Transducing units (TU) determination

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 and wild type viruses critically depend on the method of titration used.

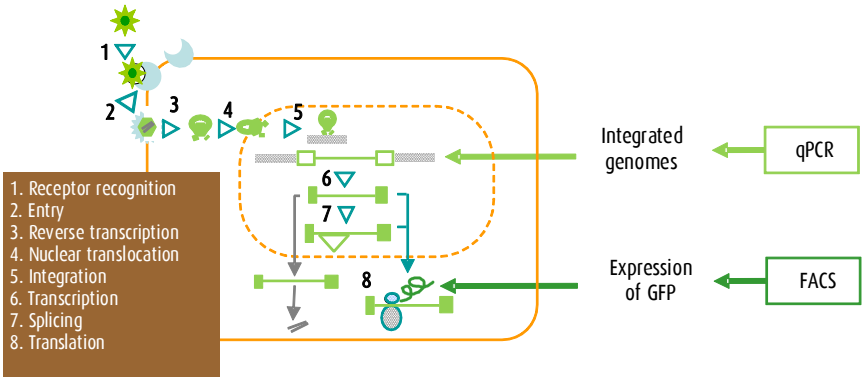
The titer can be estimated by measuring the number of efficient viral particles or transducing units.

In this case, the titer obtained only includes those particles which are able to transduce cells.

> from cell binding to viral DNA integration, the titer corresponds to the number of integrated genomes and is quantified by qPCR.

> from cell binding to protein expression, the titer corresponds to the number of transducing units and is quantified by FACS. This is a functional titer.

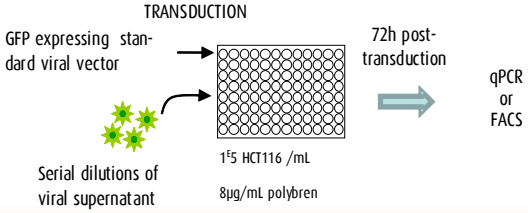
Titers determined by qPCR are normalized with our standard GFP expressing vector titrated by FACS. So, all our qPCR or FACS titers are strictly comparable for any transgene.



Both quantifications are performed after the transduction of cells and critically depend on cell and vector characteristics.

> First, the cells must be readily permissive to all steps of viral transduction. The determination of titers of a same viral batch on two different cell types exhibiting different permissiveness to lentiviral vectors may give two different viral titers even if both results are true for the respective cells.

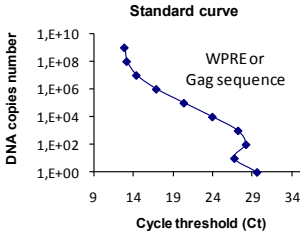
> Second, measured titers can also vary with the conditions used for titration, such as the number of target cells used, the duration of vector-cell incubation, the transduction time (3 or 5 days), the polybren concentration...

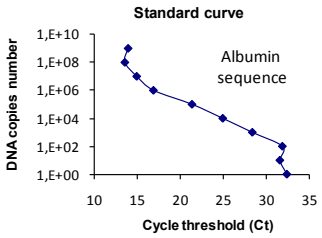


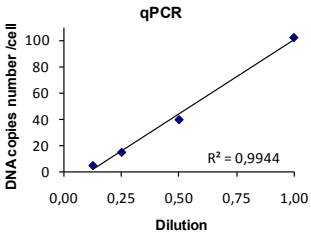
The viral titer determined by Vectalys corresponds to the number of integrated genomes or transducing units generated by transducing 1E5 HCT116 cells with one milliliter of viral supernatant in our experimental conditions: 3 days of cell culture after transduction in the presence of 8 µg/ml polybren.

Quantification of the transducing units by qPCR

Three days post-transduction, cells are harvested and genomic DNA is extracted using a genomic DNA extraction kit (Promega) for qPCR. The number of viral copies is quantified by amplifying a vector specific sequence and the number of cells is quantified by amplifying the albumin sequence known to be present in two copies per human cell. Both copy numbers are evaluated thanks to a sequence specific standard curve.







Vector specific sequence standard curve

Albumin standard curve

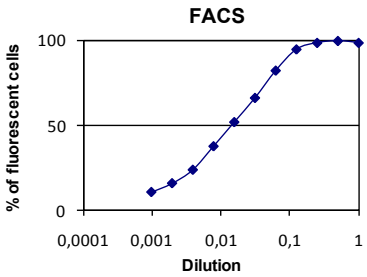
The result obtained critically depends on the conditions of the titration experiment. Indeed, the major variability may be observed during the genomic DNA extraction. The titers also depend on parameters such as oligonucleotide sequence, PCR conditions… It is therefore important to include standard controls with the samples to standardize the titers from one experiment to another.

This technique allows the determination of viral copy number integrated into the cell genome.

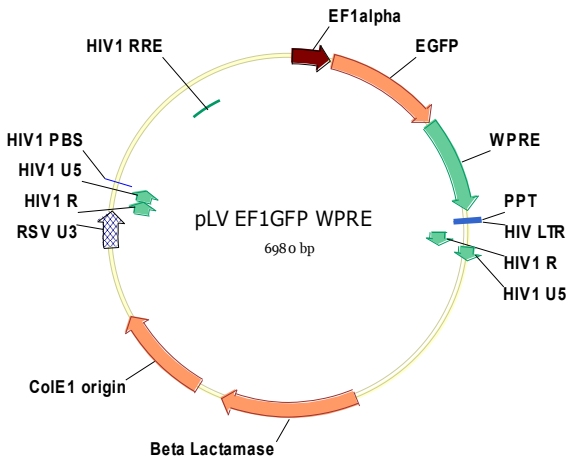
Quantification of the transducing units by FACS

Three days post-transduction, cells are harvested and the percentage of fluorescent cells is estimated by FACS.

The result obtained depends on the cassette of the vector itself. Indeed, the promoter (CMV, PGK, EF1…) and sequences stabilizing the transgene expression (WPRE, insulators…) may influence the protein expression even if the viral copy number in the target cells is the same.



The titer deduced from FACS experiments corresponds to the level of expression of the protein and may be considered as a functional titer.



A GFP expressing standard viral vector is used to correlate viral titers obtained by qPCR (biological titer) with those obtained by FACS (functional titer). It is then possible to deduce the functional titer from the biological titer obtained for other samples, if the expression cassette is the same as the Vectalys GFP expressing standard viral vector.