

WHAT IS CLAIMED IS: CLAIMS

1. A recombinant terminal deoxynucleotidyl transferase (TdT) comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 1, wherein the recombinant TdT comprises one or more amino acid substitution mutations at one or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.

~~2. The recombinant TdT of claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~3. The recombinant TdT of claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu191Ala, Glu191Arg, Glu191Asn, Glu191Asp, Glu191Cys, Glu191Gln, Glu191Gly, Glu191His, Glu191Ile, Glu191Leu, Glu191Lys, Glu191Met, Glu191Phe, Glu191Pro, Glu191Ser, Glu191Thr, Glu191Trp, Glu191Tyr, or Glu191Val.~~

4. The recombinant TdT of claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Glu191 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu191Val.

~~5. The recombinant TdT of any one of claims 1-4, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~6. The recombinant TdT of any one of claims 1-4, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys193Ala, Lys193Arg, Lys193Asn, Lys193Asp, Lys193Cys, Lys193Gln, Lys193Glu, Lys193Gly, Lys193His, Lys193Ile, Lys193Leu, Lys193Met, Lys193Phe, Lys193Pro, Lys193Ser, Lys193Thr, Lys193Trp, Lys193Tyr, or Lys193Val.~~

7. The recombinant TdT of ~~any one of claims 1-4~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Lys193 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys193Asn.

~~8. The recombinant TdT of any one of claims 1-7, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively~~

~~charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~9. The recombinant TdT of any one of claims 1-7, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu194Ala, Glu194Arg, Glu194Asn, Glu194Asp, Glu194Cys, Glu194Gln, Glu194Gly, Glu194His, Glu194Ile, Glu194Leu, Glu194Lys, Glu194Met, Glu194Phe, Glu194Pro, Glu194Ser, Glu194Thr, Glu194Trp, Glu194Tyr, or Glu194Val.~~

~~104. The recombinant TdT of any one of claims 1-7 claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Glu194 in the *Bos taurus* TdT of SEQ ID NO: 12 is Glu194Gly.~~

~~11. The recombinant TdT of any one of claims 1-10, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~12. The recombinant TdT of any one of claims 1-10, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 is Asp242Ala, Asp242Arg, Asp242Asn, Asp242Cys, Asp242Gln, Asp242Glu, Asp242Gly, Asp242His, Asp242Ile, Asp242Leu, Asp242Lys, Asp242Met, Asp242Phe, Asp242Pro, Asp242Ser, Asp242Thr, Asp242Trp, Asp242Tyr, or Asp242Val.~~

~~135. The recombinant TdT of any one of claims 1-10 claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Asp242 in the *Bos taurus* TdT of SEQ ID NO: 12 is Asp242Tyr.~~

~~14. The recombinant TdT of any one of claims 1-13, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~15. The recombinant TdT of any one of claims 1-13, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys287Ala, Lys287Arg, Lys287Asn, Lys287Asp, Lys287Cys, Lys287Gln, Lys287Glu, Lys287Gly, Lys287His, Lys287Ile, Lys287Leu, Lys287Met, Lys287Phe, Lys287Pro, Lys287Ser, Lys287Thr, Lys287Trp, Lys287Tyr, or Lys287Val.~~

16. The recombinant TdT of ~~any one of claims 1-13~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Lys287 in the *Bos taurus* TdT of SEQ ID NO: 12 is Lys287Glu.

~~17. The recombinant TdT of any one of claims 1-16, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~18. The recombinant TdT of any one of claims 1-16, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 is Phe296Ala, Phe296Arg, Phe296Asn, Phe296Asp, Phe296Cys, Phe296Gln, Phe296Glu, Phe296Gly, Phe296His, Phe296Ile, Phe296Leu, Phe296Lys, Phe296Met, Phe296Pro, Phe296Ser, Phe296Thr, Phe296Trp, Phe296Tyr, or Phe296Val.~~

19. The recombinant TdT of ~~any one of claims 1-16~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Phe296 in the *Bos taurus* TdT of SEQ ID NO: 12 is Phe296Leu.

~~20. The recombinant TdT of any one of claims 1-19, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~21. The recombinant TdT of any one of claims 1-19, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 is Met299Ala, Met299Arg, Met299Asn, Met299Asp, Met299Cys, Met299Gln, Met299Glu, Met299Gly, Met299His, Met299Ile, Met299Leu, Met299Lys, Met299Phe, Met299Pro, Met299Ser, Met299Thr, Met299Trp, Met299Tyr, or Met299Val.~~

22. The recombinant TdT of ~~any one of claims 1-19~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Met299 in the *Bos taurus* TdT of SEQ ID NO: 12 is Met299Lys.

~~23. The recombinant TdT of any one of claims 1-22, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~24. The recombinant TdT of any one of claims 1-22, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 is Thr342Ala, Thr342Arg, Thr342Asn, Thr342Asp, Thr342Cys, Thr342Gln, Thr342Glu, Thr342Gly, Thr342His, Thr342Ile, Thr342Leu, Thr342Lys, Thr342Met, Thr342Phe, Thr342Pro, Thr342Ser, Thr342Trp, Thr342Tyr, or Thr342Val.~~

259. The recombinant TdT of ~~any one of claims 1-22~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to Thr342 in the *Bos taurus* TdT of SEQ ID NO: 12 is Thr342Ser.

~~26. The recombinant TdT of any one of claims 1-25, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprises a mutation to a nonpolar amino acid, a polar amino acid, a positively charged amino acid, a negatively charged amino acid, a hydrophobic amino acid, an aromatic amino acid, an aliphatic amino acid, a small amino acid, or a hydrophilic amino acid.~~

~~27. The recombinant TdT of any one of claims 1-25, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 is His421Ala, His421Arg, His421Asn, His421Asp, His421Cys, His421Gln, His421Glu, His421Gly, His421Ile, His421Leu, His421Lys, His421Met, His421Phe, His421Pro, His421Ser, His421Thr, His421Trp, His421Tyr, or His421Val.~~

2810. The recombinant TdT of ~~any one of claims 1-25~~ claim 1, wherein the amino acid substitution mutation at the position functionally equivalent to His421 in the *Bos taurus* TdT of SEQ ID NO: 12 is His421Pro.

2911. The recombinant TdT of ~~any one of claims 1-28~~ claim 1, wherein the recombinant TdT comprises two or more amino acid substitution mutations at two or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.

~~30. The method of claim 29, wherein the two or more amino acid substitution mutations at the two or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise two or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

31. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises three or more amino acid substitution mutations at three or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

32. ~~The method of claim 31, wherein the three or more amino acid substitution mutations at the three or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise three or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

33. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises four or more amino acid substitution mutations at four or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

34. ~~The method of claim 33, wherein the four or more amino acid substitution mutations at the four or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise four or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

35. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises five or more amino acid substitution mutations at five or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

36. ~~The method of claim 35, wherein the five or more amino acid substitution mutations at the five or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise five or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

37. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises six or more amino acid substitution mutations at six or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

38. ~~The method of claim 37, wherein the six or more amino acid substitution mutations at the six or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise six or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

39. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises seven or more amino acid substitution mutations at seven or more positions~~

functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.

40. ~~The method of claim 39, wherein the seven or more amino acid substitution mutations at the seven or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise seven or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

41. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises eight or more amino acid substitution mutations at eight or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

42. ~~The method of claim 41, wherein the eight or more amino acid substitution mutations at the eight or more positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 comprise eight or more of Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

43. ~~The recombinant TdT of any one of claims 1-28, wherein the recombinant TdT comprises eight amino acid substitution mutations at eight positions functionally equivalent to Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.~~

44. ~~The method of claim 43, wherein the eight amino acid substitution mutations at the eight positions functionally equivalent to Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 are Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

45~~12~~. The recombinant TdT of ~~any one of claims 1-28~~ claim 1, wherein the recombinant TdT comprises nine amino acid substitution mutations at positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12.

46. ~~The method of claim 45, wherein the nine amino acid substitution mutations at the positions functionally equivalent to Glu191, Lys193, Glu194, Asp242, Lys287, Phe296, Met299, Thr342, and His421 in the *Bos taurus* TdT of SEQ ID NO: 12 are Glu191Val, Lys193Asn, Glu194Gly, Asp242Tyr, Lys287Glu, Phe296Leu, Met299Lys, Thr342Ser, and His421Pro, respectively.~~

~~47~~13. The recombinant TdT of ~~any one of claims 1-45~~ claim 1, wherein the recombinant TdT comprises an amino acid sequence that is at least 85% identical to SEQ ID NO: 1.

~~48.~~ ~~The recombinant TdT of any one of claims 1-45, wherein the recombinant TdT comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.~~

~~49.~~ ~~The recombinant TdT of any one of claims 1-45, wherein the recombinant TdT comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.~~

~~50~~14. The recombinant TdT of ~~any one of claims 1-45~~ claim 1, wherein the recombinant TdT comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 11.

~~51~~15. The recombinant TdT of ~~any one of claims 1-45~~ claim 1, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 12.

~~52~~16. The recombinant TdT of ~~any one of claims 1-51~~ claim 1, wherein the recombinant TdT is stable at a temperature of 47 °C or higher.

~~53.~~ ~~The recombinant TdT of any one of claims 1-51, wherein the recombinant TdT is stable at a temperature of 50 °C or higher.~~

~~54.~~ ~~The recombinant TdT of any one of claims 1-51, wherein the recombinant TdT is stable at a temperature of 55 °C or higher.~~

~~55.~~ ~~The recombinant TdT of any one of claims 1-51, wherein the recombinant TdT is stable at a temperature of 58 °C or higher.~~

~~56~~17. The recombinant TdT of ~~any one of claims 1-55~~ claim 1, wherein the terminal deoxynucleotidyl transferase activity of the recombinant TdT is at least 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, or 120%, of the terminal deoxynucleotidyl transferase activity of the *Bos taurus* TdT of SEQ ID NO: 12 at a same test temperature.

~~57.~~ ~~The recombinant TdT of claim 56, wherein the test temperature is 37 °C, 47 °C, 50 °C, 55 °C, or 58 °C.~~

~~58~~18. The recombinant TdT of ~~any one of claims 1-57~~ claim 1, wherein the recombinant TdT comprises a small ubiquitin-like modifier (SUMO) fragment.

~~59.~~ ~~The recombinant TdT of claim 58, wherein the SUMO fragment comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 13.~~

~~60~~19. The recombinant TdT of ~~any one of claims 58-59~~ claim 1, wherein the recombinant TdT comprises the SUMO fragment on the N-terminus of the recombinant TdT.

~~61.~~ ~~The recombinant TdT of claim 59, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 14.~~

~~62. The recombinant TdT of claim 59, wherein the recombinant TdT comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 15.~~

~~63. The recombinant TdT of any one of claims 58-59, wherein the recombinant TdT comprises the SUMO fragment on the C-terminus of the recombinant TdT.~~

~~64~~20. A polynucleotide encoding the recombinant terminal deoxynucleotidyl transferase (TdT) of ~~any one of claims 1-63~~ claim 1.

~~65. An expression vector comprising the polynucleotide of claim 64.~~

~~66. A host cell comprising one or more of
the recombinant terminal deoxynucleotidyl transferase (TdT) of any one of
claims 1-63,
the polynucleotide of claim 64, and
the expression vector of claim 65.~~

~~67. A kit comprising:
the recombinant terminal deoxynucleotidyl transferase (TdT) of any one of
claims 1-63, the polynucleotide of claim 64, the expression vector of claim 65, the host
cell of claim 66, or a combination thereof, and
instructions for using the recombinant terminal deoxynucleotidyl transferase, the
polynucleotide, the expression vector, the host cell, or a combination thereof.~~