UI-Indonesia

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Protocols

Chemically Competent Cells Preparation

1. Inoculate a colony of *E. coli* to 4 ml LB broth.
2. Incubate the broth overnight while shaking at 200 rpm (37 °C).
3. Add 0.1 ml of the culture to 20 ml LB broth in 50 ml conical tube.
4. Incubate the culture for 2-3 h while shaking at 200 rpm (37 °C). Incubate on ice for 30—60 min.
5. Centrifuge at 3500 rpm for 10 min (4°C). Discard the supernatants.
6. Add 100 mM $MgCl_2$ (1/5 volume of culture). Mix gently by pipetting up and down. Incubate on ice for 15—20 min.
7. Centrifuge at 3500 rpm for 10 min (4°C). Discard the supernatants.
8. Resuspend pellet with 100 mM $CaCl_2$ (1/50 volume of culture). Incubate on ice for 1 h.
9. Centrifuge at 3500 rpm for 10 min (4°C). Discard the supernatants.
10. Resuspend pellet with 100 mM $CaCl_2$ (1/100 volume of culture).
11. Aliquote 50 µl of culture to 1.5 ml tube. Cells are ready to be transformed.

Competent Cells Transformation

1. Set a heat block to 38 °C.
2. Thaw chemically competent cells on ice.
3. Pipette 50 µl of competent cells into 1.5 ml tube.
4. Add 5 µl of plasmid DNA to the tube and mix gently by pipetting up and down.
5. Incubate the tube on ice for 1 h.
6. Heat shock tube at 38°C for 90 s.
7. Incubate the bacteria on ice for 60 s.
8. Add 200 µl of SOC and recover the plasmids for 1 h while shaking at 200 rpm (37 °C).
9. Plate the suspension on agar supplemented with the appropriate antibiotics.
10. Incubate the agar containing transformants overnight (14-18 hr) at 37°C.

Restriction

1. Add 10 µl of DNA to be digested.
2. Add 10 µl of restriction buffer.

3. Add 5 µl of EcoRI.
4. Add 75 µl of ddH20
5. Incubate the restriction digest overnight at 37°C and then 80°C for 20 min to heat kill the enzymes.
6. Purify the digested DNA.
7. Add 5 µl of PstI.

Digestion

1. Add 1 µl of digested plasmid backbone
2. Add 1 µl of DNA insert
3. Add 1 µl T4 DNA ligase buffer.
4. Add 1 µl T4 DNA ligase.
5. Add water to 10 µl.
6. Incubate overnight at 16°C and hen 80°C for 20 min to heat kill the enzymes.
7. Transform with 1-2 µl of product.

Plasmid Isolation Miniprep

1. Centrifuge the cell culture at 3500 rpm for 10 min (at 4°C) to pellet the cells. Discard the supernatant.
2. Resuspend pelleted bacterial cells in 125 µl Buffer P1 that has been added by RNase A and transfer to a 1.5 ml tube. Vortex or pipette up and down the suspension until no cell clumps should be visible.
3. Add 125 µl Buffer P2 and gently invert the tube 7–8 times to mix. Do not vortex and do not allow the lysis reaction to proceed for more than 5 min.
4. Add 175 µl Buffer N3 and invert the tube immediately and gently 7–8 times.
5. Centrifuge at 12,000 rpm for 10 min (at 4°C).
6. Apply the supernatants to the QIAprep spin column by pipetting.
7. Centrifuge at 12,000 rpm for 60 s.
8. Centrifuge the supernatants at 12,000 rpm for 60 s. Discard the flow-through.
9. Wash the QIAprep spin column by adding 500 µl Buffer PB and centrifuge at 12,000 rpm for 60 s. Discard the flow-through.

10. Wash QIAprep spin column by adding 0.75 ml Buffer PE+Ethanol and centrifuge at 12,000 for 60 s. Discard the flow-through and centrifuge for an additional 60 s to remove residual wash buffer.
11. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube.
12. Add 20 µl Buffer 1/3 EB to the center of each QIAprep spin column.
13. Incubate for 60 s and centrifuge 12,000 rpm for 60 s.
14. Add 15 µl Buffer 1/3 EB to the center of each QIAprep spin column.