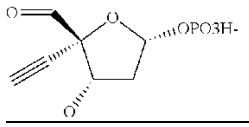
CLAIMS

We claim:

1. An engineered purine nucleoside 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:2, 6, and/or 126, ~~242, and/or 684, or a functional fragment thereof,~~ wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises ~~at least one~~ a substitution ~~or substitution set~~ at position 65 and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2, 6, and/or 126, ~~242, and/or 684.~~and wherein said engineered purine nucleoside phosphorylase

Compound 3



comprises improved activity on the substrate of compound 3 as compared to wild-type *E. coli* purine nucleoside phosphorylase.

~~2. The engineered purine nucleoside 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 purine nucleoside phosphorylase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 65, 2, 12, 12/42, 12/74/80/101/162/214/215, 12/74/101, 12/80/162/210/215, 12/80/210, 12/162, 12/165/173/210/214, 12/187, 12/210, 20/90, 21, 25, 42, 45, 69, 72, 91, 95/178/199, 105, 111, 115, 155, 162, 164, 171/176/199, 176/178/199, 176/199, 177, 178, 178/199, 179, 181, 184, 199, 202, 204, 207, and 212, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.~~

~~3. The engineered purine nucleoside 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:6, and wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 2, 2/65, 12/42/65, 12/65, 12/65/74/80/101/162/214/215, 12/65/74/101, 12/65/80/210, 12/65/162, 12/65/165/173/210/214, 21/65, 38, 42, 42/155, 42/177, 45/65, 54, 65/72, 65/91, 65/105, 65/115, 65/202, 65/212, 80, 80/95, 80/155, 80/175, 84, 91, 91/115, 95, 95/101, 95/155, 95/155/215, 95/212, 95/212/215, 101, 101/105, 101/187, 101/212, 105/155/212/215, 108, 155, 155/177/204, 155/184/212/215, 155/212, 162/199, 175, 177,~~

184/212/215, 199, 212, 212/215, and 215, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6.

4. The engineered purine nucleoside 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:126, and wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 2, 2/80/95, 2/80/95/155, 2/80/95/155/199/212, 2/80/95/199, 2/80/95/199/212, 2/80/101/155/212, 2/80/101/212, 2/80/155/177/215, 2/80/155/215, 2/80/175/199, 2/80/175/199/212/215, 2/80/177, 2/80/199/212/215, 2/80/215, 2/95, 2/95/155, 2/95/155/199, 2/95/155/199/212/215/223, 2/95/155/199/215, 2/95/155/215, 2/95/175, 2/95/199, 2/95/199/212/215, 2/95/212/215, 2/95/215, 2/101/155, 2/155, 2/155/177/212, 2/155/199, 2/199, 2/199/212/215, 2/212/215, 2/215, 28, 39, 42, 45, 53, 54/173, 57/175, 75, 80, 80/95/101/155/199, 80/95/101/155/199/215, 80/95/101/199, 80/95/155, 80/95/155/175/199/212/215, 80/95/155/199, 80/95/155/199/212, 80/95/155/199/215, 80/95/155/212/215, 80/95/177, 80/95/177/199, 80/95/177/212/215, 80/95/215, 80/101, 80/155, 80/155/177/199, 80/155/177/212/215, 80/155/199, 80/199, 80/212, 84, 85, 95, 95/155, 95/155/177, 95/155/177/199, 95/155/177/199/212, 95/155/199, 95/155/212/215, 95/177/199, 95/199, 95/212, 95/212/215, 95/215, 98, 101, 101/155/199, 101/155/199/212, 101/177/212, 101/215, 119, 123, 124, 129, 130, 131, 132, 136, 137, 139, 140, 143, 144, 148, 148/175, 150, 151, 152, 153, 155, 155/199, 155/212/215, 160, 170/203, 173, 175/212, 177, 177/199, 191, 191/237, 196, 199, 199/212, 210, 212, 212/215, 216, and 220, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO:126.

5. The engineered purine nucleoside 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:242, and wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 7, 12, 27, 28, 31, 32, 35, 37, 38, 52, 57, 85, 94, 97, 98, 100, 102, 133, 136, 143, 144, 145, 148, 149, 150, 162, 169, 170, 172, 173, 195/199, 196, 199, 207, 208, 208/238, 209, 210, 211, 212, 213, 214, 215, 217, 219, 220, 221, 223, 224, 227, 235, and 238, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 242.

6. The engineered purine nucleoside 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:684, and wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises at least one substitution or substitution set at one

~~or more positions in said polypeptide sequence selected from 10, 10/133/227, 10/145, 10/145/227, 18, 20, 26, 29, 39/98, 39/133/227, 60, 62, 63, 74, 89, 94, 97, 97/98, 108, 126, 133, 135, 145, 161, 162, 165, 167, 168, 177, 199, 208, 210, 212, 224, and 227, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 684.~~

~~7. The engineered purine nucleoside phosphorylase of Claim 1, wherein said engineered purine nucleoside 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 purine nucleoside phosphorylase variant set forth in Table 4.1, 5.1, 6.1, 7.1 and/or 8.1.~~

~~8. The engineered purine nucleoside phosphorylase of Claim 1, wherein said engineered purine nucleoside 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 NO: 2.~~

~~9~~2. The engineered purine nucleoside phosphorylase of Claim 1, wherein said engineered purine nucleoside 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 purine nucleoside phosphorylase variant set forth in the ~~even numbered~~ sequences of SEQ ID NOS: ~~4-1002~~684, 892, 894, 896, 898 and 900.

~~10~~3. The engineered purine nucleoside phosphorylase of Claim 1, wherein said engineered purine nucleoside phosphorylase comprises a polypeptide sequence forth in at least one of the ~~even numbered~~ sequences of SEQ ID NOS: ~~2-1002~~684, 892, 894, 896, 898 and 900.

~~11. The engineered purine nucleoside phosphorylase of any of Claims 1-10, wherein said engineered purine nucleoside phosphorylase comprises at least one improved property compared to wild-type *E.coli* purine nucleoside phosphorylase.~~

~~12. The engineered purine nucleoside phosphorylase of Claim 11, wherein said improved property comprises improved activity on a substrate.~~

~~13. The engineered purine nucleoside phosphorylase of Claim 12, wherein said substrate comprises compound 3.~~

~~14. The engineered purine nucleoside phosphorylase of Claim 12, wherein said substrate comprises compound 4.~~

~~15. The engineered purine nucleoside phosphorylase of Claim 12, wherein said substrate comprises compound 8.~~

~~16. The engineered purine nucleoside phosphorylase of any of Claims 11-15, wherein said improved property comprises improved production of compound 1.~~

~~17. The engineered purine nucleoside phosphorylase of any of Claims 1-12 and 15, wherein said improved property comprises improved production of compound 10.~~

~~18~~4. The engineered purine nucleoside phosphorylase of ~~any of~~ Claim~~s~~ 1~~-17~~, wherein said engineered purine nucleoside phosphorylase is purified.

~~19~~5. A composition comprising the engineered purine nucleoside phosphorylase of ~~any of~~ Claim~~s~~ 1~~-18~~.

~~20~~6. A polynucleotide sequence encoding at least one engineered purine nucleoside phosphorylase of ~~any of~~ Claim~~s~~ 1~~-18~~.

~~21~~7. A polynucleotide sequence encoding at least one engineered purine nucleoside phosphorylase of Claim 1, 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, 5, 125, 241, and/or 683, wherein the polynucleotide sequence of said engineered purine nucleoside phosphorylase comprises at least one substitution at one or more positions.

~~22~~8. A polynucleotide sequence encoding at least one engineered purine nucleoside phosphorylase of Claim 1, said polynucleotide sequence 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, 5, 125, 241, and/or 683, or a functional fragment thereof.

~~23~~9. The polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~20-22~~6, wherein said polynucleotide sequence is operably linked to a control sequence.

~~24~~10. The polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~20-23~~6, wherein said polynucleotide sequence is codon optimized.

~~25~~11. The polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~20-24~~6, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 3-1001.

~~26~~12. An expression vector comprising at least one polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~20-25~~6.

~~27~~13. A host cell comprising at least one expression vector of Claim ~~26~~12.

~~28~~14. A host cell comprising at least one polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~20-25~~6.

~~29~~15. A method of producing an engineered purine nucleoside phosphorylase in a host cell, comprising culturing the host cell of Claim ~~27 and/or 28~~13, under suitable conditions, such that at least one engineered purine nucleoside phosphorylase is produced.

~~30~~16. The method of Claim ~~29~~15, further comprising recovering at least one engineered purine nucleoside phosphorylase from the culture and/or host cell.

~~31~~17. The method of Claim ~~29 and/or 30~~15, further comprising the step of purifying said at least one engineered purine nucleoside phosphorylase.