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 $4 \times 10^5$ 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 efficiency 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