Nucleofection of marine fish cell lines

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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 nucleofection reaction.

1. Fill a 6-well plate with 1.5 ml of culture medium per well (prepare 1 well per transfection) and place it at appropriate temperature (i.e. temperature of cell culture) until used.
2. Trypsinize the cells to be nucleofected (see below) and suspend them in fresh culture medium. Determine the density of cell suspension using a Newbauer chamber.

   Trypsinization: Remove culture medium, wash cell layer with PBS and add trypsin-EDTA solution (1 ml per 10-cm dish). Monitor cell detachment microscopically and stop trypsin action by adding fresh medium supplemented with FBS.
3. Transfer appropriate volume of cell suspension (1-5 x 10^6 cells per transfection) in a clean tube and harvest the cells (90 rcf for 10 min at room temperature). Carefully discard supernatant.
4. Suspend cell pellet in 100 µl of pre-warmed Nucleofector solution then add 2 µg of appropriate vector (e.g. pmaxGFP from LONZA).

   Attention: Optimal Nucleofector solution should be previously identified by using LONZA Nucleofector optimization kit. Cells should not remain more than 15 min in Nucleofector solution to prevent cytotoxicity and improve gene transfer efficiency.
5. Transfer each cell suspension (approx. 100 µl) into a LONZA certified cuvette. Cell suspension must lie down at the bottom of the cuvette and air bubbles must be removed. Close the cuvette.
6. Select appropriate Nucleofector program and insert the cuvette into the cuvette holder. Run selected program by pressing the start button.
7. Rapidly add 500 µl of pre-warmed culture medium to the cuvette and transfer cell suspension into 6-well plate previously prepared using single-use plastic pipettes provided by LONZA.

   Attention: cells should not remain too long in the cuvette after nucleofection.
8. Incubate nucleofected cells at appropriate temperature (i.e. temperature of cell culture).

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

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; adjust pH to 7.4), trypsin-EDTA solution (1.1 mM EDTA, 0.2% trypsin in PBS) and LONZA nucleofection solutions.

Plasticware: 6-well cell culture dishes and serologic pipettes, LONZA nucleofection cuvettes and pipettes.

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

Additional information: http://www.lonzabio.com

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