

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 and/or 4, or a functional fragment thereof, wherein the polypeptide sequence of said engineered sucrose phosphorylase comprises at least one substitution or substitution set and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2 and/or 4.

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 comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from 397, 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, wherein said polypeptide sequence of said engineered sucrose 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 sucrose phosphorylase comprises at least one substitution or substitution set at one or more positions selected from 10/215/400, 158, 158/207/215, 158/207/215/301/400, 158/207/215/400, 158/207/400, 158/211/400, 158/215/301/400, 158/215/400, 158/301/400, 158/400, 205, 207, 207/215, 207/215/400, 207/400, 215/301, 215/400, 242/400, 301, 301/400, and 400, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered sucrose phosphorylase of Claim 1, 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.

5. The engineered sucrose phosphorylase of Claim 1, 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 SEQ ID NO: 2 and/or 4.

6. The engineered sucrose phosphorylase of Claim 1, wherein said engineered sucrose phosphorylase comprises a variant engineered sucrose phosphorylase set forth in SEQ ID NO: 4.
7. The engineered sucrose phosphorylase of Claim 1, 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.
8. The engineered sucrose phosphorylase of Claim 1, wherein said engineered sucrose phosphorylase comprises a polypeptide sequence forth in at least one of the even numbered sequences of SEQ ID NOS: 4-84.
9. The engineered sucrose phosphorylase of any of Claims 1-8, wherein said engineered sucrose phosphorylase comprises at least one improved property compared to wild-type *Alloscardovia omnicolens* sucrose phosphorylase.
10. The engineered sucrose phosphorylase of Claim 9, wherein said improved property comprises improved activity on a substrate.
11. The engineered sucrose phosphorylase of Claim 10, wherein said substrate comprises sucrose and/or inorganic phosphate.
12. The engineered sucrose phosphorylase of any of Claims 9-11, wherein said improved property comprises improved production of compound (1) and/or compound (3).
13. The engineered sucrose phosphorylase of any of Claims 1-12, wherein said engineered sucrose phosphorylase is purified.
14. The engineered sucrose phosphorylase of any of Claims 1-13, wherein said engineered sucrose phosphorylase is part of a multi-enzyme system for producing a nucleoside analogue.
15. A composition comprising at least one engineered sucrose phosphorylase of any of Claims 1-14.
16. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase of any of Claims 1-14.

17. 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 NOS: 1 and/or 3, wherein the polynucleotide sequence of said engineered sucrose phosphorylase comprises at least one substitution at one or more positions.

18. 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 NOS: 1 and/or 3, or a functional fragment thereof.

19. The polynucleotide sequence of any of Claims 16-18, wherein said polynucleotide sequence is operably linked to a control sequence.

20. The polynucleotide sequence of any of Claims 16-19, wherein said polynucleotide sequence is codon optimized.

21. The polynucleotide sequence of any of Claims 16-20, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 3-83.

22. An expression vector comprising at least one polynucleotide sequence of any of Claims 16-21.

23. A host cell comprising at least one expression vector of Claim 22.

24. A host cell comprising at least one polynucleotide sequence of any of Claims 16-21.

25. A method of producing an engineered sucrose phosphorylase in a host cell, comprising culturing the host cell of Claim 23 and/or 24, under suitable conditions, such that at least one engineered sucrose phosphorylase is produced.

26. The method of Claim 25, further comprising recovering at least one engineered sucrose phosphorylase from the culture and/or host cell.

27. The method of Claim 25 and/or 26, further comprising the step of purifying said at least one engineered sucrose phosphorylase.