

D. The Verification of Strain

1	Bacteria Shaking Cultivating	Picking a single bacterial colony into the ampicillin resistant liquid LB medium, cultivated overnight at 30°C.												
2	Bacteria Keeping	add 700µl bacterium solution and 700µl 50% sterile glycerin into a 1.5ml sterile centrifuge tube, storing at -20°C.												
3	Plasmid Extraction	TIANprep Mini Plasmid Kit.												
4	PCR	<p>The PCR system(using the detection primers):</p> <table style="margin-left: 40px; border: none;"> <tr> <td>PCR MIX</td> <td style="text-align: right;">12.5µl</td> </tr> <tr> <td>plasmid pKD4</td> <td style="text-align: right;">1µl</td> </tr> <tr> <td>upstream primer(10umol/L)</td> <td style="text-align: right;">1µl</td> </tr> <tr> <td>downstream primer(10umol/L)</td> <td style="text-align: right;">1µl</td> </tr> <tr> <td>sterile water</td> <td style="text-align: right;">9.5µl</td> </tr> <tr> <td>paraffin oil</td> <td style="text-align: right;">10µl</td> </tr> </table>	PCR MIX	12.5µl	plasmid pKD4	1µl	upstream primer(10umol/L)	1µl	downstream primer(10umol/L)	1µl	sterile water	9.5µl	paraffin oil	10µl
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5	AGE	To analyze if the strain is correct.												