Generation of stable cell lines via lentiviral transduction

Forked from Generation of stable cell lines via retroviral or lentiviral transduction

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Protocol Info: Elias Adriaenssens . Generation of stable cell lines via lentiviral transduction. **protocols.io** https://protocols.io/view/generation-of-stable-cell-lines-via-lentiviral-tra-cv64w9gw

Created: Jun 22, 2023

Last Modified: Jun 22, 2023

PROTOCOL integer ID: 83900

Keywords: Lentiviral transduction, Stable cell lines, HEK293T

ABSTRACT

Here, we describe a protocol to generate stable cell lines using a lentivirus system. Please note that necessary safety measures are to be taken in working with lentivirus.

GUIDELINES

References:

MATERIALS

Cell lines

- HEK293T for virus packaging and propagation
- HeLa cells

Plasmids:

- Gag/Pol plasmid
- VSV-G plasmid
- Lentiviral vectors (pHAGE-FKBP-GFP-GOI)

> **Note**
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> Note: We purify plasmids using a QIAGEN Plasmid Maxi kit following the manufacturer's protocols and ensure sterile reagents are used and mixtures prepared in tissue culture hood to avoid contamination.

Media and Reagents:

- DMEM
- 10% FBS
- 1% Penicillin-Streptomycin
- 1% non-essential amino acids
- 25 mM HEPES

Transfection media (for HEK293T cells):

- Opti-MEM I Reduced Serum Medium (Gibco)
- Lipofectamine 3000 (ThermoFisher) or PEI Max (MW 40000, Polysciences)
- Polybrene (Sigma)

SAFETY WARNINGS

Please note that necessary safety measures must be taken to work with lentivirus.

Packaging lentiviral plasmid into a lentiviral particles for in...

1 Grow HEK293T cells to 60-70% confluency in Growth media in a 6-well Petri Dish.

2 Prepare a transfection mix in a sterile 1.5 ml Eppendorf tube, containing:

- 1500 ng lentiviral vector with your gene-of-interest
- 1000 ng Gag/Pol plasmid
- 500 ng VSV-G plasmid
- 5 µL P3000 reagent (Lipofectamine 3000 kit)
- 125 µL OptiMem

3 Prepare Lipofectamine 3000 mixture in a sterile 1.5 ml Eppendorf tube, containing:

- 5 µL Lipofectamine 3000
- 125 µL OptiMem.

4 Incubate each mixture (from steps 2 and 3) separately for ~ 00:05:00 at Room temperature 5m

5 Mix the two suspensions and incubate at Room temperature for 00:15:00. 15m

6 Add the mixture drop-wise to the cells from step 1 using a P1000 sterile pipette.

7 Incubate cells at 37 °C for 24:00:00. 1d

8 Collect the supernatant from the cells (that now contains the lentiviruses) and pass it through a 0.45 µm syringe filter. If needed, add fresh growth medium and collect this too 24h later.

Lentiviral infection of HeLa cells

2w 2d

9 

Add 2 µL of polybrene (8 mg/ml) to 2 mL of lentivirus infection media from step 8 to HeLa cells seeded in 6-well plate (at 700.000/well) at 60% confluence.

10 

Incubate at 37 °C for 24:00:00. 1d

11 

Change media to fresh medium and incubate until confluency. Split three times before cells can leave the viral S2 lab.

12 Cells can now be passaged and plated for experiments or frozen down for long-term storage in liquid nitrogen (Freezing media: growth media added with 20% FBS and 10% v/v DMSO).