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We use this protocol and it's working

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Generation of stable cell lines via lentiviral transduction

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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.

ATTACHMENTS

751-1919.pdf

MATERIALS

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

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... 2d 0h 20m

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:

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

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

	A	B
	Lipofectamine 3000	5 µL
	OptiMem	125 µL

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 24:00:00 later. 1d

Lentiviral infection of HeLa cells 1d

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.

Note

Freezing media: growth media added with 20% FBS and 10% v/v DMSO.