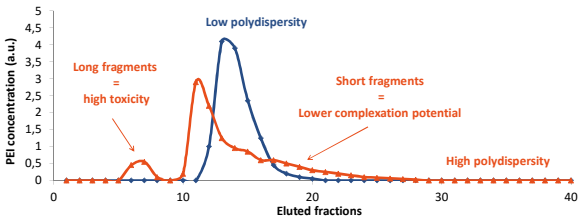
Addressing Large-Scale Manufacturing of Clinical Grade Viral Vectors Using an Optimized PEI-based Transfection Process

Mathieu Porte, Julien Depollier*, Alengo Nyamay'Antu, Géraldine Guérin-Peyrou, Patrick Erbacher
Polyplus-transfection, Bioparc, 850 Boulevard S. Brant, 67400 Illkirch, France
Presenting author

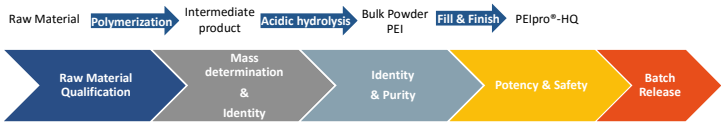
Abstract

With the progress in developing new viral vector systems guided by safety, specificity and potency considerations, several gene and cell-based therapies are now more than ever closer to being clinically approved and commercially available to treat genetic diseases.
Viral vector delivery systems, of which mainly adeno-associated viruses (AAV) and lentiviruses are produced by transient transfection of mammalian producer HEK-293 cell lines. Virus vector production using the right transient transfection method is crucial to provide the flexibility and reproducibility that is needed to scale-up from initial process development to manufacturing of high-quality grade viral vectors.
Here, we describe an optimized PEI-based virus production process for high-yielding viral vector production, compatible with different cell culture adherent and suspension systems. We further demonstrate the robust viral vector production yields, as well as the adaptability and reliability of the PEI-based transient gene expression approach to efficiently manufacture GMP-grade viral vectors at a sufficiently large scale for more advanced clinical trials, and *in fine* to drive commercialization of therapeutic vectors.

Optimized PEI for R&D to Clinical-Grade Virus Production



Optimization process of PEI polymer chemistry. Whereas long polymer fragments lead to cell toxicity and short fragments lead to lower complexation potential (in red), optimized PEI size with a low polydispersity index decreases toxicity, while increasing complexation potential (in blue) and reproducibility in transfection.



Manufacturing process of PEIpro® and PEIpro®-HQ reagents. The linear structure of PEIpro® and the manufacturing process developed by Polyplus-transfection® ensure a high, stable and reproducible amount of protonable amines available for transfection while providing a fully deacylated molecule and an extremely low polymer chain length variation.

		PEIpro®	PEIpro®-HQ
Characteristics			
1 mg/ml of Linear PEI in water		√	√
Fully synthetic		√	√
Manufacturing Process		Identical	
Quality Controls			
Identity	Molecular Weight of intermediate product	√	√
	Side chain content		√
	Assay		√
	Color, Clarity		√
	pH		√
Purity	Impurity profile		√
Safety	Endotoxin assay	√	√
	Sterility test	√	√
	Mycoplasma	√	√
Potency	Activity test	√	√
Documentation			
Certificate of Analysis		√	√
Certificate of Origin (confirming the absence of components of animal origin)		√	√
Detailed Batch Production Documentation to include in an IND or IMPD			√

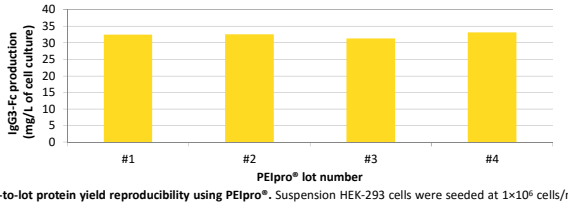
PEIpro®-HQ is a highly qualified grade of PEIpro® reagent. The quality of PEIpro® and PEIpro®-HQ are continuously assessed during the manufacturing process with suitable control testing. In comparison to PEIpro®, a more extensive number of quality controls are performed on both the bulk material and the formulated product of PEIpro®-HQ to assess **identity, purity, safety, and potency**.

Compatible with Various Production Culture Systems

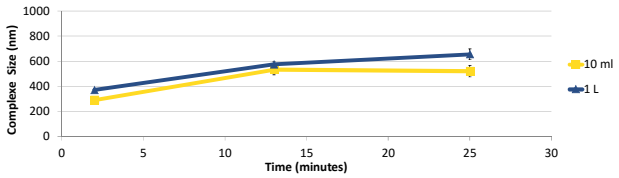
Culture medium	Productivity using PEIpro®
DMEM (Gibco®)	+++
IMDM (Gibco®)	+++
Ham's F12 (Gibco®)	+++
BalanCD® HEK293 (Irvine Scientific®)	+++
FreeStyle™ 293 (Gibco®)	+++
FreeStyle™ F17 (Gibco®)	+++
HyClone™ HyCell™ TransFx™-H (GE Healthcare™)	+++
Pro293™ (Lonza®)	++
CD 293 (Gibco®)	-

PEIpro® and PEIpro®-HQ are compatible with several serum-containing media and commercially available synthetic media for virus production in both adherent and suspension cells HEK-293 cells.

Reproducibility & Scalability

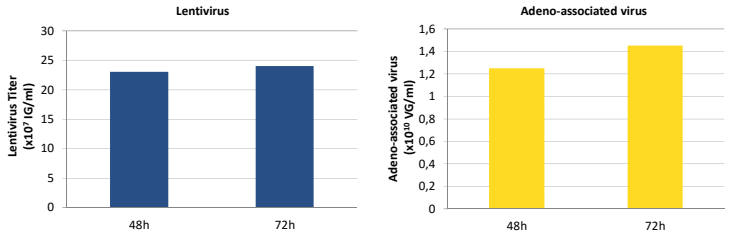


Excellent lot-to-lot protein yield reproducibility using PEIpro®. Suspension HEK-293 cells were seeded at 1×10⁶ cells/mL in serum-free medium and transfected with PEIpro® following the standard protocol. IgG3-Fc production was assayed 48 h after transfection using protein G affinity quantification (HPLC).



DNA-PEIpro® complex size is identical, independently from the volume of transfection mix preparation. Complexes were prepared with a DNA concentration of 0.01 mg per mL of complex volume at a DNA:Reagent ratio of 1:4, either in 10 mL or 1 L. The size of the complexes was then measured every ten minutes using the Zetasizer Nanometer ZS (Malvern Instrument, Malvern, UK).

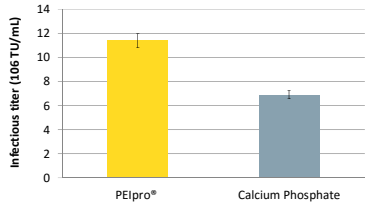
Gold standard for High Virus Production Yields



Lentivirus and AAV production in HEK-293T and HEK-293 cells grown in suspension in BalanCD® HEK293 (Irvine Scientific®). HEK-293T (lentivirus) and HEK-293 (AAV) cells were thawed directly into each medium and passaged every 3 to 4 days before going into a 2L benchtop bioreactor. Cells were seeded and cultured for 3 days before being transfected by PEIpro® (Polyplus). For transfection, four plasmids were used for lentivirus and three plasmids were used for AAV. Lentiviral and AAV titer were measured 48 and 72 hours post transfection (Data kindly provided by Généthon).

Cell culture system	Vector	Cells	Titer
CS10® / CF10®	AAV	Adherent HEK-293, HEK-293T	10^{11}-10^{13} VG / ml
Fixed-bed bioreactor (iCELLIS®)	AAV	Adherent HEK-293T	10^{14}-10^{16} Total VG
Shaker Flask	AAV	Suspension HEK-293, HEK-293T	10^{9}-10^{10} VP / ml
Bioreactor	AAV	Suspension HEK-293, HEK-293T	0.8-1.5 x10^{9}-10^{10} VG / ml
10 cm dish/75 cm2	Lentivirus	Adherent HEK-293, HEK-293T	1-2 x10^{8} TU / ml
HYPERflask®/HYPERstack®	Lentivirus	Adherent HEK-293, HEK-293T	1-2 x10^{8} TU / ml
Shaker Flask	Lentivirus	Suspension HEK-293F, HEK-293T	2x 10^{7}-10^{10} VP / ml
Bioreactor	Lentivirus	Suspension HEK-293, HEK-293T	10^{7} IG / ml

PEIpro® is the reagent of choice for virus production runs in most cell culture systems in both adherent and suspension cells Irrespective of the cell culture-based system and production scale, PEIpro® and PEIpro®-HQ have led to efficient viral vector yields superior to 10^{7} IG/mL and 10^{9} VG/mL, respectively for lentiviruses and AAV.



PEIpro® gives higher virus titers than Calcium Phosphate. Lentiviruses were produced in adherent HEK-293 cells grown in serum-free culture medium, using 15 µg DNA and 30 µL PEIpro® per 75 cm² flask. Viral titers were determined by flow cytometry of supernatants 48 h after transfection.

Conclusion

Advantages of PEIpro® and its higher quality grade PEIpro® -HQ

- A PEI optimized for transfection, suitable for virus production (and for protein production).
- Synthetic animal free reagent manufactured according to a well-established process.
- Robust product with a great lot-to-lot reproducibility and a long shelf life.
- Ideal for process development up to large-scale therapeutic viral vector production.
- Highest quality PEI available with extra Quality Controls (identity, potency, safety and purity) and supplied with extensive documentation, **PEIpro®-HQ: Ideal for use in GMP processes.**

Visit us at www.polyplus-transfection.com