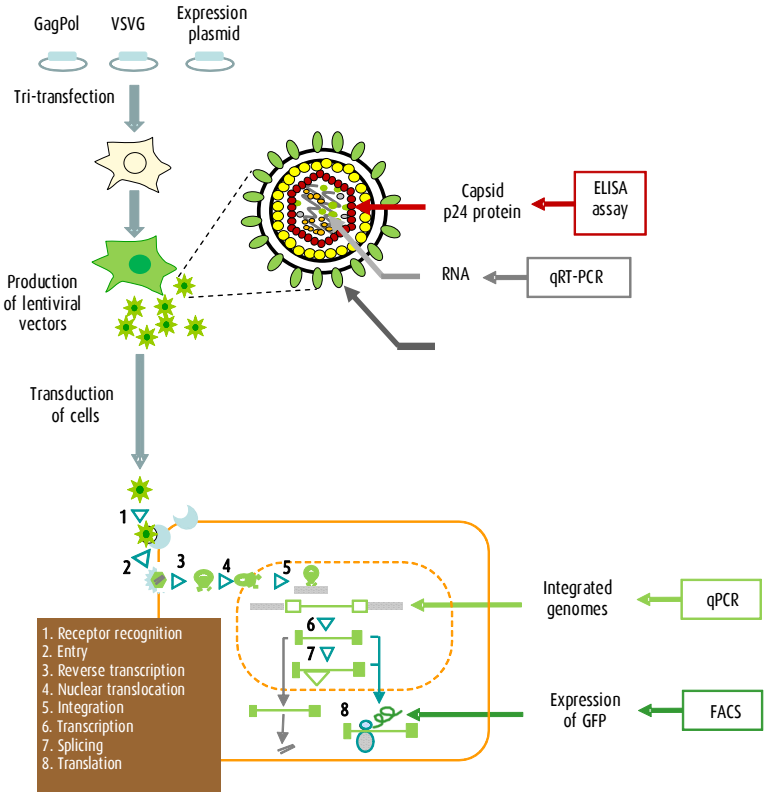
Titration of lentiviral vectors

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.

Method of titration

Titers of lentiviral vectors as well as those of wild type viruses critically depend on the method of titration.



Detection of physical particles (PP) :

Includes non-transduction-efficient particles.

> detection of viral elements directly in the viral supernatant
  - the **p24** viral capsid protein by **ELISA assay**
  - the viral **RNA** by **qRT-PCR**

Detection of vector particles able to transduce cells (TU):

Represents the efficiency of the early stage of the viral cycle:

> from cell binding to viral DNA integration

⇒ the titer represents the number of **integrated genomes** as determined by **qPCR**

> from cell binding to protein expression

⇒ the titer represents the number of **transducing units** as determined by fluorescence activated cell sorting **(FACS)**. This is a functional titer.

Titers determined by qPCR are normalized with our standard GFP expressing vector titrated by FACS. So, all our qPCR or FACS titers are strictly comparable for any transgene.

Comparison of titers following the different methods of titration for vectors produced by Vectalys

rLV-EF1-GFP	FACS	qPCR*	p24**	RNA**	PP/TU**
Crude	1^E6 TU/mL	1^E6 TU/mL	1-2^E8 PP/mL	1-2^E8 PP/mL	100-200
After purification	1^E9 TU/mL	1^E9 TU/mL	<1^E11 PP/mL	<1^E11 PP/mL	<100

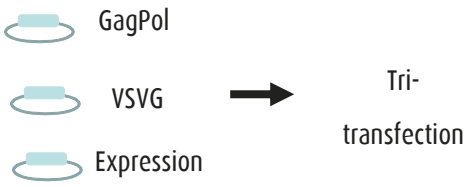
* providing the expression cassette is the same as that in our standard GFP expressing vector rLV-EF1-GFP.

** providing the production and purification processes are the same as those performed by Vectalys.

Detection of replication-competent recombinants (RCR)

The major safety concern before handling lentiviral vectors is the presence of RCRs. Ensuring their absence allows use of these vectors in different level rooms.

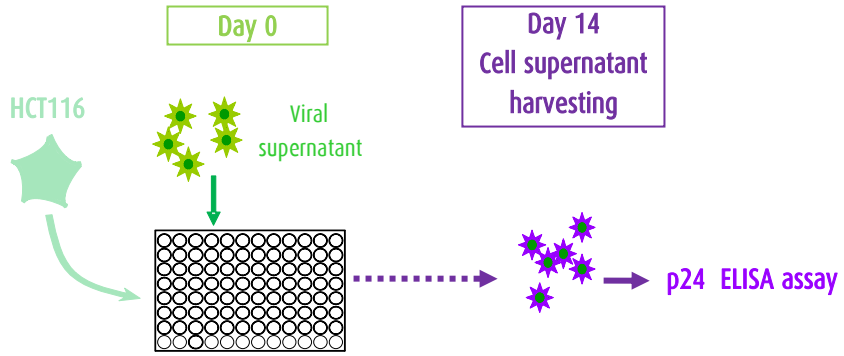
In order to avoid RCRs, we use a tri-transfection method: the viral sequences encoding the functional and structural proteins are distributed in independent expression units.



This system allows the maximization of the number of recombination events that would be required to obtain a recombinant vector able to replicate. Vectalys uses a lentiviral vector system based on the generation of vector particles from three or four separate plasmids to ensure that only replication-defective vectors are produced.

Vectalys uses p24 antigen assay to test lentiviral vector batches for the absence of RCRs.

The p24 ELISA assay is performed on transduced cell supernatant collected from stably transduced cells 14 days after transduction.



This 14 day delay is important since false positive results may be obtained by p24 ELISA assay performed during the first 14 days up to high MOI, as demonstrated by preliminary assays. These false results are not supported by PCR experiments on the transduced cell DNA demonstrating the complete absence of DNA coming from the vector itself.

Batches	At day 0		At day 6		At day 14
	TU* (FACS)	PP** (p24)	PP** (p24)	TU* (QPCR)	PP** (p24)
MOI 10	3^E4	$1,8^E7$	0	0	0
MOI 100	3^E5	$1,8^E8$	$8,7^E4$	0	0
MOI 200	6^E5	$3,6^E8$	$1,1^E5$	0	0
MOI 1000	3^E6	$3,4^E8$	$8,5^E4$	0	0
MOI 1000 40'-55°C	3^E6	$3,4^E8$	0	0	0
Negative control	0	0	0	0	0

* Transducing Units ** Physical particles