

CLAIMS

1. A chimeric terminal deoxynucleotidyl transferase (TdT) variant comprising an amino acid sequence at least 80 percent identical to the amino acid sequence defined by the formula:

5 $B_1-B_2-B_3$

wherein:

B_1 is an amino acid segment of a first species TdT without a BRCT-like region, extending from its N-terminus to an amino acid position between its VSC motif and its KMT motif inclusive, wherein B_1 comprises a substitution of methionine or functionally equivalent residue in its FMR motif, a substitution of the second arginine or functionally equivalent residue in its GGFR motif whenever B_1 includes a GGFR motif, and one or more substitutions of valine or serine or cysteine, or functionally equivalent residue, respectively, in its VSC motif whenever B_1 includes a VSC motif;

B_2 is an amino acid segment of a second species TdT extending from a C-terminus of B_1 to a C-terminus of the second species TdT, or to the N-terminus of B_3 whenever B_3 comprises one or more amino acids, wherein B_2 comprises one or more substitutions of valine or serine or cysteine, or functionally equivalent residue, respectively, in its VSC motif whenever B_2 includes a VSC motif, a substitution of the second arginine or functionally equivalent residue in its GGFR motif whenever B_2 includes a GGFR motif, a substitution of arginine or functionally equivalent residue in its TGS motif whenever B_2 includes a TGS motif, and a substitution of glutamic acid or functionally equivalent residue in its FER motif whenever B_2 includes a FER motif; and

B_3 is an amino acid segment of a third species TdT comprising from 0 to 70 amino acids and extending from a C-terminus of the third species TdT to a C-terminal amino acid of B_2 , wherein B_3 comprises a substitution of arginine or functionally equivalent residue in its TGS motif whenever B_3 includes a TGS motif, and a substitution of glutamic acid or functionally equivalent residue in its FER motif whenever B_3 includes a FER motif;

and wherein the chimeric TdT variant (i) is capable of synthesizing a nucleic acid fragment without a template and (ii) is capable of incorporating a 3'-O-blocked nucleoside triphosphate onto a nucleic acid fragment;

and wherein B₁, B₂ and B₃ are selected so that the chimeric TdT variant has from 350
5 to 410 amino acids.

2. The chimeric TdT variant of claim 1 wherein:

(i) said substitution of methionine or functionally equivalent residue in said FMR motif of B₁ is R or Q; said substitution of said second arginine or functionally equivalent residue in said GGFR motif of B₁ whenever B₁ includes a GGFR motif is L or N; and said substitution of
10 cysteine or functionally equivalent residue, in said VSC motif of B₁ whenever B₁ includes a VSC motif is G or R;

(ii) said substitution of cysteine or functionally equivalent residue in said VSC motif of B₂ whenever B₂ includes a VSC motif is R or Q; said substitution of said second arginine or functionally equivalent residue in said GGFR motif of B₂ whenever B₂ includes a GGFR
15 motif is L or N; said substitution of arginine or functionally equivalent residue in said TGS motif of B₂ whenever B₂ includes a TGS motif is P, N or A; and said substitution of glutamic acid or functionally equivalent residue in said FER motif of B₂ whenever B₂ includes a FER motif is N, L, T, or S; and

(iii) said substitution of arginine or functionally equivalent residue in said TGS motif of B₃ whenever B₃ includes a TGS motif is P, N or A; and said substitution of glutamic acid or functionally equivalent residue in said FER motif of B₃ whenever B₃ includes a FER motif is
20 N, L, T or S.

3. The chimeric TdT variant of claim 1 or 2, wherein said first species TdT and said third species TdT are the same.

25 4. The chimeric TdT variant of any one of claims 1 to 3 wherein each of said amino acid segments B₁, B₂ and B₃ has an amino acid sequence at least 90 percent identical to an amino acid segment of a sequence selected from the group consisting of SEQ ID NOs 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 and 41 and wherein:

said substitution of said methionine or its functionally equivalent residue in said FMR motif is at position 63 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 8, 11, 12 and 13; at position 62 with respect to SEQ ID NO: 5; position 64 with respect to SEQ ID NO: 9; position 61 with respect to SEQ ID NO: 10, 26, 28, 29, 30, 32, 33, 34, 36, 37, 38 and 39; position 66 with respect to SEQ ID NO: 14; position 47 with respect to SEQ ID NO: 15; and position 48 with respect to SEQ ID NO: 27, 31, 35 and 40;

said substitution of said valine or its functionally equivalent residue in said VSC motif is at position 171 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 8, 11 and 12; position 170 with respect to SEQ ID NO: 5, 32, 33, 34, 36, 37, 38, 39 and 41; position 172 with respect to SEQ ID NO: 9; position 169 with respect to SEQ ID NO: 10, 28, 29, 30, 32, 33, 34, 36, 37, 38, 38 and 41; position 180 with respect to SEQ ID NO: 13; position 174 with respect to SEQ ID NO: 14; position 154 with respect to SEQ ID NO: 15; position 168 with respect to SEQ ID NO: 26; position 156 with respect to SEQ ID NO: 27, 35 and 40; position 157 with respect to SEQ ID NO: 31;;

said substitution of said serine or its functionally equivalent residue in said VSC motif is at position 172 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 8, 11 and 12; position 171 with respect to SEQ ID NO: 5; position 173 with respect to SEQ ID NO: 9; position 170 with respect to SEQ ID NO: 10, 28, 29, 30; position 181 with respect to SEQ ID NO: 13; position 175 with respect to SEQ ID NO: 14; position 155 with respect to SEQ ID NO: 15; position 169 with respect to SEQ ID NO: 26 and position 157 with respect to SEQ ID NO: 27, 31, 35 and 40;

said substitution of said cysteine or its functionally equivalent residue in said VSC motif is at position 173 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 8, 11 and 12; position 172 with respect to SEQ ID NO: 5; position 174 with respect to SEQ ID NO: 9; position 171 with respect to SEQ ID NO: 10, 28, 29, 30, 32, 33, 34, 37, 38, 39 and 41; position 182 with respect to SEQ ID NO: 13; position 176 with respect to SEQ ID NO: 14; position 170 with respect to SEQ ID NO: 26; position 158 with respect to SEQ ID NO: 27, 31, 35, 36 and 40; and position 156 with respect to SEQ ID NO: 15;

said substitution of said second arginine or its functionally equivalent residue in said GGFR motif is at position 207 with respect to SEQ ID NOs: 2, 3, 4, 6, 7, 8, 11 and 12; position 206 with respect to SEQ ID NO: 5; position 208 with respect to SEQ ID NO: 9; position 205 with respect to SEQ ID NO: 10, 28, 29, 30, 32, 33, 34, 36, 37, 38, 39 and 41;

position 204 with respect to SEQ ID NO: 26; position 192 with respect to SEQ ID NO: 27, 31, 35 and 40; position 216 with respect to SEQ ID NO: 13; position 210 with respect to SEQ ID NO: 14; and position 190 with respect to SEQ ID NO: 15;

5 said substitution of said arginine or functionally equivalent residue in said TGSR motif is at position 325 with respect to SEQ ID NOs: 2, 7, 8, 12 and 33; position 324 with respect to SEQ ID NOs: 3, 4, 29 and 32; position 320 with respect to SEQ ID NO: 5; position 331 with respect to SEQ ID NO: 6; position 326 with respect to SEQ ID NO: 9 and 26; position 327 with respect to SEQ ID NO: 28, 30; position 323 with respect to SEQ ID NO: 10; position 328 with respect to SEQ ID NO: 11 and 14; position 338 with respect to SEQ ID NO: 13; position 10 314 with respect to SEQ ID NO: 27, 31; position 310 with respect to SEQ ID NO: 35; position 311 with respect to SEQ ID NO: 31; position 321 with respect to SEQ ID NO: 32; position 322 with respect to SEQ ID NO: 33, 34; position 323 with respect to SEQ ID NO: 36, 37, 38, 39 and 41; position 309 with respect to SEQ ID NO: 40; and position 308 with respect to SEQ ID NO: 15; and

15 said substitution of said glutamic acid or functionally equivalent residue in said FER motif is at position 328 with respect to SEQ ID NO: 2, 7, 8 and 12; position 327 with respect to SEQ ID NO: 3, 4 and 29; position 323 with respect to SEQ ID NO: 5; position 334 with respect to SEQ ID NO: 6; position 329 with respect to SEQ ID NO: 9 and 26; position 326 with respect to SEQ ID NO: 10, 36, 37, 38, 39 and 41; position 331 with respect to SEQ ID 20 NO: 11 and 14; position 341 with respect to SEQ ID NO: 13; position 317 with respect to SEQ ID NO: 27; position 330 with respect to SEQ ID NO: 28 and 30; position 312 with respect to SEQ ID NO: 40; position 313 with respect to SEQ ID NO: 35; position 314 with respect to SEQ ID NO: 31; position 324 with respect to SEQ ID NO: 32; position 325 with respect to SEQ ID NO: 33 and 34; and position 311 with respect to SEQ ID NO: 15.

25 5. The chimeric TdT variant of claim 4 wherein:

(i) said substitution of methionine or functionally equivalent residue in said FMR motif of B₁ is R or Q; said substitution of said second arginine or functionally equivalent residue in said GGFR₁ motif of B₁ whenever B₁ includes a GGFR₁ motif is L or N; and said substitution of cysteine or functionally equivalent residue, in said VSC motif of B₁ whenever B₁ includes a 30 VSC motif is G or R;

(ii) said substitution of cysteine or functionally equivalent residue in said VSC motif of B₂ whenever B₂ includes a VSC motif is R or Q; said substitution of said second arginine or

functionally equivalent residue in said GGFR motif of B₂ whenever B₂ includes a GGFR motif is L or N; said substitution of arginine or functionally equivalent residue in said TGS motif of B₂ whenever B₂ includes a TGS motif is P, N or A; and said substitution of glutamic acid or functionally equivalent residue in said FER motif of B₂ whenever B₂ includes a FER motif is N, L, T, or S; and

(iii) said substitution of arginine or functionally equivalent residue in said TGS motif of B₃ whenever B₃ includes a TGS motif is P, N or A; and said substitution of glutamic acid or functionally equivalent residue in said FER motif of B₃ whenever B₃ includes a FER motif is N, L, T or S.

6. The chimeric TdT variant of claim 4 wherein said substitution of said methionine of said FMR motif is R or Q; said substitution of said valine of said VSC motif is A; said substitution of said serine of said VSC motif is E; said substitution of said cysteine of said VSC motif is G or R; said substitution of said second arginine of said GGFR motif is L or N; said substitution of said arginine of said TGS motif is P, N or A; and said substitution of said glutamic acid of said FER motif is N, L, T or S.

7. The chimeric TdT variant of any of claims 1-6 wherein said first and second species TdTs are each mammalian TdTs.

8. The chimeric TdT variant of claim 7 wherein said first species TdT and said second species TdT are each TdTs of a species selected from group consisting of mouse, bovine, human, possum, elephant shrew, canine, mole, pika, hedgehog, tree shrew, platypus, jerboa and puma.

9. The chimeric TdT variant of claim 8 wherein said first species TdT is mouse and said second species TdT is bovine.

10. The chimeric TdT variant of claim 9 wherein said first species TdT has an amino acid sequence of SEQ ID NO: 21 and said second species TdT has an amino acid sequence of SEQ ID NO: 22.

11. The chimeric TdT variant of claim 10 having an amino acid sequence of SEQ ID NO: 23.

12. The chimeric TdT variant of claim 10 having an amino acid sequence of SEQ ID NO: 24.

13. The chimeric TdT variant of any of claims 1 to 12, wherein said 3'-O-modified nucleoside triphosphate is a 3'-O-NH₂-nucleoside triphosphate, a 3'-O-azidomethyl-nucleoside triphosphate, a 3'-O-allyl-nucleoside triphosphate, or a 3'-O-(2-nitrobenzyl)-nucleoside triphosphate.

14. A chimeric terminal deoxynucleotidyl transferase (TdT) variant comprising an amino acid sequence at least 80 percent identical to an amino acid sequence defined by the formula:

$$J_1-J_2-J_3$$

10 wherein:

J₁ is an amino acid segment of a mouse TdT without a BRCT-like region extending from its N-terminus to an amino acid position between a VSC motif and a KMT motif, inclusive, wherein J₁ comprises a substitution of methionine or functionally equivalent residue in a FMR motif, a substitution of the second arginine or functionally equivalent residue in its GGFR motif whenever J₁ includes a GGFR motif, and one or more substitutions of valine or serine or cysteine, or functionally equivalent residue, respectively, in the VSC motif whenever J₁ includes a VSC motif;

J₂ is an amino acid segment of a non-mouse TdT extending from an amino acid position located between a VSC motif and a KMT motif to an amino acid position at a TGSR motif, wherein the amino acid segment comprises a substitution of the second arginine or functionally equivalent residue in its GGFR motif whenever J₂ includes a GGFR motif, and one or more substitutions of valine or serine or cysteine, or functionally equivalent residue, respectively, in its VSC motif whenever J₂ includes a VSC motif; and

J₃ is an amino acid segment of a mouse TdT extending from an amino acid position at a TGSR motif to its C-terminal amino acid, wherein J₃ comprises a substitution of arginine or functionally equivalent residue in its TGSR motif whenever J₃ includes a TGSR motif, and a substitution of glutamic acid or functionally equivalent residue in its FER motif whenever J₃ includes a FER motif;

and wherein the chimeric TdT variant (i) is capable of synthesizing a nucleic acid fragment without a template and (ii) is capable of incorporating a 3'-O-blocked nucleoside triphosphate onto a nucleic acid fragment;

and wherein J₁, J₂ and J₃ are selected so that the chimeric TdT variant has from 350 to
5 410 amino acids.

15. The chimeric TdT variant of claim 14 wherein (i) said amino acid segment J₁ comprises an amino acid sequence selected from SEQ ID NO: 42, 71, 73 or 82; and the amino acid sequence of SEQ ID NO: 42 has a substitution of methionine at position 63, (ii) said amino acid segment J₂ comprises an amino acid sequence selected from the group consisting
10 of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, each with a substitution of arginine at position 35, and each having a substitution of cysteine at position 1, and (iii) said amino acid segment J₃ comprises an amino acid sequence of SEQ ID NO: 43 with a substitution of arginine at position 12, glutamic acid at position 15, and a substitution of arginine at position 38; or an amino acid
15 sequence of SEQ ID NO: 72.

16. The chimeric TdT variant of claim 15 wherein said substitution of methionine at position 63 is R or Q, wherein said substitution of arginine at position 35 is L or N, and wherein said substitution of cysteine at positions 1 is G or R.

17. The chimeric TdT variant of claim 15 or 16, wherein said amino acid sequence of SEQ
20 ID NO: 42 further has substitutions of alanine at position 17, leucine at position 52, glycine at position 57, methionine at position 63 and alanine at position 108.

18. The chimeric TdT variant of any one of claims 14 to 17, wherein said non-mouse TdT is bovine TdT.

19. The chimeric TdT variant of claim 18, wherein said chimeric TdT variant has an amino
25 acid sequence selected from the group consisting of SEQ ID NO: 74, 75, 76, 77, 78, 79, 80 and 81.

20. The chimeric TdT variant of any of claims 14 to 19, wherein said 3'-O-modified nucleoside triphosphate is a 3'-O-NH₂-nucleoside triphosphate, a 3'-O-azidomethyl-nucleoside triphosphate, a 3'-O-allyl-nucleoside triphosphate, or a 3'-O-(2-nitrobenzyl)-
30 nucleoside triphosphate.

21. A method of synthesizing a polynucleotide having a predetermined sequence, the method comprising the steps of:

a) providing an initiator having a free 3'-hydroxyl; and

5 b) repeating cycles of (i) contacting under elongation conditions the initiator or elongated fragments having free 3'-O-hydroxyls with a 3'-O-blocked nucleoside triphosphate and a terminal deoxynucleotidyltransferase (TdT) variant 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
10 elongated fragments having free 3'-hydroxyls, until the polynucleotide is synthesized, wherein the TdT variant a chimeric TdT variant of any of claims 1 to 20.

22. A kit for performing a nucleotide incorporation reaction comprising: a) a chimeric TdT variant according to any one of claims 1 to 20, b) one or more nucleotides, preferably one or more 3'-O-blocked nucleosides triphosphates, and c) optionally at least one nucleic acid primer.

15