NO: 31,
at position 192 with respect to SEQ ID NO: 32,
5 at position 186 with respect to SEQ ID NO: 33,
at position 243 with respect to SEQ ID NO: 34,
at position 196 with respect to SEQ ID NO: 35,
at position 253 with respect to SEQ ID NO: 36,
at position 284 with respect to SEQ ID NO: 37,
10 at position 182 with respect to SEQ ID NO: 38,
at position 187 with respect to SEQ ID NO: 39,
at position 203 with respect to SEQ ID NO: 40,
at position 224 with respect to SEQ ID NO: 41,
at position 204 with respect to SEQ ID NO: 42,
15 at position 337 with respect to SEQ ID NO: 43,
at position 296 with respect to SEQ ID NO: 44,
at position 291 with respect to SEQ ID NO: 45,
at position 218 with respect to SEQ ID NO: 46, and
at position 366 with respect to SEQ ID NO: 47,
20 wherein the PUP variant is capable of (a) synthesizing a ribonucleic acid fragment without a
template and (b) incorporating a 3'-O-azidomethyl-ribonucleoside triphosphate onto a
ribonucleic acid fragment or a 3'-O-azidomethyl-2'-deoxyribonucleoside triphosphate into a
deoxyribonucleic acid fragment.
- 25 22. The PUP variant of claim 21 wherein said substitution of said tyrosine at said position
is alanine or glycine.