I. Experiment purposes: Transformation of pGLO-Cas9 to MG1655 WT strain and MG1655 panD- mutant strain, and preparation of competent state

2. Material:
E.coli K-12 MG1655 competent cells, E.coli K-12 MG1655 panD-competent cells, pGLO-Cas9 plasmid, calcium chloride solution, glycerol

3. Steps

Transformation:
(1) Take E.coli K-12 MG1655 and E.coli K-12 MG1655 *panD*- competent cell on ice;
(2) Take pGLO plasmid pre-cooled on ice;
(3) Add pGLO plasmid to competent cells
(4) Ice bath 30min
(5) 42 ° C water bath for 90s
(6) Ice bath for 2 min;
(7) Add 1 ml of pre-warmed LB medium at 37 ° C, and incubate at 37 ° C for 1 h;
(8) Centrifuge at 4000 rpm for 5 min, discard 800 ul of supernatant, and resuspend the remaining liquid;
(9) Apply the resuspension of bacterial solution 100 ul to an LB medium plate containing ampicillin then cultured overnight.

Competent cell preparation:
(1) Pick E.coli K-12 MG1655-pGLO and E.coli K-12 MG1655 panD-pGLO single colonies and cultured in 5 ml LB liquid medium overnight, and transferred 1% to 50 ml LB liquid medium. , culture to OD_{600}=0.4
(2) 100ml is divided into 50ml centrifuge tube and pre-cooled with CaCl2 for 10min on ice;
(3) The bacteria solution was centrifuged at 4000 rpm for 4 min at 4 ° C, and the supernatant was discarded;
(4) Add 10 ml of pre-cooled 50 mM CaCl2, gently pat the suspension, centrifuge at 4000 rpm for 4 min at 5 ° C, discard the supernatant;
(5) Add 500ul of 50 mM CaCl2, resuspend, add glycerin, add 100ul per tube, store at -80 °C.

4. results
E.coli K-12 MG1655-pGLO and E.coli K-12 MG1655 panD-pGLO transformants were grown on four Amp plates in two parallel experiments. It was initially considered that the transformation was successful and the Cas9 protein function was still normal. verification. Single colonies were picked after overnight incubation and streaked onto Amp plates for storage.

Zhe Yuan 8.25