

## Plat-E Transfection and Bone Marrow Macrophage Retroviral Infection

### **Day 1**

1. Flush BMM
2. Seed  $5 \times 10^6$  cells in a 10 cm culture dish on the prior day

### **Day 2**

3. In a 1.5 ml microtube put 280  $\mu$ l of DMEM FBS free, add 18  $\mu$ l of FuGene, flick gently then add 12  $\mu$ g of plasmid DNA. Incubate at RT for 30-40 min.
4. During this incubation discard Plat-E cells SN, wash cells once with 10 ml serum free DMEM (don't point the pipet to the cells as they may be washed off the plate), add 10 ml of **serum free** DMEM and put at 37°C 5% CO<sub>2</sub>
5. Add the mixed DNA-FuGene complex by dropwise into the **serum free** culture media
6. Incubate 5-6 hours at 37°C, 5% CO<sub>2</sub>
7. Discard transfection media then add 10 ml of **complete** media (DMEM, 10%FBS)
8. Incubate at 37°C, 5% CO<sub>2</sub> O/N

### **Day 3**

9. Change media and transfer cells to 32°C, 5% CO<sub>2</sub> O/N

### **Day 4**

10. Collect virus supernatant from transfected Plat-E cells in a 15 ml falcon tube and centrifuge 10 min at 1100 rpm
11. prepare virus cocktail as follows :
  - Virus sup 5ml
  - BMM complete media 5ml
  - Polybrene (Sigma H-9268) final 4  $\mu$ g/ml
12. Aspirate culture media from cultured BMM

13. Add 10 ml virus cocktail
14. Incubate for one day at 37°C, 5% CO<sub>2</sub>
15. For transient expression use cells 24-48 hours after infection
16. For stable expression :
  - collect BMM 24 hours after infection
  - replate cells in complete media + puromycin at 2 µg/ml final for selection