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General Transfection V.1

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Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

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Protocol status: Working
We use this protocol and it's working

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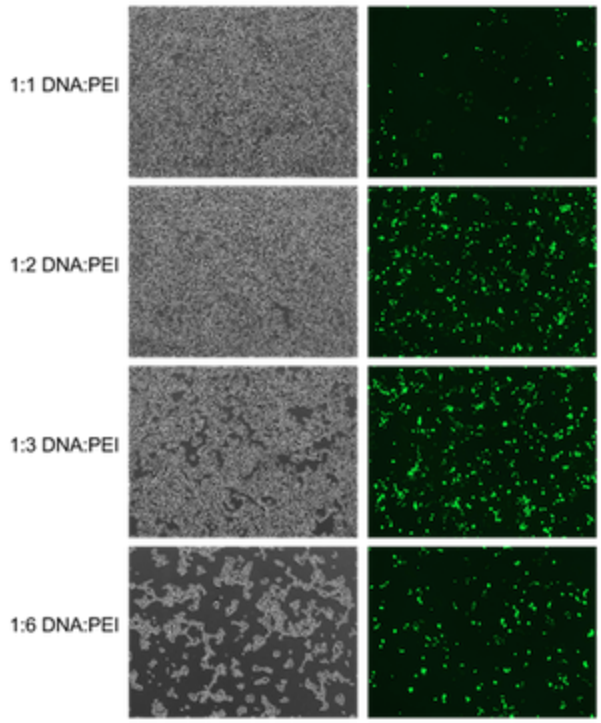
Abstract

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
- Polyethyenimine, 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 head 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.

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 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 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 1×10^6 cells in a 75 cm^2 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.

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 3.8×10^6 cells per plate in DMEM complete in 10 cm tissue culture plates.

2 Incubate the cells at 37 °C, 5% CO_2 for ~ 20:00:00.

Transfection

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

Note

For 10 mL of DMEM complete, add 10 µL of [M] 25 millimolar (mM) chloroquine 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 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.