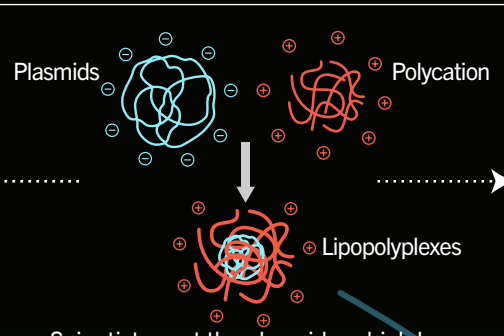
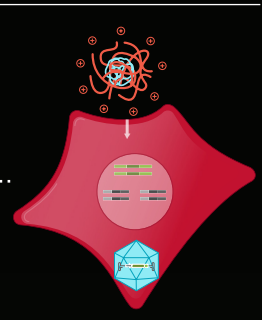
Scientists rely on the natural cell-penetrating ability of viral vectors to deliver recombinant genes for gene and cell therapies. It all starts with high quality raw materials.

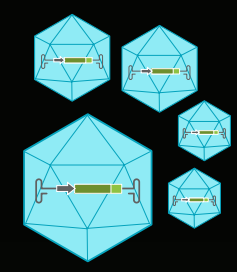
To safely produce viral vectors, scientists divide the viral genome into separate plasmids. One plasmid contains the gene of interest that is packaged into the viral particle. The remaining plasmids contain viral genes necessary for packaging and expression of the gene of interest.



Scientists coat the plasmids, which have a net negative charge, with different types of transfection reagents that condense and confer a net positive charge. When *Trans*IT-VirusGEN® reagent is complexed with DNA, lipid-polymer nanoparticles (LPNPs) are formed.



The LPNPs facilitate entry into viral production cells, usually HEK 293 cells, so that expression and assembly of the recombinant viral vectors is initiated. The viral vectors can be collected in the growth medium and/or through lysis of the viral production cells, depending on the vector type.



Animal-free, fully synthetic formulations of transfection reagents are key to Good Manufacturing Practices (GMP). The VirusGEN® Transfection Reagents and Kits from Mirus Bio are available in Research Use Only, SELECT, and GMP configurations to streamline the transition from research and development to clinical manufacturing.

MULTIPLY THIS BY ORDERS OF MAGNITUDE FOR

LARGE-SCALE TRANSFECTION

FOR VIRUS PRODUCTION

In order to meet growing clinical and commercial demands for viral vector based therapeutics, scale-up and scale-out of transfection is performed in large bioreactors. Careful consideration and optimization of the transfection steps on the large-scale will improve yield per run, ultimately expanding patient access to life-saving therapies.

Factors influencing large-scale transfections

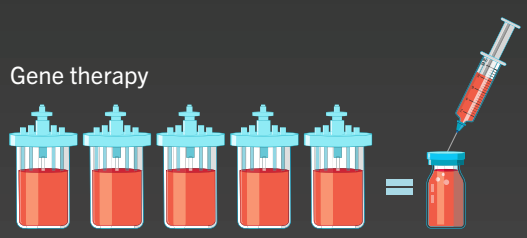
Considerations for large-scale transfection:

- Facility space and culture vessel
- Materials and Labor
- Seeding and expanding cells
- Complexation time
- Delivery of complexes to cells

Ball-park estimates for quantities required in large-transfections*

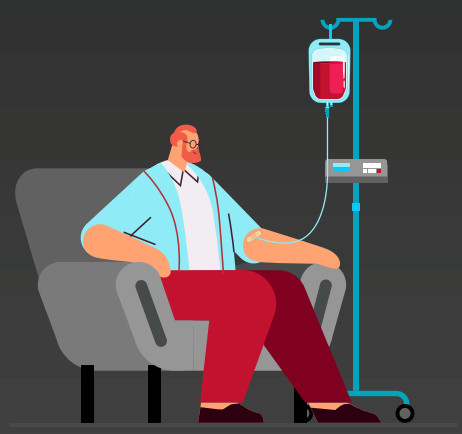
Materials	Small scale	Mid-scale	Pilot scale
DNA mass	200 µg	2 mg	200 mg
Reagent volume	300 µl	3 ml	300 ml
Culture volume	100 ml	1 L	100 L

*Exact and optimal amounts will vary by viral vector production system



For lentivirus-based cell therapy, 10^8-10^{10} transduction units are needed per dose. That means that each 100L bioreactor could produce 10-1000 doses. For AAV gene therapy, 10^{11}-10^{16} viral genomes are needed per dose. Depending on production efficiency, each dose could require production from up to five 100L bioreactors.

Transfection complexes can make up 5-10% of bioreactor volume. Avoid shear stress to maximize complex integrity.



Crude viral preps are further purified and assayed for titer and quality. In the case of cell therapies, the virus will be applied to cells ex vivo, expanded, and then reintroduced into the patient. For gene therapies, the viral vector is applied directly to the patient.

Virus particles must be purified from the cellular debris and growth medium of bioreactors. These bioreactors are typically 100 liters, producing orders of magnitude more viral particles than transfection on the benchtop.

Mirus