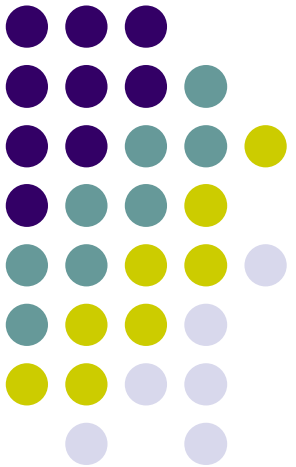
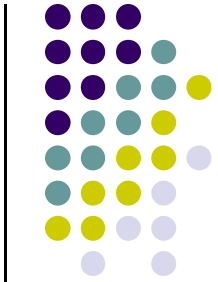
TRANSFORMATION USING CALCIUM CHLORIDE

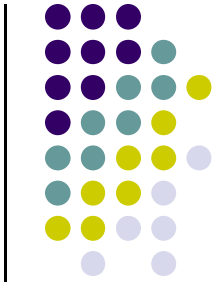


Objectives



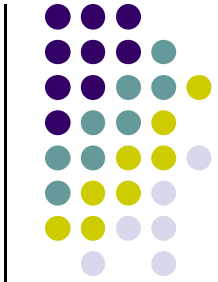
- To understand the concept and procedure of calcium chloride transformation
- to successfully transform cells to constitutively express our gene of interest (GFP in a DH5alpha cell)

BioBrick part(s)



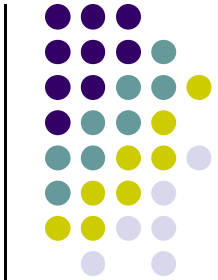
- BBaI722008 (http://partsregistry.org/wiki/index.php/Part:BBa_I13522)
  - This is a constitutively expressed GFP which can be “turned off” with tetracycline
  - This part has been tested and proved to work

Procedure



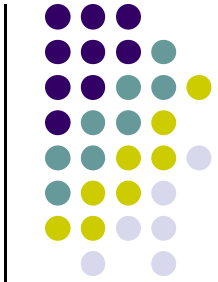
- Prepare competent cells
- Assess competency of cells
- Transform competent cells

Materials



- BioBrick Part: BBa_I13522 pTet-GFP
- Single colony of *E. coli* cells: DH5alpha
- LB medium
- CaCl2 solution, ice cold
- LB plates containing ampicillin
- Chilled 50-ml polypropylene tubes
- Beckman JS-5.2 rotor or equivalent
- 42°C water bath

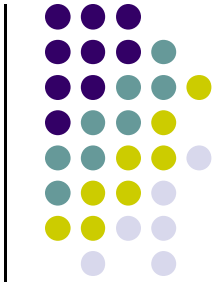
Prepare competent cells



- 1. Inoculate a single colony of *E. coli* cells into 50 ml LB medium. Grow overnight at 37°C with moderate shaking (250 rpm)
- 2. Inoculate 4 ml of the culture into 400 ml LB medium in a sterile 2-liter flask. Grow at 37°C, shaking at 250 rpm, to an OD 590 of 0.375.

- 3. Aliquot culture into eight 50-ml prechilled, sterile polypropylene tubes and leave the tubes on ice 5 to 10 min.
- 4. Centrifuge cells 7 min at 1600 × *g* (3000 rpm in JS-5.2), 4°C. Allow centrifuge to decelerate without brake.
- 5. Pour off supernatant and resuspend each pellet in 10 ml ice-cold CaCl2 solution.
- 6. Centrifuge cells 5 min at 1100 × *g* (2500 rpm), 4°C. Discard supernatant and resuspend each pellet in 10 ml ice-cold CaCl2 solution. Keep resuspended cells on ice for 30 min.
- 7. Centrifuge cells 5 min at 1100 × *g*, 4°C. Discard supernatant and resuspend each pellet in 2 ml ice-cold CaCl2 solution.
- 8. Dispense cells into prechilled, sterile polypropylene tubes (250-µl aliquots are convenient). Freeze immediately at −70°C.

Assess competency of cells



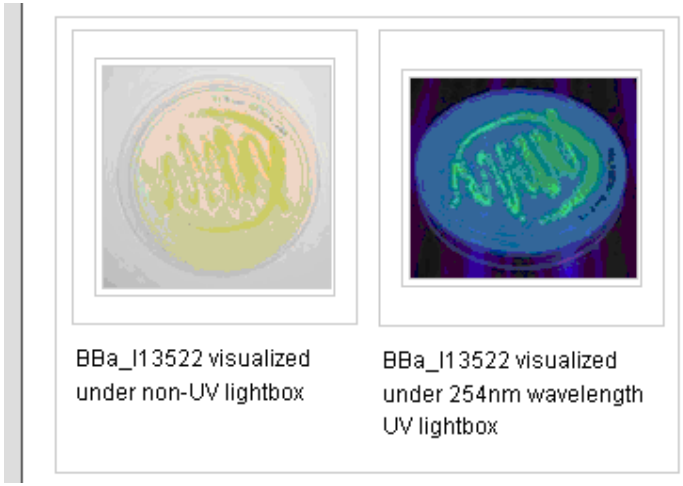
- 9. Plate appropriate aliquots (1, 10, and 25 µl) of the transformation culture on LB/ampicillin plates and incubate at 37°C overnight.
- 10. Calculate the number of transformant colonies per aliquot volume (µl) × 10

Transform competent cells

- 11.15µl of cell and 15µl of plasmaetes were taken in the tube and frozen in the ice bath for 30 minutes.
- 12. The LB broth solution was made by mixing 6.25g of LB broth powder in 250ml of DI water and autoclaving it for 15 minutes.
- 13. The tubes were placed in a water bath at 42^{o}C for 90 seconds (heat shock step).
- 14. The tubes were cooled for 15 minutes in the ice bath.
- 15.1ml of the prepared LB broth solution was added to the cells and shaken in the incubator for about an hour.
- Control was prepared by adding 1ml of the LB broth solution to the 15µl of the cells and shaking it in the incubator for about an hour.
- 15µl of the sample was transferred to the petridish and streaked with a sterilized glass rod.
- The petridishes were left in the incubator for about 12 hour for the growth.
- After 12 hours the petridishes were viewed under UV light to see the cells

Outcome of experiment

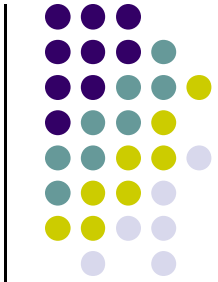
- When the colonies were hit with UV rays from the UV lamp, in 2 out of the 4 petridishes, the cells exhibited a green fluorescent color
- When tetracycline was added to the medium, the cells no longer exhibited a green fluorescent color



BBa_I13522 visualized under non-UV lightbox

BBa_I13522 visualized under 254nm wavelength UV lightbox

Skills learned in this lab exercise



- Transformation of Plasmid DNA into E.Coli cells using CaCl2, Heat Shock Transformation
  - Calcium ions help the uptake of the Plasmid DNA (making it competent)
  - Mixture of DNA and cells is heat shocked to allow entry of DNA into cells
  - Cells are grown to allow synthesis of proteins encoded in Plasmid DNA

- Lab technique