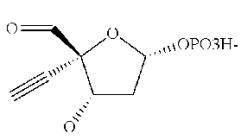
CLAIMS

We claim:

1. An engineered purine nucleoside phosphorylase comprising a polypeptide sequence having at least 85% sequence identity to SEQ ID NO:2, 6, and/or 126, wherein the polypeptide sequence of said engineered purine nucleoside phosphorylase comprises a substitution 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, 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 engineered purine nucleoside phosphorylase comprises a polypeptide sequence that is at least 85% identical to the sequence of at least one engineered purine nucleoside phosphorylase variant set forth in the sequences of SEQ ID NOS: 684, 892, 894, 896, 898 and 900.

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 sequences of SEQ ID NOS:684, 892, 894, 896, 898 and 900.

4. The engineered purine nucleoside phosphorylase of Claim 1, wherein said engineered purine nucleoside phosphorylase is purified.

5. A composition comprising the engineered purine nucleoside phosphorylase of Claim 1.

6. A polynucleotide sequence encoding at least one engineered purine nucleoside phosphorylase of Claim 1.

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.

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.

9. The polynucleotide sequence of Claim 6, wherein said polynucleotide sequence is operably linked to a control sequence.

10. The polynucleotide sequence of Claim 6, wherein said polynucleotide sequence is codon optimized.

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

12. An expression vector comprising at least one polynucleotide sequence of Claim 6.

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 6.

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

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

17. The method of Claim 15, further comprising the step of purifying said at least one engineered purine nucleoside phosphorylase.