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Passaging adherent cancer cell lines

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

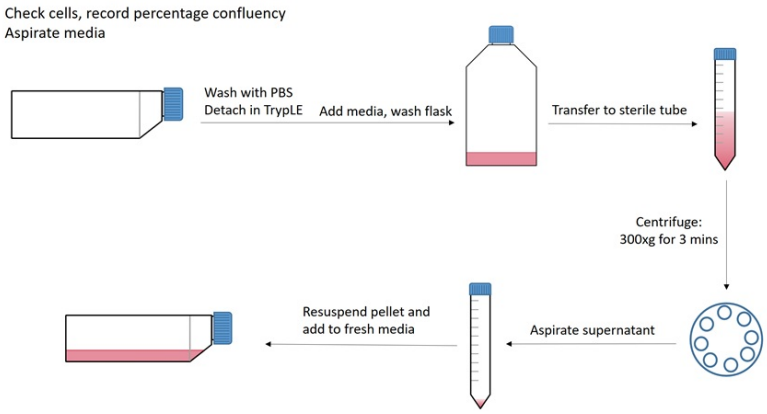
Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

This SOP is for the routine maintenance and expansion of adherent cancer cell lines. It also details how cells are harvested and counted for use in downstream protocols. This protocol is also used in expasion of Cas9 transduced cell lines, prior to banking.

Process diagram:



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dx.doi.org/10.17504/protocols.io.bgtbjwin

PROTOCOL CITATION

Emily Souster, Verity Goodwin, Adam Jackson, Charlotte Beaver, Fiona Behan, Rizwan Ansari, Mathew Garnett 2020. Passaging adherent cancer cell lines. **protocols.io** dx.doi.org/10.17504/protocols.io.bgtbjwin

COLLECTIONS

Whole genome CRISPR screening in cancer cell lines

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May 24, 2020

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37443

PARENT PROTOCOLS

Part of collection

Whole genome CRISPR screening in cancer cell lines

GUIDELINES

- Cancer cells should be passaged at ~80-90% confluent where possible. They should not be allowed to reach 100% confluence.

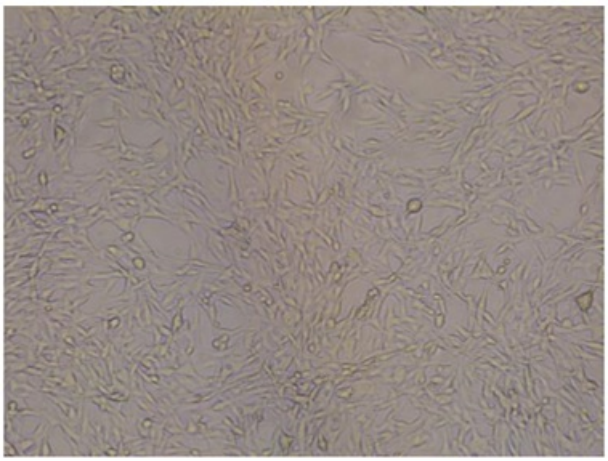

Figure 1. Adherent cancer cell line ready to be passaged (x5).

MATERIALS

NAME	CATALOG #	VENDOR
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
TrypLE™ Express Enzyme (1X), no phenol red	12604021	Thermo Fisher
Cell culture treated T75 flasks	430641	Corning
Cell Culture Treated T150 Flasks	430825	Corning
DPBS	14190	Invitrogen - Thermo Fisher
10mg/ml Blasticidin	ant-bl-1	InvivoGen

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

Microbiological Safety Cabinet (MSC)
Light Microscope
Nucleocounter/Cell counter
Pipette Boy
Stripettes
Centrifuge
Pipettes and tips

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37 °C , 5% CO_2 incubator

SAFETY WARNINGS

- Blasticidin is toxic if swallowed and harmful in contact with skin.

BEFORE STARTING

- Pre-warm complete culture media to room-temperature.
- Check the cells under a microscope and record percentage confluency. Cancer cells should be passaged when ~80-90% confluent.

Detaching and collecting cells

1 Remove the old culture media using a sterile aspirator pipette.

2 Gently wash the cells with PBS (see Table 1, column B for recommended volume). Aspirate the PBS from the flask.

Always aim to add reagents at the side/back of the flask initially to avoid dislodging any cells.

Size of flask	Volume of PBS (ml)	Volume of TrypLE (ml)	Volume of media and cells (ml)
T25	5	3	7
T75	7.5	4	12
T150	10	5	24
T525 (3 layer)	30	15	60
T875 (5 layer)	50	25	100

Table 1. Recommended volumes for different flask types.

3 Add sufficient TrypLE Express Enzyme to cover the bottom of the flask (see Table 1, column C for recommended volume). Rock the flask to allow the TrypLE to cover the surface of the cells.

4 Incubate the flask at 37 °C for 3-5 minutes. Use the microscope to check if the cells have detached. If necessary, tap the side of the flask, or return to incubator until all cells have detached.

5 Add fresh media to the flask at an equal volume to the TrypLE added in Step 3. Use the liquid in the flask to rinse the cells off the bottom of the flask. Transfer the entire volume to an appropriately sized sterile tube.

6 Spin the tube at 300 x g for 3 minutes.

Tubes should be spun inside centrifuge buckets with sealed safety caps. After spinning, the sealed bucket

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should be transferred to a microbiology safety cabinet before removing the tubes.

7 Aspirate the supernatant, taking care to avoid disturbing the cell pellet. Resuspend the pellet in an appropriate volume of fresh media (around **5 mL** - **20 mL**) and mix well to ensure a single cell suspension.

8 If a cell count is **not required**, proceed to Step 9.
To take a cell count, aliquot **100 µl** of cell suspension and count using your preferred method.

Re-seeding cells

9 Add fresh media and an appropriate volume of cell suspension to a new flask, to achieve the desired split ratio or cell number (where possible, aim for the total volume of media and cells in the flask to follow the recommeded volumes in Table 1, column D).

For example, **if splitting a T75 flask 1:2, either** add the entire cell suspension to fresh media in a T150 flask (achieving a total volume of **24 mL**), **OR** add half the cell suspension to fresh media in a T75 (achieving a total volume of **12 mL**).

The required split ratio will be dependend on the growth rate and behaviour of individual cell lines.

9.1 **If maintaining a selected population of Cas9 cells**, add blasticidin to achieve the desired concentration, as determined by: 'Blasticidin titration of cancer cell lines' protocol.

Add the required volume of 10mg/ml blasticidin depending on the flask size (Table 2), to achieve the desired final concentration.

Size of flask	Volume of media (ml)	10µg/ml Blasticidin (µl)	25µg/ml Blasticidin (µl)	50µg/ml Blasticidin (µl)	75µg/ml Blasticidin (µl)
T25	7	7	17.5	35	52.5
T75	12	12	30	60	90
T150	24	24	60	120	180
T525 (3 layer)	60	60	150	300	450
T875 (5 layer)	100	100	250	500	750

Table 2. Volume of 10mg/ml blasticidin stock required in different media volumes to achieve the final concentrations shown.

Blasticidin is toxic if swallowed and harmful in contact with skin.

10 Transfer the flask to a **37 °C** , 5% CO_2 incubator. Agitate the flask gently back and forth and side to side to ensure even distribution of cells across the flask.

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11 Inspect the cells daily and record confluency. Cancer cell lines should have a complete media change twice per week, or as needed if the media looks yellow.

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