

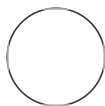


## Generation of stable cell lines via lentiviral transduction

 Forked from [Generation of stable cell lines via retroviral or 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.

### 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

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  $\mu$ L P3000 reagent (Lipofectamine 3000 kit)
-  125  $\mu$ L OptiMem

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

-  5  $\mu$ L Lipofectamine 3000
-  125  $\mu$ L OptiMem.





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

5m

 Room temperature



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  $\rightarrow$  0.45  $\mu$ m syringe filter. If needed, add fresh growth medium and collect this too 24h later.

## Lentiviral infection of HeLa cells

2w 2d

- 9 Add 2  $\mu$ L 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).