Nucleus Protocols

Assemble Base Cell

1. Prepare Stock Solutions

- ☐ Add 1 mL of mineral oil to the glass vial using a 1 mL pipette.
- ☐ Add the lipids in the above table to the glass vial on top of the mineral oil using the appropriate glass syringe.
- ☐ Briefly vortex the lipid-oil mixture for 5 seconds to mix.
- ☐ Evaporate the chloroform from the lipid–oil mixture:
    - ☐ Place glass vial in a 55°C dry bath in a fume hood.
    - ☐ Shield with aluminum foil to protect from light.
    - ☐ Evaporate uncovered for 4 hours.
- ☐ In a glass bottle, add 4 mL of chloroform using a glass 10 mL serological pipette.
- ☐ Clean syringes by rinsing with chloroform 5 times into an empty glass bottle. Store the syringes with the plunger removed inside the fume hood for (3–4) hr to allow remaining chloroform to evaporate.
- ☐ After 4 hr of incubation at 55°C in a dry bath in a fume hood, allow the lipid-oil mixture to cool to room temperature for 10-15 min.
- ☐ Return the plungers to the syringes and store them in their designated location.
- ☐ Dispose of chloroform waste following applicable chemical safety guidelines.

2. Assemble Outer Solutions

- ☐ Prepare 1.5 mL microcentrifuge tubes labeled with the appropriate reaction.
- ☐ Mix glucose stock solution and water according to the following table:

Component	Cells + deGFP DNA (µL)	Cells - deGFP DNA (µL)
Glucose (2 M)	570	570
Water	430	430
Total	1000	1000

3. Assemble Cytosol Reactions

- ☐ Remove all components listed in the Composition table above from appropriate cold storage.
- ☐ Thaw reagents on ice.
- ☐ Prepare 1.5 mL microcentrifuge tubes, on ice, to assemble reactions into.
- ☐ For a given reaction, assemble by adding the volume of reagents from the Composition table in the order listed. Pay special attention to the handling of the Cytosol components:
    - ☐ Vortex SMix: Ensure thorough mixing; 10s vortex / 10s rest on ice; should be transparent with no visible precipitate; and add to the reaction tubes.
    - ☐ Vortex or pipette mix tRNA, and add to the reaction tubes.
    - ☐ Vortex or pipette mix PMix, and add to the reaction tubes.
    - ☐ **Do NOT vortex** ribosomes: *gently* pipette mix or flick the tube, and add to the reaction tubes.
    - ☐ Add remaining reactions in the order they appear
- ☐ Mix the master mix thoroughly by pipetting up and down (10–15) times until it appears homogeneous and clear.
- ☐ Close lids on the microcentrifuge tubes and briefly spin down to eliminate bubbles.

- ☐ Pipette out 10 µL of the reaction for osmolarity check using a Vapor Pressure Osmometer before starting encapsulation.
- ☐ Hold assembled reactions on ice until ready for encapsulation.

4. Encapsulate Cytosols into Liposomes

- ☐ Set up a 1.5 mL tube rack with two 1.5 mL microcentrifuge tubes for each liposome encapsulation. Number the tubes according to the number of reactions assembled. Label the two tubes for each reaction:
    - ☐ **T**—transfer
    - ☐ **L**—liposomes
- ☐ Add 300 µL of the appropriate glucose outer solution to each of the tubes labeled **T**.
- ☐ Add 150 µL of the lipid-oil mixture (at room temperature) on top of each assembled Cytosol reaction.
- ☐ Emulsify the lipid-oil and Cytosol reaction by running the tube along a row of empty slots on the 1.5 mL tube rack. Run it down 20–30 times until the solution forms a stable emulsion with an even milky color.
- ☐ Immediately layer each emulsion over the outer solution. Slowly pipette the entire emulsion down the side of the corresponding **T** tube.
- ☐ Centrifuge **T** tubes at 9000 g for 10 min at room temperature to pellet the liposomes.
- ☐ Extract the liposomes from each **T** tube:
    - ☐ Remove the oil layer and lipid debris from the top of each **T** tube by gently pipetting with a 1000 µL pipette set to 800 µL.
    - ☐ Gently pipette mix the pellet 10-15 times with the outer solution.
    - ☐ Extract liposomes by pipetting 50-100 µL of pellet and outer solution from **T** and transfer liposome sample to the respective liposome tube **L**.
- ☐ Hold liposomes on ice until you are prepared to begin measurement.
- ☐ Pipette the liposomes into a well on a 384-well glass bottom plate. If the density appears too high under the microscope, dilute the liposomes with the outer solution for better data analysis.
- ☐ Begin measurement.
- ☐ Add a black dot to the lid of each of Cytosol component. The number of dots indicates freeze–thaw cycles.