CLAIMS

We claim:

1. An engineered uridine phosphorylase 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~~s~~: 2, ~~4, 246, 594, 776, and/or 868~~, or a functional fragment thereof, wherein the polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions selected from the group consisting of: 80, 45/51/80/188/189/241, 16, 7, 9, 14, 14/38/40/146/147/179/235/236, 14/38/86/146/147/235/236/240, 14/40, 14/40/86/147/193/236/240, 14/40/136/179/236/240, 14/40/146/235/236, 14/40/147/181/193/235, 14/40/235, 14/86/146, 14/146/147/181/240, 14/146/236/240, 14/147/193/235/236/240, 14/179/181/193/235/240, 14/235/236, 29, 31, 38/40/86/146/147/179/181, 38/40/86/147/236/240, 40, 40/43/86/146/240, 40/43/86/147/235, 40/43/146/147, 40/43/147/179/236/240, 40/43/147/179/240, 40/43/147/236/240, 40/86/146/235/236, 40/86/147/235/236/240, 40/86/179/235/240, 40/86/235/236/240, 40/146/147/240, 40/147/240, 40/235, 40/235/236/240, 40/236, 40/236/240, 42/235/236, 43/86/147/181/240, 43/146/147/235/236/240, 43/146/179/240, 43/147, 43/147/179/181, 47, 47/88, 64, 73, 80, 86, 86/136/146/147/179/181, 86/136/146/147/179/235/236, 86/147/179/181, 86/235, 86/235/236/240, 86/236/240, 86/240, 92, 97, 99, 103, 103/249, 104, 105, 106, 110, 146/147, 146/147/235/236, 146/235/240, 146/236/240, 146/240, 147/179/181, 147/235/240/249, 157, 167, 179, 179/181, 179/181/193/240, 181, 184, 216, 226, 228, 231, 233, 235, 235/236, 236, 236/240, 237, 239, 240, and 245,and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO~~s~~: 2, ~~4, 246, 594, 776, and/or 868~~; wherein said engineered uridine phosphorylase comprises at least one improved property compared to wild-type *Escherichia coli* uridine phosphorylase, and wherein said improved property:(i) comprises improved activity on one or more substrates, and wherein said one or more substrates comprises 5'-isobutyryl ribose-1-phosphate (compound **(2)**) and/or uracil (compound **(3)**), and/or (ii) comprises improved production of 5'-isobutyryluridine, compound **(1)**.

2. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence has 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, and wherein the polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 80I, 80G, 80K, 80M, 80S, 80T, 6F, 6G, 6R, 6S, 6W, 6Y, 7T, 9M, 14A, 14A/38L/40S/146E/147L/179R/235R/236A, 14A/38L/86V/146E/147L/235R/236A/240R,

14A/40D/86V/147M/193L/236A/240R, 14A/40D/235R, 14A/40N, 14A/40N/146E/235R/236A, 14A/40N/147L/181Q/193L/235R, 14A/40S, 14A/40S/136V/179R/236A/240R, 14A/86V/146E, 14A/146E/147L/181Q/240R, 14A/146E/236A/240R, 14A/147L/193L/235R/236A/240R, 14A/179R/181Q/193L/235R/240R, 14A/235R/236A, 29S, 31T, 38L/40D/86V/146E/147L/179R/181Q, 38L/40S/86V/147L/236A/240R, 40D, 40D/86V/147L/235R/236A/240R, 40D/86V/235R/236A/240R, 40D/235R, 40N/43F/86V/146E/240R, 40N/43F/86V/147L/235R, 40N/43F/146E/147L, 40N/43F/147L/179R/236A/240R, 40N/86V/179R/235R/240R, 40S/43F/147L/179R/240R, 40S/43F/147L/236A/240R, 40S/86V/146E/235R/236A, 40S/86V/147L/235R/236A/240R, 40S/146E/147L/240R, 40S/147L/240R, 40S/235R/236A/240R, 40S/236A, 40S/236A/240R, 42I/235R/236A, 43F/86V/147L/181Q/240R, 43F/146E/147L/235R/236A/240R, 43F/146E/179R/240R, 43F/147L, 43F/147L/179R/181Q, 47M, 47R, 47V, 47V/88A, 64C, 73G, 86V, 86V/136V/146E/147L/179R/181Q, 86V/136V/146E/147L/179R/235R/236A, 86V/147L/179R/181Q, 86V/235R, 86V/235R/236A/240R, 86V/236A/240R, 86V/240R, 92V, 97T, 99E, 103G/249V, 103S, 104T, 105M, 106E, 110A, 110C, 110S, 146E/147L, 146E/147L/235R/236A, 146E/235R/240R, 146E/236A/240R, 146E/240R, 147L/179R/181Q, 147L/235R/240R/249V, 157S, 167S, 179R, 179R/181Q, 179R/181Q/193L/240R, 181Q, 184R, 184S, 216M, 226R, 228A, 228G, 228K, 228L, 228R, 231I, 231P, 231T, 231W, 231Y, 233S, 235R, 235R/236A, 236A, 236A/240R, 237V, 239A, 239K, 239R, 240R, and 245S,

~~45/51/80/188/189/241, 16, 7, 9, 14, 14/38/40/146/147/179/235/236, 14/38/86/146/147/235/236/240, 14/40, 14/40/86/147/193/236/240, 14/40/136/179/236/240, 14/40/146/235/236, 14/40/147/181/193/235, 14/40/235, 14/86/146, 14/146/147/181/240, 14/146/236/240, 14/147/193/235/236/240, 14/179/181/193/235/240, 14/235/236, 29, 31, 38/40/86/146/147/179/181, 38/40/86/147/236/240, 40, 40/43/86/146/240, 40/43/86/147/235, 40/43/146/147, 40/43/147/179/236/240, 40/43/147/179/240, 40/43/147/236/240, 40/86/146/235/236, 40/86/147/235/236/240, 40/86/179/235/240, 40/86/235/236/240, 40/146/147/240, 40/147/240, 40/235, 40/235/236/240, 40/236, 40/236/240, 42/235/236, 43/86/147/181/240, 43/146/147/235/236/240, 43/146/179/240, 43/147, 43/147/179/181, 47, 47/88, 64, 73, 80, 86, 86/136/146/147/179/181, 86/136/146/147/179/235/236, 86/147/179/181, 86/235, 86/235/236/240, 86/236/240, 86/240, 92, 97, 99, 103, 103/249, 104, 105, 106, 110, 146/147, 146/147/235/236, 146/235/240, 146/236/240, 146/240, 147/179/181, 147/235/240/249, 157, 167, 179, 179/181, 179/181/193/240, 181, 184, 216, 226, 228, 231, 233, 235, 235/236, 236, 236/240, 237, 239, 240, and 245,~~ wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

3. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence of said engineered uridine phosphorylase has 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: 4, and wherein said polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions selected from 3, 3/9/216, 3/9/216/236, 3/9/235/237, 3/31/47/179/181/216, 3/31/47/179/181/237, 3/31/47/179/216, 3/31/47/179/216/237, 3/31/179, 3/31/179/181, 3/31/179/181/237, 3/31/179/216, 3/31/181/216, 3/31/181/237, 3/47/179/181/216,

3/47/179/216/237, 3/47/181, 3/179, 3/179/181, 3/179/181/216, 3/179/181/237, 3/179/216, 3/179/216/237, 3/179/237, 3/181/216, 3/181/216/237, 3/216/236/240, 9/216/236/237, 9/237, 13, 24, 31, 31/47, 31/47/179/181/237, 31/47/179/216, 31/47/181, 31/47/216, 31/179, 31/179/181, 31/179/216, 31/181, 31/181/216, 31/181/216/237, 31/181/237, 31/216, 31/216/237, 31/236/237/240, 31/237, 33, 46, 47, 47/147/181/231, 47/179/181, 47/179/181/216, 47/179/184, 47/179/216, 47/181/216, 47/181/216/237, 47/181/231, 47/216, 52, 63, 67, 83, 87/160, 92, 95, 97, 99, 100, 101, 105, 106, 108, 111, 137, 151, 152, 155, 159, 160, 170, 173, 177, 179, 179/181, 179/181/216, 179/181/216/237, 179/181/231, 179/181/241, 179/216, 179/228/231, 179/237, 181, 181/216, 181/216/237, 181/237, 183, 185, 188, 189, 191, 201, 216, 216/237, 218, 222, 228, 231, 233, 235, 235/237, 236, 236/237/240, 237, 238, 240, 241, and 248, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence of said engineered uridine phosphorylase has 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: 246, and wherein said polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions selected from 3/24/33/47/100/183/185, 3/24/33/47/100/216/228/233, 3/24/33/100/183/185/228, 3/24/33/108, 3/24/47/100, 3/24/47/100/108/111/160/185/233/241, 3/24/47/108/160/241, 3/24/47/160/189, 3/24/47/189/228/233, 3/24/47/228, 3/24/47/228/233, 3/24/95/100, 3/24/95/100/160/189/228/241, 3/24/100, 3/24/100/160/218/241, 3/24/111, 3/24/111/183/228/233/241, 3/24/111/228/233, 3/24/183/185/216, 3/24/189/233, 3/33/47/95/100/241, 3/33/47/100/108/189/216/228/233, 3/33/47/100/111/228, 3/33/47/100/111/233/241, 3/33/47/100/216, 3/33/47/108/111/233, 3/33/47/108/189/233/241, 3/33/160/233, 3/47, 3/47/95/100/108/189/233, 3/47/95/100/111/241, 3/47/95/160/189, 3/47/100/108/183/185/189/241, 3/47/100/160/185, 3/47/100/185/189/228, 3/47/108/111, 3/47/183/189/228/233, 3/47/189, 3/47/228/233, 3/95/100/160/228/233, 3/95/100/183/216/228/233, 3/95/100/183/233, 3/95/185/189/216, 3/95/189, 3/95/233, 3/160, 3/183/185/189/228/233, 3/183/189/228/233, 3/185, 3/185/189, 3/189, 24, 24/33/47, 24/33/47/228/241, 24/33/100/108/241, 24/47/95/100, 24/47/95/100/160/228/233/241, 24/47/185/216/218, 24/47/216, 24/95/183, 24/100/160/233, 24/160/183/185, 24/189/228/233, 33/47/95/100/233, 33/47/95/100/233/241, 33/47/160, 33/47/233, 33/100/183/185, 33/100/185/233, 47, 47/100/111/233, 47/100/189, 47/100/189/233, 47/108/160/228/241, 47/111, 47/160/185/189/233, 47/228/233, 95/100/183, 95/100/189, 95/100/228, 95/100/228/233, 95/100/233, 100/160/185, 100/228/233, 108, 108/183/189/233, 108/185/216/228/233, 160/233, 228, and 228/233, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 246.

5. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence of said engineered uridine phosphorylase has 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: 594, and wherein said polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions selected from 6, 6/9/29/40/100/121/126/179/181/189/237, 6/9/121/179/181, 6/46/52/63/97/121/126/179, 6/52/180/181, 6/63/126/179/242, 9, 9/40/46/97/100/106/135/179/181/207/231, 9/52/126/189/242, 9/97/100/106/126/180/207/231, 9/181/242, 29, 40, 46, 52, 52/63/126, 52/179/189, 61, 63, 97, 100, 106, 121, 126, 126/180/189, 135, 142, 179, 180, 181, 189, 201, 207, 230, 231, 236, 237, and 242, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 594.

6. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence of said engineered uridine phosphorylase has 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: 776, and wherein said polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or substitution set at one or more positions selected from 3, 3/8/22/36/181/235/250, 6, 6/45/51/81/126/226/233, 6/45/51/144, 6/45/51/188/189, 6/45/51/189/208, 6/45/51/189/228/236, 6/45/51/208/233, 6/45/149/188/189/208/233/236, 6/51/126/144/208, 6/51/126/189/208/233/236, 6/51/126/189/231/236, 6/51/126/189/233, 6/51/188/189/208/226/228, 6/51/188/189/236, 6/51/189/208/231/233, 6/51/208/226/233, 6/51/208/231, 6/126/188/231/233, 6/144/208, 6/188/189/208/228/233, 8, 8/36/143/147/235, 8/36/181/235, 8/142/147, 8/147/181/250, 8/147/235, 9, 19, 20, 22, 22/147/181/235/250, 24, 36, 36/143/147/181, 36/143/147/235, 40, 41, 43, 45, 45/51, 45/51/126/144/208/226/228, 45/51/144, 45/51/144/208/226/231/233, 45/51/188/189, 45/51/189/233, 45/51/208/226/231, 45/51/208/233, 45/51/226, 45/126/189, 45/126/189/208/226, 45/144/189/228, 45/144/226/231/233, 45/188/189, 45/188/189/208/228, 45/188/189/226/228, 45/188/189/231/233, 45/189, 45/189/208, 46, 51, 51/126/144/208, 51/126/144/226/231/233/236, 51/126/208, 51/144, 51/144/226, 51/188/189/228, 51/189, 51/189/208/226, 51/208, 51/233, 57, 58, 80, 80/135/147, 81, 81/126/144/188/208/228, 86, 103, 126, 126/144/188/189/226, 134, 135, 141, 142, 143, 143/147/235, 144, 144/188/228, 146, 147, 149, 181, 188, 188/189/233, 189, 207, 208, 208/226/233, 208/228, 208/231/233, 226, 228, 230, 231, 232, 233, 235, 236, 240, and 250, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 776.

7. The engineered uridine phosphorylase of Claim 1, wherein said polypeptide sequence of said engineered uridine phosphorylase has 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: 868, and wherein said polypeptide sequence of said engineered uridine phosphorylase comprises at least one substitution or

substitution set at one or more positions selected from 6, 6/8/9/126/147, 6/9/24/181/189, 6/9/181/189/235, 6/9/208/233/235, 6/24, 6/24/43/46/181/189, 6/24/43/126/147/189, 6/24/46/103/181/208, 6/24/46/147/240, 6/24/103/189, 6/24/126/189, 6/24/147,

6/46/126/147/181/235/240, 6/46/147/189/240, 6/103, 6/103/147/230/233, 6/103/189/235, 6/126/181/189, 6/126/181/189/235, 6/126/233/235, 6/181/230/233/235, 9/43/46/103/189/233/240, 9/46/126/147/181, 9/46/147/233, 24/43/46/147/230/235, 24/46/126, 24/46/147/189, 24/46/208/230/233/235, 24/103/126/147, 24/103/126/147/181/189/208/233, 24/147, 24/147/189/230/233, 24/181/189/230/233/235, 24/189/230, 24/208, 43/46/126/147/189/240, 43/103/189/208/233, 103/126/189/233/235, 103/147/181, 147/181/233, 189, 189/235, and 208/233, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 868.

~~8. he engineered uridine phosphorylase of Claim 1, wherein said engineered uridine phosphorylase 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 uridine phosphorylase variant set forth in 1.2, 2.2, 3.1, 4.1, 5.2, and/or 6.1.~~

8~~9~~. The engineered uridine phosphorylase of Claim 1, wherein said engineered uridine phosphorylase 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 NOs: ~~2,~~ 4, 246, 594, 776, and/or 868.

~~10. The engineered uridine phosphorylase of Claim 1, wherein said engineered uridine phosphorylase comprises a variant engineered uridine phosphorylase set forth in SEQ ID NOs: 4, 246, 594, 776, and/or 868.~~

~~11. The engineered uridine phosphorylase of Claim 1, wherein said engineered uridine phosphorylase 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 uridine phosphorylase variant set forth in the even numbered sequences of SEQ ID NOs: 4-1196~~

9~~12~~. The engineered uridine phosphorylase of Claim 1, wherein said engineered uridine phosphorylase comprises a polypeptide sequence forth in at least one of the even numbered sequences of SEQ ID NOs: 4-1196.

~~13. The engineered uridine phosphorylase of any of Claims 1-12, wherein said engineered uridine phosphorylase comprises at least one improved property compared to wild-type *Escherichia coli* uridine phosphorylase.~~

~~14. The engineered uridine phosphorylase of Claim 13, wherein said improved property comprises improved activity on one or more substrates.~~

~~15. The engineered uridine phosphorylase of Claim 14, wherein said one or more substrates comprises 5' isobutyryl ribose 1 phosphate (compound **(2)**) and/or uracil (compound **(3)**).~~

~~16. The engineered uridine phosphorylase of any of Claims 13 15, wherein said improved property comprises improved production of 5' isobutyryluridine, compound **(1)**.~~

10~~17~~. The engineered uridine phosphorylase of any one of Claims 1-9~~16~~, wherein said engineered uridine phosphorylase is purified.

11~~18~~. The engineered uridine phosphorylase of any one of Claims 1-10~~17~~, wherein said engineered uridine phosphorylase is part of a multi-enzyme system for producing a nucleoside analogue.

12~~19~~. A composition comprising at least one engineered uridine phosphorylase of any one of Claims 1-9~~16~~.

13~~20~~. A polynucleotide sequence encoding at least one engineered uridine phosphorylase of any one of Claims 1-11~~18~~.

14~~21~~. A polynucleotide sequence encoding at least one engineered uridine phosphorylase, 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, 245, 593, 775 and/or 867, or a functional fragment thereof, wherein the polynucleotide sequence of said engineered uridine phosphorylase comprises at least one substitution at one or more positions.

~~22. A polynucleotide sequence encoding at least one engineered uridine phosphorylase 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, 245, 593, 775 and/or 867, or a functional fragment thereof.~~

15~~23~~. The polynucleotide sequence of ~~any of~~ Claim~~s~~ 13 or Claim 14~~20-22~~, wherein said polynucleotide sequence is operably linked to a control sequence.

16~~24~~. The polynucleotide sequence of any one of Claims 13~~20~~-15~~23~~, wherein said polynucleotide sequence is codon optimized.

17~~25~~. The polynucleotide sequence of any one of Claims 13~~20~~-16~~24~~, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOs: 3-1195.

18~~26~~. An expression vector comprising at least one polynucleotide sequence of any one of Claims 13~~20~~-17~~25~~.

19~~27~~. A host cell comprising at least one expression vector of Claim 18~~26~~.

~~28. A host cell comprising at least one polynucleotide sequence of any of Claims 20-25.~~

20~~29~~. A method of producing an engineered uridine phosphorylase in a host cell, comprising culturing the host cell of Claim ~~27~~ 19 ~~and/or 28,~~ under suitable conditions, such that at least one engineered uridine phosphorylase is produced.

21~~30~~. The method of Claim 20~~29~~, further comprising recovering at least one engineered uridine phosphorylase from the culture and/or host cell.

~~31. The method of Claim 29 and/or 30, further comprising the step of purifying said at least one engineered uridine phosphorylase.~~