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~~a substitution at position 397 ~~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 further 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~~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.

~~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~~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.

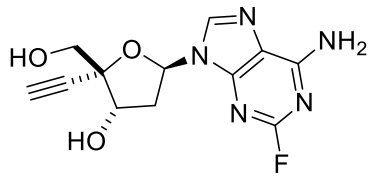
~~8. The engineered sucrose phosphorylase of Claim 1, wherein said engineered sucrose phosphorylase comprises a polypeptide sequence forth in at least one of the even numbered sequences of SEQ ID NOS: 4-84.~~

~~9~~5. The engineered sucrose phosphorylase of any one of Claims 1 to 4~~-8~~, wherein said engineered sucrose phosphorylase comprises at least one improved property compared to wild-type *Alloscardovia omnicolens* sucrose phosphorylase.

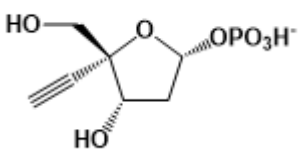
~~10~~6. The engineered sucrose phosphorylase of Claim ~~9~~5, wherein said improved property comprises improved activity on a substrate.

~~11~~7. The engineered sucrose phosphorylase of Claim ~~10~~6, wherein said substrate comprises sucrose and/or inorganic phosphate.

~~12~~8. The engineered sucrose phosphorylase of any one of Claims ~~9-11~~5 to 7, wherein said improved property comprises improved production of



compound **(1)** and/or



compound **(3)**.

~~13~~9. The engineered sucrose phosphorylase of any one of Claims 1 to 8~~ 12~~, wherein said engineered sucrose phosphorylase is purified.

~~14~~10. The engineered sucrose phosphorylase of any one of Claims 1 to 9~~ 13~~, wherein said engineered sucrose phosphorylase is part of a multi-enzyme system for producing a nucleoside analogue.

~~15~~11. A composition comprising at least one engineered sucrose phosphorylase of any one of Claims 1 to 10~~ 14~~.

~~16~~12. A polynucleotide sequence encoding at least one engineered sucrose phosphorylase of any one of Claims 1 to 10~~ 14~~.

~~17~~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~~S~~: 1 ~~and/or 3~~, wherein the polynucleotide sequence of said engineered sucrose phosphorylase comprises ~~at least one~~a substitution at a ~~one or more~~ position~~s~~ encoding amino acid position 397, numbered with reference to SEQ ID NO: 2.

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

~~19~~15. The polynucleotide sequence of any one of Claims ~~16~~12 to ~~18~~ 14, wherein said polynucleotide sequence is operably linked to a control sequence.

~~20~~16. The polynucleotide sequence of any one of Claims ~~16~~12 to ~~19~~15, wherein said polynucleotide sequence is codon optimized.

~~21~~17. The polynucleotide sequence of any one of Claims ~~16~~12 to 16~~-20~~, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOS: 3-83.

~~22~~18. An expression vector comprising at least one polynucleotide sequence of any one of Claims ~~16~~12 to 17~~-21~~.

~~23~~19. A host cell comprising at least one expression vector of Claim ~~22~~18 or ~~.~~

~~24. A host cell comprising~~ at least one polynucleotide sequence of any one of Claims ~~16~~12 to 17~~-21~~.

~~25~~20. A method of producing an engineered sucrose phosphorylase in a host cell, comprising culturing the host cell of Claim ~~23~~ 19~~and/or 24~~, under suitable conditions, such that at least one engineered sucrose phosphorylase is produced.

~~26~~21. The method of Claim ~~25~~20, further comprising recovering at least one engineered sucrose phosphorylase from the culture and/or host cell.

~~27~~22. The method of Claim ~~25~~ 20 ~~and/~~or ~~26~~21, further comprising the step of purifying said at least one engineered sucrose phosphorylase.