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Freezing cancer cell lines

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Cellular Generation and ...

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Protocol status: Working
We use this protocol and it's working

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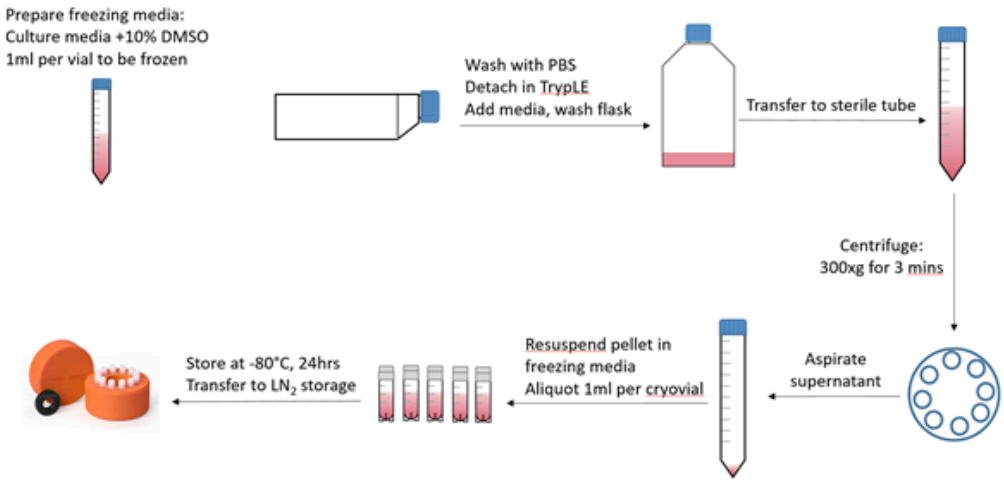
Abstract

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

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% CO_2 incubator
Centrifuge
CoolCell or appropriate freezing container
-80C freezer
Liquid Nitrogren storage

Before start

- Pre-warm complete culture media to room-temperature.
- Check the cells under the microscope and record percentage confluency. 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 1.8ml 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.