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Cas9 transduction of cancer cell lines

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1 Works for me dx.doi.org/10.17504/protocols.io.bg4ijyue

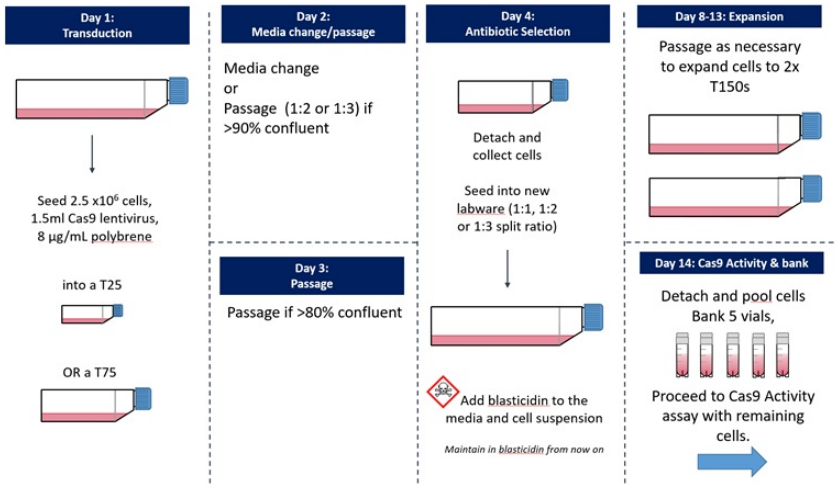
Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

The creation of stably Cas9 expressing cancer cell lines allows targeted and genome-wide gene modification using the CRISPR-Cas9 guide RNA system. The outcome of this process is the production of a cell line with greater than 75% Cas9 activity.

Process diagram:



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GUIDELINES

- We transduce cancer lines in only T25s or T75s because we use a fixed volume of Cas9 lentivirus; the transduction efficiency would be affected if this volume of virus was used in larger labware. If you think you need to use larger labware for the Cas9 transduction, be sure to adjust the volume of Cas9 lentivirus accordingly.

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
DPBS	14190	Invitrogen - Thermo Fisher
10mg/ml Blasticidin	ant-bl-1	InvivoGen
10mg/ml Polybrene	TR-1003-G	Millipore
Cas9 Lentivrus		
Cell culture treated T25 flasks	734-1532	Vwr

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
Microbiology safety cabinet (MSC)
Pipette Boy
Stripettes
Centrifuge
Pipettes and tips
Nucleocounter/Cell counter
37 °C , 5% CO_2 incubator

SAFETY WARNINGS

Lentiviral vectors can infect human cells. However they are not able to replicate, so the pathogenicity is considered negligible and the risk is managed by ensuring correct use of PPE throughout the protocol (including correct gloves, lab coat, eye protection).

All lentiviral waste should be deactivated with 1% Virkon solution for a minimum of 1 hour, preferably overnight.

BEFORE STARTING

- Pre-warm culture media to room-temperature
- Thaw an aliquot of polybrene
- Thaw an appropriate amount of Cas9 lentivirus for the number of transductions you will carry out.

Once thawed, the Cas9 virus can be freeze-thawed only once more if required.

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Day 1: Transduction

1 Detach, collect and count cells by following Steps 1-8 of protocol: Passaging adherent cancer cell lines.

2 Using the cell count from the source labware, determine the appropriate flask size for transduction.
This will be the flask size that is best able to accomodate $2.5x10^6$ cells at a confluency of 70%.

This will vary between cell lines but will generally be either a T25 or T75 flask. We do not tend to transduce in a T150 even for large cell lines.

For large cells lines that have up to $1x10^5$ cells/cm^2 at 70% confluency, a T75 should be used.
For smaller cell lines with greater than or equal to $1x10^5$ cells/cm^2 at 70%, a T25 should be used.

See Guidelines for more information.

3 Freeze 2 vials of parental cells for storage in the event that the Cas9 transduction fails and needs to be repeated.
See protocol: Freezing cancer cell lines.

4 According to the volumes in Table 1, prepare a sterile 15ml falcon tube to contain $2.5x10^6$ cells and top up to **either** 3.5ml or 13.5ml with media depending on transduction labware.

Add the remaining transduction reagents as in Table 1. Mix well and transfer to the appropriate flask.

Be aware that the total volume should not exceed **5ml for a T25** or **15ml for a T75**.

Flask	Number of Cells	Volume of Cells + Media	Volume of 10mg/ml Polybrene	Volume of Cas9 virus	Total Volume
T25	2.5x10^6	3.5ml	4µl	1.5ml	5ml
T75	2.5x10^6	13.5ml	12 µl	1.5ml	15ml

Table 1: Reagent volumes for Cas9 transduction.

Lentiviral vectors can infect human cells. Ensure correct use of PPE to reduce the risk.

5 Place the flask in a **37 °C** , 5% CO2 incubator over night and discard any remaining cells.

Day 2: Media Change

6 Aspirate media and replace with fresh media: 5ml to each T25 or 12ml to each T75.
If cells are >90% confluent, passage the cells into a T75 (1:3 from T25) or T150 (1:2 from T75), keeping all cells.

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For passage, see protocol: Passaging adherent cancer cell lines.

Day 3: Inspect

7 Inspect the cells. If >80% confluent, passage at an appropriate split ratio, keeping all cells.

For passage, see protocol: Passaging adherent cancer cell lines.

Day 4: Antibiotic Selection

8 Harvest the cells by following steps 1-7 of the protocol: Passaging adherent cancer cell lines and treat with blasticidin at the concentration determined by protocol: Blasticidin titration of cancer cell lines. See Table 2 for appropriate blasticidin volume.

For optimum selection efficiency, the cells need to be in suspension when first exposed to blasticidin. Therefore it is important that the cells are harvested and re-seeded with blasticidin at the point of selection.

-If the cells are in a T25, transfer all to a T75 (1:3 split)
-If the cells are in a T75, transfer all to a T150 (1:2 split)
-If the cells are in a T150, transfer all to one or two T150s (1:1 or 1:2 split depending on confluency and growth of the cell line).

Size of flask	Volume of media (ml)	10ug/ml Blasticidin (µl)	25ug/ml Blasticidin (µl)	50ug/ml Blasticidin (µl)	75ug/ml Blasticidin (µl)
T25	7	7	17.5	35	52.5
T75	12	12	30	60	90
T150	24	24	60	120	180

Table 2: Volumes of blasticidin and media for Cas9 antibiotic selection.

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

From this point onwarwds, Cas9 cells are maintained in media containing blasticidin- until the point at which they are screened.

Day 7: Media change

9 Inspect cells. Aspirate media and replace with fresh culture media to remove the majority of dead cells.

Day 8-13: Expansion

10 Passage cells when required using appropriate split ratios if necesary. See protocol: Passaging adherent cancer cell lines.

The aim is to expand cells to 2x confluent T150 flasks by day 14, so do not discard cells from slow growing cell lines.

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Some lines may struggle and require passages with low split ratios such as 1:1 or even temporarily downgrading by 2:1.

If cells are not >50% confluent in 2x T150 flasks by Day 14, allow to expand for another week before proceeding to the Cas9 activity assay and banking.

If cells fail to expand to this point within 4 weeks of Cas9 transduction, the cell line has failed the Cas9 transduction process due to lack of growth.

Day 14: Cas9 activity & bank

11 Detach cells from both T150 flasks according to Passaging adherent cancer cell lines protocol linked above.
Proceed directly to protocol: Assessment of Cas9 activity in Cas9 Transduced Cancer Cell Lines
Freeze 5 vials of Cas9 positive cells according to the protocol: Freezing cancer cell lines.

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