Lentiviral vectors production

Vectalys is an R&D company with a state-of-the-art technology platform for customized viral vector production.

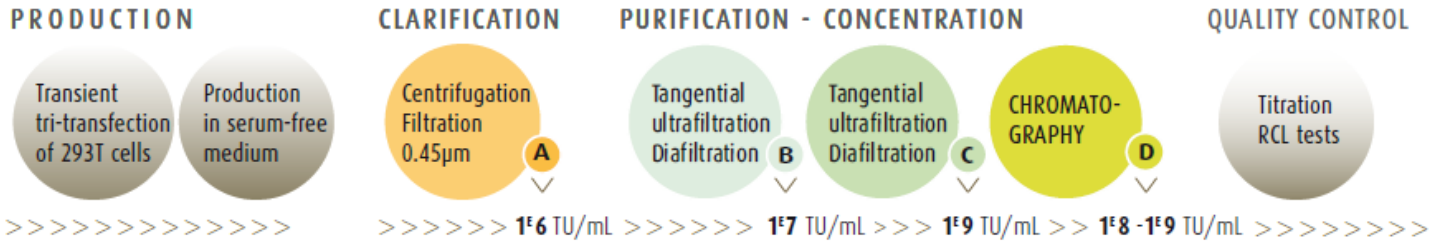
The company has developed a unique process enabling the production of high titer high purity lentiviral vectors for optimal knock-down or over expression in relevant models: primary and stem cells for target gene validation and specific tissue for animal models.

Key benefits

- 100% gene delivery achieved to primary cells such as neurons, endothelial and stem cells
- Multiple genes delivered to one cell
- High purity enabling in vivo injections and preventing cytotoxicity
- Dedicated support with scientific advice and biosafety assistance

Highly concentrated and purified lentiviral vectors

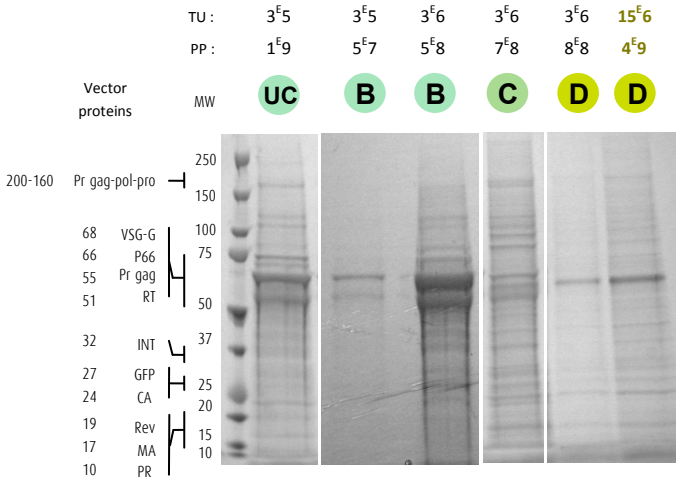
Vectalys has developed a unique process for **large-scale production** of lentiviral vectors associated to concentration and purification methods for the generation of **high titer retroviral vectors.**



Our viral vector production process provides high quality lentiviral batches at different titers:

> **High purity** : removal of major contaminants (serum and cellular proteins, plasmid and genomic DNA)

> **High titer** retroviral vectors from **1E6 TU/mL up to 1E9 TU/mL**



High purity: Viral proteins were separated by SDS-PAGE electrophoresis and visualized by coomassie blue staining

UC : classical ultracentrifugation based purification technique

	A	B	C	D
Titer (TU/mL)	**2-5E6**	**2E7-3E8**	**1-3E9**	**1E8-1E9**
Purity (PP/TU)	150-200	350-450	250-400	70-250
DNA yield		-10%	-70%	- 90%
Protein yield		-20%	-70%	- 95-98%
Application	**Immortalized cell lines**	**Primary cells Stem cells**		**Animal models**

Lentiviral vector production without serum

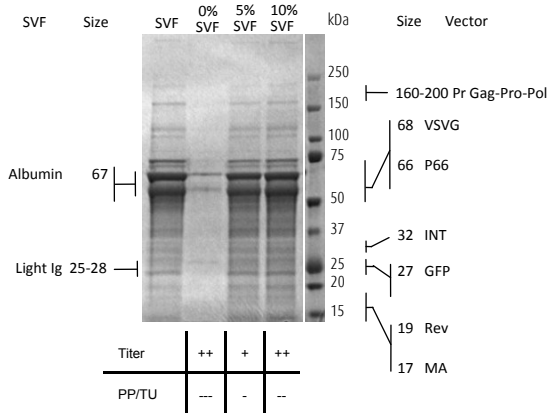
A robust process for the production of high titer viral supernatant

in **serum free medium**:

> improves the quality of the crude viral vectors :

- increases the titer (TU/mL)
- reduces the physical particles to transducing units ratio (PP/TU)

> improves the transduction efficiency of the viral vectors



15µg of total proteins were separated by SDS-PAGE electrophoresis and visualized by coomassie blue staining

Lentiviral vector purification and concentration

Vectalys has developed **upscalable** concentration and purification processes based on combined techniques to produce **highly concentrated** and **purified** lentiviral vectors.

Viral supernatant is purified by tangential ultrafiltration then purified and concentrated by chromatography:

> The *ultrafiltration process* allows the concentration of viral vectors up to 2.5E9 TU/mL. This increases the efficiency of transduction for more delicate cells such as primary and stem cells.

> The *chromatography* step greatly improves the purity of the final viral vector stock. These preparations are used for direct *in vivo* injection experiments.

		Concentration	Purification
Classical method	Ultracentrifugation	+++	-
Vectalys process	Ultrafiltration	+++	++
	Chromatography	-	+++
	Ultrafiltration and Chromatography	+++	++++

Applications

> **Customized safe lentiviral vectors for your specific application**

> **Efficient transduction of delicate cell types (i.e. primary cells, stem cells, ...)**

> **Generation of stable cell lines**

> **To develop *in vivo* animal models**

Quality control

> Production phase

- Standard production controls
- Robust production cell lines
- Drastic controls against
  - mycoplasms
  - microbial contamination

> Purification and concentration phase

- Controlled physical parameters throughout the whole process

> Lentiviral batches

- Dedicated « viral safety rooms »
- Standard controls of titration
- RCR tests
- Traceability
- Robust technical procedures

Quality assurance is guaranteed throughout the production process

References Vergnault H. et *al.* **Ultra-pure retroviral production for *in vitro* and *in vivo* applications.** Human Gene Therapy, 2009, **20** (6): 671-690.

Weber A. et *al.* **Hepatocyte Transplantation Techniques: Large Animal Models.** In: Hepatocyte Transplantation: Methods and Protocols. Humana press, 2009, vol. 481.