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 $1600 \times g$ (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 CaCl$_2$ solution.

6. **Centrifuge** cells 5 min at $1100 \times g$ (2500 rpm), 4°C. Discard supernatant and **resuspend** each pellet in 10 ml ice-cold CaCl$_2$ solution. Keep resuspended cells on ice for 30 min.

7. **Centrifuge** cells 5 min at $1100 \times g$, 4°C. Discard supernatant and **resuspend** each pellet in 2 ml ice-cold CaCl$_2$ solution.

8. **Dispense** cells into prechilled, sterile polypropylene tubes (250-µl aliquots are convenient). **Freeze** immediately at $-70^\circ$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°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.
16. 15µl of the sample was transferred to the petridish and streaked with a sterilized glass rod.
17. The petridishes were left in the incubator for about 12 hour for the growth.
18. 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.

[Images: 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