Cryopreservation and revival of marine fish cell lines

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PREAMBULE. Cell cultures should be healthy and checked microscopically before cryopreservation. Cell cultures should be pre-confluent (approx. 75% of confluence) and must therefore be subcultured accordingly 1-2 days before.

1. Trypsinize the cells to be cryopreserved (see below) and transfer them into a sterile tube. Harvest cells by centrifugation (1000 rcf for 5 min at room temperature).

   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.

2. Gently suspend cell pellet in ice-cold culture medium (1.8 ml per plate or per 3×10^6 cells) containing 10-15% dimethyl sulfoxide (DMSO).
3. Transfer 1.8 ml of cell suspension into ice-cold 2-ml cryogenic tube. Place the tube into *Mr. Frosty* and cool it down to -80ºC.
4. After 24 h at -80ºC, cryotubes are transferred into a cell container and immersed in liquid nitrogen for long-term storage. For short-term storage (less than a month), cryotubes can remain in a freezer at -80ºC.
5. Recover the cells by quickly thawing cryotube in a 25ºC water bath.
6. As soon as cell suspension is not adhering anymore to cryotube wall, quickly clean vial with 70% ethanol and transfer the cells into a 10-cm culture dish containing fresh medium.
7. Gently disperse the cells with linear movements then incubate them at appropriate temperature. Allow the cells to adhere to culture dish (approximately 6-8 h) then renew culture medium.
8. At confluence, cell cultures are sub-cultured (1:2) through trypsinization.

Apparatus: Cell incubators; a class II biological safety cabinet; a -80ºC freezer; a liquid nitrogen storage container.

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); trypsin-EDTA solution (1.1 mM EDTA, 0.2% trypsin in PBS).

Plasticware: 10-cm cell culture dishes; serologic pipettes; 2-ml cryogenic tubes; Mr. Frosty cell freezing device).

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

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