Lentivirus Production with PEI STAR™ Transfection Reagent

Introduction

Lentiviruses are a robust method to permanently integrate genes up to 6 kb into human cell lines. Third-generation lentivirus production uses four plasmids: one transfer vector plasmid containing the transgene cassette, (e.g., pLenti-MP2,) and three plasmids containing different lentiviral packaging and envelope genes (e.g., pMD2.G, pDMLg/pRRE, and pRSV-Rev). These plasmids are co-transfected into HEK293T cells, which will begin releasing infectious lentiviruses into the culture medium that can be harvested and ultimately used to permanently integrate the transgene into HEK293T or other human cell lines.

Polyethylenimine (PEI) transfection is one of the most widely used methods to co-transfect plasmids into HEK293 cells. Compared to calcium-mediated transfection, PEI is significantly more reliable. And, compared to lipid-mediated transfection, PEI is more scalable and cost-effective. PEI STAR™ has been used to prepare lentiviruses in independent, peer-reviewed literature (Almanza *et al* 2022).

This procedure describes a general method to use PEI STAR™ to prepare lentiviruses in a 10 cm dish of HEK293T cells, which can be scaled up to larger cell cultures to prepare greater numbers of lentiviruses or scaled out to prepare many different lentiviruses.

Note: This protocol can be used for second-generation lentivirus production. The copies of the envelope, packaging, and transfer plasmids should be increased to maintain the 10-µg total pDNA used for transfection in a 10 cm dish.

Materials and Equipment

- Equipment for measuring cell concentration, viability, and confluence.
- 10 cm cell culture dish.
- Incubator set to 37°C, 5% CO_2, or the most appropriate settings for the cell line.
- ~2.5 x 10^6 HEK293T cells in growth phase.
- ~30 mL cell culture medium (e.g., DMEM, 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, antibiotic).
- 2 x sterile vials, 1 mL.
- Up to 10 µg of each lentiviral packaging, envelope, and gene transfer plasmid.
- 30 µL of PEI STAR™ Solution (1 mg/mL).
- P20, P200, P1000 micropipettes and sterile tips.
- 10 mL serological pipettes and pipettor.
- Sterile centrifuge vial, 50 mL.

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Procedure

1. The day before transfection, count and split cells to seed ~2.5 x 10^6 HEK293T cells in 10 mL fresh medium. Return dish to incubator.
2. On the morning of the transfection, check that the confluence is between 60% and 70%, replace medium with 10 mL fresh medium and return the dish to the incubator.
3. In a 1 mL vial, prepare a pDNA mixture of 10 µg pDNA in 500 µL fresh medium that contains equimolar quantities of the three lentiviral packaging and envelope gene plasmids, along with two molar equivalents of the lentiviral transgene plasmid. (E.g., 1 eq. pMD2.G: 1 eq. pDMLg/pRRE: 1 eq. pRSV-Rev: 2 eq. pLenti-MP2)
4. In a separate 1 mL vial, prepare a PEI mixture that contains 25 µg PEI STAR™ in 500 µL fresh medium.
5. Combine the pDNA mixture and PEI mixture to create a transfection mixture by pipetting, using the pipette to gently mix. Allow the transfection mixture to rest without further agitation for 5 to 10 minutes. Do not use if the transfection mixture rests for more than 15 minutes.
6. Gently add the transfection mixture dropwise to the cell culture while swirling. Return the dish to the incubator.
7. Approximately 24 hours after transfection, replace the transfection medium with 10 mL fresh medium, along with any desired booster supplements (e.g., sodium butyrate, Trichostatin A, etc.). Store spent medium in a 50 mL vial at 4°C. Return the dish to the incubator.
8. At approximately 48 hours after transfection, replace the transfection medium with 10 mL fresh medium, along with any desired booster supplements (e.g., sodium butyrate, Trichostatin A, etc.). Collect spent medium in the same 50 mL vial used to collect the supernatant at 24 hours, and store at 4°C. Return the dish to the incubator.
9. At approximately 72 hours after transfection, collect the supernatant in the same 50 mL vial used in the previous steps and store at 4°C. The cells can be disposed of.
10. The viruses in the supernatant can be tested, purified, and concentrated as needed. See ‘Reference’ reading if necessary. Aliquots of lentiviruses can be stored at -80°C indefinitely.

References

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