CLAIMS

We claim:

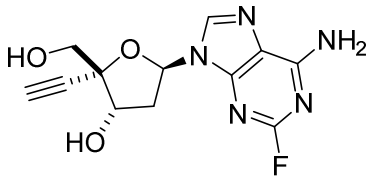
1. An engineered sucrose phosphorylase comprising a polypeptide sequence having at least 98%, 99%, or more sequence identity to SEQ ID NO: 2 wherein the polypeptide sequence of said engineered sucrose phosphorylase comprises a substitution at position 397 and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2, and wherein the engineered sucrose phosphorylase exhibits improved activity on a substrate selected from the group consisting of sucrose, sucrose-related disaccharides and/or inorganic phosphate.

2. The engineered sucrose phosphorylase of Claim 1, wherein said polypeptide sequence has at least 98%, 99%, or more sequence identity to SEQ ID NO: 2, and wherein the polypeptide sequence of said engineered sucrose phosphorylase further comprises at least one substitution at one or more positions in said polypeptide sequence selected from 7, 10, 48, 136, 158, 205, 207, 211, 215, 301, 333, 378, and 400, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

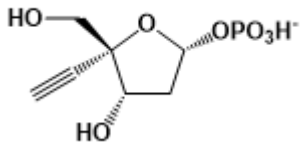
3. The engineered sucrose phosphorylase of Claim 1 or Claim 2, wherein said engineered sucrose phosphorylase comprises a polypeptide sequence that is at least 98%, 99% or more identical to the sequence of at least one engineered sucrose phosphorylase variant set forth in Table 3-1 and/or 4-1.

4. The engineered sucrose phosphorylase of any one of Claims 1 to 3, wherein said engineered sucrose phosphorylase comprises a polypeptide sequence that is at least 98%, 99% or more identical to the sequence of at least one engineered sucrose phosphorylase variant set forth in the even numbered sequences of SEQ ID NOS: 4, and 42-84.

5. The engineered sucrose phosphorylase of Claim 1, further comprising improved production of



compound **(1)** and/or



compound **(3)**.

6. The engineered sucrose phosphorylase of any one of Claims 1 to 5, wherein said engineered sucrose phosphorylase is purified.

7. The engineered sucrose phosphorylase of any one of Claims 1 to 6, wherein said engineered sucrose phosphorylase is part of a multi-enzyme system for producing a nucleoside analogue.

8. A composition comprising at least one engineered sucrose phosphorylase of any one of Claims 1 to 7.

9. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase of any one of Claims 1 to 7.

10. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase, said polynucleotide sequence comprises at least 98%, 99%, or more sequence identity to SEQ ID NO: 1, wherein the polynucleotide sequence of said engineered sucrose phosphorylase comprises a substitution at a position encoding amino acid position 397, numbered with reference to SEQ ID NO: 2.

11. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase comprising at least 98%, 99%, or more sequence identity to SEQ ID NO: 1.

12. The polynucleotide sequence of any one of Claims 9 to 11, wherein said polynucleotide sequence is operably linked to a control sequence.

13. The polynucleotide sequence of any one of Claims 9 to 12, wherein said polynucleotide sequence is codon optimized.

14. The polynucleotide sequence of any one of Claims 9 to 13, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 3, and 41-83.

15. An expression vector comprising at least one polynucleotide sequence of any one of Claims 9 to 14.

16. A non-human host cell comprising at least one expression vector of Claim 15 or at least one polynucleotide sequence of any one of Claims 9 to 14.

17. A method of producing an engineered sucrose phosphorylase in a host cell, comprising culturing the host cell of Claim 16, under suitable conditions, such that at least one engineered sucrose phosphorylase is produced.

18. The method of Claim 17, further comprising recovering at least one engineered sucrose phosphorylase from the culture and/or host cell.

19. The method of Claim 17 or 18, further comprising the step of purifying said at least one engineered sucrose phosphorylase.