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Version 2

## General Transfection V.2

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**Protocol status:** Working

**We use this protocol and it's working**

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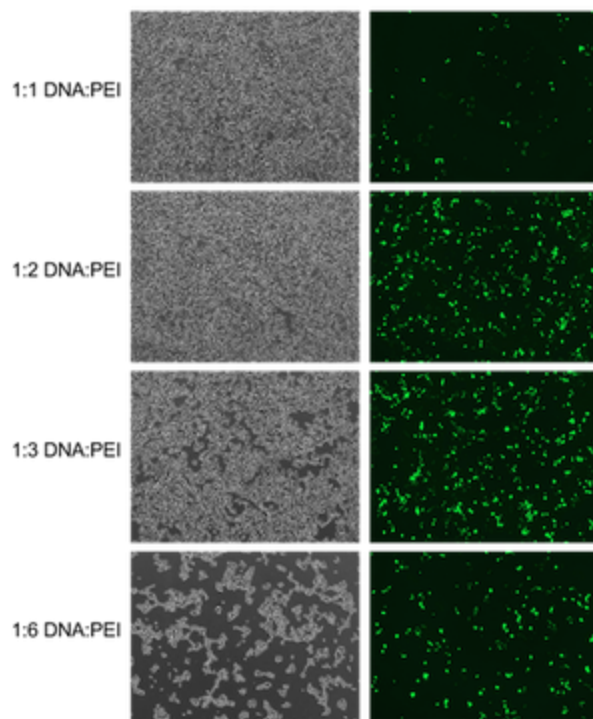
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## Abstract

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.



## 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

## Materials

### 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  $\mu$ m 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  $\mu$ m filter.



- Aliquot 50  $\mu\text{L}$  - 100  $\mu\text{L}$  and store at  $-20\text{ }^{\circ}\text{C}$  .
- Aliquots can be thawed and stored at  $4\text{ }^{\circ}\text{C}$  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.

#### Note

##### **\*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  $\mu\text{m}$  membrane.
- Aliquot 500  $\mu\text{L}$  - 1000  $\mu\text{L}$  into sterile tubes.
- Store the tubes at  $-80\text{ }^{\circ}\text{C}$  .
- After thawing the solution can be stored at  $4\text{ }^{\circ}\text{C}$  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 start

### 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  $1 \times 10^6$  cells in a 75 cm<sup>2</sup> flask in a volume of  15 mL .
  - Wednesday: Plate  $1 \times 10^6$  cells in a T75 flask in a volume of  15 mL .
  - Friday: Plate  $8 \times 10^5$  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.

#### Note

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  $3.8 \times 10^6$  cells per plate in DMEM complete in 10 cm tissue culture plates.
- 2 Incubate the cells at 37 °C , 5% CO<sub>2</sub> for ~ 20:00:00 .

## Transfection

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

### Note

For 10 mL of DMEM complete, add 10 μL of 25 millimolar (mM) cloroquine diphosphate.

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

### Note

#### **\*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).

### Note

#### **\*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  $\mu\text{L}$  of 1 mg/mL PEI, MW 25,000 Da in  386.6  $\mu\text{L}$  of OptiPro SFM per 10 cm plate.

## 5.2

|  | Rati<br>o of<br>DNA<br>:PEI | Amo<br>unt<br>of<br>DNA<br>( $\mu\text{g}$ ) | Volu<br>me<br>of 1<br>mg/<br>mL<br>PEI<br>( $\mu\text{L}$ ) |
|--|-----------------------------|----------------------------------------------|-------------------------------------------------------------|
|  | 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.