CLAIMS

1. A terminal deoxynucleotidyl transferase (TdT) variant comprising an amino acid sequence at least ninety percent identical to an amino acid sequence selected from SEQ ID NO: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 24, 25 or 26, with a substitution at position Q326 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position Q325 with respect to SEQ ID NO: 4 and 5, or at position Q332 with respect to SEQ ID NO: 7 and 9, or at position Q329 with respect to SEQ ID NO: 13 and 16, or at position Q321 with respect to SEQ ID NO: 6, or at position Q327 with respect to SEQ ID NO: 11, or at position Q324 with respect to SEQ ID NO: 12, or at position Q339 with respect to SEQ ID NO: 15, or at position Q309 with respect to SEQ ID NO: 24 and 26, or at position Q307 with respect to SEQ ID NO: 25, the amino acid Q at above positions being substituted with one of T, F, L, M, I, V and Y wherein the TdT variant (i) is capable of synthesizing a nucleic acid fragment without a template and (ii) is capable of incorporating a 3'-O-modified nucleotide onto a nucleic acid fragment.

2. The TdT variant of claim 1 further comprising one or more of:
(i) a substitution at position K265 with respect to SEQ ID NOs 3, 7, 8, 9, 10, 13 and 14, or at position K263 with respect to SEQ ID NOs 6 and 12, or at position K264 with respect to SEQ ID NO 5, or at position K266 with respect to SEQ ID NO 11, or at position K268 with respect to SEQ ID NO 16, or at position K272 with respect to SEQ ID NO 15;
(ii) a substitution at position H337 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position H336 with respect to SEQ ID NOs 4 and 5, or at position H343 with respect to SEQ ID NOs 7 and 9, or at position H340 with respect to SEQ ID NOs 13 and 16, or at position H332 with respect to SEQ ID NO 6, or at position H338 with respect to SEQ ID NO 11, or at position H335 with respect to SEQ ID NO 12, or at position H350 with respect to SEQ ID NO 15;
(iii) a substitution at position W377 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position W376 with respect to SEQ ID NOs 4 and 5, or at position W372 with respect to SEQ ID NO 6, or at position W380 with respect to SEQ ID NO 13, or at position W372 with respect to SEQ ID NO 6, or at position W383 with respect to SEQ ID NO 9, or at position W378 with respect to SEQ ID NO 11, or at position W375 with respect to SEQ ID NO 12, or at position W380 with respect to SEQ ID NO 13, or at position W390 with respect to SEQ ID NO 15;

(iv) a substitution at position A17 with respect to SEQ ID NOs 3, 4, 5, 6, 8, 10, 13, 14 and 15, or at position A18 with respect to SEQ ID NO: 11 and 16;
(v) a substitution at position G57 with respect to SEQ ID NOs 3 and 15; or
(vi) a substitution at position Q261 with respect to SEQ ID NO: 3, or at position Q262 with respect to SEQ ID NO: 6, or at position Q264 with respect to SEQ ID NO 4.

3. The TdT variant of claims 1 or 2 wherein:
(i) said substitution at position Q326 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position Q325 with respect to SEQ ID NO: 4 and 5, or at position Q332 with respect to SEQ ID NO: 7 and 9, or at position Q329 with respect to SEQ ID NO: 13 and 16, or at position Q321 with respect to SEQ ID NO: 6, or at position Q327 with respect to SEQ ID NO: 11, or at position Q324 with respect to SEQ ID NO: 12, or at position Q339 with respect to SEQ ID NO: 15 is selected from the group consisting of T, F, L, M, I, V and Y;
(ii) said substitution at position K265 with respect to SEQ ID NOs 3, 7, 8, 9, 10, 13 and 14, or at position K263 with respect to SEQ ID NOs 6 and 12, or at position K264 with respect to SEQ ID NO 5, or at position K266 with respect to SEQ ID NO 11, or at position K268 with respect to SEQ ID NO 16, or at position K272 with respect to SEQ ID NO 15 is selected from the group consisting of E, T, A and R;
(iii) said substitution of at position H337 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position H336 with respect to SEQ ID NOs 4 and 5, or at position H343 with respect to SEQ ID NOs 7 and 9, or at position H340 with respect to SEQ ID NOs 13 and 16, or at position H332 with respect to SEQ ID NO 6, or at position H338 with respect to SEQ ID NO 11, or at position H335 with respect to SEQ ID NO 12, or at position H350 with respect to SEQ ID NO 15 is selected from the group consisting of Y, F, N and D;
(iv) said substitution at position W377 with respect to SEQ ID NOs 3, 8, 10 and 14, or at position W376 with respect to SEQ ID NOs 4 and 5, or at position W372 with respect to SEQ ID NO 6, or at position W380 with respect to SEQ ID NO 13, or at position W383 with respect to SEQ ID NO 9, or at position W378 with respect to SEQ ID NO 11, or at position W375 with respect to SEQ ID NO 12, or at position W380 with respect to SEQ ID NO 13, or at position W390 with respect to SEQ ID NO 15 is R;

(v) said substitution at position A17 with respect to SEQ ID NOs 3, 4, 5, 6, 8, 10, 13, 14 and 15, or at position A18 with respect to SEQ ID NO: 11 and 16 is V, I or L;
(vi) said substitution at position G57 with respect to SEQ ID NOs 3 and 15, or at position G58 with respect to SEQ ID NO: 9 and 11 is E; and
(vii) said substitution at position Q261 with respect to SEQ ID NO: 3, or at position Q262 with respect to SEQ ID NO: 6, or at position Q264 with respect to SEQ ID NO 4 is R.

4. The TdT variant of claims 1, 2 or 3 further comprising one or more of:
(i) a substitution at position L52 with respect to SEQ ID NO: 3;
(ii) a substitution at position M63 with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 13 and 15; or at position M73 with respect to SEQ ID NO: 10; or at position M64 with respect to SEQ ID NO: 11; or at position M61 with respect to SEQ ID NO: 12; or at position M66 with respect to SEQ ID NO: 16;
(iii) a substitution at position A108 with respect to SEQ ID NOs: 3 and 12; or at position A110 with respect to SEQ ID NOs: 4, 5, 7, 8, 10, 13, 14 and 15; or at position A107 with respect to SEQ ID NO: 6; or at position A111 with respect to SEQ ID NO: 9; or at position A113 with respect to SEQ ID NO: 16;
(iv) a substitution at position L131 with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13, 14 and 15; or at position L132 with respect to SEQ ID NOs: 9 and 11; or at position L129 with respect to SEQ ID NO: 12; or at position L113 with respect to SEQ ID NO: 16;
(v) a substitution at position C173 with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13 and 14; or at position C172 with respect to SEQ ID NO: 6; or at position C174 with respect to SEQ ID NOs: 9 and 11; or at position C171 with respect to SEQ ID NO: 12; or at position C176 with respect to SEQ ID NO: 16; or at position C182 with respect to SEQ ID NO: 15;
(vi) a substitution at position R207 with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13 and 14; or at position R206 with respect to SEQ ID NO: 6; or at position R208 with respect to SEQ ID NOs: 9 or 11; or at position R205 with respect to SEQ ID NO: 12; or at position R216 with respect to SEQ ID NO: 216; or at position R210 with respect to SEQ ID NO: 16;
(vii) a substitution at position G284 with respect to SEQ ID NO: 3 or 12;
(viii) a substitution at position E289 with respect to SEQ ID NO: 3; or at position E294 with respect to SEQ ID NO: 5, or at position E290 with respect to SEQ ID NO: 6; or at position

E292 with respect to SEQ ID NO: 7; or at position E295 with respect to SEQ ID NOs: 10 and 14; or at position E298 with respect to SEQ ID NOs: 13 and 16;
(ix) a substitution at position R325 with respect to SEQ ID NO: 3, 10 and 14; or at position R324 with respect to SEQ ID NOs 4 and 5; or at position R320 with respect to SEQ ID NO: 6; or at position R331 with respect to SEQ ID NOs: 7 and 9; or at position R323 with respect to SEQ ID NO: 12; or at position R328 with respect to SEQ ID NOs: 13 and 16; or at position R338 with respect to SEQ ID NO: 15;
(x) a substitution at position E328 with respect to SEQ ID NOs: 3, 8, 10 and 14; or at position E327 with respect to SEQ ID NOs: 4 and 5; or at position E334 with respect to SEQ ID NOs: 7 and 9; or at position E329 with respect to SEQ ID NO: 11; or at position E326 with respect to SEQ ID NO: 12; or at position E331 with respect to SEQ ID NOs: 13 and 16; or
(xi) a substitution at position R351 with respect to SEQ ID NO: 3; or at position R353 with respect to SEQ ID NOs: 4 and 5; or at position R354 with respect to SEQ ID NO: 8 and 14; or at position R355 with respect to SEQ ID NO: 11; or at position R352 with respect to position 13.

5. The TdT variant of any one of the previous claims further comprising one or more of:
(i) a substitution Q37E with respect to SEQ ID NOs: 3, 5, 7, 9 and 15;
(ii) a substitution D41R with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13, 14 and 15, or D40R with respect to SEQ ID NO: 6, or D42R with respect to SEQ ID NO: 9 and 11, or D44R with respect to SEQ ID NO: 16, or D39R with respect to SEQ ID NO:12;
(iii) a substitution C59R with respect to SEQ ID NOs: 3, 5, 7, 13 and 15, or C58R with respect to SEQ ID NO: 6, or C60R with respect to SEQ ID NO: 10, or C62R with respect to SEQ ID NO:16;
(iv) a substitution L60D with respect to SEQ ID NOs: 3, 7, 10 and 15, or L59D with respect to SEQ ID NO:6;
(v) a substitution S94R with respect to SEQ ID NOs: 3, 12 and 15;
(vi) a substitution G98E with respect to SEQ ID NO: 3;
(vii) a substitution S119A with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13, 14 and 14, or S118A with respect to SEQ ID NO: 6, or S120A with respect to SEQ ID NOs 9 and 11, or S122A with respect to SEQ ID NO: 16;

(viii) a substitution S146E with respect to SEQ ID NOs: 3, 4, 5, 8, 10 and 14, or S147E with respect to SEQ ID NO: 11, or S144E with respect to SEQ ID NO: 12;
(ix) a substitution Q149R with respect to SEQ ID NO: 3;
(x) a substitution F193Y with respect to SEQ ID NOs: 3, 4, 5, 7, 8, 10, 13 and 14, or F192Y with respect to SEQ ID NO: 6, or F194Y with respect to SEQ ID NO: 11, or F191Y with respect to SEQ ID NO: 12, or F196Y with respect to SEQ ID NO: 16;
(xi) a substitution V199M with respect to SEQ ID NO: 3, 4, 5, 7, 8, 10, 13, 14 and 15, or V198M with respect to SEQ ID NO: 6, or V200M with respect to SEQ ID NOs: 9 and 11; or
(xii) a substitution M201V with respect to SEQ ID NOs: 3, 4, 5, 8, 10 and 13, or M202V with respect to SEQ ID NO: 11, or M199V with respect to SEQ ID NO: 12.

6. The TdT variant of any one of the previous claims comprising the following combination of substitutions with respect to SEQ ID NO: 3:
(i) A17V + Q37E + D41R + L52F + G57E + M63R + S94R + G98E + A108V + S119A + L131R + S146E + Q149R + C173G + R207L + K265T + G284P + E289V + R325P + Q326F + E328N + H337D + R351K + W377R;
(ii) A17V + Q37E + D41R + L52F + G57E + M63R + S94R + G98E + A108V + S146E + Q149R + C173G + F193Y + V199M + M201V + R207L + K265T + G284P + E289V + R325A + Q326F + E328N + R351K;
(iii) L52F + A108V + R351K + A17V + Q37E + D41R + G57E+ C59R + L60D + M63R + S94R + G98E + S119A + L131R + S146E + Q149R + C173G + R207L + K265T + G284P + E289V + R325A + Q326F + E328N;
(iv) L52F + A108V + R351K + A17V + Q37E + D41R + G57E + C59R + L60D + M63R + S94R + G98E + K118Q + S119A + L131R + S146E + Q149R + C173G + R207L + K265T + G284P + E289V + R325A + Q326F + E328N + W377R;
(v) A17V + Q37E + D41R + L52F + G57E + C59R + L60D + M63R + S94R + G98E + A108V + S119A + L131R + S146E + Q149R + C173G + R207L + F259S + Q261L, G284P + E289V + R325A + Q326F + E328N + R351K + W377R;
(vi) A17V + Q37E + D41R + L52F + G57E + C59R + L60D + M63R + S94R + G98E + A108V + S119A + L131R + S146E + Q149R + C173G + R207L + E257D + F259S + K260R + Q261L + G284P + E289V + R325A + Q326F + E328N + R351K + W377R;

(vii) A17V + D41R + L53F + G57E + C59R + L60D + M63R + S94R + G98E + K118Q + S119A + L131R + S146E + Q149R + C173G + R207L + K265T + G284P + E289V + R325A + Q326F + R351K + W377R;
(viii) A17V + D41R + L52F + G57E + C59R + L60D + M63R + S94R + G98E + A108V + S119A + L131R + S146E + Q149R + R207L + K265T + G284P + E289V + R325A + Q326F + R351K;
(ix) A17V + L52F + M63R + A108V + C173G + R207L + K265T + G284P + E289V + R325P + E328N + R351K;
(x) A17V + D41R + L52F + G57E + M63R + S94R + G98E + A108V + S146E + Q149R + C173G + M184T + R207L + K209H + G284L + E289A + R325V + E328K + R351K;
(xi) A17V + L52F + G57E + M63R + A108V + C173G + R207L + K265T + G284P + E289V + R325P + E328N + R351K;
(xii) A17V + L32T + Q37R + D41R + L52F + G57E + C59R + L60D + M63R + S67A + S94R + G98E + A108V + S119A +L131R + S146E + Q149R + V171A + S172E + C173R + V182I + S183E + R207L + K209H + M210K + T211I + E223G + A224P + E228D + Q261L + G284P + E289V + R325A + Q326F + E328N + R351K + D372E; or
(xiii) comprising SEQ ID NOs: 27, 28, 29, 30 or 31.

7. The TdT variant of any one of claims 1 to 6having amino acid sequence at least ninety percent identical to SEQ ID NO: 4 and further having substitutions at positions M63, R207, R324 and E327.

8. The TdT variant of claim 7, comprising the combination of substitutions M63R+ R207L+E227N.

9 The TdT variant of any one of claims 1 to 6, having amino acid sequence at least ninety percent identical to SEQ ID NO: 24 and further having substitutions at positions M47, R190, R308 and E311.

10. The TdT variant of claim 9, comprising the combination of substitutions M47R+ R190L+E311N.

11. The TdT variant of any one of claims 1 to 6 having amino acid sequence at least ninety percent identical to SEQ ID NO: 26 and further having substitutions at positions M46, R190 and E311.

12. The TdT variant of claim 11, comprising the combination of substitutions M46R+ R190 L+ E311N.

13. The TdT variant of any one of claims 1 to 6, having amino acid sequence at least ninety percent identical to SEQ ID NO: 25 and further having substitutions at positions R184, R306 and E309.

14. The TdT variant of claim 13, comprising the combination of substitutions R184L+R306A+E309N.

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

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

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 TdT variant according to any one of claims 1 to 14, 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 the polynucleotide is formed.

16. The method of claim 15, wherein said 3'-O-blocked 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.

17. A kit for performing a nucleotide incorporation reaction comprising:

a) a variant of TdT according to any one of claims 1 to 14,

b) one or more nucleotides, preferably one or more 3'O-modified nucleotides, and

c) optionally at least one nucleic acid primer.