CLAIMS

We claim:

1. An engineered phenylalanine ammonia lyase 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, 4, 8, 106, 252, 446, 482, 516, 618, 714, 830, 894, and/or 988, or a functional fragment thereof~~, wherein said engineered phenylalanine ammonia lyase comprises a substitution at position 104 and at least one substitution ~~or substitution set~~ selected from positions 220, 222, and 359 in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~2, 4, 8, 106, 252, 446, 482, 516, 618, 714, 830, 894, 988, and/or 1140, respectively~~.

~~2. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase comprises 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: 4, or a functional fragment thereof, and wherein said engineered phenylalanine ammonia lyase comprises at least one substitution or substitution set at one or more positions selected from 80/99/104/175/220/359, 80/104, 80/104/105/172, 80/104/105/172/175/222/359, 80/104/105/172/220/222, 80/104/105/220, 80/104/105/220/222/416, 80/104/105/222, 80/104/172/175, 80/104/172/175/220/310/359, 80/104/172/222, 80/104/359/416, 84, 90, 99/104/105/172/175/220/222, 100, 101, 104, 104/105/175, 104/172/310/359, 104/175/213/222/359, 104/175/220/222, 104/220/222/359, 104/359, 107, 108, 110/419, 175/315, 219, 219/540, 220, 347, 360, 363, 405, 416, 418, 423, 450, 451, and 452, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.~~

~~3~~2. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase ~~comprises 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: 8, or a functional fragment thereof, and wherein said engineered phenylalanine ammonia lyase~~ further comprises at least one substitution or substitution set at one or more positions selected from 20/306/564, 74/80/105/107/394/420, 74/83/102/105/107/111/222/394/416, 74/97/105/106/107, 74/102/105/106/107/175/394, 74/102/105/106/107/175/394/421, 80/84/99/104/105/107/219, 80/102/105/107/304, 83/102/105/106/107/416/420/421, 83/102/105/394/416/420, 84, 84/99, 84/99/104/105/219, 84/107, 97/102/105/106/107/111/394/420/421, 97/102/105/107/111/175/304/421/424, 97/102/111/175/222/420/421, 97/105/107/111/222/421/424, 97/105/107/111/394/416/421, 99/105/107, 102, 102/105/107/222/304/307/394/421/424,

102/105/107/222/304/394/421/424, 102/105/107/304/424, 102/105/107/394/416/424, 102/107/111/222/394, 102/107/420/424, 103, 104, 105, 105/106/107/420/421, 105/107, 105/107/111, 105/107/111/304, 105/107/111/394/420/424, 105/107/222/304/416, 105/111/219, 105/175/219, 105/219, 106, 107, 107/111/209/222/304, 107/222/304, 107/291, 107/421, 175, 216, 219, 220, 222/421/424, 304/394/416/420, 306, 359, 394, 395, 413, 416, 418, and 420, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 8.

~~4~~3. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 106, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 3, 3/550, 4, 5, 6, 7, 10, 14, 22, 22/76, 24, 25, 40, 75, 76, 76/561, 84/107/175/219, 84/107/219, 102/107/219/410, 107, 107/216/410, 107/219, 107/220, 212, 219/220, 219/220/410, 220/359, 220/410, 286, 301, 303, 410, 502, 544, 566, and 567, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~106~~8.

~~5~~4. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 252, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 3/4/5/7/76/84/107/307, 3/5/107/307/566, 3/7/76/107/307/566, 3/7/84/307, 24/76/107/307, 24/84/107/307, 76, 76/84/107, 76/84/107/307, 76/84/107/307/502, 76/84/107/502, 76/107, 76/107/307, 76/307, 76/307/502, 84/107/307, 84/107/307/502, 84/301/307/566, 84/307, 107/301/502, 107/307, 107/307/566, 107/502, 107/502/566, 307, 307/502, and 307/566, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~252~~8.

~~6~~5. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 446, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 3/4/6/7/76, 3/4/6/7/303, 3/4/7, 3/4/7/76, 3/6/502, 3/7/76, 7/303, 40/303, 76, 76/502, 82, 100, 102, 171, 174, 216, 218, 219, 222, 222/509, 303, 303/502, 304, and 345, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~446~~8.

~~7~~6. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 482, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 3/4/7, 3/7, 4/7, 4/7/216/218, 7, 7/40, 7/76/82/174/222/303, 7/76/174/222, 7/76/219/303, 7/82, 7/216/218, 40/82, 47, 66, 76/82/216/218, 76/216, 76/216/219, 82, 112, 171, 174/222, 209, 216, 219, 219/345, 222, 268, 271, 331, 366, 428, 437, 443, 460, 474, 503, 524, 538, and 543, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~482~~8.

~~8~~7. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 516, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 4, 4/304, 6, 7, 9, 16, 20, 25, 40, 40/437, 44/47, 44/47/94/509, 44/94/270/554, 44/94/554, 47, 47/76, 47/76/345, 47/94/509, 47/195/554, 47/428, 51/106, 76, 76/271, 76/345, 82, 84, 94/149, 94/195, 94/554, 98, 98/460, 109, 112/524, 271/345, 271/428, 302, 303, 304, 306, 349, 358, 410, 413, 416, and 524, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~516~~8.

~~9~~8. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 618, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 4/47/76/82/94, 7/44/94/98, 7/47/76/82, 7/76/554, 7/94/98, 16/40/76, 16/44/76/98, 16/44/94/98/524, 25/54/68/72, 25/54/68/72/158/339, 25/54/68/72/209/212/339/517, 25/54/68/158/339/517, 25/54/72/339/517, 25/54/158/209/212/339/551, 30/68/72/207/209/212/339/495/517, 40/44/76/304/509, 40/44/98/304, 40/76/304/437, 40/76/554, 44/76/94/112/304, 44/76/112, 44/94/271/304/437/554, 47/76/82/94/271, 47/76/82/271/304, 47/76/94/271, 47/76/94/271/306/375/524/554, 47/76/304/306/554, 47/76/304/524/554, 47/94, 47/94/271, 47/94/271/304/554, 49/114/240/521, 54/68/158/209/212/495/517, 68/72/158/209/212/339/495/551, 68/72/158/517, 68/158/209/495/517/551, 76, 76/271/304/554, 76/304/437, 82, 82/554, 94/98, 94/98/306, 94/98/509, 94/98/524, 94/554, 98/270/304/554, 119, 294, 357, 400, 516, 527, and 565, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~618~~8.

~~10~~9. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 714, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 25, 25/40/158/209/304/410/517, 25/54/271/517, 25/158/209/410, 25/306/339, 25/410, 40/47/68/94/98/410/517, 40/68/460/517, 47/98/339/410, 54/68/72/98/209/517, 68, 68/339/517, 72/94/158/339/410/460/517, 72/158/209/410/517, 83, 94/158/209/339/410, 100, 129, 158, 158/207/339/410, 158/209/410/517, 207/410, 207/410/460/517, 220, 317, 339, 394, 410, 410/517, 416, 460, 460/517, and 517, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~714~~8.

~~11~~10. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 830, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 25/83/158/220/517, 25/83/220/416, 25/158/209/220/517, 25/158/220, 25/158/220/517, 25/220/339, 25/220/517, 25/410/416/517, 40, 45, 54/59, 54/285, 83, 83/209/220/410/517, 83/339/410, 119, 158/220/271/517, 209, 220, 220/410/416/517, 220/517, 244, 246, 271, 271/410/416/517, 293, 304, 339, 368, 400, 410, 410/416/517, 424, 459, 479, 520, 525, 537, 562, and 565, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~830~~8.

~~12~~11. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 894, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 25/40/45/209/424, 25/40/424, 25/45/54/73/246/424, 25/54/73/209/424/520, 40/47/54/214/503, 40/54/209/214/244/339/520, 40/54/214/244/339/503, 40/209/246/424, 54, 54/209/214/244, 54/209/214/244/339/503, 54/424, 54/424/520, 209/503, 227, 246, 246/424, 274/311, 410, 411, 413, and 424, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~894~~8.

~~13~~12. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 988, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 40, 40/54/214/421/424, 40/90/421/424, 40/214, 40/214/424, 40/421/424, 40/424, 54/214, 66, 90/214/424, 106/227, 106/227/244, 106/227/244/554, 106/227/554, 214, 214/421, 339, 421/424, 424, 454, 463, 464, 474, and 54340, 40/54/214/421/424, 40/90/421/424, 40/214, 40/214/424, 40/421/424, 40/424, 54/214, 66, 90/214/424, 106/227, 106/227/244, 106/227/244/554, 106/227/554, 214, 214/421, 339, 421/424, 424, 454, 463, 464, 474, and 543, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~988~~8.

~~14~~13. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 988, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 36, 40/66/227, 40/66/227/244/410/424, 40/66/410/474, 40/410/411/424, 47, 66, 66/214/374/410/474, 66/214/424, 66/214/437/474, 66/227, 66/227/244/424/543, 66/227/424, 66/339, 66/339/410/543, 66/339/474, 66/370, 66/410/424/454/527, 66/424, 66/463/464, 66/543, 102, 104, 105, 154, 214/244/543, 214/374/424, 227, 227/244/411/424, 227/339/413/437, 244/411, 339, 394, 410, 410/411/424, 413, 421, 424, 517, 524, and 554, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~988~~8.

~~15~~14. The engineered phenylalanine ammonia lyase of Claim 1, ~~wherein said engineered phenylalanine ammonia lyase comprises 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: 1140, or a functional fragment thereof, and~~ wherein said engineered phenylalanine ammonia lyase further comprises at least one substitution or substitution set at one or more positions selected from 36/47/424/517/554, 47/214/413/524/563, 47/410/524, 47/524, 47/554, 214, 214/424, 410, 410/517/554, 410/554, 424/517/554, 517/524/554, and 554, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: ~~1140~~8.

~~16. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase 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, 4, 8, 106, 252, 446, 482, 516, 618, 714, 830, 894, 988, and/or 1140, or a functional fragment thereof.~~

~~17~~15. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered

phenylalanine ammonia lyase comprises the SEQ ID NO: 4, 8, 106, 252, 446, 482, 516, 618, 714, 830, 894, 988, 1140, or a functional fragment thereof.

18. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase 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 phenylalanine ammonia lyase variant set forth in Table 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 11.1. 12.1, 13.1, 14.1, 15.1, 18.1, and/or 19.1.

19. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase 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 phenylalanine ammonia lyase variant set forth in the even numbered sequences of SEQ ID NOS: 4-1222.

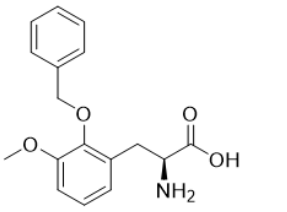
20. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase comprises a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered phenylalanine ammonia lyase variant set forth in the even numbered sequences of SEQ ID NOS: 4-1222.

21. The engineered phenylalanine ammonia lyase of Claim 1, wherein said engineered phenylalanine ammonia lyase comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOS: 4-1222.

2216. The engineered phenylalanine ammonia lyase of any of Claims 1-21, wherein said engineered phenylalanine ammonia lyase comprises a polypeptide sequence that exhibits at least one improved property compared to wild-type *Anabaena variabilis* phenylalanine ammonia lyase.

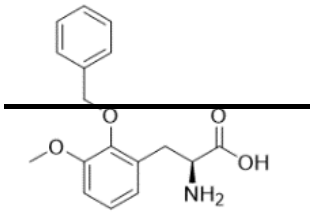
23. The engineered phenylalanine ammonia lyase of Claim 22, wherein said improved property comprises improved production of compound 2.

2417. The engineered phenylalanine ammonia lyase of Claim 2216, wherein said improved property comprises improved production of

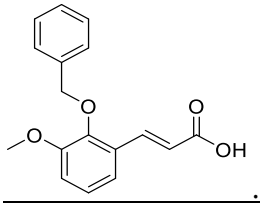


.

~~25~~18. The engineered phenylalanine ammonia lyase of Claim ~~22~~16, wherein said improved property comprises improved utilization of

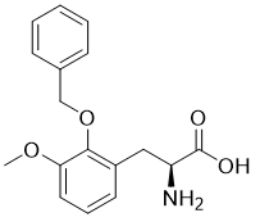


.

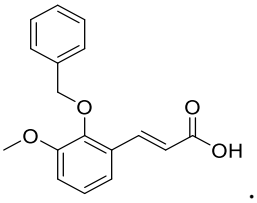


.

~~26~~19. The engineered phenylalanine ammonia lyase of Claim ~~22~~16, wherein said improved property comprises improved production of



from



.

~~27~~20. The engineered phenylalanine ammonia lyase of Claim ~~22~~16, wherein said improved property comprises improved enantioselectivity.

~~28~~21. The engineered phenylalanine ammonia lyase of Claim ~~22~~16, wherein said improved property comprises improved stability.

~~29~~22. The engineered phenylalanine ammonia lyase of ~~any of~~ Claim~~s~~ 1~~-28~~, wherein said engineered phenylalanine ammonia lyase is purified.

~~30. A composition comprising at least one phenylalanine ammonia lyase provided in any of Claims 1-29.~~

~~31~~23. ~~An engineered~~ A polynucleotide sequence encoding at least one engineered phenylalanine ammonia lyase of ~~any of~~ Claim~~s~~ 1~~-30~~.

~~32~~24. An engineered polynucleotide sequence encoding at least one engineered phenylalanine ammonia lyase of Claim 1, wherein 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, 3, 7, 105, 251, 445, 481, 515, 617, 713, 829, 893, 987, and/or 1139, wherein the polynucleotide sequence of said engineered phenylalanine ammonia lyase comprises at least one substitution at one or more positions.

~~33~~25. The engineered polynucleotide sequence of Claim ~~31 or 32~~23, wherein 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, 3, 7, 105, 251, 445, 481, 515, 617, 713, 829, 893, 987, and/or 1139.

~~34~~26. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~29-31~~23, wherein said polynucleotide sequence comprises SEQ ID NO: 1, 3, 7, 105, 251, 445, 481, 515, 617, 713, 829, 893, 987, and/or 1139.

~~35~~27. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~29-32~~23, wherein said polynucleotide sequence comprises a sequence set forth in the odd-numbered sequences set forth in SEQ ID NOS: 3-1221.

~~36~~28. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~31-35~~23, wherein said polynucleotide sequence is operably linked to a control sequence.

~~37~~29. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~21-36~~23, wherein said engineered polynucleotide sequence is codon optimized.

~~38~~30. An expression vector comprising at least one polynucleotide sequence of ~~any of~~ Claims ~~31-37~~23.

~~39~~31. A host cell comprising at least one expression vector of Claim ~~38~~30.

~~40~~32. A host cell comprising at least one polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~31-37~~23.

~~41~~33. A method of producing an engineered phenylalanine ammonia lyase in a host cell, comprising culturing the host cell of Claim ~~39 and/or 40~~32, in a culture medium under suitable conditions, such that at least one engineered phenylalanine ammonia lyase is produced.

~~42~~34. The method of Claim ~~41~~33, further comprising the step of recovering at least one engineered phenylalanine ammonia lyase from said culture medium and/or said host cell.

~~43~~35. The method of Claim ~~41 and/or 42~~33, further comprising the step of purifying said at least one phenylalanine ammonia lyase.