SAFETY WARNINGS

Please wear gloves for the experiment.

1. The entire plasmid was amplified reversely by PCR using primers with the fragment sequence that you want to replace.

2. The temple plasmids in the PCR process were digested by DpnI enzyme.

3. The digested product was transferred into DH5α. Overnight culture them at 37°C.

4. To determine whether the vector was constructed successfully, colony PCR and enzyme digestion were done.

5. Select the positive results for sequencing and the final results were obtained.