Escherichia Coli Transformation Experiment Guide

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Abstract

Escherichia Coli, also known as *E. coli,* is the most widely studied prokaryotic model organism, and an important species in the field of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Typically, *E. coli* expression is the first choice for protein expression and protein product in *E. coli is* fast, convenient, well established and always with high yields.

Experiment Principle

Plasmid DNA or recombinant DNA adhered to the surface of bacterial cells; 42 °C heat treatment for a short time to promote the absorption of DNA and then cultivate a generation in the non-selective medium; when the antibiotic gene on the plasmid expressed, it can be placed in the medium containing antibiotics.

Experimental Materials

Plasmid DNA, recombinant DNA

Reagents, kits

LB medium, Distilled water, IPTG, X-gal, Ampicillin

Equipment

Vortex mixer, Micro-pipettes, Pipette tip, Centrifuge tube, Double-sided micro-centrifuge tube rack, Dry air bath, Constant temperature water bath, Ice maker, Constant temperature shaker, Petri dishes, Clean bench, Alcohol lights, Glass sticks, Constant temperature incubator

Operating Method

1. Adjust the temperature of the constant temperature water bath to 42°C

2. Geta tube (100 μl) of the competent bacteria from the -70 °C ultrafilter freezer and

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immediately melt with a finger and insert it into ice and ice for 5 to 10 minutes

3. Add 5 µl of the attached plasmid mixture (DNA content of no more than 100 ng), gently shakeandplace on ice for 20 min

4. Gently shakeand insertinto the 42 °C water bath 1 ~ 2 min for heat shock, and then quickly put back to the ice; put it aside for 3 ~ 5 min

5. Add 500 µl of LB medium (without antibiotics) to each of the tubes in a clean bench and mix them gently onto a shaker of 37 °Cfor 1 h

6. In the clean bench, take the above conversion mixture 100-300 µl, respectively, to the appropriate solid LB plateculture dish containing antibiotics; coat evenly with alcohol lamp burned glass

7. If the carrier and host bacteria are suitable for blue-white screening, drop 40 µl of 2% X-gal, 8 µl of 20% IPTG on the plate and coat evenlywith alcohol lamp burned glass

8. Mark on a coated dish and place in a 37 °Cincubator for 30 to 60 min until the liquid on the surface penetrates into the culture medium and thenplace in the 37 °C incubator overnight

9. Spray 70% ethanol on the bacteria-contaminated table, dry the table, write an experimental report

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Protocol

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