Recombinant Adeno-Associated Virus (rAAV) Production with PEI STAR™ Transfection Reagent in Suspension HEK293 Cells

Introduction

Recombinant adeno-associated viruses (rAAVs) are a relatively safe and reliable tool for gene transfer and stable expression in targeted human tissues. rAAV production typically starts with three plasmids: one with a transgenic cassette containing the gene of interest and two with AAV packaging genes. These plasmids are co-transfected into HEK293 cells, which will generate infectious rAAVs containing a copy of the transgene. A few days after transfection, rAAVs can be harvested from the cells, purified, and used to insert genes into specific tissues.

Polyethylenimine (PEI) transfection is one of the most widely used methods to co-transfect the rAAV plasmids into HEK293 cells. PEI is more scalable and cost-effective than lipid-mediated transfection, and significantly more reliable than calcium-mediated transfection. PEI STAR™ has been shown to be an effective option for creating highly infectious rAAVs in independent, peer-reviewed literature (Trivedi *et al* 2021).

This procedure describes a general method to use PEI STAR™ to prepare rAAVs in a 50 mL suspension of HEK293 cells. The methods can be scaled out to prepare multiple rAAVs or scaled up to prepare greater quantities of a desired rAAV. Compared with transfection of adherent HEK293 cells, transfection of suspension HEK293 cells is easier to scale up.

The pDNA and PEI concentrations do not need to be increased when using a high-density cell culture. The transfection should be performed at 3.3 x 10^6 cells / mL instead of 1.1 x 10^6 cells / mL, but the final pDNA concentration should still be 1 µg / mL and the final PEI concentration should still be 2.5 µg / mL.

Note: If the AAV genes are on one plasmid instead of two, the plasmid containing the AAV genes should be mixed with the transgene cassette plasmid in equimolar amounts to obtain a pDNA mixture with a concentration of 20 µg / mL, and a final pDNA concentration of 1 µg / mL in the cell culture.

Materials and Equipment

- Equipment for measuring cell concentration and viability.
- Shaker flask, 125 mL.
- Incubator set to 37°C, 5% CO_2, 125 rpm, or the most appropriate settings for the cell line.
- 5.0 x 10^6 suspension adapted HEK293 cells in growth phase.
- 50 mL HEK293 medium.
- 2 sterile vials, 5 mL.
- Up to 50 µg of transfection-grade plasmids (pDNA) containing transgene cassette and rAAV packaging genes.
- 125 µL of PEI STAR™ solution (1 mg/mL).
- P20 and P200 micropipettes with sterile tips.
- Pipette controller with serological pipette, 5 mL.

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Procedure

1. Four days prior to the transfection, count viable cell density of a cell culture and use fresh media to dilute to 0.6 x 10^6 viable cells / mL.
2. On the day of transfection, dilute and aliquot the cell culture to obtain 45 mL of a subculture with a viable cell density of 1.1 x 10^6 cells / mL.
3. Prepare a pDNA mixture in a vial by combining equimolar amounts of all plasmids to be co-transfected in fresh media to obtain 2.5 mL of a mixture with a total pDNA concentration of 20 µg / mL.
4. Prepare a PEI mixture in a vial by diluting 125 µL of PEI STAR™ solution (1.0 mg / mL) with fresh media to obtain 2.5 mL of a mixture with a PEI concentration of 50 µg / mL.
5. Prepare the transfection mixture by combining the pDNA and PEI mixtures and briefly vortex for a few seconds. Allow the transfection mixture to rest for 5 to 10 minutes before use. *Do not use transfection mixture more than 15 minutes after mixing.*
6. Transfect the 45 mL cell culture by slowly adding the 5 mL transfection mixture while swirling. Return the cell culture to incubation.
7. rAAVs can be harvested and purified 48 to 96 hours post-transfection.

References

Huang *et al* (2013) AAV2 production with optimized N/P ratio and PEI-mediated transfection results in low toxicity and high titer for *in vitro* and *in vivo* applications. *J.Virol.Methods* ***193*** 270. PMID: 23791963.

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