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Freezing cancer cell lines

Emily Souster[1], Verity Goodwin[1], Adam Jackson[1], Charlotte Beaver[1], Rizwan Ansari[1], Fiona Behan[1], Mathew Garnett[1]

[1]Wellcome Sanger Institute

1 Works for me dx.doi.org/10.17504/protocols.io.bgtyjwpw

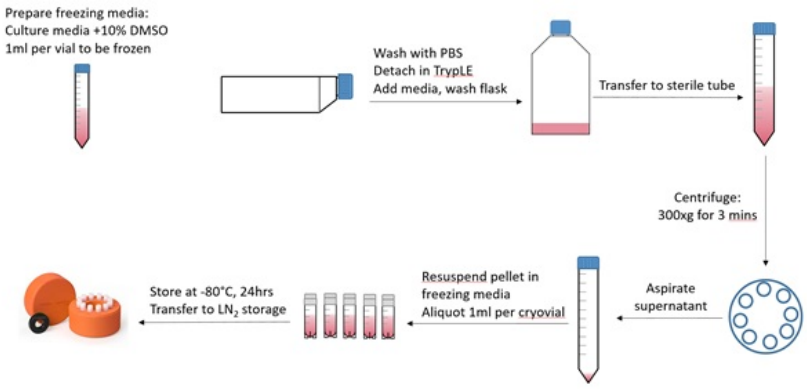
Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

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

Process diagram:



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PROTOCOL CITATION

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COLLECTIONS

Whole genome CRISPR screening in cancer cell lines

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PROTOCOL INTEGER ID

37464

PARENT PROTOCOLS

Part of collection

Whole genome CRISPR screening in cancer cell lines

GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
TrypLE™ Express Enzyme (1X), no phenol red	12604021	Thermo Fisher
Nunc™ Biobanking and Cell Culture Cryogenic Tubes, 1.8mL, 48mm, external thread, printed	375418	Thermo Fisher
DMSO	D2650	Sigma Aldrich
DPBS	14190	Invitrogen - Thermo Fisher

MATERIALS TEXT

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 STARTING

- 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.

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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.

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.

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