Int-h (E174K) and its derivative Int-h/218 (E174K/E218K) have been described and were shown to catalyze intermolecular recombination reactions at least as efficiently as the corresponding intramolecular recombination reactions in human cells. Although the presence of arm-site sequences have been shown to increase the recombination of core-sites by Int-h/218 in vivo, given the absence of an attB site in the human genome, recombination reactions occur in non-cognate sites in an essentially random manner.

This makes it difficult to engineer cell lines in a controlled, reproducible fashion.

Therefore, there remains a need to provide mutant integrases having greater efficiency and specificity in catalyzing site specific recombination reactions.

Summary

In one aspect, there is provided a lambda integrase comprising at least one amino acid mutation selected from the group consisting of I43F, E319G and D336V.

In another aspect, there is provided a lambda integrase comprising an amino acid mutation at at least one of positions 336, 319 and 43 of the lambda integrase as set forth in SEQ ID NO: 1

In another aspect, there is provided a nucleic acid molecule comprising a nucleotide sequence encoding the lambda integrase as disclosed herein.

In another aspect, there is provided a nucleic acid molecule. The nucleic acid molecule includes a nucleotide sequence encoding a mutant as described herein.

In a further aspect, there is provided a host cell. The host cell includes a nucleic acid molecule as described herein.

In another aspect, there is provided a method of recombining a nucleic acid of interest into a target nucleic acid, the method comprising contacting a targeting nucleic acid comprising the nucleic acid of interest with the target nucleic acid in the presence of a lambda integrase as disclosed herein.

In yet another aspect, there is provided a method of recombining a nucleic acid of interest into a target nucleic acid. The method includes contacting a targeting nucleic acid comprising the nucleic acid of interest with

the target nucleic acid in the presence of a mutant as described herein.

In another aspect, there is provided a sequence specific recombination kit comprising:

a. a targeting nucleic acid into which a nucleic acid of interest can be inserted, and

b. a lambda integrase as disclosed herein or a nucleic acid as disclosed herein.

In yet a further aspect, there is provided a sequence specific recombination kit. The kit includes a targeting nucleic acid into which a nucleic acid of interest can be inserted, and a mutant as described herein.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "mutant" refers to a protein arising as a result of a mutation or a recombinant DNA procedure.

The term "Int" or "integrase" refers to the lambda phage integrase protein.

As used herein, "nucleic acid" refers to any nucleic acid in any possible configuration, such as linearized single stranded, double stranded or a combination thereof. Nucleic acids may include, but are not limited to DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA) , analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, cDNA synthetic DNA, a copolymer of DNA and RNA, oligonucleotides, and PNA (protein nucleic acids) . DNA or RNA may be of genomic or synthetic origin and may be single or double stranded. A respective nucleic acid may furthermore contain non-natural nucleotide analogues and/or be linked to an affinity tag or a label.

As used herein, nucleotides include nucleoside mono-, di-, and triphosphates. Nucleotides also include modified-nucleotides, such as, but not limited to, phophorothioate nucleotides and deazapurine nucleotides and other nucleotide analogs.

Claims

1. A lambda integrase comprising an amino acid mutation at at least one of positions 336, 319 and 43 of the lambda integrase as set forth in SEQ ID NO: 1.

2. The lambda integrase according to claim 1, wherein the lambda integrase comprises an amino acid mutation at positions 43, 319 and 336 of the lambda integrase as set forth in SEQ ID NO: 1; optionally wherein the amino acid residue isoleucine at sequence position 43 is replaced by an aromatic amino acid; optionally wherein the aromatic amino acid is selected from the group consisting of phenylalanine, tyrosine and tryptophan.

3. The lambda integrase according to claims 1 or 2, wherein the amino acid residue glutamate at sequence position 319 is replaced by glycine.

4. The lambda integrase according to any one of claims 1 to 3, wherein the amino acid residue aspartate at sequence position 336 is replaced by a hydrophobic amino acid; optionally wherein the hydrophobic amino acid is an aliphatic amino acid; optionally wherein the aliphatic amino acid is selected from the group consisting of isoleucine, leucine and valine.

5. The lambda integrase according to any one of claims 1 to 4, wherein the lambda integrase comprises amino acid mutations I43F, E319G and D336V in the lambda integrase as set forth in SEQ ID NO: 3.

6. The lambda integrase according to claim 1, wherein the lambda integrase comprises an amino acid mutation at position 336 of the lambda integrase as set forth in SEQ ID NO: 1; optionally wherein the amino acid residue aspartate at sequence position 336 is replaced by a hydrophobic amino acid; optionally wherein

the hydrophobic amino acid is an aliphatic amino acid; optionally wherein the aliphatic amino acid is selected from the group consisting of isoleucine, leucine and valine.

7. The lambda integrase according to claim 6, wherein the lambda integrase comprises the amino acid mutation D336V in the lambda integrase as set forth in SEQ ID NO: 2.

8. The lambda integrase according to any one of claims 6 or 7, wherein the amino acid residue glutamate at sequence position 319 is replaced by glycine; optionally wherein said lambda integrase comprises the amino acid mutations E319G and D336V in the lambda integrase as set forth in SEQ ID NO: 3.

9. A nucleic acid molecule comprising a nucleotide sequence encoding the lambda integrase according to any of claims 1 to 8.

10. The nucleic acid molecule according to claim 9, wherein the nucleic acid molecule is operably linked to a regulatory sequence to permit expression of the nucleic acid molecule; optionally wherein the regulatory sequence comprises a promoter sequence.

11. The nucleic acid molecule according to claims 9 or 10, wherein the nucleic acid molecule is located in a vector.

12. A host cell containing a nucleic acid molecule according to any one of claims 9 to 11.

13. A method of recombining a nucleic acid of interest into a target nucleic acid, the method comprising contacting a targeting nucleic acid comprising the nucleic acid of interest with the

target nucleic acid in the presence of a lambda integrase as defined in any one of claims 1 to 8.

14. The method according to claim 13, wherein the target nucleic acid comprises DNA; optionally wherein the target nucleic acid comprises genomic DNA; optionally wherein the target nucleic acid comprises a sequence selected from the group consisting of an attH sequence (SEQ ID NO: 7) and an attH4X sequence (SEQ ID NO: 31); optionally wherein the targeting nucleic acid is a vector; optionally wherein the targeting nucleic acid comprises a sequence selected from the group consisting of an attPH sequence (SEQ ID NO: 8) and an attP4X sequence (SEQ ID NO: 9).

15. The method according to claims 13 or 14, wherein the sequence specific recombination is performed in the presence of one or more cofactors; optionally wherein the cofactors is selected from the group consisting of XIS, FIS and IHF.

16. The method according to any one of claims 14 or 15, wherein the genomic DNA is comprised in a cell.

17. A sequence specific recombination kit comprising:

a. a targeting nucleic acid into which a nucleic acid of interest can be inserted, and

b. a lambda integrase according to any one of claims 1 to 8 or a nucleic acid as claimed in any one of claims 9 to 11.

18. The kit according to claim 17, further comprising at least one reagent for inserting said nucleic of interest into said targeting nucleic acid; optionally wherein said targeting nucleic acid comprises a sequence selected from the group consisting of an attPH sequence (SEQ ID NO: 8) and an attP4X sequence (SEQ ID NO: 9); optionally wherein said targeting nucleic acid comprises a sequence selected from the group

consisting of an attH sequence (SEQ ID NO: 7) and an attH4X sequence (SEQ ID NO: 31).

19. The kit according to claims 17 or 18 further comprising buffer (s) and/or instructions for recombining said nucleic acid of interest with a given target nucleic acid.

20. The kit according to any one of claims 17 to 19 further comprising at least one reagent for determining a successful sequence specific recombination event; optionally wherein said reagent is a primer pair.