

CLAIMS

We claim:

1. An engineered transaminase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NOS: 2, 4, 8, 366, and/or 650, or a functional fragment thereof, wherein said engineered transaminase comprises at least one substitution or substitution set 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, 366, and/or 650.
2. The engineered transaminase 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% sequence identity to SEQ ID NO: 4, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 21/163/286/291/314/316/323/383/388/408/414/416, 18, 20, 21, 21/23/56/146, 21/23/56/146/432, 21/23/146/417, 21/23/395/417/432, 21/53/56, 21/53/417, 21/56/395, 21/417/432, 23, 23/53, 23/53/56, 23/53/56/146/395, 23/53/395, 23/53/417, 23/53/432, 23/56, 23/56/395, 23/56/395/417, 23/395/417, 23/417, 23/417/432, 53, 53/56, 53/146/417, 53/395, 56, 56/74/241/286/314/316/323, 56/86/163/314/316/383/414/416/422, 56/86/286/314/414/416, 56/86/314/316/323/394/414/422, 56/146/417, 56/146/432, 56/147, 56/163, 56/163/286/316/323/383/394, 56/286/314/316/323/422, 56/286/383, 56/323, 56/323/383, 56/323/383/394, 56/383, 56/395, 74/81/286/316/323/383, 74/85/86/163/286/316/323/394, 74/85/314/316/414/416, 74/86/163/316, 74/86/316/323/383/394, 74/88/286/316/323/383, 74/88/323/383, 74/163/286/316/383/394/416, 74/163/314/316, 74/163/314/316/323/394, 74/163/314/323/383/414/416, 74/286, 74/286/316/323, 74/286/394/416, 74/314/323/383/394/414, 74/316/323/394, 85/86/88/163/323/383/394, 85/86/163/314/323/394/414, 85/286, 85/286/323, 86, 86/88/163/323/383/414/422, 86/383/394, 88, 88/163/286/383, 88/286/316/323, 88/286/316/323/383/414/416, 88/316/323, 146, 146/147/395/417, 146/395, 146/395/417, 146/417, 147/395/417/432, 147/417, 149, 157, 163, 163/222/286/316/323/383/394, 163/286, 163/286/314/316/323/414/416, 163/286/314/323/394, 163/286/316/323/394/416, 163/286/414, 163/314/316/394, 163/314/323/394, 163/314/383, 163/314/414, 163/316/323, 163/323, 163/383, 164, 199/417, 259, 260, 284, 286, 286/314/323/383, 286/314/394, 286/316/323/383/414/416, 286/316/383/394, 286/316/394/414/416, 286/323, 286/323/383/414, 286/323/416, 286/383, 286/416, 314/316, 314/316/323, 314/316/323/383/422, 314/316/323/394, 314/316/394, 314/323/383/394, 314/383, 314/383/414/422, 315, 316, 316/323/383/394, 316/323/394/414/416, 316/414/422, 323, 323/383, 323/383/394/414/416, 323/394, 383, 395, 395/417, 395/417/432, 400, 401, 403, 404, 405, 406, 408, 415,

417, 417/432, 420, and 422, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

3. The engineered transaminase 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% sequence identity to SEQ ID NO: 4, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 74/81/286/316/323/383, 163/286/314/316/323/414/416, 163/286/314/323/394, 286/314/323/383, 286/316/323/383/414/416, 315, and 408, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered transaminase 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% sequence identity to SEQ ID NO: 8, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 5, 18/23/149/260/383/395/401/416, 18/23/149/383, 18/163/164, 21, 21/163/315/316, 21/163/323/408, 21/408, 23/56/86/149/163/164/383/401/416, 23/86, 23/149/260, 23/149/284/383/395, 23/163/164/383, 23/163/164/401/416, 24, 42, 42/110, 42/187/272, 42/187/324/363/366, 42/187/353, 42/272/291, 42/272/291/363, 42/272/324/363/366, 42/272/363/410, 42/272/410, 42/291/313/363/410, 42/291/363, 42/291/363/366, 42/353, 42/363, 46, 66, 77, 86/149/163/164/383/395/401, 86/149/395, 86/163/164/260/383, 86/383, 107, 110, 110/187, 110/187/253/410, 134, 138, 149/164/260/383/395/401, 149/260/383, 149/416, 163/259/323/408, 163/259/408, 163/315/316, 164/260/401, 164/316/383/401, 167, 186, 187, 187/253/363/366, 187/272/324/363/410, 187/272/363, 187/272/363/366/410, 187/291, 189, 191, 195, 199, 203, 210, 211, 248, 259/307, 260/395/401, 272, 272/353, 272/363/366, 272/410, 277, 291, 305, 309, 315, 342, 343, 351, 354, 358, 361, 362, 363, 363/366, 365, 367, 383, 383/401, 383/416/422, 385, 388, 389, 392, 395, 396, 401, 404, 405, 408, 410, 416, 417, 439, 443, 447, 450, and 451, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 8.

5. The engineered transaminase 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% sequence identity to SEQ ID NO: 8, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 18/23/149/383, 21/163/323/408, 272, 291, and 383, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 8.

6. The engineered transaminase 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% sequence identity to SEQ ID NO: 366, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 24, 24/42/66/291, 24/42/291/362, 24/66/163/191/362/383/388, 24/66/191/199/260/291/351, 24/66/191/199/291, 24/66/191/260/408, 24/66/260/291/383/388/408, 24/66/291/342/383, 24/66/291/365, 24/66/342/365/388/408, 24/77/291, 24/107/163/191/291/351/383/388, 24/107/291/351/365/388, 24/163/351/383, 24/191/291/365, 24/199/260/351/362/383, 24/199/260/362/383/388, 24/260/362/383/388, 24/291, 24/291/342/351/383, 24/291/362/388, 24/291/408, 24/383/388, 24/388, 25, 28, 33, 42/191/408, 42/199/291/383, 42/291/351/362/365/383/388, 42/291/351/362/383/408, 42/291/383/388, 66/82/291/383, 66/163/191/365/383, 66/199/351/383, 66/291, 66/291/362/365/383, 66/291/383/388, 66/383, 77/291, 77/383/388, 86, 107/191/199/365/383/388, 107/191/291/383, 148, 153, 163/291/362/365/383/388, 163/291/383/388, 163/383, 191/199/365/383/388, 191/260/388, 191/291, 191/291/342/362/365, 191/351/383/388, 199/260/383, 199/291, 260, 260/291/365/383/408, 260/365/383, 291, 291/351/383/388, 291/351/383/388/408, 291/362/365, 291/365/388, 291/383, 314, 315, 316, 319, 342/362, 351/383/388, 362, 362/388, 383, 383/388, 396, 397, 405, 406, 413, 419, and 423, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 366.

7. The engineered transaminase 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: 366, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 24/66/191/199/291, 24/66/291/365, 163/291/362/365/383/388, 163/291/383/388, 191/291/342/362/365, 291, and 291/383, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 366.

8. The engineered transaminase 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% sequence identity to SEQ ID NO: 650, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 10, 13, 13/24/108/163, 13/24/108/163/311, 13/24/133/199/311, 13/24/163, 13/24/199/311, 13/108, 13/108/199, 13/108/311, 13/199, 13/311, 14, 14/24/108, 14/24/108/133, 14/24/108/199, 14/24/199, 14/108, 14/108/133/311, 14/108/311, 14/311, 24, 24/163, 24/163/199, 35, 72, 73, 78, 95, 101, 108, 108/199, 114, 154, 163, 169, 175/316, 199, 199/311, 226, 293, 311, 316, 382, 383, and 386, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 650.

9. The engineered transaminase 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% sequence identity to SEQ ID NO: 650, wherein said engineered transaminase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 14/108/133/311, 24/163/199, 72, 78, 316, and 383, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 650.

10. The engineered transaminase of Claim 1, wherein said engineered transaminase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the sequence of at least one engineered transaminase variant set forth in Table 2-1, 2-2, 3-1, 3-2, 4-1, 4-2, 5-1, and/or 5-2.

11. The engineered transaminase of Claim 1, wherein said engineered transaminase is a variant engineered transaminase provided in Table 2-1, 2-2, 3-1, 3-2, 4-1, 4-2, 5-1, and/or 5-2.

12. The engineered transaminase of Claim 1, wherein said engineered transaminase is a variant engineered transaminase set forth in SEQ ID NOS: 2, 4, 8, 366, and/or 650.

13. The engineered transaminase of Claim 1, wherein said engineered transaminase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the sequence of at least one engineered transaminase variant set forth in the even numbered sequences of SEQ ID NOS: 6-936.

14. The engineered transaminase of Claim 1, wherein said engineered transaminase comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOS: 6-936.

15. The engineered transaminase of any of Claims 1-14, wherein said engineered transaminase comprises at least one improved property compared to wild-type *V. fluvialis* transaminase.

16. The engineered transaminase of Claim 15, wherein said improved property comprises improved activity on a substrate.

17. The engineered transaminase of Claim 16, wherein said substrate comprises compound (1).

18. The engineered transaminase of any of Claims 15, wherein said improved property comprises improved thermostability.

19. The engineered transaminase of any of Claims 1-18, wherein said engineered transaminase is purified.
20. A composition comprising the engineered transaminase of any of Claims 1-19.
21. A polynucleotide sequence encoding at least one engineered transaminase of any of Claims 1-19.
22. A polynucleotide sequence encoding at least one engineered transaminase of any Claims 1-19, 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 NOS: 1, 3, 7, 365, and/or 649, and wherein the polynucleotide sequence of said engineered transaminase comprises at least one substitution at one or more positions.
23. A polynucleotide sequence encoding at least one engineered transaminase 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: 2, 4, 8, 366, and/or 650, or a functional fragment thereof.
24. The polynucleotide sequence of any of Claims 21-23, wherein said polynucleotide sequence is operably linked to a control sequence.
25. The polynucleotide sequence of any of Claims 21-24, wherein said polynucleotide sequence is codon optimized.
26. An expression vector comprising at least one polynucleotide sequence of any of Claims 21-25.
27. A host cell comprising at least one expression vector of Claim 26.
28. A host cell comprising at least one polynucleotide sequence of any of Claims 21-25.
29. A method of producing an engineered transaminase in a host cell, comprising culturing the host cell of Claim 27 and/or 28, under suitable conditions, such that at least one engineered transaminase is produced.

30. The method of Claim 29, further comprising recovering at least one engineered transaminase from the culture and/or host cell.

31. The method of Claim 29 and/or 30, further comprising the step of purifying said at least one engineered transaminase.