Bio Bharati Life Science Pvt. Ltd.

Bacterial Transformation

Cat# BB-MTK030S (5reactions)
Cat# BB-MTK030 (10 reactions)

Background and principle: By definition transformation is a process of genetic alteration in a bacterium associated with an uptake of a naked exogenous DNA. To uptake a naked DNA a bacterium must be in a special state called 'competent'. Usually a bacterium is not competent for transformation. Competency is induced by treating bacteria grown to logarithmic phase with divalent cation at low temperature. The exact mechanism of competence of bacteria is not understood. It is thought that the competent bacteria carry a special conformation in phospholipid bilayer in the outer membrane which enables bacteria to uptake DNA when incubated with naked DNA and briefly treated with heat shock at 42ºC. Because not all competent cells can uptake the DNA a selection step following the treatment is necessary to select the transformants.

Escherichia coli popularly known as *E. coli* most routinely is a bacterium of choice in research laboratories practicing recombinant DNA technology for transformation. An essential ingredient of 'recombinant DNA technology' is a circular DNA called 'plasmid'. Normally the plasmid DNA carries a replication origin recognized by the bacterial replication machinery thus the plasmid can be replicated (copied) and maintained during the subsequent generations with the replication of host bacterial genome. Usually the plasmid carries a gene conferring resistance to an antibiotic in which the host bacteria is sensitive, allowing the transformants to be selected on a growth medium containing the antibiotic. α-complementation provides another level of selection of transformants which scores for β-galactosidase activity, an enzyme, encoded by lacZ gene harbored in the plasmid such as pUC18. Transformants in this case produce bright blue colored colony when plated on a medium supplemented with X- gal and isopropyl thiogalacyoside (IPTG). IPTG, a non-hydrolysable galactose derivative acts as an inducer of β-galactosidase. The enzyme substrate X-gal is a synthetic and colorless galactose derivative.

β-galactosidase functions as a tetramer, where each monomer is composed of two parts alpha and omega. The omega fragment in the absence of alpha part is inactive but supplying alpha in *trans* can restore β- galactosidase activity as tetramer. α-complementation is carried out in *E. coli* host with a deletion of alpha part of lacZ gene. Thus, the expression of lacZ alpha part present in the pUC18 in the host cell complements for functional β-galactosidase.

Components of the kit

Materials	Quantity	Store at
Plasmid DNA (10 ng / µl)	25 µl (pUC18)	-20 ºC
Ampicillin (100 mg / ml)	600 µl	-20 ºC
0.1 M $CaCl_2$	36 ml	4 ºC
Host (Modified *E.coli*)	Stab culture (DH5 alpha cell)	RT
IPTG (1M)	400 µl	-20 ºC
X-Gal (100mg/ml)	400 µl	-20 ºC
LB Broth	12.5 gm	RT
Agar	10 gm	RT
1.5 ml vials	40	RT

Materials Required:

Equipment:

Centrifuge (preferably refrigerated), Incubator, 37°C shaker, Spectrophotometer.

Glassware: Conical flask, Petri plates, Pipettes, Spreader.

Reagent: Distilled water.

Other Requirements: Capped Centrifuge tubes.

Preparation of competent cells

Day1:

1. Streak *E. coli* strain from the agar stab in a LB agar plate without any antibiotic; allow the cell to grow over night at 37ºC.

Day 2:

2. Inoculate a single colony in a 2 ml LB in a test tube without antibiotic. Allow the organism to grow at 37ºC with shaking (~200 rpm) over night (O/N).

Day 3:

3. Measure the OD_{600nm} of overnight (O/N) culture.

4. Inoculate 50 ml LB medium with 500 µl of O/N culture in 250 ml conical flask.

5. Grow the cells at constant rotation at 37ºC, (Generation time of *E. coli* is 20 min).

6. As soon as the OD_{600nm} reaches to approximately 0.3, place the flask on ice until the cell culture reaches the temp of ice (30 min).

7. Transfer cells into a 50 ml capped sterile centrifuge tubes. Centrifuge the culture at a low speed (3000 rpm) at 4ºC for 8 min to collect the cell pellet. Discard the supernatant/medium.

8. Resuspend the cell pellet in 10 ml ice cold 0.1 M $CaCl_2$ by slow swirling. Incubate on ice for 30 min. (Work in the cold room- or handle the cells in such a way that the temp of cell suspension stays close to ice temp). Care must be taken not to remove the tubes from ice during resuspension.

9. Collect cells by centrifugation as before. Resuspend cells in 7 ml 0.1M $CaCl_2$, incubate on ice for 30 min.

10. Centrifuge to collect cells as above. Resuspend the cell pellet in 0.5 ml 0.1M $CaCl_2$.

11. Make 100µl aliquot of competent cells in sterile 1.5 ml tubes.

12. Competent cells are now ready and should be used immediately for the transformation experiment, as the efficiency of transformation drops on storage at temperature higher than -70°C.

Transformation

Thaw two tubes (aliquots) of competent cell (100 µl) on ice (mark them as A and B). Add 2 µl (10 ng / µl) of plasmid DNA into one aliquot (A), and 2 µl of sterile water to the second aliquot (B). Mix by gentle tapping (3 to 4 times) with finger.

1. Incubate on ice for 10 min.

Heat shock

2. Transfer the tubes to a water bath pre-set at 42ºC,incubate for 45 seconds.

3. Transfer the tubes immediately to ice, incubate for5 min.

Expression

4. Add 0.9 ml of LB medium (without any antibiotic) in each tube and incubate preferably with gentle shaking (~200 rpm) for 1 hr at 37ºC. This is to allow bacteria to recover and express the antibiotic resistance.

5. Label four LB -Amp Plates with X-Gal and IPTG (previously spread) as A1, B1, and C1& D1.

6. Plate 25µl, 50µl & 100µl of the transformation mix respectively from tube A on plate A1, B1 and C1 respectively with a sterile glass spreader and plate 100µl of transformation mix from tube B on plate labeled as D1(for control) . Incubate the plate at 37^{o}C O/N.

7. Transfer the plates at 4^{o}C for a few hours until blue colony appears. Count for blue colonies, only Ampicillin resistant colonies (three experimental plates). The cells without the DNA should not grow any colony.

NOTE

As X-gal is light sensitive, it is recommended to keep the plates wrapped with aluminum foils.

Transformation Efficiency is expressed as:

No. of Transformants / µg of DNA.

Calculation: Transformation Efficiency is: No. of colonies x 1000 ng =_____/µg Amount of DNA plated (in ng)

For example: Amount of DNA transformed = 100 ng Volume of culture plated = 25 µl (of 1 ml)Thus, amount of DNA plated = 2.5 ng.

If no. of colonies observed on plating 2.5 ng = 250Transformation Efficiency = 250 x 1000 ÷ 2.5 = 100,000 = 1 x 10^{5} / µg

Results and Discussion: pUC18 carries Ampicillin resistant gene. As a result on transforming the competent cell with this plasmid antibiotic resistance is conferred on the host and only transformed cells grow on LB-Amp plates whereas no colonies will be found on the plate D1 because instead of plasmid, water was added to the competent cells. So, D1 plate is the negative control of this experiment. Number of colonies will gradually increase from plates A1 to C1.

Blue colonies indicate successful transformation process. Using calcium chloride method for preparation of competent cells, the expected transformation efficiency on transforming 100 ng of pUC18 is approximately 1 x 105/µg of DNA. Transformation Efficiency lower than this may be attributed to improper conditions during preparation of competent cells. e.g. higher temperature.

Appendix:

Preparation of LB Agar/broth (1 liter): Dissolve 25 g of media in 800 ml of distilled water. Adjust the pH to 7.0 with 5N NaOH (if necessary) and make up the volume to 1000 ml. Sterilize by autoclaving. For LB agar, add 1.5% agar and autoclave.

Ampicillin Preparation: Dissolve 100 mg of Ampicillin in 1 ml sterile water to get a stock concentration of Ampicillin 100 mg/ml. Store at 4°C for 2 weeks. Use the antibiotic within this period.

For Ampicillin LB media: Add Ampicillin to LB broth or LB-Agar at a final concentration of 100 µg/ml, when the temperature of the media is around 40-45°C.

Preparation of LB-Amp-Growth Plates with X-Gal and IPTG:

After Ampicillin is added to the media, add 25 µl each of X-Gal and IPTG for every 20 ml of LB agar. Mix well and pour media into required number of plates.

Adapted from internet