CLAIMS

We claim:

1. An engineered phosphopentomutase comprising a polypeptide sequence having at least 85% or more sequence identity to SEQ ID NO: 4, wherein the polypeptide sequence of said engineered phosphopentomutase comprises a substitution of D173A and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

2. The engineered phosphopentomutase of Claim 1, wherein said polypeptide sequence has at least 85% or more sequence identity to SEQ ID NO:2, and wherein the polypeptide sequence of said engineered phosphopentomutase further comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 65, 77/118/147/154/231/357, 98, 99, 114, 117, 118/147/154/225/231, 118/147/154/225/233/257/357, 118/147/154/225/257/357, 118/147/154/231/309, 118/147/225/231/257/309/357, 118/147/225/231/357, 118/147/225/234/257/357, 118/147/231, 118/147/231/257/308, 118/147/231/257/309/357, 118/154/225/231/357, 118/154/225/234/257/357, 118/154/231, 118/154/231/257/309/357, 118/154/231/257/357, 118/154/231/309/338/357, 118/154/231/357, 118/231, 118/231/257/357, 145, 146, 147, 147/154/225/231/309/357, 147/231, 147/231/257/357, 147/231/309/357, 147/231/357, 153, 153/231, 154/231/309, 154/231/309/357, 154/231/354/357, 225/231/257/309/357, 231, 233, 314, and 357, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

3. The engineered phosphopentomutase of Claim 1, wherein said polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 4, and wherein said polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions selected from 64/98/99/146/179/231/261/357, 65/67/99/114/117/135/147/154/231/233, 65/67/99/114/117/135/147/154/231/257, 65/67/99/114/135/147/179/231/233, 65/67/99/114/135/154/231/263, 65/67/99/117/135/147/179/231/233/263, 65/67/99/117/135/154/179/231/233, 65/97/99/117/135/147/154/231/257, 65/97/99/135/154/179/231/233/263, 65/97/99/231/257, 65/99/114/117/135/154/231, 65/99/114/117/135/231, 65/99/114/135/147/154/231/233, 65/99/114/135/147/154/231/257, 65/99/114/135/147/179/231/233/257, 65/99/114/135/147/231/233/257/263, 65/99/114/135/231/233/263, 65/99/114/147/154/179/231, 65/99/114/231/233, 65/99/135/154/179/231/233/263, 67/99/114/117/135/147/231/233, 67/99/114/117/135/147/231/257, 67/99/114/135/147/179/231/233/263, 67/99/114/135/147/231/257/263, 67/99/114/135/154/231/257/263, 67/99/117/135/154/179/231, 98/99/146/153/179/231/357, 98/99/146/153/231/261/357, 98/99/146/153/231/261/357/397, 98/99/146/153/231/357,

98/99/146/179/231/261/357, 98/99/146/179/231/261/357/397, 98/99/146/179/231/357, 98/99/146/179/231/357/397, 98/99/146/231, 98/99/146/231/261/357, 98/99/146/231/261/357/397, 98/99/146/231/357, 98/99/146/261/357/397, 98/99/146/357, 98/99/231/261/357/397, 98/99/231/357/397, 98/99/357, 98/146/179/231/261/357, 98/146/231/357, 98/146/231/357/397, 98/153/231/357/397, 99/146/153/179/231/261/357, 99/146/153/231/261/357/397, 99/146/179/231/261/357, 99/146/179/231/261/357/397, 99/146/179/231/357, 99/146/231/261/357, 99/146/231/261/357/397, 99/146/231/357, 99/146/261/357/397, and 100, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 118, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions selected from 64/99/114/231/357, 65, 67, 87/225, 99/114, 99/114/147/231/261/397, 99/114/147/231/357, 99/114/147/257, 99/114/147/261, 99/114/153, 99/114/153/231/357, 99/114/153/261, 99/114/153/261/357, 99/114/231, 99/114/231/261, 99/114/231/357, 99/114/257/261/357, 99/114/357, 99/231/257/261, 99/257, 114/231/257/261, 114/231/357/397, 114/257/357, 114/392, 154, 211, 211/316, 225, 234/310, 257, 257/261, 257/357, 263, 350, 357, and 359, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 118.

5. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 266, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions selected from 65/225, 65/225/263, 97, 118, 143/194, 147, 156, 160, 172, 176, 188, 192, 194, 207, 211, 217, 225, 227, 236, 238, 257, 257/261, 261, 263, 306, and 397, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 266.

6. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 266, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions selected from 116/238, 118/147/192/238, 118/147/225/257, 118/147/238/257/357, 118/156/192/194/231/257, 118/194/238, 118/231/257/357, 118/238, 118/257/357, 147/194/217/225/236/257, 147/225/238/357, 147/236/238/257, 156/192/217/236/257, 156/217/236/257/357, 156/236/238, 192/238, 192/238/357, 225/238/357, 227, 231/257, 236, 236/238/257, 238/257, and 257, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 266.

7. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 420, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions selected from 12, 94, 118/147/227/238/257/357, 118/151, 118/236/357, 121, 142, 148, 150, 151, 172, 181, 185, 186, 189, 193, 199, 206, 218, 235, 237, 239, 256, 266, 267, 281, 300, 308, and 383, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 420.

8. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 420, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 98/151/172/235/256/266/267, 98/256, 108/235/267, 148/150/172/235, 148/151/172/235/256, 148/151/235/256/301, 148/151/235/267, 150/151/172/235/260, 150/151/235, 150/172/235/267/301, 150/235, 150/267, 151, 151/172/235, 151/172/235/256/301, 151/235/260, 151/256, 151/267/301, 172, 172/235, 172/235/237/256/267, 172/267/301/312, 235, 235/256/267, 235/256/267/307, 235/260, 235/267, 235/267/301, 256, 260, 267, 267/301, 301, 307, and 312, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 420.

9. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 562, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 21, 118, 120, 151, 155, 220, 225, and 357, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 562.

10. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 562, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 21/66/120/155, 21/117/120/139, 21/117/120/151/155/225, 21/117/120/222, 21/117/222/225, 21/120, 21/120/139, 21/120/151, 21/120/151/155/222/225, 21/120/151/222/225, 21/120/151/225/316, 21/120/155, 21/120/222/357, 21/139/151/357, 21/139/357, 21/151/155/222/225, 21/225/357, 21/357, 50/357, 117/120/151/155, 117/120/151/222, 117/120/151/222/225, 117/120/225, 117/139/151/225, 117/151/155/222/357, 117/155/222/357, 117/220/225, 120, 120/139, 120/139/151, 120/139/151/222, 120/139/222, 120/139/357, 120/151/155, 120/151/155/222/225, 120/151/155/225, 120/151/222,

120/151/222/225, 120/151/225, 120/155, 120/155/222, 120/155/225, 120/155/357, 120/220, 120/220/222/357, 120/220/225, 120/222, 120/225, 120/225/357, 120/225/401, 120/357, 139/357, 151/155, 155/222/225/357, 220/357, 222/357, 298/357, and 357, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 562.

11. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 656, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 6, 8, 39, 46, 53, 108, 133, 151, 160, 192, 196, 200, 217, 225, 236, 239, 251, 257, 272, 284, 335, 341, 368, 369, 391, and 397, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 656.

12. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 790, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 8, 8/53, 8/53/120/225/257/272, 8/53/146/151/225/227/257, 8/53/151/192/227/357/369/397, 8/53/151/225, 8/53/151/225/227, 8/53/151/225/227/257/272/369, 8/53/151/257/272/357, 8/53/151/272/369, 8/53/151/357, 8/53/257/272/357, 8/53/369, 8/120/151, 8/151, 8/151/192/227/257/369, 8/151/192/227/357/397, 8/151/225/257/357/397, 8/151/225/357, 8/151/227/257/272, 8/151/257/272/397, 8/225/257/272, 8/225/257/357/369, 8/272, 8/357, 9, 53/151, 53/151/272, 53/151/357, 53/151/357/369, 53/192, 53/257/272/357, 53/257/357, 53/272, 53/397, 99, 118, 120, 151/192/257, 151/225/357, 151/227/257, 151/257, 151/357/369, 172, 227/272/357/369, 235, 238, 256, 257, 257/272, 257/357, 355, 357, and 357/397, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 790.

13. The engineered phosphopentomutase of Claim 1, wherein the polypeptide sequence of said engineered phosphopentomutase has at least 85% or more sequence identity to SEQ ID NO: 846, and wherein the polypeptide sequence of said engineered phosphopentomutase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 6, 7, 9/108/118/172/192/238/355, 9/108/118/172/235/238/256/257/355, 9/108/118/192/256/257/355, 9/108/192/256/257/355, 9/118, 9/118/172/192/257/355, 9/118/172/192/355, 9/118/172/235/238/257, 9/118/172/256/355, 9/118/235/256/355, 9/118/355, 9/172/192/235/238/256/355, 9/172/192/235/256, 9/172/192/355, 9/192/235/256, 9/192/235/355, 9/192/355, 9/235/238/256/355, 9/235/256/257/355, 9/256/355, 9/355, 17, 22, 22/62, 52, 58, 62, 65, 68, 76, 82, 87, 92, 108/118/172/235/238/256/257/355, 108/118/172/256/257/355, 108/172/192/235/238/256/355, 108/172/192/238/256/355, 114, 118,

118/172/235, 118/172/256/257, 118/235/256/257/355, 118/256, 118/355, 126, 133, 137, 139, 151, 155, 172/192/355, 172/235/238/256/257/355, 172/256/257/355, 172/257/355, 180, 188, 189, 192/235/256/355, 192/355, 200, 217, 219, 221, 225, 231, 235, 235/256/257/355, 251, 257, 266, 286, 287, 288, 296, 308, 324, 327, 332, 333, 334, 335, 337, 338, 341, 355, 363, 389, 391, and 393, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 846.

14. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises a polypeptide sequence that is at least 85% or more identical to the sequence of at least one engineered phosphopentomutase variant set forth in Table 4.1, 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 12.1, 12.2, 13.1, 14.1, and/or 15.1.

15. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises a polypeptide sequence that is at least 85% or more identical to SEQ ID NO: 2, 4, 118, 266, 420, 562, 656, 790, and/or 846.

16. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises a variant engineered phosphopentomutase set forth in SEQ ID NO: 4, 118, 266, 420, 562, 656, 790, and/or 846.

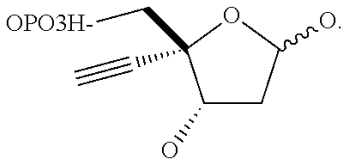
17. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises a polypeptide sequence that is at least 85% or more identical to the sequence of at least one engineered phosphopentomutase variant set forth in the even numbered sequences of SEQ ID NOS: 8-1152.

18. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises a polypeptide sequence set forth in at least one of the even numbered sequences of SEQ ID NOS: 8-1152.

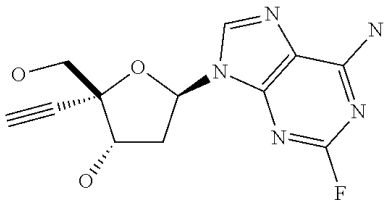
19. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase comprises at least one improved property compared to wild-type E.*coli* phosphopentomutase.

20. The engineered phosphopentomutase of Claim 19, wherein said improved property comprises improved activity on a substrate.

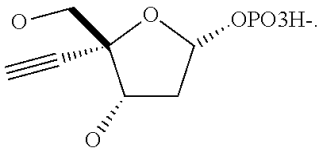
21. The engineered phosphopentomutase of Claim 20, wherein said substrate comprises compound 4:



22. The engineered phosphopentomutase of Claim 19, wherein said improved property comprises improved production of compound 1:



and/or compound 3:



23. The engineered phosphopentomutase of Claim 1, wherein said engineered phosphopentomutase is purified.

24. A composition comprising at least one engineered phosphopentomutase of Claim 1.

25. A polynucleotide sequence encoding at least one engineered phosphopentomutase of Claim 1.

26. A polynucleotide sequence encoding at least one engineered phosphopentomutase of Claim 1, said polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOS: 1, 3 117, 265, 419, 561, 655, 789, and/or 845, wherein the polynucleotide sequence of said engineered phosphopentomutase comprises at least one substitution at one or more positions.

27. A polynucleotide sequence encoding at least one engineered phosphopentomutase of Claim 1, comprising at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOS: 1, 3 117, 265, 419, 561, 655, 789, and/or 845, or a functional fragment thereof.

28. The polynucleotide sequence of Claim 25, wherein said polynucleotide sequence is operably linked to a control sequence.

29. The polynucleotide sequence of Claim 25, wherein said polynucleotide sequence is codon optimized.

30. The polynucleotide sequence of Claim 25, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 7-1151.

31. An expression vector comprising at least one polynucleotide sequence of Claim 25.

32. A host cell comprising at least one expression vector of Claim 31.

33. A host cell comprising at least one polynucleotide sequence of Claim 25.

34. A method of producing an engineered phosphopentomutase in a host cell, comprising culturing the host cell of Claim 32, under suitable conditions, such that at least one engineered phosphopentomutase is produced.

35. The method of Claim 34, further comprising recovering at least one engineered phosphopentomutase from the culture and/or host cell.

36. The method of Claim 34, further comprising the step of purifying said at least one engineered phosphopentomutase.