

CLAIMS

1. A UvsX recombinase polypeptide comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1, which UvsX recombinase polypeptide comprises an amino acid substitution mutation at the position functionally equivalent to position 256 in the Enterobacteria phage RB49 UvsX of SEQ ID NO: 1, the UvsX recombinase polypeptide having increased recombinase activity compared to a T4 UvsX recombinase polypeptide having SEQ ID NO: 8 or a RB49 UvsX recombinase polypeptide having all of the amino acid sequence of SEQ ID NO: 1 except for a His to Ser substitution at amino acid position 63 of SEQ ID NO: 1.
2. A UvsX recombinase polypeptide according to Claim 1, in which the substitution mutation comprises a mutation to a charged residue.
3. A UvsX recombinase polypeptide according to Claim 1, in which the substitution mutation comprises a mutation to a basic residue.
4. A UvsX recombinase polypeptide according to Claim 1, in which the amino acid substitution mutation replaces the residue at the position functionally equivalent to position 256 in the polypeptide of SEQ ID NO: 1 with a lysine (Lys).
5. A UvsX recombinase polypeptide according to Claim 1, in which the UvsX recombinase polypeptide further comprises a substitution mutation at a position functionally equivalent to position 63 in the polypeptide of SEQ ID NO: 1.
6. A UvsX recombinase polypeptide according to Claim 5, in which the substitution mutation replaces the residue at a position functionally equivalent to position 63 in the RB49 UvsX polypeptide of SEQ ID NO: 1 with a serine (Ser).
7. A UvsX recombinase polypeptide according to Claim 1, further comprising the addition of one or more glutamic acid residues at the C-terminus; the addition of one or more aspartic acid residues at the C-terminus; or a combination thereof.
8. A UvsX recombinase polypeptide according to Claim 1, in which the UvsX recombinase polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 2.
9. A UvsX recombinase polypeptide comprising the amino acid sequence SEQ ID NO: 2.

10. A UvsX recombinase polypeptide comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and comprising a semiconserved domain, the semiconserved domain comprising all of SEQ ID NO: 3, 4 or 5 except for a substitution at position 7 of SEQ ID NO: 3, 4, or 5 to any residue other than Phe, Pro, Asp, Glu or Asn, the UvsX recombinase polypeptide having increased recombinase activity compared to a T4 UvsX recombinase polypeptide having SEQ ID NO: 8 or a RB49 UvsX recombinase polypeptide having all of the amino acid sequence of SEQ ID NO: 1 except for a His to Ser substitution at amino acid position 63 of SEQ ID NO: 1.
11. A UvsX recombinase polypeptide according to Claim 10, in which the substitution at position 7 comprises a mutation to a charged residue.
12. A UvsX recombinase polypeptide according to Claim 11, in which the substitution at position 7 comprises a mutation to Lys.
13. A UvsX recombinase polypeptide comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and comprising a semiconserved domain, the semiconserved domain comprising all of SEQ ID NO: 6 or 7 except for a substitution at position 12 of SEQ ID NO: 6 or 7 to any residue other than Phe, Pro, Asp, Glu or Asn, the UvsX recombinase polypeptide having increased recombinase activity compared to a T4 UvsX recombinase polypeptide having SEQ ID NO: 8 or a RB49 UvsX recombinase polypeptide having all of the amino acid sequence of SEQ ID NO: 1 except for a His to Ser substitution at amino acid position 63 of SEQ ID NO: 1.
14. A UvsX recombinase polypeptide according to Claim 13, in which the substitution at position 12 comprises a mutation to a charged residue.
15. A UvsX recombinase polypeptide according to Claim 14, in which the substitution at position 12 comprises a substitution to Lys.
16. A UvsX recombinase polypeptide comprising all of the amino acid sequence of SEQ ID NO: 1 except for a proline (Pro) to lysine (Lys) substitution at amino acid position 256.
17. A UvsX recombinase polypeptide according to Claim 9, further comprising the addition of one or more glutamic acid residues at the C-terminus; the addition of one or more aspartic acid residues at the C-terminus; or a combination thereof.

18. A UvsX recombinase polypeptide according to Claim 1, in which the substitution mutation replaces the residue at the position corresponding to position 256 of the polypeptide of SEQ ID NO: 1 with any residue other than Phe, Pro, Asp, Glu or Asn.
19. A UvsX recombinase polypeptide according to Claim 1, in which the amino acid substitution mutation replaces the residue at the position functionally equivalent to position 256 of the polypeptide of SEQ ID NO: 1 with a lysine (Lys) and further comprising an amino acid substitution mutation replacing the residue at a position functionally equivalent to position 63 of the polypeptide of SEQ ID NO: 1 with serine (Ser).
20. A UvsX recombinase polypeptide according to Claim 1, in which recombinase activity is increased in the presence of a single stranded template nucleic acid compared to the T4 UvsX recombinase polypeptide having SEQ ID NO: 8 or the RB49 UvsX recombinase polypeptide having all of the amino acid sequence of SEQ ID NO: 1 except for a His to Ser substitution at amino acid position 63 of SEQ ID NO: 1.
21. A UvsX recombinase polypeptide according to Claim 1, the UvsX recombinase polypeptide having improved seeding and/or amplification on a solid support compared to the T4 UvsX recombinase polypeptide having SEQ ID NO: 8 or the RB49 UvsX recombinase polypeptide having all of the amino acid sequence of SEQ ID NO: 1 except for a His to Ser substitution at amino acid position 63 of SEQ ID NO: 1.
22. A UvsX recombinase polypeptide according to Claim 3, in which the basic residue comprises lysine (Lys), arginine (Arg), or histidine (His).
23. A UvsX recombinase polypeptide according to Claim 1, the UvsX recombinase polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.
24. A UvsX recombinase polypeptide according to Claim 1, the UvsX recombinase polypeptide comprising an amino acid sequence that is at least 99% identical to SEQ ID NO: 1.