Objectives:

- To prepare competent cells
- To perform transformation and
- To learn the concept of $\alpha$-complementation.

Principle:

Bacterial transformation is a process in which bacteria manage to uptake or bring in free/external DNA from the environment/surrounding medium. This is a very basic technique that is routinely used in a molecular biology lab. The purpose of this technique is to introduce a foreign plasmid DNA into bacteria and to use these bacteria to amplify the plasmid DNA.

The ability to take up DNA efficiently by most bacteria is limited in nature. However, bacterial cells can be artificially induced to take up DNA by treating them with calcium chloride. Culture of such cells that are capable of taking up DNA is said to be competent. The conditions required to produce competence vary from species to species.

The phenomenon of competence has not been understood very well. It appears to result from changes in the cell wall of bacteria and is probably associated with the synthesis of cell wall material at a particular stage of the life cycle of bacteria. In the course of developing competence, receptors of some kind are either formed/activated on the cell wall, which are responsible for initial binding of the DNA. The complex thus formed is resistant to DNases. Cells are then exposed briefly to a temperature of 42°C – “heat shock”, wherein pores are created and DNA is taken up. Immediate chilling on ice ensures closure of pores. Cells are then said to be transformed. These cells are then screened for transformants/recombinants.
Screening of transformants:

Selection of cells containing foreign DNA is done based on the selection marker carried by this DNA. Example, pUC plasmid has ampicillin resistance factor that enables only transformed cells to grow on LB-Amp plates. Non-transformants, which are ampicillin sensitive, do not produce colonies on the selective medium. Transformants and non-transformants are therefore easily distinguished.

Screening of recombinants:

Identification of recombinants among the transformed cells is generally done by insertional-inactivation. With most cloning vectors, insertion of DNA fragment into the plasmid destroys the integrity of one of the genes present on the molecule. As a result, the characteristic coded by the inactivated gene is no longer displayed by host cells and this is called insertional-inactivation.

For example, pUC18 is a high copy number plasmid of size 2686 bp, with Col E1 origin of replication. It carries a 54 bp polycloning region and ampicillin resistance marker, along with coding information for the first 146 amino acids (amino terminal) of β-galactosidase (Lac Z) gene. Some strains of E. coli bear a deletion at the amino terminal end of Lac Z gene and thus synthesize an inactive C-terminal fragment. On transforming such competent bacterial strains with pUC18, the host and plasmid encoded fragments associate to form an enzymatically active protein. This type of complementation is known as α-complementation. Lac bacteria that result from α-complementation can be recognized as they form blue colour colonies in presence of X-gal (chromogenic substrate for β-galactosidase) and IPTG (inducer for the expression of the enzyme). However, insertion of a fragment of foreign DNA into the polycloning site of plasmid results in production of an amino terminal fragment that is not capable of α-complementation. Hence, cells carrying recombinant plasmid will form white colonies. This is also referred to as Blue-White Screening.
Kit Description:

In this kit, pUC18 & modified E.coli strain are supplied. Modified E.coli cells will be made competent by calcium chloride method and transformed with pUC18 DNA. Transformed cells will then be plated on LB agar having ampicillin, X-gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside) and IPTG (Isopropyl-β-D-Thiogalactopyranoside). Due to antibiotic resistance, conferred by the plasmid DNA on the host, colonies will be seen on the antibiotic plate and will also appear blue due to α-complementation.

KT07 : Kit is designed to carry out 5 transformation experiments using competent cells prepared from a single lyophilized vial supplied.

KT07A : Kit is designed to carry out 20 transformation experiments. Two lyophilized vials are supplied, each vial to be used to prepare competent cells for 10 transformation experiments.

Duration of experiment: Experiment is carried out over a span of 4 days, approximate time taken on each day is indicated below:

Day 1 : 2 hours (Preparation of media and revival of host)
Day 2 : 10-15 minutes (Inoculation of media for competent cell preparation)
Day 3 : 6 hours (Preparation of competent cells and Transformation)
Day 4 : 45 minutes (Observation and Interpretation)

Materials Provided:
The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 6 months of arrival.

<table>
<thead>
<tr>
<th>Materials</th>
<th>KT07 Quantity</th>
<th>KT07A Quantity</th>
<th>Store</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100 mg</td>
<td>2 X 100 mg</td>
<td>4°C</td>
</tr>
<tr>
<td>0.1 M CaCl_2</td>
<td>25 ml</td>
<td>75 ml</td>
<td>4°C(Sterile)</td>
</tr>
<tr>
<td>Host (Modified E.coli)</td>
<td>1 vial</td>
<td>2 vials</td>
<td>4°C</td>
</tr>
<tr>
<td>IPTG</td>
<td>1 ml</td>
<td>2 X 1.5 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>Plasmid DNA</td>
<td>25 µl</td>
<td>0.1 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>X-Gal</td>
<td>1 ml</td>
<td>2 X 1.5 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>LB Broth</td>
<td>15 g</td>
<td>50 g</td>
<td>RT</td>
</tr>
<tr>
<td>Agar</td>
<td>5 g</td>
<td>20 g</td>
<td>RT</td>
</tr>
<tr>
<td>1.5 ml vials</td>
<td>10 Nos.</td>
<td>25 Nos.</td>
<td>RT</td>
</tr>
</tbody>
</table>

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, Crushed ice, Cuvette (of 1 cm path length), Tips, Micropipette, Water bath.
Note:

- Read the entire procedure before starting the experiment.
- All microbiological procedures should be done under aseptic conditions.
- Revive the strain as soon as the lyophilized vial is opened.
- Prepare competent cells within 3 days of reviving the strain.
- Carry out transformation as soon as the competent cells are prepared.
- Pre-cool tubes, pipettes, 0.1 M CaCl₂ solution and centrifuge tubes, prior to preparation of competent cells.
- Calcium chloride solution supplied is sterile, handle under aseptic conditions.
- Always place the centrifuge tubes containing calcium chloride on ice during resuspension.
- Storage of competent cells may result in poor/no transformants.
- Always cover X-Gal with aluminium foil, as it is light sensitive.
- Ensure that all the required components are ready prior to starting the experiment.
- Concentration of plasmid supplied is 20 ng/µl.
- For preparation of media, antibiotic, etc., refer appendix.

Procedure:

Preparation of Competent Cells:
(For 5 Transformation experiments)

Day 1: Revival of Host:
1. Break open the lyophilized vial, add 0.1 ml of LB media.
2. Streak (in duplicate) a loopful of suspension onto LB plates and incubate at 37°C overnight.

Day 2:
3. Inoculate a single colony into 5 ml of LB medium and incubate at 37°C (in a shaker), overnight.

Day 3: Preparation of Competent Cells
4. Inoculate 1 ml of overnight culture into 100 ml LB medium (in a 1 litre conical flask) and incubate at 37°C in a shaker. Grow until the OD A₆₀₀ reaches 0.23-0.26, this takes about 2-3 hours.
5. Chill the culture flask on ice for 10-20 minutes.
6. Transfer the culture aseptically into sterile centrifuge tubes and spin down at 6000 rpm for 8 minutes, preferably in a refrigerated centrifuge at 4°C or spin at Room temperature (RT).
7. Discard the supernatant and to the cell pellet, add approximately 15 ml of cold 0.1M CaCl₂ solution provided, aseptically. Suspend the cell pellet gently in the solution using a pre-chilled pipette. Care must be taken not to remove the tubes from ice during resuspension.
8. Place the tube on ice for 30 minutes.
9. Centrifuge at 6000 rpm for 8 minutes either at 4°C or RT.
10. Discard the supernatant and resuspend gently in 0.6 ml of cold 0.1 M CaCl₂ solution.
11. Aseptically aliquot 100 µl of competent cells into 6 pre-chilled vials, take care not to remove the centrifuge tubes/vials from ice.
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 Procedure:
13. Add 5 µl (100 ng) of the plasmid DNA to 5 aliquots of 100 µl of competent cells. Gently tap and incubate on ice for 20 minutes (for the DNA to bind to cells). The remaining one aliquot will not be transformed.
14. Heat shock the cells by placing the vial(s) in 42°C water bath for 2 minutes, then return vials to ice to chill for 5 minutes.
15. Add 1 ml of LB broth aseptically to the vial(s) and incubate at 37°C (in a shaker) for an hour. This is to allow bacteria to recover and express the antibiotic resistance.
16. Label three LB-Amp. plates with X-Gal and IPTG as a, b & c. Pipette 100 µl of LB broth on to each plate. Add 25, 50 and 100 µl of transformed cells to plates a, b & c respectively. Mix well and spread thoroughly using a pipette or spreader.
17. Repeat step 16 for the other 4 aliquots of competent cells transformed.
18. Plate 100 µl of competent cells that has not been transformed, to check for any cell contamination. Label this as control plate/non-transformed plate.
19. Incubate the plates at 37°C overnight.

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 = 250
   Transformation Efficiency = \( \frac{250 \times 1000}{2.5} \)
                             = 1 x 10⁵/µg
Observation:
Record your observations as follows:

<table>
<thead>
<tr>
<th></th>
<th>Growth</th>
<th>No. of colonies</th>
<th>Transformation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Plate or non-transformed plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformed Plate (a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformed Plate (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformed Plate (c)</td>
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<td></td>
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</tbody>
</table>

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

Interpretation:
- On transforming competent cells with pUC18, antibiotic resistance is conferred on the host as this plasmid carries the gene for ampicillin resistance. As a result, only transformed cells grow on LB-ampicillin unlike non-transformed cells.
- These transformed colonies appear blue on LB-Amp-X-Gal-IPTG plate due to α-complementation i.e., active β-galactosidase produced cleaves X-gal to give the blue colour on IPTG induction.
- Using calcium chloride method for preparation of competent cells, the expected transformation efficiency on transforming 100 ng of pUC18 is approximately $1 \times 10^5$ of DNA. Efficiency lower than this may be attributed to improper conditions during preparation of competent cells. eg., temperature higher than 4°C.

Appendix:
Preparation of LB Agar/broth (1 litre): 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 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 agar at a final concentration of 100 µg/ml, when the temperature of the media is around 40-45°C.

Preparation of LB-Amp. plates with X-Gal and IPTG: After ampicillin is added to the media, add 40 µl each of X-Gal and IPTG for every 20 ml of LB agar. Mix well and pour media into required number of plates.

Following aliquots of media are required for 5 transformation experiments:
- LB Broth 4 x 5 ml; 1 x 100 ml
- LB Agar 50 ml
- LB Agar/Amp/X-Gal/IPTG 300 ml

Note: Prepare 100 ml of LB broth in a 1 litre conical flask.
### Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GeNei™ Transformation</strong> teaching Kit</td>
<td>1 Pack</td>
<td>KT07</td>
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<tr>
<td>(Consumables for 5 experiments)</td>
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<td></td>
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<tr>
<td><strong>GeNei™ Transformation</strong> teaching Kit</td>
<td>1 Pack</td>
<td>KT07A</td>
</tr>
<tr>
<td>(Consumables for 20 experiments)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Email:**

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