Luciferase and β-galactosidase assays in procyclic forms

Transient transfection

- Count cells. You should have $2x10^7$ cells per transfection at $\sim 7.5x10^6$/ml.
- Centrifuge cells at 1,800 rpm (speed 4) for 10 min at 4°C.
- Discard supernatant and keep pellet on ice.
- Resuspend in 5 ml cytomix to wash cells: keep cold.
- Centrifuge cells at 1,800 rpm (speed 4) for 10 min at 4°C.
- Resuspend in appropriate amount of Cytomix. You need 500 μl per transfection.
- Pipette 10 μg (not more than 50 μl) of DNA into each cuvette.
- Immediately add 500 μl of Cytomix and cells to cuvette.
- Electroporate: Voltage 1.6, Resistance 2.5 kV. Resistance timing R2.
- Pipette 550 μl of cell into 5 ml of SDM-79 and incubate for 16–22 h at 26 °C.

Preparations for assay

- 1x lysis buffer from 5x stock (cell culture lysis reagent, Promega) at RT.
- Calibrate Turner, TD-20e Luminometer by inserting empty tube before turning it on.
- Thaw luciferase substrate (Promega: this should be stored at –70°C). Substrate should be at room temperature when performing assay.
- Pipette 45 μl aliquots of luciferase substrate into assay tubes and keep dark.

Harvesting cells

- Transfer all 5 ml of cells into 15 ml Falcon tubes.
- Centrifuge cells at 1,800 rpm (speed 4) for 10 min at 4°C.
- Discard supernatant and keep pellet on ice.
- Resuspend pellet in 1 ml of cold PBS and transfer to Eppendorf tube.
- Centrifuge for 2–3 min at 7,000 rpm in cold room.
- Aspirate supernatant and keep pellet on ice.

Luciferase assay

- Resuspend pellet in 100 μl lysis buffer. **Proceed to next step immediately**.
- Add 10 μl of lysed cells to 45 μl luciferase substrate, tap tube on table to move mixture to bottom of tube and insert into luminometer immediately.
- Good luciferase activity from a PARP promoter regulated gene should give values ~ 850.

β-galactosidase assay

- Resuspend pellet in 100 μl lysis buffer. 10μl of lysed cells are used for luciferase assay.
- Centrifuge the remaining 90 μl of lysed cells at 17,900 g at 4 °C for 4 min.
- Add 30 μl of cell lysate supernatant to a mixture of 260 μl 0.1 M sodium phosphate, 6.6 μl 10x CPRG (Roche), and 3 μl 100x Mg^{2+} solution (0.1 M $MgCl_2$, 4.5 M β-mercaptoethanol)
- Incubate for 8 h at 37 °C.
- Add 500 μl of 1 M Tris to terminate reaction.
- Transfer all 800 μl to a disposable cuvette and measure absorbance at 570 nm (Novaspec II spectrophotometer).