A. The Preparation of Targeting Vector

<table>
<tr><td>1</td><td>The Design of the Primers</td><td>a. The knockout primers:
agcacttatctggagtttgttatgccacattcactgtGTGTAGGCTGGAGCTGCTTC
gtcatcatgcaaccagcgactaaccgcagttaaagcaATGGGAATTAGCCATGGTCC
(The part of lowercase letters is the kan resistance gene and the capital one is the LysA homology arms)
b. The detection primers:
TAGTAGTCCGACGCTGGTACGTCG
TTGCATAGACTCGACATAAATCGA</td></tr>
<tr><td>2</td><td>PCR</td><td>Amplify the targeting vector, using the plasmid pKD4 as template.
The PCR system:
PCR MIX 12.5μl
plasmid pKD4 1μl
upstream primer(10umol/L) 1μl
downstream primer(10umol/L) 1μl
sterile water 9.5μl
paraffin oil 10μl
The PCR processes:<table><tr><td>Denaturation</td><td>94℃</td><td>20s</td><td rowspan="3">33 circulations</td></tr><tr><td>Annealing</td><td>52℃</td><td>20s</td></tr><tr><td>Elongation</td><td>72℃</td><td>5min</td></tr><tr><td>Final elongation</td><td>72℃</td><td>5min</td><td>1 circulations</td></tr></table></td></tr>
<tr><td>3</td><td>AGE</td><td>To analyze whether the PCR product is correct.
a. The preparation of the agarose gel
1*TAE 30mL
agarose 300mg
genecolour I™ 3μl
heating 2min
clotting in the gel container
b. Electrophoresis
110V 35min
c. Analysis
Gel-Imaging System
d. Gel extraction
Gel Extraction Kit</td></tr>
<tr><td>4</td><td>Purification</td><td>To eliminate the false positives of pKD4
a. Enzyme digestion
System: 17μl extraction product, 1μl Dpn1, 2μl 10*buffer, 37℃, 1h
b. AGE</td></tr>
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