CLAIMS

WHAT IS CLAIMED IS:

1. A method for constructing a gene mutation library, comprising

(1) synthesizing a pool of oligonucleotide sequences comprising mutant nucleotide(s) at one or more mutation sites;

(2) performing PCR amplification on the oligonucleotides in the synthesized pool of the oligonucleotide sequences comprising the mutant nucleotide(s);

(3) performing linear PCR amplification by using a plasmid comprising methylated sites and the gene to be mutated or a part thereof as a template and using two strands of oligonucleotides in the pool of the amplified oligonucleotide sequences obtained in step (2) as primers to respectively extend to the head and tail ends of the gene to be mutated or a part thereof by controlling the extension time of the PCR reaction, wherein the molar ratio of the primers to the template in step (3) is 15 : 1 to 100 : 1;

(4) using an endonuclease that recognizes and cleaves the methylated site to cleave the template plasmid present in the amplified system of step (3); and

(5) adding a forward primer and a reverse primer respectively corresponding to both ends of the gene to be mutated or the part thereof to the system obtained in step (4) and performing PCR amplification to obtain the gene mutation library comprising a plurality of genes containing the mutant nucleotide(s) or a part thereof.

2. The method according to claim 1, wherein purification is not performed during steps (1)-(5).

3. The method according to claim 1, further comprising

(6) recovering and purifying the amplified product of step (5) to obtain a final product.

4. The method according to claim 3, further comprising

(7) sequencing the final product obtained in step (6) to verify sequence and/or detect gene mutation distribution.

5. The method according to claim 4, wherein the sequencing is Sanger sequencing and/or high-throughput sequencing.

6. The method according to any one of claims 1-5, wherein the length of the oligonucleotide sequences in the pool of the oligonucleotide sequences synthesized in step (1) is

60-170 bp.

7. The method according to claim 6, wherein the length of the oligonucleotide sequences in the pool of the oligonucleotide sequences synthesized in step (1) is 80-90 bp.

8. The method according to any one of claims 1-7, wherein the pool of the oligonucleotide sequences synthesized in step (1) comprises mutant nucleotide(s) at 1 to 10 mutation sites.

9. The method according to any one of claims 1-8, wherein the mutation sites are either adjacent or non-adjacent.

10. The method according to any one of claims 1-9, wherein the amino acid sequence encoded by a gene comprising the mutant nucleotide(s) has at least one amino acid difference compared to the amino acid sequence encoded by the gene to be mutated.

11. The method according to claim 10, wherein the at least one amino acid difference is selected from: substitution, addition or deletion.

12. The method according to claim 11, wherein the at least one amino acid difference is substitution.

13. The method according to claim 12, wherein the substitution is to substitute the original amino acid with an amino acid having different properties from the original amino acid.

14. The method according to claim 13, wherein the properties are selected from: acidity and alkalinity, polarity, charge characteristic and side chain group.

15. The method according to any one of claims 1-14, wherein two primers respectively corresponding to both ends of the oligonucleotide sequences comprising the mutant nucleotide(s) at one or more mutation sites are used in the PCR amplification in step (2).

16. The method according to any one of claims 1-15, wherein the number of cycles of the PCR amplification in step (2) is 10-25 cycles.

17. The method according to claim 16, wherein the number of cycles of the PCR amplification in step (2) is 15-18 cycles.

18. The method according to claim 17, wherein the number of cycles of the PCR amplification in step (2) is 16 cycles.

19. The method according to any one of claims 1-18, wherein the DNA polymerase used in the PCR amplification in step (2) is a high-fidelity DNA polymerase.

20. The method according to any one of claims 1-19, wherein the molar ratio of the primers to the template in step (3) is 15 : 1 to 50 : 1.

21. The method according to claim 20, wherein the molar ratio of the primers to the template in step (3) is 18 : 1 to 25 : 1.

22. The method according to claim 21, wherein the molar ratio of the primers to the template in step (3) is 20 : 1

23. The method according to any one of claims 1-22, wherein the number of cycles of the PCR amplification in step (3) is 10-20 cycles.

24. The method according to claim 23, wherein the number of cycles of the PCR amplification in step (3) is 14-16 cycles.

25. The method according to claim 24, wherein the number of cycles of the PCR amplification in step (3) is 15 cycles.

26. The method according to any one of claims 1-25, wherein the DNA polymerase used in the PCR amplification in step (3) is a high-fidelity DNA polymerase.

27. The method according to claim 26, wherein the DNA polymerase used in the PCR amplification in step (3) is a high-fidelity Phusion DNA polymerase.

28. The method according to any one of claims 1-27, wherein the plasmid is extracted from bacteria that methylate the plasmid.

29. The method according to claim 28, wherein the plasmid is extracted from *E. coli*.

30. The method according to claim 29, wherein the plasmid is extracted from *E. coli* TOP10 strain.

31. The method according to any one of claims 1-30, wherein the endonuclease in step (4) is DpnI, MspJI or FspEI.

32. A method for analyzing the relationship between an amino acid mutation in a protein and the properties, regulation and/or function of the protein, comprising the following steps:

(1) constructing a gene mutation library of a gene encoding the protein using the method according to any one of claims 1-31;

(2) comparing the properties, regulation and/or function of the protein encoded by a mutant gene in the gene mutation library with the properties, regulation and/or function of the unmutated protein; and

(3) analyzing the relationship between the amino acid at the mutation position and the properties, regulation and/or function of the protein.