CLAIMS

We claim:

1. An engineered acetate kinase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 2, or a functional fragment thereof, wherein said engineered acetate kinase comprises a substitution at position 298 in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

2. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 2, or a functional fragment thereof, and wherein said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions selected from 12/259, 15, 16, 18, 23, 25, 26, 31, 32, 39, 40, 41, 44, 47, 47/411, 50, 51, 55, 58/135, 59, 64, 70, 76, 76/232/262, 76/232/364/386, 76/273, 85, 92, 96/119, 101, 102, 104, 107, 108, 116, 122, 135, 135/392, 136, 137, 140, 141, 143, 144, 145/400, 152, 154, 159, 161, 164, 183, 191, 194, 197, 215, 216, 222, 224, 229, 232, 236, 251, 256, 258, 259/284, 260, 261, 263/391, 268, 273, 274, 276, 283, 284, 286, 287, 288, 289, 290, 292, 297, 298/405, 299, 300, 301, 302, 303, 304, 305, 308, 310, 312, 314, 316, 317, 322, 323, 337, 346, 347, 348, 349, 351, 352, 352/405, 354, 355, 356, 362, 366, 368, 371, 372, 373, 374, 375, 376, 377, 378, 380, 386, 390, 391, 392, 398, 399, and 407, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

3. The engineered acetate kinase of Claim 1 or Claim 2, comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 12, or a functional fragment thereof.

4. The engineered acetate kinase of any one of Claims 1 to 3, and wherein said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions selected from 15/154/191/297/298/301//352/391, 15/154/297/298/348/352/355/391, 15/191/297/298/301/348/352/355/391, 15/191/298/352/355, 15/297/298/355, 29/154/191/298/348/355, 70/154/191/298/348/352/355/391, 70/154/191/298/352, 70/154/297/298/348/355, 70/191/297/298/352/391, 154/191/297/298, 154/191/297/298/301/352/355, 154/191/297/298/352, 154/191/298/301/348/352/391, 154/191/298/348/352, and 154/297/298, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 12.

5. The engineered acetate kinase of any one of Claims 1 to 4, wherein said engineered acetate kinase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered acetate kinase variant set forth in Table 2-1, Table 3-1, and/or Table 4-1.

6. The engineered acetate kinase of any one of Claims 1 to 5, wherein said engineered acetate kinase comprises a variant engineered polypeptide set forth in SEQ ID NO: 12.

7. The engineered acetate kinase of any one of Claims 1 to 6, wherein said engineered acetate kinase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered acetate kinase variant set forth in the even numbered sequences of SEQ ID NOS: 2-696.

8. The engineered acetate kinase of any one of Claims 1 to 7, wherein said engineered acetate kinase comprises at least one improved property compared to wild-type *M. thermatoga* acetate kinase.

9. The engineered acetate kinase of Claim 8, wherein said improved property comprises improved activity on a substrate, as compared to a wild-type acetate kinase; optionally wherein said substrate comprises adenosine diphosphate and acetyl phosphate.

10. The engineered acetate kinase of Claim 9, wherein said improved property comprises improved production of adenosine triphosphate, as compared to a wild-type acetate kinase.

11. The engineered acetate kinase of any one of Claims 1 to 10, wherein said engineered acetate kinase is purified.

12. The engineered acetate kinase of any one of Claims 1 to 11, wherein said engineered acetate kinase is part of a multi-enzyme system for producing a nucleoside analogue.

13. A composition comprising at least one engineered acetate kinase of any one of Claims 1 to 12.

14. A polynucleotide sequence encoding at least one engineered acetate kinase of any one of Claims 1 to 12.

15. A polynucleotide sequence encoding at least one engineered acetate kinase, wherein 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 NO: 1, wherein the polynucleotide sequence of said engineered acetate kinase comprises a substitution at a position encoding amino acid position 298, numbered with reference to SEQ ID NO: 2.

16. The polynucleotide sequence of Claim 14 or Claim 15, wherein said polynucleotide sequence is operably linked to a control sequence.

17. The polynucleotide sequence of any one of Claims 14 to 16, wherein said polynucleotide sequence is codon optimized.

18. The polynucleotide sequence of any one of Claims 14 to 17, wherein said polynucleotide comprises an odd-numbered sequence of SEQ ID NOS: 1-695.

19. An expression vector comprising at least one polynucleotide sequence of any one of Claims 14 to 18.

20. A host cell comprising at least one expression vector of Claim 19 or at least one polynucleotide sequence of any one of Claims 14 to 18.

21. A method of producing an engineered acetate kinase in a host cell, comprising culturing the host cell of Claim 20, under suitable conditions, such that at least one engineered acetate kinase is produced.

22. The method of Claim 21, further comprising recovering at least one engineered acetate kinase from the culture and/or host cell.

23. The method of Claim 21 or Claim 22, further comprising the step of purifying said at least one engineered acetate kinase.