HiPer® Transformation Teaching Kit

Product Code: HTBM017

Number of experiments that can be performed: 10

Duration of Experiment: 4 days

Day 1- Preparation of media and revival of *E. coli* Host
Day 2- Inoculation of host strain from a single colony
Day 3- Protocol
Day 4- Observation and calculation of transformation efficiency

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store *E.coli* Host at 2-8°C
- Store Plasmid DNA, Ampicillin, X-Gal and IPTG at -20°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To prepare competent cells and transform plasmid DNA.

Introduction:

Bacterial transformation is a process which involves genetic alteration of bacteria by incorporation and stable expression of a foreign genetic material from the environment or surrounding medium. Since DNA is a very hydrophobic molecule, it will not normally pass through a bacterial cell membrane. In order to uptake the foreign DNA, the bacterial cells must first be made competent. Competence is the ability of a cell to take up extracellular DNA from its environment. There are different methods of carrying out transformation, e.g. chemical transformation, electroporation, gene gun, liposome mediated transfer and microinjection. Chemical transformation includes the usage of Calcium chloride ($CaCl_2$). This mode of transformation is easy to perform and requires minimum number of equipments.

Principle:

For the incorporation of plasmid into a cell, bacteria must first be made "competent". This process includes the treatment of cells with bivalent calcium ions in ice-cold condition. As a result small pores are formed on the cell membrane, which makes it permeable. The plasmid DNA may adhere to the surface of the cell and uptake is mediated by a pulsed heat shock at 42°C. A rapid chilling step on ice ensures the closure of the pores. These cells are allowed to propagate and selection of transformants can be done by growing the cells on a selective media which will allow only the plasmid containing cells to grow.

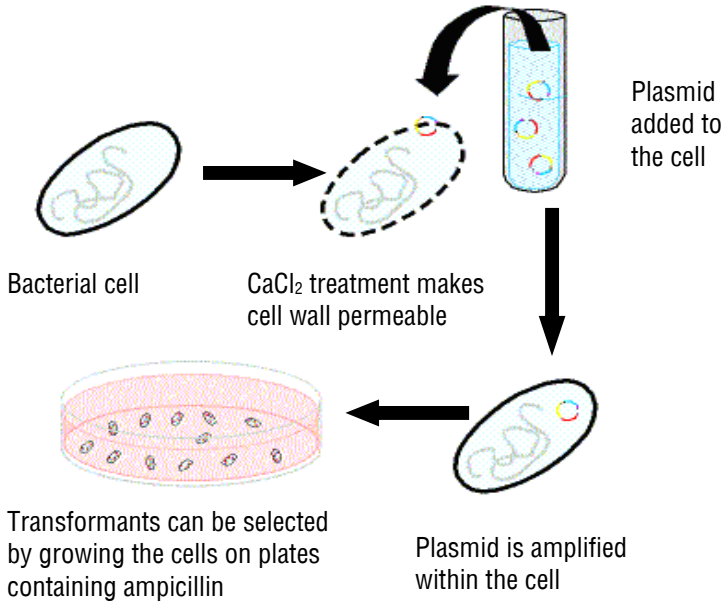

Fig 1: The process of bacterial transformation includes treatment of cells with $CaCl_2$, which makes cells permeable, and plasmid DNA can enter the cell.

Plasmids are extrachromosomal DNA element capable of independent replication inside a suitable host. Plasmids encode a wide variety of genes, including those required for antimicrobial resistance. These genes act as selective markers when a transformation experiment is carried out.

The *E. coli* plasmid pUC19 encodes a gene that can be used as a selectable marker during a transformation experiment. pUC19 has ampicillin resistance marker that enables only transformed cells to grow on LB – Ampicillin plates. Transformants, thus having the ability to grow on ampicillin plates can be selected. This process of direct selection of recombinants is called insertional-inactivation. pUC19 also carries the *N*-terminal coding sequence for β-galactosidase of the *lac* operon. The *E. coli* host strain has a deletion at the amino terminal end of the LacZ gene, which codes for β-galactosidase. When pUC19 is transformed into the competent host cells, the truncated products from both complement each other and as a result enzymatically active β-galactosidase is produced. This is called α-complementation. The tranformants turn blue on X-gal and IPTG containing plates due to the production of β-galactosidase. X-gal is the chromogenic substrate of β-galactosidase and IPTG acts as the gratuitous inducer for the expression of this enzyme.

Kit Contents:

This kit can be used to prepare competent cells by $CaCl_2$ method and perform transformation using pUC19.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials provided	Quantity 10 expts	Storage
1	MB104	Ampicillin	0.03 g	-20°C
2	M1245	Luria Bertani (LB) Broth	50 g	R T
3	MB053	Agar Powder, Bacteriological	20 g	R T
4	TKC027	*E. coli* Host	1 No.	2-8°C
5	TKC026	Plasmid DNA	0.022 ml	-20°C
6	TKC065	1M Calcium chloride (Sterile)	40 ml	R T
7	TKC261	X-Gal	2.2 ml	-20°C
8	TKC262	IPTG	1.1 ml	-20°C
9	PW1139	Collection Tubes, Polypropylene (2.0 ml)	20 Nos.	R T

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Other requirements: Micropipettes, Tips, 50 ml Centrifuge Tubes, Water bath (42°C), 37°C Incubator, 37°C Shaker, Centrifuge, UV Transilluminator, Crushed ice, Sterile double distilled water, Sterile loop and spreader.

Storage:

HiPer® Transformation Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store *E.coli* Host at 2-8°C. Plasmid DNA, Ampicillin, X-Gal and IPTG should be stored at -20°C. All other kit contents can be stored at room temperature (15-25°C)

Important Instructions:

1. Read the entire procedure carefully before starting the experiment. The entire procedure should be carried out under sterile conditions.

2. **Preparation of 0.1M Calcium chloride (sterile) (400 ml):** To prepare 400 ml of 0.1M Calcium chloride, take 40 ml of 1M sterile Calcium chloride and add 360 ml of sterile distilled water. Store this solution at 2-8°C.

3. Prior to the preparation of competent cells, pre-chill the tubes, 0.1M Calcium chloride solution and centrifuge tubes. Set the centrifuge at 4°C and water bath at 42 °C.

4. Transformation should be carried out as soon as possible after the competent cells are prepared. Storage of competent cells leads to poor or no transformants.

5. Concentration of plasmid is 50 ng/µl.

6. **Preparation of LB (Luria Bertani) broth (55 ml):** Dissolve 1.38 g of LB media in 55 ml of distilled water. Sterilize by autoclaving.

7. **Preparation of LB (Luria Bertani) agar plates (20 ml):** Dissolve 0.5 g of LB media and 0.3 g of agar in 20 ml of sterile distilled water. Sterilize by autoclaving and pour on sterile petriplate.

8. **Preparation of Ampicillin:** Dissolve 30 mg of ampicillin powder in 600 µl of sterile double distilled water to give a concentration of 50 mg/ml.

9. **Preparation of LB (Luria Bertani) Agar plates containing Ampicillin, X-Gal and IPTG (100 ml):** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 °C. Add 100 µl of ampicillin, 200 µl of X-Gal and 100 µl IPTG to 100 ml of autoclaved LB agar media, mix well and pour on sterile petriplates.

Procedure:

Day 1:

1. Open the vial containing culture and resuspend the pellet with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto LB agar plate.
3. Incubate overnight at 37^{0}C. Streak a loopful of culture from the stab *E. coli* Host) on to LB Agar plate.

Day 2:

1. Inoculate a single colony from the revived plate in 1 ml LB broth.
2. Incubate at 37°C overnight.

Day 3:

1. Take 50 ml of LB broth in a sterile flask. Transfer 1 ml of overnight grown culture into this flask.
2. Incubate at 37°C shaker at 300 rpm for 3-4 hours till the $O.D_{600}$ reaches ~ 0.6.

A) Preparation of Competent Cells:

Note: Prepare competent cells within 3 days of reviving the strain.

1. Transfer the above culture into a prechilled 50 ml polypropylene tube (not provided).
2. Allow the culture to cool down to 4°C by storing on ice for 10 minutes.
3. Centrifuge at 5000 rpm for 10 minutes at 4°C.
4. Decant the medium completely. No traces of medium should be left.
5. Resuspend the cell pellet in 30 ml prechilled sterile 0.1 M Calcium chloride solution.
6. Incubate on ice for 30 minutes.
7. Centrifuge at 5000 rpm for 10 minutes at 4°C.
8. Decant the calcium chloride solution completely. No traces of solution should be left.
9. Resuspend the pellet in 2 ml prechilled sterile 0.1M Calcium chloride solution.
10. This cell suspension contains competent cells and can be used for transformation.

B) Transformation of cells:

1. Take 200 µl of the above cell suspension in two 2.0 ml collection tubes and label them as 'control' and 'transformed'. Add 2 µl of plasmid DNA to the tube labeled as transformed and mix well.
2. Incubate both the tubes on ice for 30 minutes.
3. Transfer them to a preheated water bath set at a temperature of 42°C for 2 minutes (heat shock).
4. Rapidly transfer the tubes to ice-bath. Allow the cells to chill for 5 minutes.
5. Add 800 µl of LB Broth to both the tubes. Incubate the tubes for 1 hour at 37°C to allow the bacteria to recover and to express the antibiotic resistance marker encoded by the plasmid.

6. Take four LB agar plates containing ampicillin, X-Gal, IPTG and label them as control, A, B and C. Plate 200 µl of culture from the 'control' tube and plate it on the corresponding plate with a sterile spreader. Plate 50 µl, 100 µl and 200 µl of cell cultures from the 'transformed' tube on to the plates labeled as A, B and C, respectively.

7. Store at room temperature till the plates are dry.

8. Incubate the plates overnight at 37°C.

Observation and Result:

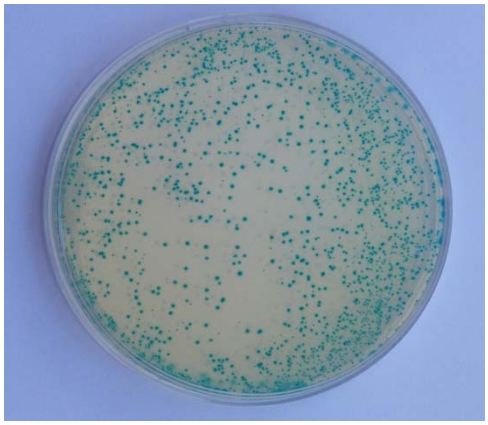

Fig 2: Transformant plate with blue colonies

After incubation, observe the plates for bacterial growth and count the number of visible colonies. Calculate the efficiency of transformation.

Record your observations as follows:

Sr. No.	Plate	Growth	Number of colonies	Transformation Efficiency
1	Control plate			
2	Transformed plate (A)			
3	Transformed plate (B)			
4	Transformed plate (C)			

Denote +ve when you observe bacterial growth, -ve when there is no growth

Calculation of transformation efficiency:

Transformation efficiency is defined as the number of cells transformed per microgram of supercoiled plasmid DNA in a transformation reaction.

Transformation Efficiency = Number of colonies x 1000 ng/ Amount of DNA plated (ng) = cells/µg

Concentration of plasmid DNA provided - 80-100 ng/microlitre

Interpretation:

On transformation of cells with pUC19 plasmid, antibiotic resistance is conferred on the host as this plasmid carries gene for ampicillin resistance. As a result, those cells that grow in presence of ampicillin are transformed cells. The transformed colonies are blue on X-Gal, IPTG plates due to α-complementation.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Few or no transformants	Cold chain not maintained properly during the procedure	Always keep the cells on ice & make sure the centrifuge machine is cold throughout the experiment
		Heat shock treatment not done accurately	Follow the procedure, with exact time duration for heat shock treatment
		Ampicillin concentration used is not correct	While making the plates add accurate amount of ampicillin
		Plates were not incubated at proper temperature for sufficient time	Incubate the plates at 37°C for minimum 18 hours
2	Transformant colonies are not blue in colour	X-Gal and IPTG are inactivated	Add X-Gal and IPTG to the LB agar media after it cools down to 40-45 °C
		X-Gal and IPTG are not stored properly	Always store X-Gal and IPTG properly as mentioned in the brochure
3	Observed bacterial growth on control plate	Ampicillin is inactivated	Add ampicillin to the LB agar media after it cools down to 40-45°C
4	Contamination observed on plates	Sterility not maintained during the experiment	Make sure that the entire procedure is performed aseptically

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com

Storage temperature

Do not use if package is damaged

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