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PEI transfection protocol for imaging of transient expression of target protein

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We use this protocol and it's working

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Abstract

How to transfect U2OS cells with PEI, for transient expression of GPF-fused protein.

Materials

- U2OS cells (RRID: CVCL_0042)
- 1.5ml tubes (Eppendorf, #0030 120.086)
- 12-well plates (Lab solute, #7696791)
- Coverslips (Carl Roth, #YX03.1)
- Centifuge 5810R (#5811000015, Eppendorf)
- Safety cabinet (Thermo, #Safe S2020 1.2)
- Automated cell counter (Biorad, #TC20)
- Trypan blue solution for cell counting (Sigma, #T8154)
- Counting Slides for cells (Biorad, #145-0011)
- Serologicla Pipettes (Greiner, cellstar)
- 50ml Falcon tubes (Corning, #352070)
- 15ml falcon tube (Corning, #352096)
- Incubator (Thermo, #BBD 6220, CO2 Incubator), settings: 5% CO2, 95%rH, 37°C

- Milli-Q water system (Merck, Advantage A10)
- Poly-D-Lysine (Gibco, #A38904-01)
- PEI (polyethylenimine, MW 25 kDa, Polyscience, #23966)
- DMEM (Sigma, #D6429)
- Opti-MEM (Gibco, #31985-047)
- Formaldehyde (Carl Roth, # CP10.1)
- DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride, Thermo Fisher #D1306)
- Permafluor mounting medium (#041300, Menzel)

Day 1

1 From a healthy 80% confluent U2OS cells (RRID:CVCL_0042), seed 50 000 cells into a 12-well plate with cover glasses (either ⌀ 12mm)

2 Prepare PEI stock solution as the following
- dissolve 100mg of PEI (polyethylenimine, MW 25 kDa, Polyscience, 23966) in 90ml warm sterile ddH2O in a glass beaker
- add HCl dropwise (37%) to pH 2.0 (a few drops should be enough)
- stir until everything is dissolved (~10min)
- adjust to pH 7.0 with NaOH (1M)
- adjust final volume to 100ml
- filter and store aliquots at -20°C for long term storage

3 Prepare the following transfection solution in a 1.5ml tube:
1:3 (µg DNA:µg PEI) in 100µL total of DMEM or Opti-MEM (without serum and anitbiotics!)

+ 1µg DNA (plasmid containing candidate of choice, optionally fused to a fluorescent protein like GFP or RFP)
+ 3µg PEI (prepare 1mg/ml stock solution in sterile ddH2O)
Add up to 100µl DMEM (Sigma, D6429) or Opti-MEM (Gibco, 31985-047)

3.1 Vortex quickly

3.2 Incubate transfection solution 15min at RT

4 Add the ~100µl transfection solution to the well (drop-wise) and gently swirl
(depending on the candidate protein, 20-30% of the transfection solution is enough to transfect the cells, reducing the risk of inducing artifacts of overexpression)

5 Place cells back to incubator at 37°C

Day 2

6 Replace media with complete DMEM media.
(Incubation time with PEI might affect the degree of expression of the candidate protein, and might vary depending on the candidate)

Imaging after 12h or up to 48h

7 Time of expression after PEI removal might vary depending on the candidate protein. Usually, after 12h to 48h the cells are ready to be imaged

Fixing cells

8
- Wash cells three times with PBS
- Fix with 4% formaldehyde (v/v) in PBS for 10 min (prepared freshly!)
- Incubated 5 min with DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride, Thermo Fisher D1306, 0.02 µg/µl in PBS) at RT (note: do not perform this step on ice!)
- Wash 3 times with PBS

- Mount coverslips in Permafluor mounting medium using glass slides (041300, Menzel)
- Let dry at room temperature overnight

- Store samples at 4°C in the dark until further analysis by microscopy

note: If lysotracker was added prior fixation, the fluoresence will decrease over time. Imaging is best after the mounting media has dried.