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, SEQ ID NO: 12, and/or SEQ ID NO: 600, or a functional fragment thereof, wherein said engineered acetate kinase comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2, SEQ ID NO: 12, and/or SEQ ID NO: 600.~~

1. ~~The~~ An engineered acetate kinase ~~of Claim 1,~~ 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 ~~at least one~~a substitution ~~or substitution set~~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.

~~3~~2. The engineered acetate kinase of Claim ~~2~~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 ~~298,~~ 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.

~~4~~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~~, wherein said engineered acetate kinase comprises at least one substitution or substitution set in said~~

~~polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ~~

~~ID NO: 12~~.

~~5~~4. The engineered acetate kinase of any one of Claims 1 to 3 ~~2~~, ~~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: 12, 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 ~~15/70/154/297/352/355,~~ 15/154/191/297/298/301//352/391, ~~15/154/191/297/355,~~ 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, ~~23/101/102/122/140/143/316/372, 23/101/102/140, 23/101/374, 23/122/316/372/374, 23/122/316/374/395, 23/140/316/374,~~ 29/154/191/298/348/355, ~~51/101/102/135/242/316/374, 51/101/316, 70/154/162/191/297/301/355/391, 70/154/191/297/352,~~ 70/154/191/298/348/352/355/391, 70/154/191/298/352, 70/154/297/298/348/355, ~~70/154/297/352/355,~~ 70/191/297/298/352/391, ~~101/102/122/140/142/316/372/374, 101/136/242/372, 102/135/140/316, 102/136/140/142/143/316, 102/142/316, 122/140/142/164/242, 122/142/316, 122/143/242/374, 135/136/140/142/143/242/316/372/374, 135/140/143/242/374/395, 135/140/316, 136/242, 142/316, 142/316/372/374, 142/316/374, 143/316,~~ 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.

~~6. The engineered acetate kinase of Claim 1, 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: 600, or a functional fragment thereof, wherein said engineered acetate kinase comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 600.~~

~~7. The engineered acetate kinase of Claim 2, 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: 600, or a functional fragment thereof, and wherein said engineered acetate kinase comprises at least one substitution or substitution set at one or more positions selected 23/242/374, 23/374, 70/374, 242/298, 242/374, 298/374, and 374, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 600.~~

~~8~~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.

~~9. The engineered acetate kinase of Claim 1, 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 SEQ ID NO: 2.~~

~~10. The engineered acetate kinase of Claim 1, 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 SEQ ID NO: 12.~~

~~11~~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.

~~12. The engineered acetate kinase of Claim 1, 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 SEQ ID NO: 600.~~

~~13. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase comprises a variant engineered polypeptide set forth in SEQ ID NO: 600.~~

~~14~~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.

~~15. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase comprises a polypeptide sequence forth in the even numbered sequences of SEQ ID NOS: 2-696.~~

~~16~~8. The engineered acetate kinase of any one of Claims 1 to 7~~-15~~, wherein said engineered acetate kinase comprises at least one improved property compared to wild-type *M. thermatoga* acetate kinase.

~~17~~9. The engineered acetate kinase of Claim ~~8~~16, wherein said improved property comprises improved activity on a substrate, as compared to a wild-type acetate kinase; optionally~~.~~

~~18. The engineered acetate kinase of Claim 17,~~ wherein said substrate comprises adenosine diphosphate and acetyl phosphate.

~~19~~10. The engineered acetate kinase of Claim ~~16~~9, wherein said improved property comprises improved production of adenosine triphosphate, as compared to a wild-type acetate kinase.

~~20~~11. The engineered acetate kinase of any one of Claims 1 to 10~~-19~~, wherein said engineered acetate kinase is purified.

~~21~~12. The engineered acetate kinase of any one of Claims 1 to ~~-20~~11, wherein said engineered acetate kinase is part of a multi-enzyme system for producing a nucleoside analogue.

~~22~~13. A composition comprising at least one engineered acetate kinase of any one of Claims 1 to 12~~-21~~.

~~23~~14. A polynucleotide sequence encoding at least one engineered acetate kinase of any one of Claims 1 to 12~~-21~~.

~~24~~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, ~~, SEQ ID NO: 11, and/or SEQ ID NO: 599,~~ wherein the polynucleotide sequence of said engineered acetate kinase comprises ~~at least one~~a substitution at a ~~one or more~~ position encoding amino acid position 298, numbered with reference to SEQ ID NO: 2~~s~~.

~~25. The polynucleotide sequence of Claim 23, encoding at least one engineered acetate kinase 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 NO: 1, or a functional fragment thereof.~~

~~26. The polynucleotide sequence of Claim 23, encoding at least one engineered acetate kinase 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 NO: 11, or a functional fragment thereof.~~

~~27. The polynucleotide sequence of Claim 23, encoding at least one engineered acetate kinase 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 NO: 599, or a functional fragment thereof.~~

~~28~~16. The polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~23~~14 or Claim ~~27~~15, wherein said polynucleotide sequence is operably linked to a control sequence.

~~29~~17. The polynucleotide sequence of any one of Claims ~~23~~14 to ~~28~~16, wherein said polynucleotide sequence is codon optimized.

~~30~~18. The polynucleotide sequence of any one of Claims ~~23~~14 to ~~29~~17, wherein said polynucleotide comprises an odd-numbered sequence of SEQ ID NOS: 1-695.

~~31~~19. An expression vector comprising at least one polynucleotide sequence of any one of Claims ~~23~~14 to ~~30~~18.

~~32~~20. A host cell comprising at least one expression vector of Claim ~~31~~19 or~~.~~

~~33. A host cell comprising~~ at least one polynucleotide sequence of any one of Claims ~~23~~14 to ~~30~~18.

~~34~~21. A method of producing an engineered acetate kinase in a host cell, comprising culturing the host cell of Claim ~~32~~ 20~~and/or 33~~, under suitable conditions, such that at least one engineered acetate kinase is produced.

~~35~~22. The method of Claim ~~34~~21, further comprising recovering at least one engineered acetate kinase from the culture and/or host cell.

~~36~~23. The method of Claim ~~34~~ 21 ~~and/~~or ~~35~~Claim 22, further comprising the step of purifying said at least one engineered acetate kinase.