CLAIMS

We claim:

1. An engineered acetate kinase comprising a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NOs: 2, or a functional fragment thereof, wherein the polypeptide sequence of said engineered acetate kinase comprises at least one substitution at position 294 selected from the group consisting of 294L/F, or substitution set comprising a substitution at position 294 and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NOs: 2.

2. The engineered acetate kinase of Claim 1, wherein said polypeptide sequence has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, and wherein the polypeptide sequence of said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 4/142/242, 12/76/164/284, 12/76/232, 15, 23, 39, 41, 47, 51, 52, 54, 57, 76, 76/164/232/262/386, 76/164/262/284, 76/232/262/284/386, 76/232/364/386, 76/262, 76/262/273, 76/262/364, 76/273, 76/284/299, 76/364, 130, 135/284, 135/392, 145/400, 191, 232, 232/386, 275, 285, 288, 290, 292, 297, 298, 298/377, 298/405, 343, 372, 373, 374, 376, 391, and 392, 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, wherein said polypeptide sequence of said engineered acetate kinase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, and wherein said polypeptide sequence of said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions selected from 4/142/242, 12/76/164, 15, 23, 39, 41, 44, 45, 47, 47/411, 48, 50, 51, 53, 55, 57/135, 58, 58/135, 61, 76, 76/142/299/386, 76/164, 76/164/232/262/386, 76/164/262, 76/164/273, 76/164/287/364, 76/164/386, 76/232, 76/232/259/364, 76/232/262, 76/232/273/299/311/386, 76/232/364/386, 76/262, 76/262/273, 76/262/284/287/386, 76/262/284/364, 76/262/287, 76/262/364, 76/273, 76/284/299, 76/284/311/386, 76/364, 76/386, 130, 132, 135/284, 135/392, 145/400, 164/232/284/287, 191, 220, 220/360, 241, 246, 262/284/287, 273/311/313/364, 275, 279, 283, 285, 287, 288, 289, 290, 292, 297, 298,

298/405, 299, 311, 311/364/386, 332, 340, 343, 343/388, 347, 364, 372, 373, 374, 375, 376, 391, 392, and 405, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

4. The engineered acetate kinase of Claim 1, wherein said polypeptide sequence of said engineered acetate kinase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, and wherein said polypeptide sequence of said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions selected from 14, 14/46, 14/46/47, 14/46/75/293/296/342, 14/46/284/342/371, 14/46/293, 14/50/231/283/296, 14/75, 14/284/371, 14/310/391, 14/373, 22/46/296/373, 22/75/342, 22/231/283/373, 22/283/293/296, 22/296, 22/296/373, 22/372/373, 38/283/296/373, 38/296/373, 40/289/372/373, 46, 46/47/284/293, 46/47/284/390/391, 46/47/284/391, 46/50/54/342/373, 46/50/75/373, 46/50/283/296/297/373, 46/50/293/296, 46/50/293/297, 46/284/371, 46/293, 46/371/390/391, 47/75/284/293/371, 47/284/371/391, 47/293/310/371, 47/342/391, 50/75/283/293/296, 50/75/296, 50/75/373, 50/231/283/342, 50/231/373, 50/268/289/372/373, 50/283/293/296, 50/283/293/342/373, 50/289/372/373, 50/293/296, 50/372, 75/284/391, 75/293/296/373, 75/293/342, 75/293/391, 75/342/371, 231/283/342, 231/293/296/373, 231/296/342, 231/342/373, 283/293/296, 283/293/296/342, 283/296, 283/296/297/342, 283/296/342/373, 283/296/373, 283/356, 284/293/310/342/371, 284/293/390, 284/293/390/391, 284/342/371, 284/342/390, 284/390, 289, 289/372/373, 293, 293/296, 293/296/297, 293/296/342/373, 293/296/373, 293/342/373, 293/371, 293/390, 296/297, 296/342, 310/342/391, 310/371/391, 342, 342/371/391, 371, 371/390, 372/373, 373, and 390/391, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

5. The engineered acetate kinase of Claim 1, wherein said polypeptide sequence of said engineered acetate kinase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, and wherein said polypeptide sequence of said engineered acetate kinase further comprises at least one substitution or substitution set at one or more positions selected from 14/38/50/75/293/296, 14/342/391, 38/46/50/75/373, 46/293, 47/342/391, 50/75/283/293/296, 50/75/296, 50/103/296/342, 50/231/373, 50/268/289/372/373, 50/283/293/296, 50/283/293/342/373, 50/293, 50/293/296, 50/372/373, 75/283/296, 75/293/296/297, 75/293/391, 75/342/371, 75/390/391, 283/296, 289, 289/372, 289/372/373, 293/342/373, 293/342/391, 293/371, 296, 296/342, 310/342/391, 342, 372, and

372/373, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

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

7. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase comprises a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of at least one engineered acetate kinase variant set forth in the even numbered sequences of SEQ ID NOs: 6, 202, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 360, 362, 364, 366, 368, 374, 382, 388, 390, 392, 396, 438, 440, 446, 448, 522, 526, 534, and 542.

8. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase comprises at least one improved enzyme property compared to wild-type *Thermotoga maritima* acetate kinase.

9. The engineered acetate kinase of Claim 8, wherein said improved enzyme property comprises improved activity on a substrate.

10. The engineered acetate kinase of Claim 9, wherein said substrate comprises Sp-3'Fluoro-3'-deoxyguanosine-5'-(1-thio)-diphosphate (F-thioGDP) and/or Sp-2'F-ara-adenosine-5'-(1-thio)-diphosphate (F-thioADP).

11. The engineered acetate kinase of Claim 8, wherein said improved property comprises improved production of Sp-3'Fluoro-3'-deoxyguanosine-5'-(1-thio)-triphosphate (F-thioGTP) and/or Sp-2'Fluoro-ara-adenosine-5'-(1-thio)-triphosphate (F-thioATP).

12. The engineered acetate kinase of Claim 1, wherein said engineered acetate kinase is purified.

13. A composition comprising at least one engineered acetate kinase of Claim 1.

14. The engineered acetate kinase of Claim 1, wherein the polypeptide sequence of said engineered acetate kinase comprises a substitution of M294L and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NOs: 2.

15. The engineered acetate kinase of Claim 1, wherein the polypeptide sequence of said engineered acetate kinase comprises a polypeptide sequence set forth in SEQ ID NOs: 6, 202, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 360, 362, 364, 366, 368, 374, 382, 388, 390, 392, 396, 438, 440, 446, 448, 522, 526, 534, and 542.