

CLAIMS

We claim:

1. An engineered adenylate kinase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, wherein the polypeptide sequence of said engineered adenylate kinase comprises a substitution at position 42 and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

2. The engineered adenylate kinase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, and wherein the polypeptide sequence of said engineered adenylate kinase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 34/35/51, 34/35/51/53/138, 34/35/51/53/172, 34/35/51/76, 34/35/51/76/172/202, 34/35/51/119, 34/35/51/119/172, 34/35/51/172, 34/35/52/53/119/172, 34/35/52/76/119, 34/35/53/76, 34/35/53/202, 34/35/76, 34/35/76/119/172, 34/35/119, 34/35/138, 34/35/138/172, 34/51, 34/51/52/53/76, 34/51/53/76/172, 34/51/53/138, 34/51/53/172, 34/51/76, 34/51/119/138/172, 34/51/131, 34/51/202, 34/76/138/172, 34/119, 34/119/172, 34/138/172, 35/51, 35/51/52/53, 35/51/52/53/76/119/172/202, 35/51/52/53/119, 35/51/53/138/172, 35/51/53/172, 35/51/53/202, 35/51/76, 35/51/76/131, 35/51/76/138, 35/51/76/172, 35/51/119, 35/51/119/138, 35/51/119/138/172, 35/51/119/172, 35/51/172, 35/51/172/202, 35/51/202, 35/52/53/119, 35/52/53/119/138, 35/53/119/131/202, 35/53/119/138, 35/76, 35/76/119, 35/76/119/138, 35/76/119/138/172, 35/76/172, 35/119, 35/119/138, 35/119/172, 35/138/172, 35/172, 35/172/186, 35/172/202, 51, 51/52/53/172/202, 51/53, 51/53/119, 51/53/172, 51/76/119, 51/76/119/172, 51/119/138, 51/119/172/202, 51/138, 51/172, 52/53/76/172/202, 52/76/172, 52/138/172, 53/76/202, 53/172, 76, 76/119, 76/119/172, 76/172/202, 119/138/172, 119/172, 138/202, 172/202, and 202, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

3. The engineered adenylate kinase of Claim 1, wherein said polypeptide sequence of said engineered adenylate kinase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, and wherein said polypeptide sequence of said engineered adenylate kinase comprises at least

one substitution or substitution set at one or more positions selected from 1/2/104, 16, 29, 41, 46, 49, 50, 51, 61, 65, 72, 74, 76, 77, 79, 81, 108, 110, 127, 152, 191, 214, 218, and 222, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered adenylate kinase of Claim 1, wherein the polypeptide sequence of said engineered adenylate kinase further comprises at least one substitution or substitution set at one or more positions selected from 16, 16/29/49/104/108/191/218, 16/29/49/104/108/210, 16/29/49/104/191, 16/29/49/104/191/218, 16/29/49/108, 16/29/49/108/191, 16/29/49/108/210, 16/29/49/191, 16/29/49/191/210, 16/29/76/210, 16/29/104/108/191/218, 16/29/104/210, 16/29/108/191, 16/49, 16/49/76/104/191, 16/49/104/108, 16/49/104/108/191, 16/49/104/108/210, 16/49/104/191, 16/49/104/210, 16/49/108/210, 16/49/108/218, 16/49/191, 16/49/191/210, 16/81/104, 16/104/191/210, 16/108, 16/108/191/210, 16/108/218, 16/191/210, 29, 29/49/77/81/108, 29/76/77/104/191/210, 29/76/104/108, 29/104/108/191/210, 29/104/108/191/218, 29/108, 29/108/191, 29/108/191/210, 29/108/210, 29/49/76/77/81/191/210, 29/49/77/81/104/108/191, 29/49/81/108, 29/49/104/108, 29/49/104/108/191, 29/49/104/108/191/210, 29/49/104/108/191/210/218, 29/49/104/108/210, 29/49/104/191, 29/49/104/191/210, 29/49/108/191, 29/49/108/191/210, 29/49/108/191/210/218, 29/49/108/191/218, 29/49/108/210, 29/49/191, 29/49/191/210, 29/49/191/210/218, 29/49/210/215, 29/76/191/210, 29/104, 29/104/108, 29/104/108/191/210, 29/104/108/210, 29/104/191, 29/104/191/210, 29/104/191/210/218, 29/104/210, 29/108, 29/108/191, 29/108/191/210, 29/108/191/210/218, 29/108/191/218, 29/108/210, 29/191/210, 49/76/81/108/210, 49/108/191/210/218, 77/108/191, 108/191/210, 49, 49/76/77/81/104/191/210, 49/76/81/104/108, 49/76/108/191, 49/76/108/191/210, 49/81/104/108, 49/81/108, 49/104/108, 49/104/108/180/191, 49/104/108/191, 49/104/108/191/210, 49/104/108/210, 49/104/191, 49/104/191/210, 49/104/218, 49/108, 49/108/191, 49/108/191/210, 49/108/191/210/218, 49/108/210, 49/191, 49/191/210, 49/210, 76/77/81/108/191, 76/77/81/108/191/210, 76/77/81/210/218, 76/81/108, 76/81/108/191/218, 77/81/108/191, 104/108/191, 104/108/191/210, 104/108/191/210/218, 104/108/210, 104/108/218, 104/191, 104/191/210, 108/191, 108/191/210, 108/191/218, and 108/210, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

5. The engineered adenylate kinase of Claim 1, wherein the polypeptide sequence of said engineered adenylate kinase further comprises at least one substitution or substitution set at one or more positions selected from 16, 16/29, 16/29/51/110/191, 16/29/51/191, 16/29/51/214, 16/29/119/191, 16/29/119/191/214, 16/29/191, 16/51/107, 16/51/107/110/214, 16/51/110/202/214, 16/51/191/202, 16/51/191/202/214, 16/51/214, 16/107/110/119, 16/107/110/119/191/202/214, 16/107/110/191, 16/107/191/214, 16/110/119, 16/110/191, 16/191, 16/191/202, 16/214, 29, 29/46/79/107/110/202/214, 29/46/110/202/214, 29/46/110/214, 29/46/191, 29/51/107/110/191, 29/51/107/110/191/214, 29/51/107/110/202/214, 29/51/107/119/191, 29/51/110/191/202, 29/51/119/191/214, 29/51/191, 29/51/191/202, 29/51/191/214, 29/74/79/107/214, 29/74/107/191, 29/74/191, 29/107/119/191, 29/110/119/191, 29/119, 29/191/214, 51, 51/107/110, 51/107/110/119, 51/107/110/191, 51/107/110/191/214/218, 51/107/119/191/214, 51/107/214, 51/110/191, 51/110/214, 51/191, 51/191/202/214, 51/191/214, 51/202, 51/214, 74/79/107/110/191/202, 107/110/119/191, 107/110/119/207/214, 107/110/123/191/202, 110/191/202/214, 119, 119/191, 191/202, and 191/214, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

6. The engineered adenylate kinase of Claim 1, wherein said engineered adenylate kinase comprises the amino acid sequence of SEQ ID NO: 230.

7. The engineered adenylate kinase of Claim 1, wherein said engineered adenylate kinase comprises a polypeptide sequence set forth in at least one of the sequences of SEQ ID NOs: 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782,

784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844, 846, and 848.

8. The engineered adenylate kinase of Claim 1, wherein said engineered adenylate kinase comprises improved activity on 2'F-ara-adenosine-5'-(1-thio)-monophosphate (F-thioAMP) and/or Sp-2'F-ara-adenosine-5'-(1-thio)-triphosphate (F-thioATP) as compared to the engineered adenylate kinase of SEQ ID NO: 4.

9. The engineered adenylate kinase of Claim 1, wherein said engineered adenylate kinase is purified.

10. A composition comprising at least one engineered adenylate kinase of Claim 1.

11. A polynucleotide sequence encoding at least one engineered adenylate kinase of Claim 1.

12. An expression vector comprising at least one polynucleotide sequence of Claim 11.

13. A host cell comprising at least one expression vector of Claim 12.

14. A host cell comprising at least one polynucleotide sequence of Claim 11.

15. A method of producing an engineered adenylate kinase in a host cell, comprising culturing the host cell of Claim 13 in a culture medium under suitable conditions such that at least one engineered adenylate kinase is produced.

16. The method of Claim 15, further comprising recovering the engineered adenylate kinase from the culture and/or the host cell.

17. The method of Claim 15, further comprising purifying said engineered adenylate kinase.