Applied Biology and Bioengineering (BT520)

EXPERIMENT- 5

TRANSFORMATION OF COMPETENT CELLS WITH PLASMID DNA

Aim: Transformation of competent cells with plasmid DNA

Principle: The uptake of the DNA by the cells is called transformation. The transformation can be brought about by heat shock treatment as well as by electroporation. The DNA is added to competent cells on ice. During a heat shock at 42℃ the cells are transformed. Once the *E. coli* cells are transformed, the DNA can be extracted easily and amplified. The selection of the right colonies is done on LB medium containing ampicillin because only those transformed cells will grow which contain plasmid that has ampicillin resistance gene.

Requirements:

1. *DH5*α host cells
2. Plasmid DNA
3. SOC medium
4. Luria Bertani Agar medium
5. Ampicillin
6. Perti plates

Media Compositions: All the Ingredients are in (g/L)

LB Agar Medium

Bactotryptone	: 10g	
Bacto Yeast extract	: 5g	
Sodium Chloride	: 10g	
Agar	: 20g	adjust pH to 7.2

SOC medium

Bactotryptone	: 2%
Bacto Yeast extract	: 0.5%
Sodium Chloride	: 10 mM
KCl	: 2.5 mM
$MgCl_2$	: 10 mM
$MgSO_4$	: 10 mM
Glucose	: 20 mM

Make volume to 1 L with distilled water and autoclave.

Ampicillin (Stock 100mg/ml) Final concentration required is 100 µg/ml.

Equipment

1. Autoclave
2. BOD incubator shaker (37°C)
3. Water bath (42°C)
4. Ice flaker

Wares

1. Micropipettes
2. Glass spreaders
3. Sterile Eppendorf tubes (1.5 ml)
4. Ice buckets
5. Sterile tips (yellow + blue)
6. Floating rack for tube (1.5 ml)
7. Sterile petri plates

Day 1:

1. Take 200 µl of freshly prepared competent cells in 1.5 ml tube and keep on ice for 10 min.
2. Take 10 µl (2-3 ng) of Plasmid DNA and add to 200 µl of competent cells, gently mix by the micropipette and incubate on ice for 30 min, undisturbed.
3. Subject the mixture (DNA+ cells) to a heat shock treatment at 42℃, for exactly 40 sec and place back on ice for 5 min.
   (Subject the 200 µl competent cells without mixing with DNA, to the same treatment serving as negative control)
4. Add 800µl of SOC medium (pre-warmed 42℃) to the tube containing cells and DNA.
5. Incubate the mixture in incubator shaker at 37℃, 200 rpm for 1 h.
6. Centrifuge the cells at 1600 rpm at 25℃ (room temperature) for 10 min, remove carefully 800 µl of the supernatant from the top.
7. Resuspend the cells in the remaining 200 µl supernatant in the same micro tube.
8. Take the cells in the laminar hood and pour the 200 µl cells using sterile micro tips on LB agar plates (containing ampicillin, 100 µg/ml final concentration) and spread the cells with a sterile glass spreader.
9. Dry the plates for 15 min in the Laminar hood.
10. Invert the plates and incubate in incubator at 37℃ for overnight.

Day 2:

11. Count the number of transformed cells and compare with control.

12. Calculate the transformation efficiency as follows.

$$\frac{y \times z}{x} = \text{cfu/µg DNA}$$

where

x = ng of plasmid DNA used for transformation
y = No. of colonies observed
z = dilution factor of DNA or cells
cfu = colony forming unit

Observation:

Result: