CLAIMS

What is claimed is:

1. An engineered terminal deoxynucleotidyl transferase comprising a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference sequence selected from SEQ ID NO: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, 5246, or a functional fragment thereof, and at least one amino acid residue difference relative to the reference sequence.

2. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises residue differences relative to the reference sequence of SEQ ID NO: 2 at a set of amino acid positions selected from:

80/121/185/315;

80/121/185/190/289/290/293/313/342/499;

80/121/185/190/289/290/293/313/315/336/342/359/391/414/470/474/499/522/523;

80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/470/474/499/522/523;

80/121/174/185/190/196/244/266/273/284/288/289/290/293/313/315/317/324/336/342/352/359/391/394/397/401/419/428/431/462/470/474/499/522/523;

80/106/121/185/190/205/289/290/293/313/315/336/342/359/391/470/474/499/522/523;

80/106/121/185/190/205/289/290/293/313/342/470/474/499/523;

80/106/121/185/190/244/289/290/293/307/342/359/470/474/499;

80/121/131/185/190/205/244/289/290/293/313/315/336/342/359/391/414/470/474/499/522/523;

80/121/131/185/190/205/244/289/290/293/313/336/342/359/391/414/470/474/499/522/523;

80/121/131/185/190/289/290/293/313/342/470/474/499/522/523;

80/121/174/179/185/190/236/244/288/289/290/293/313/315/317/336/342/359/363/391/394/408/426/462/470/474/499/522/523;

80/121/174/185/186/190/236/244/273/284/288/289/290/293/313/315/317/336/342/352/359/391/394/395/419/428/431/462/470/474/499/522/523;

80/121/174/185/190/193/196/244/273/284/288/289/290/293/297/313/315/317/324/336/342/352/359/376/380/391/394/401/415/419/428/431/435/441/462/470/474/499/522/523;

80/121/174/185/190/193/244/273/284/288/289/290/293/297/313/315/317/336/342/352/359/391/394/415/419/428/431/462/470/474/499/522/523;

80/121/174/185/190/236/244/273/282/284/288/289/290/293/313/315/317/336/342/352/359/391/394/395/419/428/431/462/470/474/499/522/523;

80/121/174/185/190/244/273/284/288/289/290/293/313/315/317/336/342/352/359/391/394/419/428/431/462/470/474/499/522/523;

80/121/174/185/190/244/273/284/288/289/290/293/313/315/317/336/342/359/391/394/428/431/462/470/4

74/499/522/523;
80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/352/359/391/394/419/428/431/462/470/474/499/522/523;
80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/352/359/391/394/428/431/462/470/474/499/522/523;
80/121/174/185/190/244/284/288/289/290/293/313/315/317/336/342/359/391/394/428/431/462/470/474/499/522/523;
80/121/185/190/196/244/289/290/293/313/315/317/336/342/359/391/470/474/499/522/523;
80/121/185/190/201/289/290/293/313/342/470/474/499/522;
80/121/185/190/244/273/289/290/293/313/315/317/336/342/352/359/391/419/435/470/474/499/522/523;
80/121/185/190/244/289/290/293/300/313/315/317/336/342/359/391/470/474/499/522/523;
80/121/185/190/244/289/290/293/313/315/317/336/342/359/380/391/401/419/470/474/499/522/523;
80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/392/470/474/499/522/523;
80/121/185/190/244/289/290/293/313/315/317/336/342/359/391/395/470/474/499/522/523;
80/121/185/190/244/289/290/293/313/336/342/359/391/414/470/474/499/522/523;
80/121/185/190/289/290/293/313/336/342/359/391/470/474/499/522/523;
80/121/190/289/290;
80/185/236/289/293;
121/185/190/213/289/290/293; and
185/289/290/293.

3. The engineered terminal deoxynucleotidyl transferase of Claim 1, wherein the polypeptide sequence comprises at least one substitution set comprising substitutions as compared to the reference sequence of
(i) SEQ ID NO: 36 at amino acid positions selected from:
174/244/273/284/288/315/317/336/352/359/391/394/419/428/431/462/470/474/522/523;
174/244/284/288/315/317/336/359/391/394/428/431/462/470/474/522/523;
244/315/317/336/359/391/470/474/522/523; and
336/359/391/470/474/522/523; and/or any combination thereof;
(ii) SEQ ID NO: 8 at amino acid positions selected from:
129/196, 173, 183, 186, 193, 195, 196, 263, 266, 268, 281, 282, 297, 300, 303, 316, 318, 320, 324, 343, 360, 392, 395, 397, 411, 415, 417, 421, 454, 456, 477, 481, and 492, and/or any combinations thereof;
(iii) SEQ ID NO: 16 at amino acid positions selected from:
186, 186/236/318, 186/236/395, 186/282/318, 193/196, 193/196/266/324/376/380, 193/196/297, 193/196/297/324/376/380/401/415/435/441, 193/196/324, 193/196/324/397/401/441, 193/196/376/380, 193/297/324/376/435, 193/297/324/380, 193/297/415, 193/435, 196, 196/266, 196/266/324/397/401,

196/297/324/435, 236/282, 236/282/395, 236/318/481, 266/297/380/397/401, 282, 282/318, 282/481, 297/380/401/441, 297/435, 318/395, 376/401/441, 415, and 435/441, and/or any combinations thereof;

(iv) SEQ ID NO: 24 at amino acid positions selected from: 12, 13, 14, 17, 18, 20, 21, 22, 23, 24, 26, 27, 29, 30, 31, 33, 34, 35, 37, 41, 53, 57, 58, 61, 92, 94, 97, 101, 102, 103, 104, 105, 106, 107, 108, 124, 126, 133, 135, 137, 138, 139, 140, 141, 142, 144, 145, 147, 149, 150, 152, 153, 154, 155, 156, 156/294, 159, 160, 161, 162, 163, 7/135, 12, 13, 14, 15, 16, 17/131, 18, 20, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 44, 45, 46, 57, 65, 77, 85, 89, 93, 94, 97, 101, 102, 103, 105, 106, 108, 109, 110, 119, 123, 124, 126, 130, 131, 132, 133, 134, 135, 137, 138, 139, 149, 150, 153, and 156, and/or any combinations thereof;

(v) SEQ ID NO: 268 at amino acid positions selected from: 14/53/300, 14/53/419, 106/300/415/419/456, 140, and 300/395/419, and/or any combinations thereof;

(vi) SEQ ID NO: 648 at amino acid positions selected from: 12/14/34, 12/14/34/37/94/140/141/145, 12/14/34/37/106/140/142/150/152/153, 12/14/34/37/141/142, 12/14/34/37/142/145, 12/14/34/37/142/161, 12/14/34/37/150, 12/14/34/37/150/153, 12/14/34/140/142, 12/14/34/140/150, 12/14/34/142/150/153, 12/14/92/94, 12/14/94/150/152, 12/14/106/107/141/142, 12/14/106/108/140/141/145/150, 12/14/106/108/152, 12/14/141/142, 12/14/150/152/153, 12/14/153, 12/34/92/140, 12/34/150/152, 12/37/94/141/150/152/153, 12/37/140/141/150/162, 12/161, 14, 14/31/34/37/140/141/145/161/162, 14/34/37, 14/34/37/145, 14/34/37/152, 14/34/94/106/108/141, 14/34/150/153, 14/106, 14/140, 14/141/161, 14/142, 14/142/161/162, 14/153, 14/161, 20/21/24/33/58/104/106/124/155/156, 20/21/33/58/101/104/106/124/155, 20/21/58/104/106/155/156, 20/33/104/106/124/156, 20/58/101/104/106/156, 20/58/101/106, 20/101/106/156, 21/33/58/101/106, 21/33/58/106/155/156, 21/33/101/104/106, 21/33/106, 21/58/101/104/106/155, 21/58/106/155/156, 21/101/104/106/156, 21/104/106, 21/104/106/124, 21/104/106/156, 30/33/58/104/106/155/156, 30/33/101/106/156, 30/33/104/106/155/156, 30/101/104/106/155/156, 30/104/106/155, 30/104/106/155/156, 30/106/155, 33/58/104/106, 33/101/104/106/155, 34/37, 34/37/92, 34/37/140/141/142/145, 34/37/141/142, 34/37/150/153, 34/92/94/141/142, 34/141/142/145, 34/150/152/153, 37/92/142, 37/141/142, 37/153, 58/101/104/106/156, 58/101/106/155, 58/104/106/155/156, 92/94/106/142/145, 101/104/106, 101/104/106/155/156, 101/104/106/156, 101/106, 101/106/124/155, 101/106/155/156, 104/106, 104/106/124, 104/106/155, 104/106/155/156, 104/106/156, 106/107/108/142/220, 106/108/140/141/152/153, 106/108/140/142/150/153, 106/156, 140/141/142, 140/145, 140/145/150/152, 141, 141/152, and 161, and/or any combinations thereof;

(vii) SEQ ID NO: 660 at amino acid positions selected from: 16/29/30, 16/29/30/33/153, 16/29/30/101/104, 16/30/104, 16/33, 29/30, 58, 92/94/108/141/155/392, 92/101/137/155/476, 94/101/156/476, 101, 101/104, 101/137/155, 101/141/155/156, 108, 195, 197, 204/342, 205, 236/297, 258, 261, 262, 264, 268, 269, 276, 278, 280, 281, 282, 290, 291, 297, 300, 303, 306, 308, 309, 310, 312, 315, 316, 342, 344, 353, 360, 385, 391, 410, 413, 419, 421, 448, 454, 456, 473, 476, 515, 525, and/or any combinations thereof;

(viii) SEQ ID NO: 882 at amino acid positions selected from 175, 179, 196, 199, 201, 203, 208, 272, 273, 275, 313, 314, 317, 319, 321, 322, 324, 325, 329/462, 350, 376, 379, 394, 397, 403/462, 404, 406, 408, 457, 461, 462, 469, 477, 481, 484, 491, 492, and 495, and/or any combinations thereof, wherein the amino acid positions are numbered with reference to SEQ ID NO: 882;
(ix) SEQ ID NO: 1100 at amino acid positions selected from 101/137/264/476/525 and 264, and/or any combinations thereof;
(x) SEQ ID NO: 1336 at amino acid positions selected from 61, 152/503, 160, 162, 165, 177, 200, 200/425, 213, 217, 219, 223, 236, 246, 248, 292, 292/411, 295, 326, 329, 330, 333, 334, 338, 340, 340/438, 363, 369, 370, 372, 373, 383, 400/401/402, 425, 427, 435, 435/503, 437, 440, 441, 442, 443, 444, 446, 459, 460, 488, 490, 501, 502, 503, 504, and 506, 15/199/203/394, 15/199/394, 28/344/353/395, 195/199/203/278/297/394, 195/199/203/278/314/353/394, 195/203/278/394/395, 195/203/297/314/394, 195/203/297/394/419, 195/203/394, 195/278/297/394, 195/278/297/394/395, 195/314/344, 195/394/395, 199/203/297/394/395, 203/278/297/394, 203/297/314/394/395, 203/310/314/394/395/419, 203/344/394, 203/353, 203/394, 203/394/395, 297/394, 314/394/395, 344/394/395, 353, and 394, and/or any combinations thereof;
(xi) SEQ ID NO: 1348 at amino acid positions selected from 3/175/213/313/325/340/457/481/485, 3/307/321/340/353/406/408/445, 148/175/201/457/485, 175/201/333/412/425/457/485, 175/325/397, 175/333, 175/333/369/481, 175/333/485, 175/485, 199/307/321/340/406/408/445/484, 201/213/333/344/397/425/481/485, 201/333/344/457/481, 201/333/481, 201/406/408/462/484/502, 213, 213/333/397, 307/333/340/408/445/462/502, 307/373/406/408/484, 321/333/340, 325/333/369/425, 325/425/457/481, 333, 333/344/369/397, 333/344/369/485, 340/484, 344/485, 353/406, 373, and 406/408, and/or any combinations thereof;
(xii) SEQ ID NO: 1596 at amino acid positions selected from 161, 162, 163, 165, 171, 177, 179, 188, 200, 205, 208, 231, 233, 251, 252, 253, 260, 261, 277, 306, 307, 321, 325, 327, 329, 330, 353, 368, 370, 371, 376, 380, 382, 393, 400, 402, 405, 406, 407, 408, 410, 413, 414, 419, 426, 441, 442, 446, 460, 464, 484, 488, 490, 495, 506, 508, 518, 520, 160/219/460/503/506, 160/165/203/205/219/353/406/408/442/446/460/488, 160/165/205/219/406/408/441/442/446, 160/203/205/406/408/441/442/446, 160/219/330/406/408/442/446/484, 205/307/406/408/441/442/446/460/488, 252/333, 406/408/441/442/446, 406/408/442/446219/307/326, 406/408, 406/408/442/446, and 406/408/490, and/or any combinations thereof;
(xiii) SEQ ID NO: 1654 at amino acid positions selected from 14/53/300, 14/53/419, 106/300/415/419/456, 140, 160, 160/163/165/203/205/219/353/414/441/460/488, 160/165/203/205/219/353/460/488, 160/165/179/203/205/208/219/353/376/413/414/441/488, 160/165/179/203/307/376/413/495, 160/165/203/205/219/353/460/488, 160/165/203/205/307/371/376/413/414, 160/165/203/208/371/376/413/414, 160/165/205, 160/165/205/208, 160/165/205/219/441, 160/165/205/414/441/495, 160/165/307/353/413/414/441/488/495, 160/179/203/205/208/219/371/414/484/506,

160/179/208/219/307/413/414/503/506, 160/179/208/307/371/376/414, 160/179/307/376/441/488/503, 160/203/205/208/219/414/460/506, 160/203/205/208/413/460/484/488, 160/203/205/441, 160/203/208/307/353/495, 160/203/208/371/413/414, 160/203/208/413/414/441/484, 160/203/326/353/413/414/484/495, 160/205/208/219/326/441/484/488/503, 160/208/326/376/414/441/484/488, 160/208/371/441/484/506, 160/208/414/441/452/480/488/495, 160/219/307/371/506, 160/219/330/484, 165/179/203/205/219/414/418/441/488/503, 165/179/203/205/484/503, 165/179/205/413/441, 165/179/208/353/413/414/503, 165/203/205, 165/203/205/307/414/441/484/495/503/506, 165/203/205/484/488, 165/203/208/326/376/503, 165/205, 165/208/326/413/414/484/495/506, 179, 179/203/205, 179/203/208/326/353/376/484, 179/205/208/353/414/441/460/484/488, 179/205/353, 179/208/353/460, 179/353, 203/205/208/307/330/353/441/460/503/506, 203/205/208/307/441, 203/205/208/353, 203/208/219/376/441, 203/208/219/441, 203/208/326/353, 203/413/503/506, 205/208/307/353/376/413, 205/208/414, 205/219/307/353, 205/307, 205/307/376/414/441/495, 205/307/441/460/488, 205/326/488/503/506, 208/488/506, 300/395/4193/160/205/208/219/307/353/371/376/413/414/441/488, 326/353/371/376/414, and 441, and/or any combinations thereof;

(xiv) SEQ ID NO: 1830 at amino acid positions selected from 163/179/277/338/340, 163/414/441, 171/200/334/406/490, 177, 194, 196, 266, 267, 268, 269, 273, 273/501, 274, 277, 278, 292/406, 297, 298, 299, 301, 302, 309, 312, 347, 359, 390, 392, 394, 407, 408, 413, 416, 454, 468, 473, 477, 479, and 493, and/or any combinations thereof;

(xv) SEQ ID NO: 1950 at amino acid positions selected from 163/169, 185, 186, 192, 193, 194, 197, 198, 245, 251, 258, 259, 261, 263, 271, 274, 278, 280, 284, 286, 290, 291, 297, 304, 306, 308, 316, 347, 352, 359, 362, 378, 393, 396, 398, 399, 405, 407, 409/414, 410/414, 411/414, 413/414, 414, 414/415, 414/417, 415, 455, 465, 466, 468, 494, and 509, and/or any combinations thereof;

(xvi) SEQ ID NO: 2008 at amino acid positions selected from 3, 8, 9, 11, 12, 15, 16, 26, 26/27, 36, 38, 39, 50, 58, 62, 70, 76, 79, 81, 90, 116, 147, 151, 157, 158, 158/274/411/413/414, 185/274/413/414, 185/411/413/414, 189, 196, 205, 206, 246, 248, 249, 253, 255, 262, 263/411/413/414/468, 263/413/414, 274/286/411/413/417/468, 274/411/417/468, 274/411/468, 274/413/414/417/468, 274/468, 278/411/413/468, 307, 314, 318, 324, 353, 397, 408, 410, 411/413/417, 411/413/417/468, 411/413/468, 411/414, 413, 413/414, 413/414/468, 413/417/468, 413/468, 414, and 468, 469, 473, 480, 481, 491, and 493, and/or any combinations thereof;

(xvii) SEQ ID NO: 2254 at amino acid positions selected from 197/407/455, 197/455, 284/398/466, 362/407/455, 396/398/410/466, 398/466, 399/411/416, and 466, and/or any combinations thereof;

(xviii) SEQ ID NO: 2514 at amino acid positions selected from 26/90/94/248/261/266/362/455, 26/90/246, 26/90/246/248, 26/90/246/248/261, 26/90/246/248/362/455, 26/90/246/248/455, 26/90/246/266/362, 26/90/246/362, 26/90/246/455, 26/90/248, 26/90/248/266/455, 26/90/248/455, 26/90/248/455/459, 26/90/266, 26/90/362/455, 26/173/248, 26/246, 26/246/248/362, 26/246/248/362/455, 26/246/248/455, 26/248, 26/248/261/266, 26/248/261/266/362/455, 26/248/266/362, 26/248/362/455,

26/248/455, 26/362, 26/362/455, 58/197/249/407/410, 62/249, 90/246/248, 90/246/248/261/266/362/455, 90/246/248/266/362/455, 246/248, 246/248/362, 246/266/455, 248, 248/266/362, 248/362/455, 248/455, and 362, and/or any combinations thereof;

(xix) SEQ ID NO: 2524 at amino acid positions selected from 9/16/62/157/246/249/362, 11/58/227/246, 12/16/158/246/248/249, 30/189/261/266/353/465/468, 47/326, 30/189/266, 30/261/266/353/468, 30/266, 30/266/303, 30/266/353, 38, 38/81/318, 38/197, 39, 39/79, 58/157/158/362, 70/353, 79/81, 81, 189, 189/261, 189/353, 246/249, 261/353, 266/307/353/468, 266/353/468, 266/468, 169, 175, 179, 191/413, 200, 203, 292, 293, 304/329, 325, 327/406, 329, 340, 353/459, 373, 379, 382, 402, 403, 404, 406, 427, 429, 459, 461, 484, 490, 495, 504, 506, and 508, and/or any combinations thereof;

(xx) SEQ ID NO: 2638 at amino acid positions selected from 11/30/79/189/480, 30/58/79/189/307/480, 79/189/307/410, and 79/307, and/or any combinations thereof;

(xxi) SEQ ID NO: 2804 at amino acid positions selected from 169/304/340/402/427, 169/304/340/427/429/504, 169/304/340/429/506, 169/304/340/504, 169/304/402/403/427/506, 169/304/403/427/504, 169/304/427/504/506, 169/304/504, 169/340/402/403/427/429/504, 169/340/402/403/427/504/506, 169/340/402/504, 169/340/427, 169/340/506, 169/402/403/504/506, 169/402/427/429/504, 169/402/504, 169/402/504/506, 169/403/427/506, 266/327/329/404/410, 292/327/329/468, 304, 304/340/402, 304/340/402/403/427/429/504/506, 304/340/402/403/504/506, 304/340/402/403/506, 304/340/402/427/504/506, 304/340/402/506, 304/340/403/427/429/504/506, 304/340/427, 304/402/403, 304/402/403/427/504, 304/402/403/429, 304/402/403/504/506, 304/402/403/506, 304/403/504, 304/504, 304/504/506, 327, 327/329, 327/329/379/404/406/410, 327/329/404/410, 327/329/465/484, 327/382/406/410/484, 327/410/484, 340, 340/402/403, 340/402/427/429/506, 340/402/429/504/506, 340/402/504, 340/403/504, 340/504, 340/506, 379/382/468, 379/404/410, 379/410, 379/465/468/484, 379/468, 402/403/427/504/506, 402/403/429/504, 402/427/504, 402/504, 403/427, 427, 484, 504, 504/506, and 506, and/or any combinations thereof;

(xxii) SEQ ID NO: 2812 at amino acid positions selected from 30, 186, 188, 198, 231, 233, 235, 243, 248, 253, 264, 266, 278, 287, 297/440, 326, 366, 367, 368, 370, 396, 414, 433, 435, 437, 439, 442, 444, 446, 485, 499, 503, 515, 520, 525, 30/179/200/373/403, 30/179/373/379, 30/184/246/325/379/429/495, 30/184/246/327/329/459, 30/184/246/379/404, 30/184/246/459/461/495/500/504, 30/200/373, 30/200/373/379, 30/246/325/327/329/404/461, 30/246/325/379/404/427/429/461, 30/246/427/459/461, 30/325/327, 30/325/327/379/404/429/495, 30/327/404/504, 30/329/379, 30/373, 30/373/403, 30/379/429/459/461, 30/379/459/461/504, 30/403/441/460, 200/373/379, 246/325/329/379/461, 246/327/404/461/495, 327/329/379/504, 327/459/461/495, 353/403, 373/379, 373/379/403, 379/403/406/468, and 403/441, and/or any combinations thereof;

(xxiii) SEQ ID NO: 2956 at amino acid positions selected from 140/142/153/177/427/434/441/444/461/502, 140/142/153/427/484, 140/142/177, 140/142/177/441, 140/142/365/373/404/427/484, 140/142/373/427/484, 140/148/161/177/404, 140/150/153/365/373/427/484, 140/150/177/404/436/441/484/502, 140/161/177/404/427/484,

140/177/404, 140/177/404/484, 142/150/158/177/427/445, 142/150/177/404, 142/153/177/441/444, 142/177/373, 142/177/373/441, 142/461/484/502, 150/177, 153, 153/161/325/404/427/441/484, 153/484, 177, 177/365/427/434, 325/427, 427, and 484, and/or any combinations thereof;

(xxiv) SEQ ID NO: 3174 at amino acid positions selected from 122/473, 189, 190, 193, 194, 196, 263, 264, 266, 267, 268, 269, 273, 273/501, 274, 278, 279, 281, 297, 298, 299, 300, 301, 302, 304, 309, 312, 315, 347, 350, 352, 353, 359, 390, 392, 394, 407, 408, 410, 411, 413, 414, 416, 436, 454, 468, 472, 473, 477, 479, 480, 493, 186/188/248/253/365/366/444/445, 186/231/248/253/484, 186/231/248/365/366/368/484, 186/231/248/366/368/444, 186/231/248/484, 186/231/368/416/441/442/444/484/485, 186/231/441/444/445, 186/484/485, 188/231/248/253/365/441/442/444/445/484, 198/243/264/431/441, 198/243/396/414/431/433/441/499, 198/243/396/414/433/437/441/515, 198/264/266/414, 198/264/396/414/433/441/515, 198/266/396/414/433/441/499/501, 198/266/414/433/441/515, 198/266/437/441/499, 198/414/431/441/499/520, 198/414/433/441/515/520, 231/248/441/484, 243/264/515/520, 243/396/414/433/441/515/520, 243/414/433/437/441, 243/414/437/441/515, 248/442/444/445/484, 264/266/396/414/433/441/515, 264/266/414/441/499/501/515/520, 264/414, 264/414/441, 264/433/441, 266/437/441/499/515/520, 365/366/441/484/485, 396/414/441, 396/414/441/515, and 414/441/520, and/or any combinations thereof;

(xxv) SEQ ID NO: 3222 at amino acid positions selected from 186/194/248/396, 186/198/243/248/366/368/394/501, 186/231/243/368/394/485/499, 186/231/248/485, 186/231/366/368/394/485, 186/243, 186/243/248/366/368/394, 186/243/248/394/484/485, 186/243/484/520, 186/248, 186/365, 186/365/366/368/394/499, 186/365/366/394/485, 186/366/368/394/396/484, 186/366/368/394/396/484/485, 186/394/396/485, 194/198/243/366/368/499, 194/515/520, 198/231/243/248/485, 198/243/248/365/394/501, 198/248/394/396/484/485/499, 198/394/396/484/485/499, 198/394/396/499/515/520, 198/394/499/501, 231/365/368/394/499/520, 231/368/394, 231/484/485/499/501, 243/248/394/396/484/485, 243/484, 243/484/485/499, 248/365/366/368/394/484/520, 248/394/484, 365/366/368/394, 365/368/394/396/520, 394/396, and 394/499, and/or any combinations thereof;

(xxvi) SEQ ID NO: 3670 at amino acid positions selected from 168, 198, 267, 273, 273/493, 273/493/499, 273/309/493/499, 273/309/413/499, 273/499, 274/299/408/416, 274/408/416, 274/416, 288/299/416, 298/299/416, 301, 303/396, 307, 308, 308/361, 309, 309/413, 309/499, 313, 372, 392, 397, 413, 413/493/499, 413/499, 415, 416, 419, 451, 452, 456, 472, 473, 475, 493/499, and 528, and/or any combinations thereof;

(xxvii) SEQ ID NO: 3674 at amino acid positions selected from 194/196/390, 194/196/390/394/460/480, 194/196/390/394/480, 194/196/390/454/480, 194/196/390/480, 194/196/394/454/480, 194/196/454, 194/390, 194/394, 194/394/454, 194/394/454/480, 196, 196/390, 196/390/394, 196/390/394/454, 196/390/394/454/480, 196/390/394/480, 196/390/454, 196/394, 196/394/454, 196/394/454/480,

196/394/480, 196/454, 297/470/473, 297/473/493, 390, 390/394, 390/394/454, 390/394/454/480, 390/394/480, 390/454, 390/480, 394, 394/480, and 454, and/or any combinations thereof;

(xxviii) SEQ ID NO: 3796 at amino acid positions selected from 198, 198/267/313/451/475/494/499, 198/267/314/451, 198/267/314/475, 198/267/409/451, 198/267/475, 198/451/493, 208/308, 208/308/461, 267, 267/314/328/451/494/499, 267/451, 267/451/494/499, 308, 314/328/451/499, 314/451, 328/409/451, 328/451, 409, 409/475/494, 451, 451/493/494, 451/493/499, 451/494, and 475, and/or any combinations thereof;

(xxix) SEQ ID NO: 2 or 660 at amino acid positions selected from 26, 30, 38, 79, 81, 90, 92, 94, 101, 108, 137, 140 141, 142, 153, 155, 160, 163, 165, 177, 184, 189, 194, 196, 201, 203, 205, 213, 219, 231, 246, 248, 258, 263, 264, 266, 267, 301, 304, 307, 314, 318, 325, 333, 340, 344, 353, 362, 379, 390, 392, 394, 395, 396, 397, 398, 402, 403, 406, 408, 410, 411, 413, 414, 416, 425, 427, 429, 433, 434, 441, 442, 444, 446, 451, 455, 460, 461, 466, 468, 476, 481, 484, 485, 488, 495, 499, 501, 502, 506, 515, 525, 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/194/196/201/203/205/213/219/231/248/258/263/264/266/267/301/304/307/314/318/325/333/340/344/353/362/379/390/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/451/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525, 26/30/38/79/81/90/92/94/101/108/137/140/141/142/153/155/160/163/165/177/184/189/201/203/205/213/219/231/248/258/263/264/266/304/307/314/318/325/333/340/344/353/362/379/392/394/395/397/398/402/403/406/408/410/411/413/414/416/425/427/429/433/434/441/442/444/446/455/460/461/466/468/476/481/484/485/488/495/499/501/502/506/515/525, 26/38/79/81/90/92/94/101/108/137/141/155/160/163/165/189/201/203/205/213/219/246/248/258/263/264/304/307/314/318/333/340/344/353/362/392/394/395/396/397/398/402/403/406/408/410/411/413/414/425/441/442/446/455/460/461/466/468/476/481/485/488/506/525, 92/94/101/108/137/141/155/160/163/165/201/203/205/213/219/258/263/264/314/333/344/353/392/394/395/397/406/408/411/413/414/425/441/442/446/460/461/468/476/481/485/488/525, and 92/94/101/108/137/141/155/201/213/264/314/333/344/392/394/395/397/406/408/425/442/446/461/476/481/485/525, and/or any combinations thereof;

(xxx) SEQ ID NO: 3870 at amino acid positions selected from 165, 169, 171, 173, 175, 176, 179, 183, 187, 191, 192, 195, 197, 199, 200, 203, 204, 210, 257, 259, 267, 291, 293, 295, 301, 319, 325, 340, 341, 342, 374, 387, 398, 399, 403, 404, 406, 429, 480, 481, 483, 484, 490, 491,493, 494, 495, 521, 507, 508, 509, and 522, and/or any combinations thereof;

(xxxi) SEQ ID NO: 3918 at amino acid positions selected from 3, 7/400/459/504, 164, 173/367/459/500, 184, 203, 203/367/459, 203/367/459/500/501, 203/400/459/501, 203/459, 203/459/499/504, 203/459/500, 215, 218, 294, 335, 335/402/481/484, 335/402/512, 335/481/484, 335/481/493, 335/481/512, 336, 338, 339, 367, 367/459/500, 370, 373, 376, 380, 384, 390, 395, 395/402, 395/402/481/484, 395/481, 395/481/512, 395/484, 400, 400/459, 400/459/499/500/504, 400/459/500/501, 400/459/501/504, 400/501, 400/504, 402, 402/481, 402/481/484, 402/481/484/512, 402/481/493, 402/484/493, 402/512, 458, 459,

459/501, 460, 481, 481/512, 484, 485, 493, 499, 500, 501, 504, 512, 515, and 516, and/or any combinations thereof;

(xxxii) SEQ ID NO: 4266 at amino acid positions selected from 95/428/480, 163/190, 163/203/366, 163/328/363/480, 163/363/480/485, 172/174/178/340, 178, 188, 190/202/203/363/366/480/483/485, 190/202/203/480, 190/480/485, 192, 192/498/499/503, 202, 202/203/328/362/363/366/428/480/485/498/499/503, 202/203/485, 203/328/363/428/483, 203/328/428, 203/328/480/485, 203/362/366, 203/498/499/503, 272, 280, 280/498/499/503, 296, 297, 299, 299/498/499/503, 301, 301/503, 308, 308/503, 311, 328/428, 328/480/483, 328/485, 343, 346, 349, 358, 359/498/499/503, 362/363, 362/363/366/428/480/483/498/499/503, 392/498/499/503, 406, 407, 410, 411, 418/498/499/503, 418/503, 419/503, 465, 471, 472/498/499/503, 473, 480/483, 491, and 498/499/503, and/or any combinations thereof;

(xxxiii) SEQ ID NO: 4558 at amino acid positions selected from 94/365/367, 172/174/178/401/403, 172/174/178/401/403/507, 172/174/178/402/508, 172/174/401/403/507, 172/178, 172/178/401, 174/178, 178/401/403, 178/402/403, 318/375/380, 324/379/405/483, 340, 340/394, 365, 365/367/428, 365/389/394, 367, 375/376, 375/379/483, 375/380, 375/380/400/483, 375/380/483, 376/483, 379/483, 389/394, 394, 401, 401/402/403/507, 402/403, 405/483, and 483, and/or any combinations thereof;

(xxxiv) SEQ ID NO: 4442 at amino acid positions selected from 174/296/299, 182, 185/190, 189/190, 190, 190/193, 192/280, 192/402/507, 257, 259, 260, 281, 289, 296/299, 305, 306, 307, 308, 312, 313, 316, 318, 327, 374, 381, 394, 395, 402, 402/507, 404, 405, 414, 432, 451, 455, 460, 461, 476/480, 480, 480/481, 493, 494, and 522, and/or any combinations thereof;

(xxxv) SEQ ID NO: 4654 at amino acid positions selected from 190, 190/197/308, 190/308/380/405, 190/375, 190/375/380, 190/380/405, 190/405/406, 272/301/393/394/480, 272/318/480/483, 301/394/480, 318, 375, 375/380, 375/405, 375/405/406, 380, 394, 394/480, and 480/483, and/or any combinations thereof;

(xxxvi) SEQ ID NO: 4850 at amino acid positions selected from 189, 189/193/207/307/353, 190/322, 193, 193/307, 261/322/421, 297/298/300/392, 297/300, 297/300/328, 298/300/328, 298/300/328/395, 298/300/360, 298/300/392/395, 298/300/392/395/492, 298/300/395, 298/300/481, 300, 300/392/395, 319, 322, 392, 421, and 492, and/or any combinations thereof;

(xxxvii) SEQ ID NO: 4856 at amino acid positions selected from 10/413, 260, 268, 302/307, 317, 353, 354, 362, 364, 392, 393, 394, 395, 397, 402, 404, 412, 413, 419, 436/512, 460, 477, 486, 490, 495, and 518, and/or any combinations thereof;

(xxxviii) SEQ ID NO: 4904 at amino acid positions selected from 190, 190/287/300/302, 190/300/477/490, 194/300/302/413, 194/300/302/481, 297/298/308/392/395, 298/392/525, 300, 300/317, 300/490, and 395, and/or any combinations thereof;

(xxxix) SEQ ID NO: 5002 at amino acid positions selected from 11/523, 190/194, 194, 194/198, 202, 208, 264, 273, 273/347/354, 274, 281, 290, 298/300/302, 298/302/392/393/394/433, 298/302/392/394, 298/392/393/394, 298/392/393/394/477, 298/392/394/490, 298/393/394/395/433/477, 298/393/394/477/495, 298/394/433, 300/302/303, 308/402/460, 309, 313, 314, 324, 352, 359, 360, 361, 392/393/394/433, 392/393/394/477, 392/393/394/477/495, 392/393/394/490, 392/394, 392/394/395, 392/394/433/477, 392/394/433/495, 392/394/477/495, 392/394/495, 393/394, 393/394/433/477/490, 394/477, 394/490, 405, 408/413, 411/413, 413, 460/525, 463, 466, 467, 472, 473, 477, 492, 523, and 526, and/or any combinations thereof;
(xl) SEQ ID NO: 5028 at amino acid positions selected from 82/194/198/313, 194, 194/198, 194/198/208/313, 194/198/309, 194/198/313, 194/198/411, 194/208/411, 194/309, 194/313, 194/411, 198, 198/208, 198/208/309/411, 198/208/313/411, 273/274, 274, 274/281/526, 274/359/526, 274/523, 309, 309/313/411, 324/526, 411, 466, 466/526, 523, and 526, and/or any combinations thereof;
(xli) SEQ ID NO: 5192 at amino acid positions selected from 96/295, 169, 176, 177, 179, 184, 187, 193, 195, 197, 197/307, 198, 199, 200, 203, 292, 295, 300/394, 304, 325, 326, 326/380, 329, 373, 376, 377, 383, 394, 403, 409, 430, 485, 508, and 520/526, and/or any combinations thereof; or
(xlii) SEQ ID NO: 5246 at amino acid positions selected from 177/198/200, 177/200/203, 177/200/203/295, 177/200/295/326, 180, 184/198/200/203/295, 184/200/295/326, 190/198/200/203, 190/200/203/295/380, 190/200/295, 197, 198, 198/200, 198/200/203, 198/200/203/295, 200/326, 200/380, 203, 203/380, 233, 252, 295, 336, 364, 365, 367, 381, 384, 441, 459, and 485, and/or any combinations thereof.

4. The engineered terminal deoxynucleotidyl transferase of any one of Claims 1-3, wherein (i) the polypeptide sequence further comprises an N-terminal truncation of 1-156 amino acids; and/or (ii) the engineered terminal deoxynucleotidyl transferase polypeptide is fused with a second polypeptide; optionally, wherein the second polypeptide has inorganic pyrophosphatase (IPP) activity, further optionally wherein (a) the second polypeptide with IPP activity comprises an amino acid sequence selected from SEQ ID NO: 3942 and 3944; or (b) the engineered terminal deoxynucleotidyl transferase polypeptide fused with the second polypeptide comprises a sequence selected from SEQ ID NO: 5468, 5470, 5472, and 5474.

5. The engineered terminal deoxynucleotidyl transferase of any one of Claims 1-4, (i) wherein said engineered terminal deoxynucleotidyl transferase is capable of template-independent synthesis; or

(ii) having at least one improved property, as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase; optionally wherein said improved property is selected from increased thermostability, increased activity at elevated temperatures, increased soluble expression or isolated protein yield, decreased by-product formation, increased specific activity on one or more NTP-3'-O-RBG or natural or modified NTP substrates, increased incorporation efficiency in extension of oligo acceptor substrates, and increased activity on one or more oligo acceptor substrates.

6. The engineered terminal deoxynucleotidyl transferase of any one of Claims 1-5, wherein
(i) said engineered terminal deoxynucleotidyl transferase comprises increased soluble expression or isolated protein yield as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase;
(ii) said engineered terminal deoxynucleotidyl transferase comprises increased thermal stability as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase; or
(iii) said engineered terminal deoxynucleotidyl transferase comprises increased activity on one or more NTP-3'-O-RBG or natural or modified NTP substrates as compared to a wild-type or reference terminal deoxynucleotidyl transferase or template-independent polymerase; and/or
(iv) said terminal deoxynucleotidyl transferase is purified.

7. A polynucleotide sequence encoding at least one engineered terminal deoxynucleotidyl transferase of any one of Claims 1-6.

8. A polynucleotide sequence comprising at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 1, 7, 11, 15, 23, 35, 53, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and 5245, and/or or a functional fragment thereof, wherein said polynucleotide sequence encodes an engineered polypeptide comprising at least one substitution at one or more amino acid positions; optionally wherein
(i) said polynucleotide sequence encodes at least one engineered terminal deoxynucleotidyl transferase comprising a sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638, 2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and 5246;
(ii) said sequence comprises SEQ ID NOs: 7, 11, 15, 23, 35, 53, 267, 647, 659, 881, 1099, 1335, 1347, 1595, 1653, 1829, 1949, 2007, 2253, 2513, 2523, 2637, 2803, 2811, 2955, 3173, 3221, 3669, 3673, 3795, 3869, 3917, 4265, 4441, 4653, 4849, 4855, 4903, 5001, 5027, 5191, and 5245;

(iii) said polynucleotide sequence is operably linked to a control sequence; or
(iv) said polynucleotide sequence is codon-optimized.

9. An expression vector comprising at least one polynucleotide sequence of any one of Claims 7 or 8.

10. A host cell comprising at least one expression vector of Claim 9.

11. A method of producing an engineered terminal deoxynucleotidyl transferase polypeptide in a host cell comprising culturing a host cell of Claim 10, under suitable culture conditions, such that at least one engineered terminal deoxynucleotidyl transferase is produced; optionally wherein the method further comprises

(i) recovering at least one engineered terminal deoxynucleotidyl transferase from the culture and/or host cells; and/or
(ii) the step of purifying said at least one engineered terminal deoxynucleotidyl transferase.

12. A composition comprising at least one engineered terminal deoxynucleotidyl transferase of any one of Claims 1-6.

13. A method for template-independent synthesis of an oligonucleotide, the method comprising:

a) providing at least one terminal deoxynucleotidyl transferase or template-independent polymerase;
b) providing at least one oligo acceptor substrate, wherein the oligo acceptor substrate comprises a 3'-OH, or an equivalent thereof; and
c) contacting the oligo acceptor substrate, the terminal deoxynucleotidyl transferase or template-independent polymerase, and one or more nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBG under conditions sufficient for the addition of the nucleotide, modified nucleotide, or nucleotide-3'-O-RBG to the 3' end of the oligo acceptor substrate.

14. The method of Claim 13, wherein
(i) the method further comprises (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position of the oligonucleotide product;
(ii) the method further comprises e) deactivating unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs;
(iii) the method further comprises an optional step (f) of removing excess nucleoside and/or excess

inorganic phosphate or pyrophosphate from the reaction;
(iv) the method further comprises repeating steps (a)-(c) or (a)-(d) or (a)-(e) or (a)-(f) until a desired oligonucleotide sequence is obtained;
(v) the method further comprises (g) cleaving or releasing the growing or completed oligonucleotide chain;
(vi) step (d) deblocking the oligonucleotide formed in step (c) at the protected 3'-O-position and step (e) deactivating the unreacted nucleotide triphosphates, modified nucleotide triphosphates, or NTP-3'-O-RBGs occur simultaneously;
(vii) a phosphatase is used to deblock the NTP-3'-O-RBG and to deactivate unreacted NTP-3'-O-RBGs by removing at least one of the 5' phosphates, preferably wherein the phosphatase comprises an alkaline phosphatase set forth in SEQ ID NO: 3922, 3924, 3926, 3928, 3930, 3932, or 3934;
(viii) step (c) optionally includes contacting the oligo acceptor substrate, the terminal deoxynucleotidyl transferase or template-independent polymerase, and either a nucleotide triphosphate, or a modified nucleotide triphosphate, or an NTP-3'-O-RBG with a phosphatase, such as an inorganic pyrophosphatase, to convert pyrophosphate to inorganic phosphate, preferably wherein the phosphatase comprises an inorganic pyrophosphatase set forth in SEQ ID NO: 3936, 3938, 3940, 3942, 3944, 3946, or 3948;
(ix) the method further comprises an NTP-3'-O-RBG comprising a carbonitrile, phosphate, carbonate, carbamate, ester, ether, borate, nitrate, sugar, phosphoramidate, phenylsulfenate, or sulfate;
(x) the nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG comprise a 2' modification, preferably wherein the 2' modification comprises a 2'-F, 2'-O-alkyl, 2'-O-methoxyethyl, or a locked or constrained ethyl, and optionally wherein (a) the modification is 2'-F and the 2' modified nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG is selected from: 2'-fluoro-2'-deoxyadenosine-5'-triphosphate, 2'-fluoro-2'-deoxycytidine-5'-triphosphate, 2'-fluoro-2'-deoxyguanosine-5'-triphosphate, and 2'-fluoro-2'-deoxyuridine-5'-triphosphate; or (b) the modification is 2'-O-alkyl and the 2' modified nucleotide triphosphate, the modified nucleotide triphosphate, or the NTP-3'-O-RBG is selected from: 2'-O-methyladenosine-5'- triphosphate, 2'-O-methylcytidine-5'-triphosphate, 2'-O-methylguanosine-5'-triphosphate, 2'- O-methyluridine-5'-triphosphate, and 2'-O-methylinosine-5'-triphosphate;
(xi) the oligonucleotide chain comprises one or more phosphorothioate linkages;
(xii) the oligonucleotide, oligonucleotide sequence, or oligonucleotide chain comprises RNA;
(xiii) the oligonucleotide, oligonucleotide sequence, or oligonucleotide chain comprises DNA;
(xiv) the terminal deoxynucleotidyl transferase or template-independent polymerase comprises one or more engineered terminal deoxynucleotidyl transferases, optionally wherein (a) the engineered terminal deoxynucleotidyl transferase of any one of claims 1-54; (b) the engineered terminal deoxynucleotidyl transferase comprises a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference sequence of SEQ ID NO: 2, 8, 12, 16, 24, 36, 54, 268, 648, 660, 882, 1100, 1336, 1348, 1596, 1654, 1830, 1950, 2008, 2254, 2514, 2524, 2638,

2804, 2812, 2956, 3174, 3222, 3670, 3674, 3796, 3870, 3918, 4266, 4442, 4654, 4850, 4856, 4904, 5002, 5028, 5192, and/or 5246, or a functional fragment thereof, and comprises an amino acid residue difference or set of amino acid residue differences in its polypeptide sequence relative to the reference sequence, wherein the amino acid residue difference or set of differences is selected from the amino acid differences listed in: Tables 5.1, 6.2, 7.2, 8.2, 9.2, 10.2, 11.2, 12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 26.3, 26.4, 27.2, 27.3, 27.4, 27.5, 28.1, 28.2, 28.3, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2, 36.2, 37.2, 38.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2 46.2, 47.2, 48.2, 49.2, 50.2, 51.2, 52.2, 53.2, 54.2, 55.2, 56.2, 56.3, 56.4, 61.2, 63.2, 64.2, 65.2, 66.2, 67.2, 68.2, 69.2, 70.2, 71.2, 72.2, 73.2, 74.2, 75.2, 76.2, 77.2, 78.2, 79.2, and 80.1; or (c) the engineered terminal deoxynucleotidyl transferase comprises a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity to an amino acid sequence selected from the even-numbered sequences SEQ ID NO: 4-1960, 2004-3920, 4048-5466, and 5476;

(xv) (a) the terminal deoxynucleotidyl transferase or template-independent polymerase is immobilized, optionally wherein the method further comprises a column solid support for immobilization of the terminal deoxynucleotidyl transferase or template-independent polymerase; or the method further comprises a batch method with a solid support for immobilization of the terminal deoxynucleotidyl transferase or template-independent polymerase;

(b) the oligoacceptor substrate and subsequent oligonucleotide products are immobilized; or

(c) neither the oligoacceptor substrate nor the terminal deoxynucleotidyl transferase of template-independent polymerase are immobilized; and/or

(xvi) the method further comprises an aqueous liquid phase.

15. The engineered terminal deoxynucleotidyl transferase of any one of Claims 1-6, wherein the engineered terminal deoxynucleotidyl transferase is immobilized.