Plasmid cloning in E. coli strain DH5α

PSR

DH5α is one of the best strains for proliferating the different plasmids (vectors). For this purpose, the plasmid introduced to DH5α by different methods such as electroporation and heat shock method. In contrast to electroporation, heat shock method is very simple method with relatively high efficiency.

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Essential materials:

- LB medium
- deionized H2O: 950 mL
- tryptone: 10 g
- yeast extract: 5 g
- NaCl: 10 g

Note: for solid LB medium, 15 g Agar adds to 1 liter LB broth.

- Appropriate antibiotic based on bacterial selection marker on the plasmid.
- pipet Pasteur
- Plasmid of intersted
- DH5α competent cell: For easily introducing the plasmids into the DH5α, it is essential to competent the membrane of DH5α by $CaCl_2$ 0.1 M. For this goal, one single of DH5α on the solid LB petri dish is picking up by crystal tip and is transport to 5 mL fresh LB broth in a 50 mL falcon. Another falcon with 5 mL LB is also use for control. These falcons incubate 12-16 hours (an overnight (O/N)) in 37˚C and 200 RPM. After finishing the time, the control falcon must be clear and the other falcon must be milky. Then, 1 mL from this falcon add to 9 mL fresh LB broth in new falcon 50 and incubate in the above condition for 2- 3 hours. In the next step, DH5α is aliquot in 2 mL micro tubes and centrifuge in 3000 RPM for 3 minute. Then, the supernatant is decanted in waste and 300 µL cold $CaCl_2$ add to the bacterial plate and with finger mix very slowly. In this step, competent cells is ready to use, but for storage in -80˚C, it is essential to centrifuge in the same above condition and decant supernatant and the adding the mixture of cold $CaCl_2$ 0.1 M- Glycerol (3- 1 ratio) into the plates. After mixing with fingers, the tubes can storage for a few years in -80˚C.

Note: all of cited ceases must be perform in sterile laminar flow.

Detailed procedure

Two tubes of competent cell placed on ice for 30 min.

1. Add 2-5 µL of the plasmid to one of competent cell tube and with finger mix slowly. The other tube is used for control.

2. Place the tubes on ice for 30 min.

3. Plac the tubes on 42˚C water bath for exactly 90 second and transfer on ice for 2-5 min.

Important note: the tubes should not be shaking on water bath.

4. Add 500 µL of LB broth to the both tubes and incubate for 1 hour in 37˚C and 200 RPM.

5. Centrifuge the tubes for 5 min at 3000 RPM. Decant supernatant and add 200 µL of fresh LB to the tubes. Use your fingers for mixing bacterial plates.

6. Ready two agar solid LB petridishes contain appropriate antibiotic.

- Pour plasmid tube into one of solid LB petri dish and label as Plasmid. The other tube also pours into the other petri dish and label as control. For spreading bacteria on petri dishes use pipet Pasteur.

7. Incubate the petridishes at 37˚C for 12- 16 hours. If all of cases performed correctly, bacterial colonies that contain the plasmid of interested growth on the petridish that labeled as plasmid.

Good luck!
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