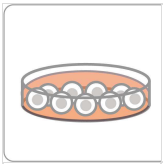

Aug 05, 2021

Generation of stable cell lines using Lentiviral vectors

Forked from Generation of stable cell lines using Lentiviral vectors

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ABSTRACT

Generation of a stable cell line refers to**the process of developing homogenous populations of cells that demonstrate expression of a transfected gene insert**. The transfected gene integrates into the genome of the host cell, and as a result, are able to express the transfected genetic material.

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FORK NOTE

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GUIDELINES

Production and working with Virus-like particles is always performed in a Bio Safety Lab 2 (BSL-2 lab)

BSL-2: Here, it is permitted to work with organisms that cause diseases. However, the lab is only accessible to people who work there and know the procedures. Doors are always closed during work and windows cannot be opened. The entire area is set up for efficient cleaning and disinfection. All waste is disinfected.

MATERIALS TEXT

Materials

- Sterile 1,5 mL Eppendorf tubes
- Sterile 50 mL Falcon tubes
- 96-well plates
- 24-well plates
- 6-well plates
- T25 flasks
- T75 flasks
- Pipetboy
- Sterile pipets (5 mL, 10mL, 25 mL)
- Micro-pipets
- Sterile Pipet tips (10µL, 100 µL, 1000 µL)
- Incubator
- Microscope
- Cell Counter
- Multichannel pipet

Reagents

- DMEM medium 2/5/8% FCS
- OptiMEM
- Gentamycin
- Selection (Puromycin, Hygromycin, Blasticidin, …)
- PBS
- Cells

SAFETY WARNINGS

Be careful not to mix bleach with alcohol-derived reagents. The key ingredient in household bleach is sodium hypochlorite. Sodium hypochlorite reacts with ethanol, isopropyl alcohol, and other types of alcohol to make chloroform ($CHCl_3$), hydrochloric acid (HCl), and other compounds, such as dichloroacetate or chloroacetone.

BEFORE STARTING

- Desinfect the flow with 70 % Ethanol before starting any work there.
- Always wear to sets of gloves and a sterile lab coat.
- Desinfect bottles and tip boxes with 70 % Ethanol, before entering the flow and after the work is finished.
- Desinfect gloves regularly with 70 % Ethanol.
- Waste is collected in a beaker filled with bleach

Day 0

1 SEEDING CELLS

1.1 Count the cells with a cell counter (keep the range of correct measurement of the device into account).

1.2 Based on the cell count, dilute the cells to seed a suitable amount in a 96-well plate. *For example, you want to put 20.000 cells in 200 µL medium in each well of a 96-well. The cell counter says you have 500.000 cells/mL. How do you proceed?*

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- Figure out the dilution factor first. You start from 500.000 cells/mL, which needs to be diluted to 20.000 cells/200 µL.
  ---> 20.000 cells/200 µL = 100.000 cells/1.000 µL = 100.000 cells/mL
  ---> 500.000 cells/mL // 100.000 cells/mL = 5 (=dilution factor).
- 200 µL is needed for each well (96 wells in total). This means a total of 19,2 mL of cell suspension is required. Make 25 mL just to be sure there is enough.
- Put in a 50 mL falcon, 20 mL medium and 5 mL 500.000 cells/mL, and mix
- Put the cell suspension in a white tray and use a multichannel to put 200 µL into each well.

1.3 Leave the cells **Overnight** in the incubator on **37 °C** at **5 % (v/v)** CO_2 (temperature and/or CO_2 concentration may change depending on the cell type).

Day 1

2 LENTIVIRAL TRANSDUCTION

2.1 Thaw the vector you want to transduce your cells with

2.2 Inspect the well plate macroscopically and microscopically. *Did the color change of the medium? Is the color change the same in each well? Do the cells look healthy?*

2.3
1. Use a new 96 well plate to make vector dilutions
   - Fill the wells with 100 µL medium
   - Add 50 µL vector to the first well and mix by pipetting up and down.
   - Start a serial dilution by taking 50 µL of the first well and adding it to the second well. Mix again by pipetting up and down.

- Incubate 2-3 days in the incubator 37 °C at 5% CO_2

Day 3/4

3 SELECTION OF CELLS

3.1 Usually, a vector with a selection gene (puromycin, hygromycin, blasticidin, ...) is used to transduce the cells. To check how well the cells were transduced, in other words,. how good the vector works. Cells are transferred to a 24-well plate and are given medium with selection. Only transduced cells will survive. Depending on how good the vector works, cells transduced with more diluted vector will also survive.
- More concentrated vector could also be toxic to cells. Check each well for which vector dilution is the best. Depending on the purpose of the experiments you want to conduct, you keep cells transduced with a higher/lower vector dilution.
- always take along 2 wells of non-transduced cells during the selection process. Put one well on selection and the other on regular medium. All the non-transduced cells should die off (*if this is not the case, increase the concentration of the selection*).

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Other methods of separating the transduced cells from non-transduced cells is via Fluorescence Activated Cell Sortingt (FACS). If the vector carries a reporter gene (*for example eGFP, mCherry,...*) or a cluster of differentiation (*for example tCD34, CD4, CD8,...*), they can be sorted via FACS.

Day ?

4 FURTHER GROWTH OF THE CELL LINE

4.1 When the cells with the optimal vector dilution are selected, they can be used to grow in bigger volumes.

4.2 Before cells can leave the BSL-2 environment, the cells should be free of viral particles. To test this a p24-assay is performed on medium that is on the cells.

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