CLAIMS

We claim:

1. An engineered sucrose 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 or a functional fragment thereof, 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.

2. The engineered sucrose 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 sucrose phosphorylase further comprises at least one substitution or substitution set 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 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 sucrose phosphorylase variant set forth in Table 3-1 and/or 4-1.

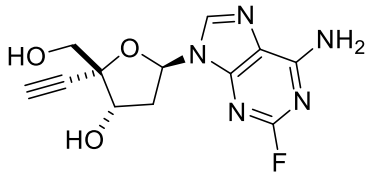
4. The engineered sucrose phosphorylase of any one of Claims 1 to 3, wherein said engineered sucrose phosphorylase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered sucrose phosphorylase variant set forth in the even numbered sequences of SEQ ID NOS: 4-84.

5. The engineered sucrose phosphorylase of any one of Claims 1 to 4, wherein said engineered sucrose phosphorylase comprises at least one improved property compared to wild-type *Alloscardovia omnicolens* sucrose phosphorylase.

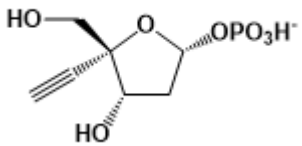
6. The engineered sucrose phosphorylase of Claim 5, wherein said improved property comprises improved activity on a substrate.

7. The engineered sucrose phosphorylase of Claim 6, wherein said substrate comprises sucrose and/or inorganic phosphate.

8. The engineered sucrose phosphorylase of any one of Claims 5 to 7, wherein said improved property comprises improved production of



compound **(1)** and/or



compound **(3)**.

9. The engineered sucrose phosphorylase of any one of Claims 1 to 8, wherein said engineered sucrose phosphorylase is purified.

10. The engineered sucrose phosphorylase of any one of Claims 1 to 9, wherein said engineered sucrose phosphorylase is part of a multi-enzyme system for producing a nucleoside analogue.

11. A composition comprising at least one engineered sucrose phosphorylase of any one of Claims 1 to 10.

12. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase of any one of Claims 1 to 10.

13. A polynucleotide sequence encoding at least one engineered sucrose 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 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.

14. A polynucleotide sequence encoding at least one engineered sucrose 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 NO: 1, or a functional fragment thereof.

15. The polynucleotide sequence of any one of Claims 12 to 14, wherein said polynucleotide sequence is operably linked to a control sequence.

16. The polynucleotide sequence of any one of Claims 12 to 15, wherein said polynucleotide sequence is codon optimized.

17. The polynucleotide sequence of any one of Claims 12 to 16, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 3-83.

18. An expression vector comprising at least one polynucleotide sequence of any one of Claims 12 to 17.

19. A host cell comprising at least one expression vector of Claim 18 or at least one polynucleotide sequence of any one of Claims 12 to 17.

20. A method of producing an engineered sucrose phosphorylase in a host cell, comprising culturing the host cell of Claim 19, under suitable conditions, such that at least one engineered sucrose phosphorylase is produced.

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

22. The method of Claim 20 or 21, further comprising the step of purifying said at least one engineered sucrose phosphorylase.