

*T*ransfection of marine fish cell lines using polyethylenimine (PEI)

PREAMBULE. Cell cultures should be pre-confluent (approx. 75% of confluence) and must therefore be subcultured accordingly 1-2 days before. Volumes and quantities given below are for a single transfection reaction.

1. The day before the transfection: Seed cells in a 12-well plate at 5×10^4 cells per well and culture them for 12-16 h in appropriate culture conditions.
2. The day of the transfection: Prepare a fresh solution of PEI by mixing stock solution and serum-free culture medium in appropriate proportions (see note below). Each transfection reaction requires 100 μ L of diluted PEI solution.

Highest nontoxic concentration of PEI should be determined for each cell line prior to transfection. Example: VSa13 cells are transfected using 25 mg/L of PEI.

3. For each transfection: Place 1.5 μ g of vector DNA in a sterile 1.5-mL microcentrifuge tube and add serum-free medium up to a final volume of 50 μ L.
4. Mix 100 μ L of freshly prepared PEI solution with 50 μ L of DNA solution and homogenize by pipetting up and down 5 times.
5. Incubate PEI-DNA complex for 15 min at room temperature.
6. Meanwhile, renew culture medium with 850 μ L/well of serum-free medium.
7. Add PEI-DNA complex (150 μ L) to each well and incubate cells for 8 h in appropriate culture conditions.
8. Renew culture medium containing PEI-DNA complex with 1 mL/well of complete medium.

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Apparatus: Cell incubators; a class II biological safety cabinet.

Cell culture medium: Dulbecco's modified Eagle medium (DMEM); Leibovitz's medium (L15).

Medium supplements: Fetal bovine serum (FBS); L-glutamine; antibiotics; fungizone.

Solutions: phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM Na_2HPO_4 , 1.23 mM KH_2PO_4 ; pH 7.4). Stock solution of polyethylenimine 25 kDa (40 g/L in sterile water; storage at -80°C)

Plasticware: 12-well cell culture dishes and serologic pipettes.

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Braga D, Laizé V, Tiago DM, Cancela ML (2006) Enhanced DNA transfer into fish bone cells using polyethylenimine. *Molecular Biotechnology* 34(1):51-54