Freezing cancer cell lines

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Cellular Generation and Phenotyping

ABSTRACT

This protocol outlines routine banking of cancer cell lines and Ca9 transduced cancer lines.

Process diagram:
GUIDELINES

- As a guideline, we usually bank 5 cryovials from a 70% confluent T150 flask, each containing 1ml cell suspension.

MATERIALS

Falcon™ 15mL Conical Centrifuge Tubes Fisher Scientific Catalog #14-959-53A

TrypLE® Express Enzyme (1X), no phenol red Thermo Fisher Catalog #12604021

Nunc® Biobanking and Cell Culture Cryogenic Tubes, 1.8mL, 48mm, external thread, printed Thermo Fisher Catalog #375418

DMSO Sigma Aldrich Catalog #D2650

DPBS Invitrogen - Thermo Fisher Catalog #14190

Select an appropriate culture media for your cell line. Common culture medias used for cancer cell lines are serum supplemented Advanced DMEM F-12 or RPMI, in the presence of pen-strep.

Equipment

- Light Microscope
- Microbiological Safety Cabinet (MSC)
- Pipette Boy
- Stripettes
- Pipettes and tips
- 37 °C, 5% CO₂ incubator
- Centrifuge
- CoolCell or appropriate freezing container
- -80°C freezer
- Liquid Nitrogen storage

BEFORE START INSTRUCTIONS

- Pre-warm complete culture media to room-temperature.

- Check the cells under the microscope and record percentage confluence. Cancer cells should be banked when ~70% confluent.
1. Prepare 1 mL freezing media per vial as follows: complete culture media + 10% DMSO.

2. Detach and collect cells from a flask, by following Steps 1-6 of the protocol: Passaging adherent cancer cell lines.

3. Aspirate the supernatant, taking care to avoid disturbing the cell pellet. Resuspend the pellet in an appropriate volume of freezing media depending on the number of vials being frozen. Mix well to ensure a single cell suspension.

   For example, if 5 vials are being frozen from a T150, resuspend the cell pellet in 5 mL of freezing media.

4. Transfer 1 mL aliquots of the cell suspension to pre-labelled 1.8 ml cryovials.

5. Place vials in a ‘CoolCell’ or appropriate freezing container and store at -80 °C overnight.

   **Note**

   Appropriate freezing containers will ensure that the liquid freezes at a controlled rate of around -1 °C per minute at -80 °C.

6. Transfer the vials to liquid nitrogen for long-term storage.