Sugden Lab.

Transfection by Lipofectamine

(using Invitrogen Lipofectamine™)

1. The day before transfection plate cells into 6 cm dish so that they are 80~90% confluent the day of transfection.

2. Dilute 3 µg DNAs and 1 µg reporter DNAs (fluorescence proteins) into OptiMEM or DMEM without FBS and mix gently.

3. In the other tube dilute 6-10 µl Lipofectamine™ into 300 µl OptiMEM or DMEM without FBS and incubate within 5 min at r.t.

4. Combine diluted DNAs and diluted Lipofectamine reagent, mix gently and incubate for 15~45 min at r.t. to allow DNA-loposome complexes to form.

5. While complex are forming replace the medium on the cells with 3 ml OptiMEM or DMEM without FBS.

6. For each transfection, add 600 µl medium contains complexes dropwise onto the cells and incubate for 4~6 h at 37˚C in a CO_2 incubator.

7. Following incubation, Add 3 ml DMEM contains 20% FBS into plate without removing the trasnfeciton mixture. I toxicity is a problem, remove the transfection mixture and replace it with complete growth medium.

8. Replace the medium with fresh, complete medium at 18~24 h following the start of transfection if continued cell growth required.

9. Harvest cells and determine the transfection efficiency by % of fluorescence protein-positive cells 24~72 h after the start of transfection. Passage the cells 1:10~1:20 into the selective medium for the reporter gene transfected.