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General Transfection [↗](#)

Addgene the Nonprofit Plasmid Repository¹

¹Addgene

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Works for me

dx.doi.org/10.17504/protocols.io.4xqgxm



Addgene the nonprofit plasmid repository

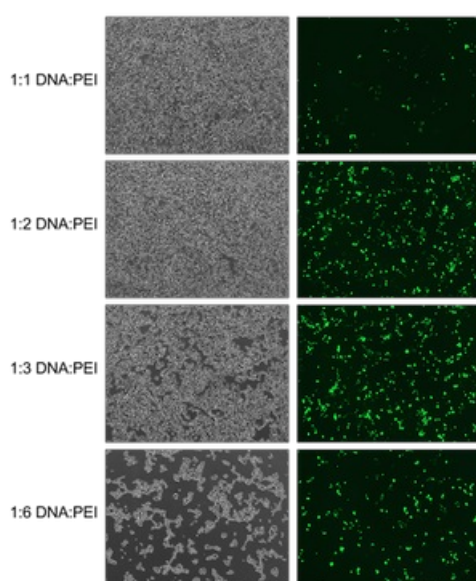
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ABSTRACT

This protocol is for general transfection. To see the full abstract and additional resources, visit the [Addgene protocol page](#).

Sample Data



Legend: Lenti-X 293T cells were transfected using 1:1, 1:2, 1:3 and 1:6 ug of pRosetta:ug of PEI. The 1:2 and 1:3 ratios provided high transfection efficiencies as can be seen here by the amount of green fluorescent protein expression (green in the right panels) with a limited effect on cell growth.

EXTERNAL LINK

<https://www.addgene.org/protocols/transfection/>

GUIDELINES

Workflow Timeline

Day 0: Seed Lenti-X 293T cells (this cell line is optimized for production of lentiviral vectors)

Day 1 (pm): Transfect Cells

Day 2 (am): 18h post transfection - Remove media, replace with fresh media

Day 3 or more (am): Observe fluorescence, harvest cells, or perform your experiment

Reagents

- DMEM high glucose
- L-alanyl-L-glutamine (or alternative stable glutamine)
- Heat-inactivated FBS
- Low serum medium such as Opti-MEM or Opti-Pro SFM
- Chloroquine diphosphate
- Polyethylenimine, linear MW 25,000 Da
- Microcentrifuge tubes
- 10 cm dishes
- Pipettes
- Pipette tips
- Hydrochloric acid
- Sodium hydroxide
- 0.22 um polyethersulfone filter
- Syringes for filtering

Equipment

- Biosafety cabinet
- Pipetman
- Pipettors
- Incubator
- Fluorescence microscope

Reagent Preparation

1. DMEM Complete: 10% v/v FBS and 4 mM L-alanyl-L-glutamine

- To a **500 ml** bottle of DMEM high glucose, add **55 ml** of heat inactivated FBS and **11 ml** of 200 mM L-alanyl-L-glutamine. Store at **4 °C**.

Pro-Tip Different brands and lots of FBS can promote or inhibit transfection. Test a variety of brands and lots of FBS to find one suitable with your protocols. FBS can be purchased already heat inactivated or it can be inactivated in the lab by heating to **56 °C** for **00:30:00**.

2. 25 mM chloroquine diphosphate

- Dissolve **0.129 g** of chloroquine diphosphate salt into **10 ml** of sterile water.
- Filter sterilize through a 0.22 um filter.
- Aliquot **50 µl** - **100 µl** and store at **-20 °C**.
- Aliquots can be thawed and stored at **4 °C** prior to use. Thawed aliquots should be discarded after 1-2 months.

3. 1 mg/mL polyethylenimine, linear MW 25,000 Da (PEI)

- Dissolve **100 mg** of powder into **100 ml** of deionized water.
- While stirring, slowly add hydrochloric acid until the solution clears.
- Check the pH of the solution
- Use hydrochloric acid or sodium hydroxide to adjust the pH to 7.0. Typically the solution will be basic and will need adjustment with hydrochloric acid first.



Pro-Tip

The pH of this solution will drift pretty rapidly upon addition of acid or base. Add only a few drops at a time, allow them to mix and recheck the pH to prevent over or undershooting the desired pH.

- Allow the solution to mix for 00:10:00 and then recheck the pH to ensure that it has not drifted.
- Filter the solution through a 0.22 um membrane.
- Aliquot 500 µl - 1000 µl into sterile tubes.
- Store the tubes at -80 °C .
- After thawing the solution can be stored at 4 °C for up to 2 months. After 2 months, discard the tube and thaw a new working stock.

SAFETY WARNINGS

See SDS (Safety Data Sheet) for safety warnings and hazards.

BEFORE STARTING

Considerations Before You Start

- The health of the packaging cell line is critical for obtaining high levels of virus.
- Lenti-X 293T cells should be split 3 times a week:
 - Monday: Plate 1×10^6 cells in a 75 cm² flask in a volume of 15 ml .
 - Wednesday: Plate 1×10^6 cells in a T75 flask in a volume of 15 ml .
 - Friday: Plate 8×10^5 cells in a T75 flask in a volume of 15 ml .
- Do not add antibiotics to the media.
- The optimal mass DNA:mass PEI ratio will need to be empirically determined for each new batch of 1 mg/mL PEI prepared.



There may be variation between batches of PEI depending on the user, quantities of chemical used, volumes, pH adjustment etc. Consequently, each batch needs to be validated and the best ratio of mass DNA:mass PEI determined.

Seeding cells

- 1 Seed 293T packaging cells at 3.8×10^6 cells per plate in DMEM complete in 10 cm tissue culture plates.
- 2 Incubate the cells at 37 °C , 5% CO₂ for ~ 20:00:00 .

Transfection

- 3 Gently aspirate media, add 10 ml fresh DMEM complete containing 25 Micromolar (µM) chloroquine diphosphate and incubate ~ 05:00:00 .



For 10 ml of DMEM complete, add 10 µl of 25 Milimolar (mM) chloroquine diphosphate.

- 4 Dilute **18.9 µg** of DNA into **500 µl** of Opti-Pro SFM.



Pro-Tip

Endotoxins can inhibit transfection, therefore, plasmid DNA purification should include an endotoxin removal step. For high quality plasmid DNA, the plasmid should also be propagated in an endonuclease negative *E. coli* strain such as NEB stable.

- 5 Dilute 1:3 (ug DNA:ug PEI) in **500 µl** total of OptiPro SFM (per 10 cm plate).



Pro-Tip

The ratio of ug DNA:ug PEI needs to be empirically determined. Once a batch of PEI is prepared, transfect cells with a fluorescent plasmid using a variety of ratios. Check the cells 1-2 days after transfection to determine what ratio gives the highest percentage of GFP positive cells.

5.1 Step 5 example:

56.7 µl of 1 mg/mL PEI, MW 25,000 Da in **386.6 µl** of OptiPro SFM per 10 cm plate.

5.2

Ratio of DNA:PEI	Amount of DNA (µg)	Volume of 1 mg/mL PEI (µL)
1:1	18.9	18.9
1:2	18.9	37.8
1:3	18.9	56.7
1:4	18.9	75.6
1:5	18.9	94.5
1:6	18.9	113.4

Refer to this table for a possible range of ratios to test.

- 6 Gently add the diluted PEI to the diluted DNA. Add the diluted PEI dropwise while gently flicking the diluted DNA tube. Incubate the mixture **00:15:00** - **00:20:00** at **Room temperature**.

- 6.1 Gently add the diluted PEI to the diluted DNA.

- 6.2 Add the diluted PEI dropwise while gently flicking the diluted DNA tube.

- 6.3 Incubate the mixture **00:15:00** - **00:20:00** at **Room temperature**.

- 7 Carefully transfer the transfection mix to the Lenti-X 293T packaging cells. Add the transfection mix dropwise being careful not to dislodge the cells.

- 8 Incubate the cells for 🕒 **18:00:00** , or until the following morning.
- 9 The following morning, carefully aspirate the media. Replace the media with 🧴 **15 ml** of DMEM complete.
- 10 Incubate the cells 🕒 **24:00:00** - 🕒 **48:00:00** before checking for protein expression.



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