2024 NEFU_China

RED homologous recombination

1. Construction of Homologous Recombination Fragment:

• Design and construct a DNA fragment with up-kan-down regions (5’ to 3’) that will replace the up-guaB-down fragment in the plasmid. Ensure that the kanamycin resistance gene (kan) is properly flanked by sequences homologous to the regions upstream (up) and downstream (down) of the guaB gene in the plasmid.

2. Preparation for Electroporation:

• Prepare competent E. coli cells suitable for electroporation.

• Mix the desired plasmid and the constructed up-kan-down DNA fragment with the competent cells in a sterile 1.5 mL microcentrifuge tube.

3. Electroporation:

• Set the electroporator to 1400V and a time constant of 3ms.

• Subject the mixture to electroporation to facilitate the entry of the up-kan-down DNA fragment into the bacterial cells. The high voltage will allow the DNA to penetrate the phospholipid membrane of the bacteria.

4. Recovery and Selection:

• Immediately after electroporation, add 1 mL of LB medium to the cells and incubate at 37°C for 1 hour to allow recovery.

• After recovery, plate 100 μL of the transformed cells onto an agar plate containing the appropriate antibiotic (e.g., kanamycin) to select for cells that have incorporated the up-kan-down fragment.

5. Growth and Verification:

• Incubate the plates overnight at 37°C.

- Add 50 μL of xanthine to the plates to test for conditional growth, ensuring that the correct insertion and knockout of the guaB gene have occurred.

6. Analysis:

- After incubation, analyze the bacterial colonies for successful homologous recombination by PCR and sequencing.
- Confirm the knockout of the guaB gene and the correct integration of the kanamycin resistance gene.