Experiment (2): Competent Cells Formation and Transformation of Competent Cells with recombinant plasmid DNA

Aims:

- Making a competent cells using calcium chloride method.
- Transformation of the competent cells with recombinant plasmid DNA using chemical transformation method.

Introduction:

Molecular cloning is an important tool to understand the structure, function and regulation of individual genes and their products. It is a cell-based technique used to create copies of certain DNA fragments using a vector carrying the DNA of interest, which eventually inserted to a host cell (usually bacteria) and self-replicate. The cell-based DNA cloning involve four steps:

1. *Construction of recombinant DNA molecules:* which is the insertion of DNA fragment into a cloning vector (e.g. plasmid). This step is facilitated by cutting the target DNA and replicon molecules with specific restriction endonucleases before joining the different DNA fragments using the enzyme DNA ligase.

- PAUSE AND THINK ➔ What are different important features that the cloning vectors should have?

2. *Transformation:* which is the introducing of the recombinant DNA into bacterial cells (the host) and in which the recombinant DNA amplified using bacterial DNA replication machinery.
3. *Selective propagation of cell clones:* which is the plating of clones on selection media to allow screening.
4. *Isolation of recombinant DNA.*

Bacteria are able to take up DNA from their environment (exogenous DNA) in three ways: *conjugation, transformation, and transduction*. Only transformation is the direct uptake of DNA, since conjugation requires cell-cell contact via a sex pilus and transduction requires a bacteriophage intermediary to transfer DNA from one cell to another. For a bacterial cell to take up DNA from its surroundings, it must be in a special physiological state called **competence**. It defines as the ability of the cell to undergo transformation (the ability of a cell to take the DNA from the environment). There are two classes of competent cells: *Natural competence:* a genetically specified ability of bacteria that is occur under natural condition, and *artificial competence:* when cells in laboratory cultures are treated to be permeable to DNA.

Transformation is a very basic technique that is used on a daily basis in a molecular biological laboratory. In 1971-1973 recombinant DNA technology was developed as gene cloning to be its major application. Transformation can be done using different methods including: electroporation (or Electropermeabilization) and less the less efficient method called Chemical transformation.

Transformation efficiency is a quantitative value that describes how effective you were at getting plasmid DNA into your competent cells. The number represents how many cells were transformed per microgram (µg) of plasmid DNA used. This calculation requires two values: the number of cells that were successfully transformed and the amount of plasmid DNA used for the transformation.

Principle:

Introduction of recombinant plasmid into cells is achieved by the transformation of competent cells. In the chemical transformation method the competent cells are prepared by treating the cell with a divalent cation like calcium chloride solution which help the cells to take up the DNA plasmid by increasing the bacterial cells membranes permeability [renders them competent to take up DNA]. Once the cells are made competent, the plasmid DNA is mixed with the cells. The competent cells are then subjected to heat shock, which allows the DNA to enter the cells. The cells are then plated onto a LB agar plate containing appropriate antibiotic to be able to count the transformed colonies only (which they are colonies containing transformed cells containing the recombinant DNA), each colony on an antibiotic plate presents a single transformation event. The recombinant plasmid can be amplified as well.

Materials:

Chemical

$CaCl_2$, LB medium, NaCl, Tryptone, Yeast extract**,** Ampicillin

Preparation of solutions

1) LB medium

To 950 ml of deionize H_2O add 10g Tryptone, 5g yeast extract, and 10g NaCl. Shake until the solution dissolve. Adjust pH to 7.0 with 5N NaCl. Adjust the volume to 1L with deionize H_2O. Sterilize by autoclave for 20 minutes at 15 psi on liquid cycle.

2) 50 M $CaCl_2$ solution:

Dissolve 7.4g of CaCl2.2H2O in d.H2O, make up the volume up to 1L by d.H2O. Autoclave the solution then store it at 4°C

3) Ampicillin:

Dissolve ampicillin sodium salt in AnalaR water to a concentration of 100 mg/ml. Filter the solution through a 0.2µm filter. It should be added to a final concentration of 100 µg/ml. Store at 20 °C**.**

Equipment and Glassware

Microfuge centrifuge, electronic balance, water bath, roller, plate, incubator, microcentrifuge tube, centrifuge tube, Pasteur pipette, micropipette, tips.

Protocol:

A) Competent cells formation:

1. Centrifuge 10-15 ml of bacterial sample for 7 minutes at 3000 rpm at 4°C. allow centrifuge to decelerate without break.
2. Discard the supernatant and resuspend each pellet gently in 10 ml ice cold $CaCl_2$ solution.
3. Centrifuge the cells for 5 minutes at 2500 rpm at 4 °C.
4. Discard the supernatant and resuspend each pellet in 10 ml ice cold $CaCl_2$ solution.
5. Keep resuspended cells on ice for 10 minutes.
6. Centrifuge the cells 5 minutes at 2500 rpm at 4 °C.
7. Discard the supernatant and resuspend each pellet in 2 ml ice cold $CaCl_2$ solution.

B) Transformation of competent cells with DNA:

1. Transfer 100 ng of plasmid DNA in a volume of 25 µl into clean microcentrifuge tube and place it on ice.
2. Rapidly add 100 µl of competent cells immediately into microcentrifuge tubes containing the plasmid DNA. Gently swirl tubes to mix, then place them on ice for 10 minutes.
3. Store the remaining competent cells at -80 °C.
   Note: Competent cells should be used immediately after thawing. Remaining cells should be discarded rather than refroze it.
4. Using the thermomixer, heat shock the cells by placing the tubes into a 42 °C for 45 sec.
5. Add 1 ml LB medium (without antibiotic).
6. Place each tube on the thermomixer at 250 rpm for 20-60 minutes at 37°C.
7. Plate aliquot of transformation culture on LB/Ampicillin or other appropriate antibiotic containing plates.
8. When plates are dry, incubate 24 to 48 hours at 37 °C.
9. A negative control should also be included that contains cells with no added plasmid DNA.
10. Calculate the transformation efficiency.

Results:

- **Transformation efficiency calculation:**

Count white colonies as recombinant transformants and test for insert. Calculate the transformation efficiency in terms of the number of colony-forming units (CFU) per microgram of transforming DNA as follows:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies}}{\text{Amount of DNA plated [µg]}}$$

$$= \_\_\_\_\_ \text{ CFU/µg}$$

References:

1. Campbell N, Reece JB. (2005) Biology. 7th edition. Pearson.
2. Cohen S.N., Chang A.C.Y., and Hsu L. 1972. Nonchromosomal antibiotic resistance in bacteria: Genetic transformation of Escherichia coli by R-factor DNA.Proc. Natl. Acad.Sci. 69: 2110-2114.
3. Sambrook,J., Fritsch, E.F. andManiatis, T. (2001) Molecular Cloning. A laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press.