CMBL – CRJ/JJR
8/10/07

Protocol: Transfecting NIH 3T3s with GFP-Actin

Materials:

- 3T3 Fibroblasts (seeded in 35 mm dishes or a 6-well plate)
- FuGENE 6 transfection reagent (Roche Applied Science Cat# 11 815 091 001)
- pAcGFP1-Actin vector (Clontech Cat.# 632453)
- DMEM (Invitrogen #11995-065)
- Water bath
- Serological Pipets
- Pipet aid
- Pipette tips
- 20 μL, 200 μL, 1000 uL pipetter
- Microscope
- Sterile polystyrene tube
- Timer
- Waste beaker
- Biohazard Bag
- 70% ethanol
- Kimwipes
- Markers
- Gloves

Procedure:

Adding serum-free DMEM to cells

1. Heat DMEM to 37^{o}C in a water bath
2. Prepare the hood:
   - spray and wipe the hood surface with 70% ethanol
   - place the following materials into the hood: waste beaker, tube rack, polystyrene tube, pipetting materials, pre-warmed DMEM
   - tape a biohazard bag to the front of the hood
3. Observe the cells under microscope – check for contamination and note cell confluence
4. Place the cells in the hood
5. Remove the cell culture media, and add 2 mL of DMEM (without serum or Pen/Strep)
6. Label the dish (initials) and place the dish back in the incubator

Diluting FuGENE 6 with DMEM

7. Aseptically place FuGENE 6 and GFP-Actin DNA in the hood
8. Pipette 91 uL of DMEM into a sterile polystyrene tube
9. Pipette 9 uL of FuGENE 6 directly into the DMEM
   - **Avoid** direct contact between FuGENE and the tube wall
10. Tap to mix
11. Incubate for 5 min @ room temp

Forming FuGENE/DNA complex

12. Add 5.3 uL of pAcGFP1-Actin vector (1 ug) to the dilute FuGENE
13. Tap to mix
14. Incubate for 15 min @ room temp

15. Remove the dish of cells from the incubator
16. Add the FuGENE/DNA complex to the cells in a drop-wise fashion
17. Gently swirl dish to mix
18. Label the dishes (GFP-Actin, date)
19. Place the cells back in the incubator (add serum containing medium in 6-24 hrs)
20. Clean the hood and place all material that contacted cells/medium in a biohazard waste bag; clean the waste beaker by adding bleach for ~15 min. before washing