

WHAT IS CLAIMED IS:

1. An engineered terminal deoxynucleotidyl transferase comprising a polypeptide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a reference sequence of SEQ ID NO: 5296, or a functional fragment thereof, wherein the engineered terminal deoxynucleotidyl transferase comprises at least one residue difference at position 68 in its polypeptide sequence, and wherein said engineered terminal deoxynucleotidyl transferase or functional fragment thereof has terminal deoxynucleotidyl transferase activity, and wherein the amino acid positions of the polypeptide sequence are numbered with reference to SEQ ID NO: 5296.

2. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 65, 65/184/187, 82/184/220/386, 154, 154/184, 154/187/220/350/386, 154/293, 154/350, 154/386, 184/187, 184/293, 187/220/350, 187/293, 220, 220/293, 293, 293/350, 293/350/386, 350, and 386, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5296.

3. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 157, 160, 186, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 206, 209, 210, 211, 227, 229, 230, 233, 234, 235, 236, 237/381, 238, 241, 242, 243, 247, 253, 258, 260, 267, 272, 278, 282, 286, 288, 290, 292, 297, 298, 299, 327, 331, 368, 373, and 381, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5296.

4. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 160, 186, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 209, 210, 211, 229, 230, 233, 234, 235, 236, 237/381, 238, 241, 243, 244, 247, 253, 260, 278, 286, 288, 292, 297, 298, 299, 327, 331, 368, 373, and 381, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5296.

5. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 26, 26/60/200, 26/60/200/203/292, 26/60/203/229/234/290, 26/200/203, 26/200/203/229, 26/200/290, 26/203/234/290/292, 60, 60/200/203/290, 60/203, 157/162, 157/368, 162/242/368, 200, 200/203/229/234, 200/203/290, 200/206, 200/234/290, 200/290/292/360, 203, 203/229, 229/360, and 360, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5628.

6. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 26, 26/60/200, 26/60/200/203/229/234/267, 26/60/200/203/229/267, 26/60/200/203/229/267/290, 26/60/200/203/234/267, 26/60/200/203/292, 26/60/200/234/290, 26/60/203/229/234/290, 26/60/229/267, 26/60/290, 26/200, 26/200/203, 26/200/203/229, 26/200/290, 26/229/234/360, 53/157/278/327/331, 53/162/327/331/368, 60, 60/200/203/290, 60/200/229/234, 60/200/234, 60/200/234/267/290/292, 60/200/234/290, 60/203, 157/162, 157/162/241/242/260, 157/162/241/242/327/331, 157/162/241/278/331/368, 157/162/241/331, 157/162/242/260/331, 157/162/331, 157/327/331, 162/241/278/327/331, 162/241/331, 162/242/368, 200, 200/203/229/234, 200/203/290, 200/206, 200/234/290, 200/290/292/360, 210/211/242, 229/360, 241/242/368, 242/331, 260/327, 260/331, 260/368, 331, 360, and 368, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5628.

7. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 217, 226, 239, 256, 264, 273, 275, 315, 325, 328, 339, 342, 342/363, 344, 345, 346, 350, 351, 352, 353, 361, 363, 364, 365, 366, 367, 371, 374, 376, 380, 383, and 388, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5628.

8. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 113/355, 134, 169/390, 217, 226, 239, 256, 264, 265, 271, 275, 313, 314, 315, 321, 325, 328, 332, 339, 342, 342/363, 344, 345, 346, 348, 349, 350, 351, 352, 353, 354, 356, 360, 361, 362, 363, 364, 365, 366, 367, 369, 370, 371, 374, 376, 377, 378, 380, 382, 383, 384, 385, 386, 388, and 391, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5628.

9. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 184/188/200/203/211/242, 184/188/200/203/242/297, 184/188/200/203/290/297/368, 184/188/203/290/297, 184/188/211/242/290/368, 184/189, 184/189/206/297/368, 184/189/297, 184/200, 184/206/242/290/297, 184/211, 184/242, 184/242/297/368, 184/290/297, 184/290/368, 188/211, 188/211/242/290/297/368, 192/193/211/242/297/368, 192/193/290/297/368, 193/194, 194, 194/242, 194/242/290, 200, 200/203, 200/203/290/368, 203/206/368, 203/242/290/297/368, 203/297, 206/290, 211, 211/368, 242/290, 242/290/297, 297, and 368, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5630.

10. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 184, 184/188/200/203/211/242, 184/188/200/203/242/297, 184/188/200/203/290/297/368, 184/188/203/290/297, 184/188/211/242/290/368, 184/189, 184/189/206/297/368, 184/200, 184/242,

184/242/297/368, 184/290/297, 184/290/368, 188/211/242/290/297/368, 189/200, 192/193/211/242/297/368, 192/193/290/297/368, 194/242, 200, 200/203, 200/203/290/368, 203/206/368, 203/297, 206/290, 206/297, 211/368, 242/290, 242/290/297, 297, and 368, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5630.

11. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 56, 57, 59, 63, 65, 66, 73, 128/296, 140, 144/220, 149, 151, 159, 209/211, 210/211, 219, 219/300, 230, 261, 272, 273, 275, 278, 292, 324, 327, 331, 345, 364, 371, and 373, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5632.

12. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 56, 57, 59, 61, 65, 66, 73, 74/272, 75, 79, 128, 128/296, 134, 140, 144, 145, 149, 151, 155, 156, 157, 158, 159, 200/202/203, 209/211, 210/211, 211, 219, 219/300, 228, 230, 242, 242/244, 261, 262, 270, 272, 273, 275, 278, 292, 319, 322, 324, 327, 339, 344, 345, 364, 371, 373, and 381, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5632.

13. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 65, 65/70/155/209/228, 65/128/209/371/373, 65/151, 65/155/209, 65/155/209/352/353, 65/159, 65/209/219/352/353, 65/209/352/353/371, 65/228/352/353/373, 65/228/371/373, 65/352/353/371, 128/159/209/352/353, 128/209, 128/209/219, 128/209/219/352/353/371, 128/209/228/371, 128/209/371, 128/209/371/373, 128/209/373, 128/338/352/353/373, 151/209, 151/209/371, 151/209/373, 151/228/352/353/373, 151/228/373, 151/352/353, 151/352/353/371/373, 155, 155/209, 155/228/352/353, 159/209/352/353, 208/209, 209, 209/219/352/353/371, 209/219/371, 209/228, 209/352/353/371, 209/371, 209/371/373, 209/373, 219/228, 219/352/353/371/373, 219/371/373, 228, 228/371, 228/371/373, 228/373, 352/353, 352/353/371, 371, 371/373, and 373, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5636.

14. The engineered terminal deoxynucleotidyl transferase of Claim 1, further comprising a residue difference(s) or a residue difference set(s) at amino acid positions selected from 55/65/151, 65, 65/70/155/209/228, 65/70/228/373, 65/128, 65/128/155/209/371, 65/128/159/209/371/373, 65/128/209/371, 65/128/209/371/373, 65/128/219/373, 65/128/373, 65/151, 65/155/209, 65/155/209/352/353, 65/159, 65/209/219/352/353, 65/209/352/353/371, 65/219/371, 65/228/352/353/373, 65/228/371, 65/228/371/373, 65/352/353, 65/352/353/371, 65/373, 70/155/352/353, 128, 128/155/209, 128/159, 128/159/209/352/353, 128/209, 128/209/219, 128/209/219/352/353/371, 128/209/228/371, 128/209/371, 128/209/371/373, 128/209/373, 128/219/352/353, 128/219/371/373, 128/228/352/353, 128/228/371, 128/228/373, 128/352/353,

128/352/353/373, 128/371, 128/371/373, 128/373, 151, 151/155, 151/155/209, 151/155/209/219/228/371/373, 151/155/209/373, 151/155/219/352/353/371, 151/155/352/353, 151/155/352/353/371/373, 151/155/352/353/373, 151/209, 151/209/371, 151/209/371/373, 151/209/373, 151/219, 151/219/371, 151/228/352/353/373, 151/228/373, 151/352/353, 151/352/353/371/373, 151/371, 151/373, 155, 155/209, 155/219, 155/228/352/353, 155/228/371, 155/228/371/373, 155/352/353/373, 155/371, 155/371/373, 155/373, 159/209/352/353, 209, 209/219/352/353/371, 209/219/371, 209/228, 209/352/353/371, 209/371, 209/371/373, 209/373, 219, 219/228, 219/352/353/371/373, 219/352/353/373, 219/371, 219/371/373, 228/352/353, 228/371, 228/371/373, 228/373, 352/353, 352/353/371, 352/353/373, 371, 371/373, and 373, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 5636.

15. An engineered terminal deoxynucleotidyl transferase comprising a polypeptide sequence selected from the even-numbered sequences of SEQ ID NO: 5624-6766.

16. The engineered terminal deoxynucleotidyl transferase of Claim 1, having at least one improved property, as compared to wild-type terminal deoxynucleotidyl transferase or a reference engineered terminal deoxynucleotidyl transferase, wherein said improved property is selected from increased thermostability, increased activity at elevated temperatures, increased soluble expression, decreased by-product formation, and increased conversion of substrates to products.

17. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the engineered terminal deoxynucleotidyl transferase comprises at least one residue difference selected from 53T, 65L, 68R, 159R, 211V, 217R, 224V, 271H, 271P, 272A, 273P, 275Q, 278G, 331K, 341R, and 391L.

18. A polynucleotide sequence encoding at least one engineered terminal deoxynucleotidyl transferase of any one of Claims 1-17.

19. The polynucleotide sequence of Claim 18, wherein said polynucleotide sequence encodes at least one engineered terminal deoxynucleotidyl transferase comprising a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 5296, 5628, 5630, 5632, and/or 5636.

20. The polynucleotide sequence of Claim 19, wherein said sequence comprises SEQ ID NOs: 5295, 5627, 5629, 5631, and/or 5635.

21. An expression vector comprising at least one polynucleotide sequence of Claim 18.

22. A host cell transformed with at least one expression vector of Claim 21.

23. An *in vitro* method of producing an engineered terminal deoxynucleotidyl transferase polypeptide in a host cell comprising culturing a host cell of Claim 22, under suitable culture conditions, such that at least one engineered terminal deoxynucleotidyl transferase is produced.

24. A composition comprising at least one engineered terminal deoxynucleotidyl transferase of any one of Claims 1-17.