Time saving tools for the generation of stable cell lines

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Most of the transfection issues are the gene transfer efficiency into hardly to transfect cells (lymphoid and myeloid cell lines, primary and stem cells). Using lentiviral vectors, the expression of the sequence of interest (cDNA, shRNA, miRNA) is stable due to the vector DNA integration into genomic DNA bringing permanent cell line at once. This saves us from having to transfect targeted cells for bioproduction or screening (CHO, HEK,…) whenever we want to produce the same protein. Hence lentiviral vectors allow time, money and energy saving. In vivo applications are an emerging and important area. Lentiviral vectors meet this new demand by providing a single tool from *in vitro* validation to *in vivo* injection.

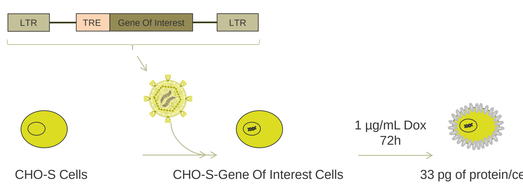
Money and time saving: We can generate stable cell lines for bioproduction in few days after one transduction only. Just thaw this new cell line to produce the protein of interest without restarting the laborious gene transfer process from plasmid production to cell transfection.

We produce customized expression cassette in which type of promoters (constitutive, inducible, cell-specific, ubiquitous…), number of transgenes and the presence or not of a reporter can be designed according to any applications.

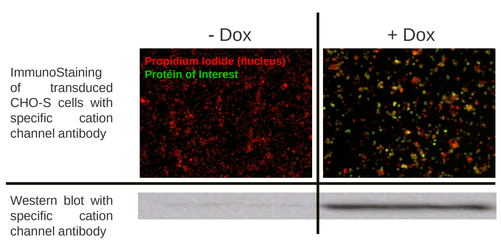
We can shorten the timeline for developping stable cell lines silencing transgene without any antibiotic needs

We can manage both % of positive cells and transgene expression level by controlling the M.O.I

Hardly to produce protein : Bioproduction of a cation channel in order to determine its 3D structure by X-ray crystallography. Since in a consitutive expression system this protein gave rise to cell mortality, we produced it using an inducible expression system (TRE promoter) in CHO-S cells.

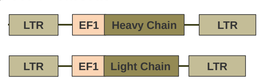


72h after induction with Doxycycline, the Protein of Interest expression was validated by Western blot and Immunofluorescence on CHO-S transduced:



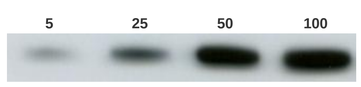
IgG Bioproduction: Bioproduction can be performed by either two lentiviral Vectors each expressing one IgG chain, or one lentiviral vector expressing both IgG chains using a multicistronic expression system:

a) Two lentiviral vectors for two monocistronic expression cassette

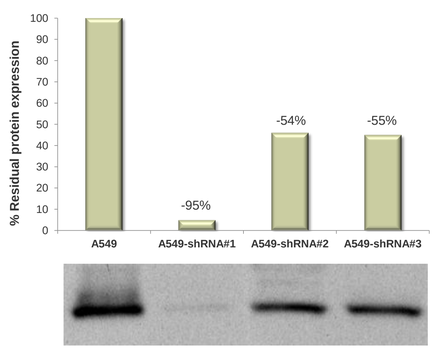


b) One lentiviral vector for one polycistronic expression cassette allowing a stoechiometric expression between two transgenes

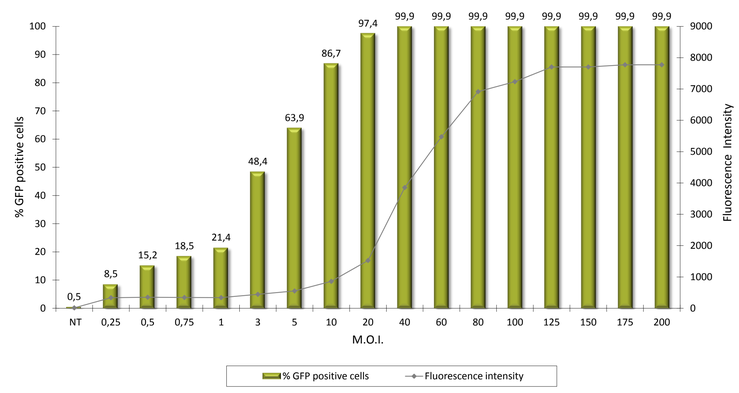
Heavy chain was detected by western blot on supernatant of CHO cells transduced by polycistronic expression cassette at differents M.O.I.

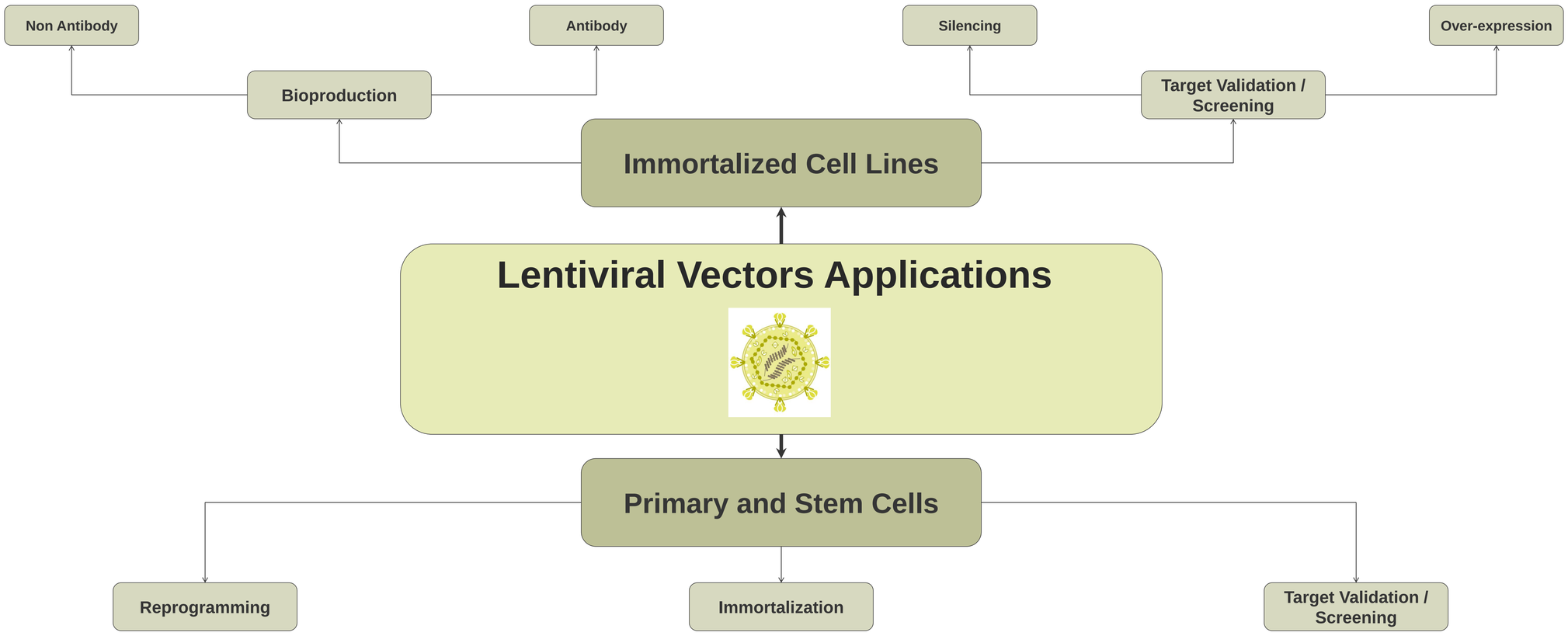


A549 cells were transduced by shRNA-expressing lentiviral vectors silencing the target gene. One week post-transduction, cells were trypsinized and total proteins were extracted. Western blot was carried out to quantify the % of residual protein target expression.

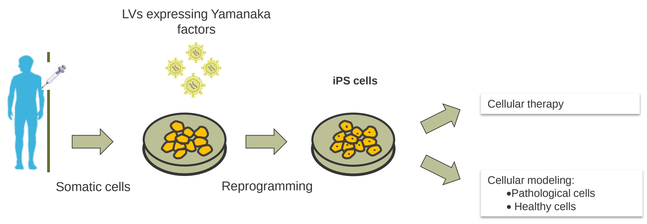


MRC5 cells were transduced by GFP-expressing lentiviral vectors using a range of M.O.I. from 0.25 to 200. Transduction analysis was performed by cytometry five days after transduction.



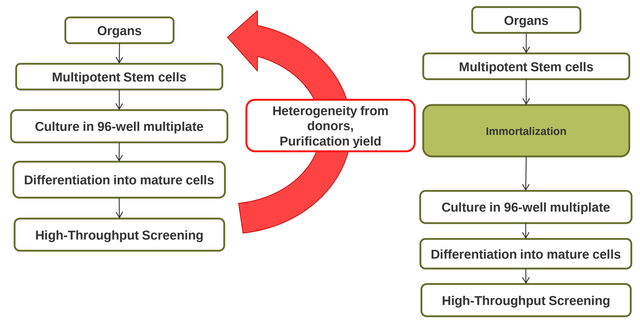


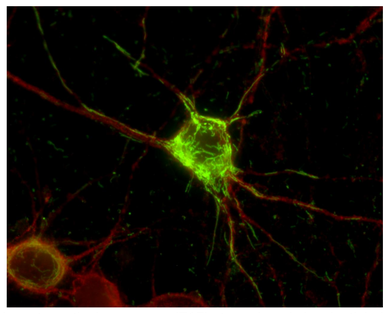
Vectalys has signed a licensing agreement with Kyoto University to provide services to generate iPS cells for its customers.



Customer publication using our lentiviral vectors:

- **Rashid S et al. J Clin Invest, 2010**
- **Lapillonne H et al. Haematologica, 2010**
- **Banito A et al. Genes Dev, 2009**
- **Vallier L et al. Stem cells, 2009**





Embryonic rat cortical neurons (E17) transduced by lentiviral vectors expressing a green fluorescent reporter gene. Transgenes were expressed specifically into mitochondria. Neurons are immunostained in red by detection of Synapsin.

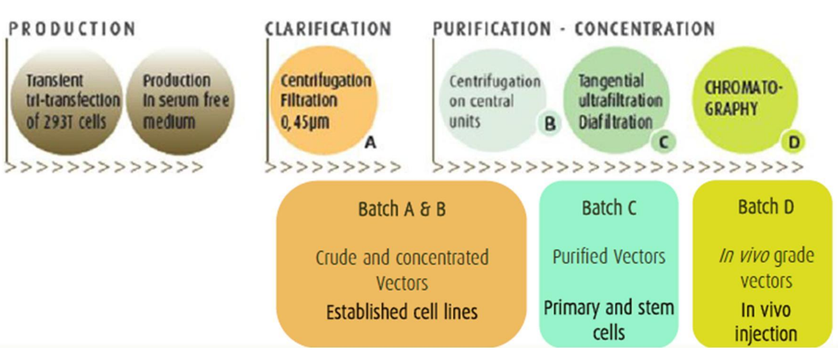
Our highly purified and concentrated lentiviral vectors allow, at low M.O.I., to obtain an higher quality/quantity ratio of iPS clones compared to conventional protocols.

Our highly purified and concentrated lentiviral vectors allow to transduce 100% of primary and stem cells.

Our highly purified and concentrated lentiviral vectors do not affect the viability and the proliferation of your target cells. Due to the high purification level of lentiviral vectors, the cell phenotype is preserved.

Why Vectalys lentiviral vectors?

Vectalys has developped different purification levels adapted to several applications:



Vectalys highly concentrated and purified lentiviral vectors batches show several advantages:

- **Allow to save Time and Money for the generation of stable cell lines used in Bioproduction, Target validation or HTS.**
- **Allow to transduce 100% of primary and stem cells.**
- **Do not affect the viability, the proliferation and preserve the phenotype of your target cells.**
- **Enable to manage a dose effect of expression level and percentage of positive cells by controlling the M.O.I.**
- **Are equally efficient in all cell types and for any transgenes.**
- **Eliminate needs for antibiotic resistance selection steps on primary cells.**