CLAIMS

1. 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

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 elongated fragments having free 3'-hydroxyls, until the polynucleotide is synthesized, wherein a first TdT variant elongates the initiator or elongated fragments with a 3'-O-blocked nucleoside triphosphate selected from a first set of 3'-O-blocked nucleoside triphosphates and a second TdT variant, different from the first TdT variant, elongates the initiator or elongated fragments with a 3'-O-blocked nucleoside triphosphate selected from a second set of 3'-O-blocked nucleoside triphosphates, and wherein the first TdT variant elongates the initiator or elongated fragments with 3'-O-blocked nucleoside triphosphates from the first set with greater efficiency than the second TdT variant and the second TdT variant elongates the initiator or elongated fragments with 3'-O-blocked nucleoside triphosphates from the second set with greater efficiency than the first TdT variant.

2. The method of claim 1, wherein a third TdT variant, different from the first TdT variant and from the second TdT variant, elongates the initiator or elongated fragments with a 3'-O-blocked nucleoside triphosphate selected from a third set of 3'-O-blocked nucleoside triphosphates, and wherein the first TdT variant elongates the initiator or elongated fragments with 3'-O-blocked nucleoside triphosphates from the first set with greater efficiency than the second TdT variant or the third TdT variant, the second TdT variant elongates the initiator or elongated fragments with 3'-O-blocked nucleoside triphosphates from the second set with greater efficiency than the first TdT variant or the third TdT variant, and the third TdT variant elongates the initiator or elongated fragments with 3'-O-blocked nucleoside triphosphates from the third set with greater efficiency than the first TdT variant or the second TdT variant.

3. The method of claim 1 or 2, wherein said first set comprises 3'-O-blocked deoxyadenosine triphosphate, 3'-O-blocked deoxycytidine triphosphate, and 3'-O-blocked deoxythymidine triphosphate and said first TdT variant comprises an amino acid sequence that has at least 90% identity with the amino acid sequence of SEQ ID NO: 1 and comprises the following combination of substitutions: A17V + L52F + G57E + M63R + A108V + K147R + C173G + R207L + M210Q + + R325V + E328K + N345E + R351K.

4. The method of claim 3 wherein said first TdT variant comprises the following combination of substitutions: A17V + Q37E + D41R + L52F + G57E + M63R + S94R + G98E + A108V + S146E + K147R + Q149R + C173G + M184T + R207L + K209H + M210Q + G284L + E289A + R325V + E328K + N345E + R351K.

5. The method of anyone of claims 1 to 4, wherein said second set comprises 3'-O-blocked deoxyguanosine triphosphate and said second TdT variant comprises an amino acid sequence that has at least 90 % identity to the amino acid sequence of SEQ ID NO: 1 and comprises the following combination of substitutions: A17V + L52F + G57E + M63R + I76V + A108V + C173G + R207L + F259E + Q261R + K265G + R325V + E328N + R351K.

6. The method of claim 5 wherein said second TdT variant has the following combination of substitutions: A17V + Q37E + D41R + L52F + G57E + M63R + I76V + S94R + G98E + A108V + S146E + Q149R + C173G + M184T + R207L + K209H + F259E + Q261R + K265G + G284L + E289A + R325V + E328N + R351K.

7. A terminal deoxynucleotidyltransferase (TdT) comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 99% identity to the full length amino acid sequence set forth in SEQ ID NO:1, and (ii) has the combination of substitutions A17V + L52F + G57E + M63R + A108V + K147R + C173G + R207L + M210Q + R325V + E328K + N345E + R351K, as compared to SEQ ID N°1.

8. The TdT according to claim 7, comprising the combination of substitutions A17V + Q37E + D41R + L52F + G57E + M63R + S94R + G98E + A108V + S146E + K147R + Q149R +

C173G + M184T + R207L + K209H + M210Q + G284L + E289A + R325V + E328K + N345E + R351K, as compared to SEQ ID NO:1.

9. A terminal deoxynucleotidyltransferase (TdT) comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 99% identity to the full length amino acid sequence set forth in SEQ ID NO:1, and (ii) has the combination of substitutions A17V + L52F + G57E + M63R + I76V + A108V + C173G + R207L + F259E + Q261R + K265G + R325V + E328N + R351K, as compared to SEQ ID NO:1.

10. The TdT according to claim 9, comprising the combination of substitutions A17V + Q37E + D41R + L52F + G57E + M63R + I76V + S94R + G98E + A108V + S146E + Q149R + C173G + M184T + R207L + K209H + F259E + Q261R + K265G + G284L + E289A + R325V + E328N + R351K, as compared to SEQ ID NO:1.

11. Kit for practicing a method of synthesizing a polynucleotide having a predetermined sequence, wherein the kit comprises
- a first TdT variant according to claims 7 or 8,
- a second TdT variant, according to claims 9 or 10 and
- the first and second sets of 3'-O-blocked nucleoside triphosphates, wherein said dNTPs are nonoverlapping.

12. Kit according to claim 11, wherein the first set of dNTPs comprises 3'-O-blocked deoxyadenosine triphosphate, 3'-O-blocked deoxycytidine triphosphate, and 3'-O-blocked deoxythymidine triphosphate and the second set comprises 3'-O-blocked deoxyguanosine triphosphate.