SEED 2009

Day 6: Transformation

Learning Objective: Understand transformation of a plasmid into a host strain to complete the cloning process. Gain practical experience with the importance of selection and antibiotic resistance.

Background: Transformation is a process by which cells pickup foreign DNA from their surroundings and incorporate it into the cytoplasm. Transformation a common method for introducing plasmids into a host cell. For a transformation to be successful, the cells must be ***competent***. There are two ways in which cells can be competent, ***electrocompetent*** and ***chemically competent***. For electrocompetent cells, a high voltage is passed through the media in a process called electroporation. For chemically competent cells, the cells are prepared in a proper salt solution and heat shocked to allow DNA to enter the cell.

Following transformation, cells are grown in rich media to allow them to recover from their fragile, damaged state and to build up selective resistance (if they contain the target DNA). Following this period, the cells are plated on selective media so that only cells with the targeted DNA grow. Single monoclonal colonies can then be selected which contain the target DNA.

The exercise: You will transform the plasmids you ligated this morning into chemically competent cells. Only products which were properly ligated will replicate within a cell and confer resistance to ampicillin. You will then plate these transformations to obtain clones. A positive and negative control will help to determine if the transformation and/or ligation was a success.

Materials:
Chemically Competent Cells
Ligation Product
Sterile Water
pUC19 Control Vector
Heat Block
Ice Bucket

Protocol

1. Keep cells on ice at ALL times, and allow to defrost. NEVER vortex or use the pipette to mix competent cells.

2. To a new clean microcentrifuge tube, add 5 uL sterile water and label as a negative control. To another tube, at 5 uL of the pUC19 control vector and label as a positive control.

3. To each of the ligation mixtures and controls, VERY GENTLY add 25 uL of chemically competent cells. Do NOT pipette up and down to mix. Each tube contains exactly 50 uL, so be very careful with the volume.

4. Incubate on ice for 30 minutes.

5. Place tubes in 42C incubator for exactly 30 seconds.

6. Remove the tubes and place them on ice.

7. Add 250 uL of SOC media to each tube. Be very careful to use proper sterile technique as there is no antibiotic selection at this point!

8. Incubate the tubes at 37C for 30 minutes.

9. Plate all of the transformation mixture onto an LB-Agar plate with Ampicillin, IPTG, and X-Gal. Grow overnight at 37C.