Transfection of MCF-7 with Lipofectamine 2000

(Maartje Vogel version 120903)

Please check http://www.nki.nl/nkidep/vansteensel for updated versions of this protocol.

One day before transfection

- Harvest cells from exponential phase.
- Seed 4×10^5 cells per well (of a 6-well plate) in 2 ml DMEM complete medium (10% FCS + antibiotics (e.g pen/strep)).
- Rock the plate after seeding.

Cells should be ~90% confluent at the time of transfection.

Day of transfection

Consider the following (control) transfections:

> … x Plasmid(s) encoding the Dam protein
> … x Plasmid(s) encoding the Dam-fusion protein (your protein of interest fused to Dam).
>
> Controls:
> 1 x pH2B-GFP (to determine the transfection efficienty by FACS).
> 2 x no plasmid (control in methylation PCR and neg. control for FACS).
>
> 1 x pIND-(V5)-EcoDam and
> 1 x pIND-Cbx1-V5-EcoDam (serve as a positive control in the methyl-PCR and hybridizations).
>
> 1-2 x pIND (empty vector, serves as a control in the methyl PCR).

- For each transfection sample, prepare in separate 1.5 ml tubes:
  1) 2 μg DNA in 100 μl Opti-MEM-1 (RT).
  2) 6 μl Lipofectamine 2000 in 100 μl Opti-MEM-1 (RT). Mix the Lipofectamine gently before use.
- Incubate 5 min. at RT (proceed to next step within 30 min. after diluting the lipofectamine)
- Combine the two solutions, mix gently and incubate for 20 min. at RT (The mixture may appear cloudy).
- In the mean time: wash the cells with DMEM (w/o serum and antibiotics).
- After the 20 min. incubation: add 800 μl DMEM (w/o serum and antibiotics) to the DNA-Lipofectamine mixture, mix gently and add this to the cells.
- Incubate for 5 hours in a 37°C incubator.
- Carefully add 1 ml of DMEM (with **20**% FCS, w/o antibiotics) to the cells (don't remove the transfection mixture).
- Incubate o/n in a 37°C incubator.

First day after transfection

- The next morning: replace transfection mixture with DMEM complete medium.

Second Day after transfection

- 48 hours after start of transfection harvest the cells by trypsinization.
- Add ~1.7 ml DMEM complete and transfer cells to a 2 ml tube and spin 5 min. at 300×*g*.

For FACS samples:

- Remove supernatant, resuspend cells in PBS.
- Transfer the cells to a FACS tube and spin 5 min. at 300×*g*.
- Remove supernatant and resuspend cells in ~0.5 -1ml PBS.
- Keep cells on ice.
- Perform FACS assay.

For the other samples:

- Remove supernatant as much as possible.
- Continue with the gDNA isolation or store the cell-pellet at -80°C.

Genomic DNA isolation

Isolate the gDNA with the DNeasy Tissue kit. Follow the 'DNeasy Protocol for Cultured Animal Cells'.

- Include RNase A in step 1.
- Elute gDNA in 1×200 µl + 1×100 µl buffer AE in a 2 ml tube.
- Measure concentration of gDNA.

Ordering information

Lipofectamine 2000	Invitrogen	Cat. No.: 11668-027
Opti-MEM I	Invitrogen	Cat. No.: 31985-047
DNeasy Tissue Kit (50)	Qiagen	Cat. No.: 69504
RNAse A solution 100 mg/ml	Qiagen	Mat. No.: 19101