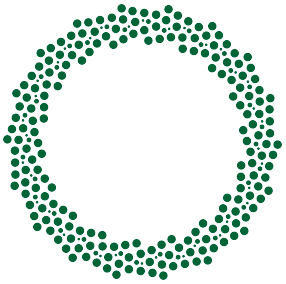

Nucleus Protocols

Protocol

Reporter: deGFP (Cells)

1. Overview

This protocol show you how to validate the functionality of the Reporter Module `pOpen-deGFP` in cells using the POPC:cholesterol Membrane Module.

1.1. Cytosol Reaction Setup

Table 1. *Reaction setup.*

Component	Cells + deGFP DNA [µL]	Cells - deGFP DNA [µL]
SMix	12	12
tRNA	4	4
PMix	4.8	4.8
Ribosomes	7.2	7.2
RNase Inhibitor	2	2
`pOpen-deGFP` DNA	0.95	0
Optiprep	1.33	1.33
Water	7.72	8.67
Total master mix volume [µL]	40	40

1.2. Protocol

1.2.1. *Prepare Stock Solutions*

Prepare lipids in mineral oil

- Add 1 mL of mineral oil to the 1.8 mL glass vial using a 1 mL pipette.

> **Warning**
>
> Work inside of a fume hood when handling chloroform and lipids.

- Add the lipids 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 hours to allow remaining chloroform to evaporate.
- After 4 hours 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 minutes.

> **Note**
>
> The lipid–oil mixture can be used immediately after cooling to room temperature or stored at 4°C for up to one week. Protect from light by storing in an opaque container or wrapping the vial with aluminum foil.

- Return the plungers to the syringes and store them in their designated location.
- Dispose of chloroform waste following applicable chemical safety guidelines.

1.2.2. *Assemble Outer Solutions*

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

Table 2. *Preparation of outer solutions. These values are approximates and may vary based on the measured osmolarity of your inner solution.*

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

> **Note**
>
> These outer solution concentrations may vary and should be based on the measured osmolarity of your inner solution.

1.2.3. *Assemble Cytosol Reactions*

- Remove all components listed in the Reaction Setup table above from appropriate cold storage.
- Thaw reagents on ice.
- Prepare 1.5 mL microcentrifuge tubes, on ice, to assemble reactions into.

> **Tip**
>
> Prepare the reaction on ice or a cold block to prevent protein expression from starting during assembly. This ensures the plate reader captures the complete fluorescence kinetics for deGFP expression.

- For a given reaction, assemble by adding the volume of reagents from the 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.

Critical

Adjust the outer solution concentration so its osmolarity is 100–120 units lower than the inner solution when measured on a Wescor EliTech Vapro 5600 Vapor Pressure Osmometer.

- Hold assembled reactions on ice until ready for encapsulation.

1.2.4. *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 uL of the appropriate glucose outer solution to each of the tubes labelled **T**.
- Add 150 uL 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.

Tip

While running the tubes on the tube rack, hold the cap firmly to prevent it from coming off during vigorous mixing.

- 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.

Tip

Align the hinges of each T tube towards the outside of the centrifuge rotor, so that the final pellet location will be indicated by the tube hinge since the liposome pellet may not always be visible.

- 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 uL pipette set to 800 uL.
    - Gently pipette mix the pellet 10-15 times with the outer solution.
    - Extract liposomes by pipetting 50-100 uL of pellet and outer solution from **T** and transfer liposome sample to the respective liposome tube **L**.

Critical

Critical: Do not transfer the entire solution. It is important to avoid transferring the top of the solution which may contain a residual oil layer.

- 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.

Return reagents to their appropriate storage locations.

- Add a black dot to the lid of each of Cytosol component. The number of dots indicates freeze–thaw cycles.