From a versatile and integrated platform for the manufacturing of lentiviral vectors-based nanoparticles for *in situ* gene delivery towards an open solution to accelerate advanced therapies to the clinics

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BioProcess International by informa Europe

A GMP-compatible process for VSV-G+ and VSV-G-deficient LVs

The current 3rd generation lentivectors (LVs) bioproduction process developed at Alaya.bio is GMP-compatible for all types of vectors:

- Intracellular & surface expressed Genes of Interest (GOIs) with transgene sizes ranging from 2 to 8.9 kb
- With or without VSV-G envelope
- Integrative or non-integrative
- Mono- or bicistronic GOIs
- Fluorescent LVs (p17-GFP fusion)

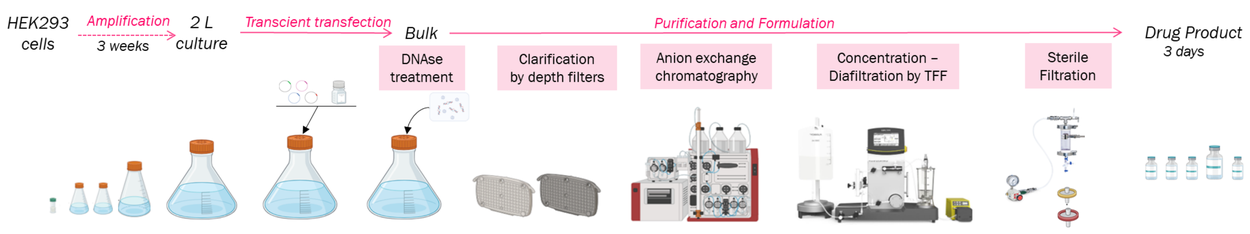
✓ USP process in suspension cells can be performed in bioreactors

VSV-G pseudotyped lentiviral vector

- VSV-G envelope protein which confers broad tropism
- Produced by transfection of four plasmids
- Low transduction in non-activated/non-dividing-cells

VSV-G-deficient lentiviral vector

- Envelope protein: none
- Produced by transfection of three plasmids
- Deficient for transduction



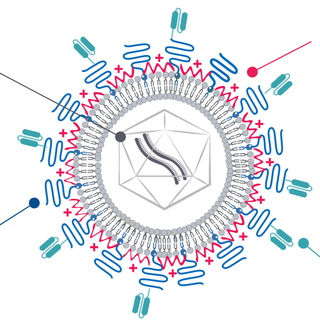
Targeting nanoparticles (TNPs) platform for *in situ* gene delivery

Optimized Lentiviral Vector

- ✓ Deficient for transduction (no VSV-G)
- ✓ Transduction restored through polymer coating
- ✓ Efficacy: 10-fold higher than VSV-G+
- ✓ Safety: no off-target

Shielding polymers

- ✓ Stabilization of NPs
- ✓ Conjugation of targeting agent

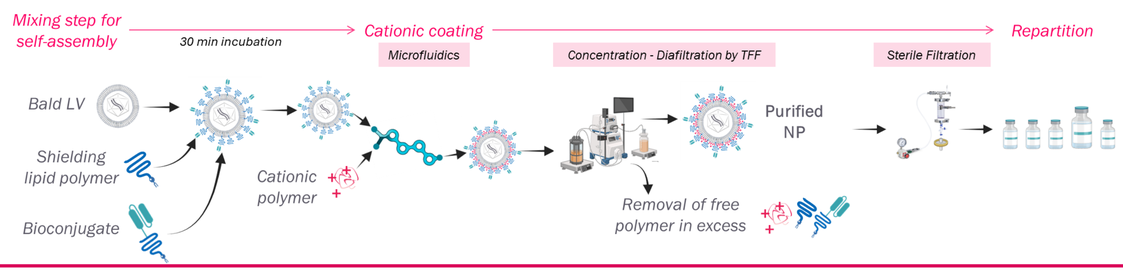


Cationic polymers

- ✓ Biodegradable & pH-responsive
- ✓ Electrostatic interactions with LV surface
- ✓ Endosomal escape

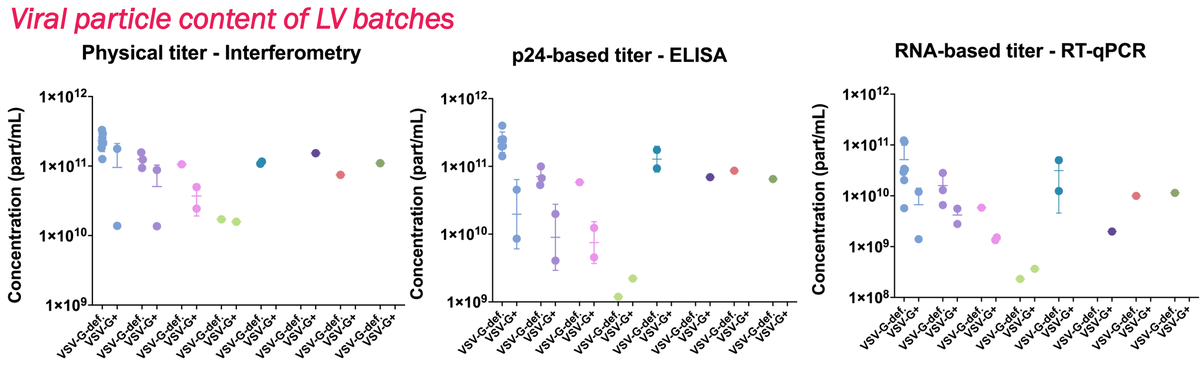
- ✓ Grafted to polymer for T-cell targeting
- ✓ High receptor affinity (nM range)
- ✓ Cell specificity

Anti-CD3 cell Targeting Agents

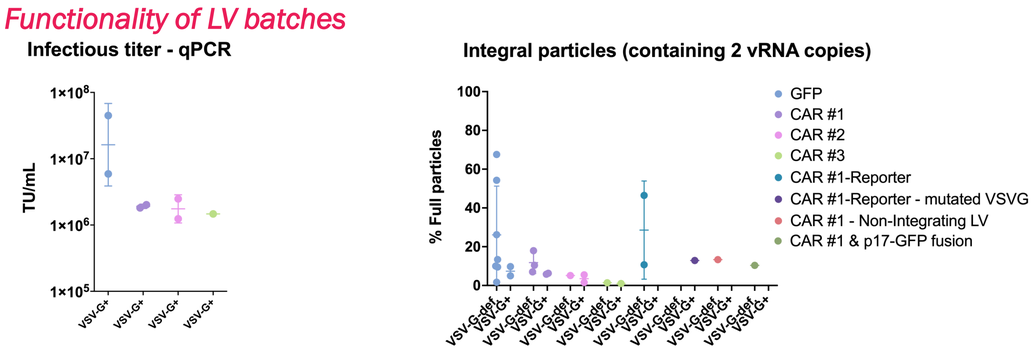


QCs of 24 R&D batches at the 2 L scale

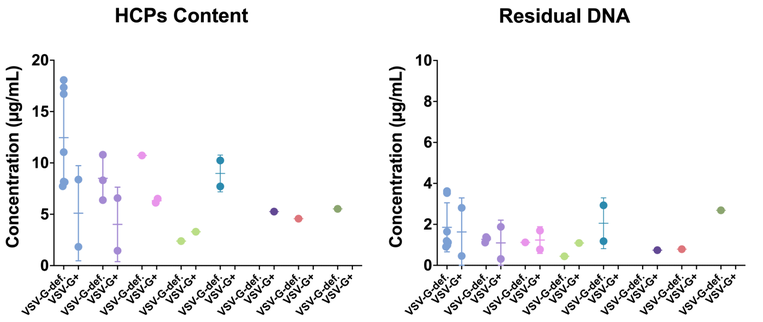
Viral particle content of LV batches



Functionality of LV batches



Process-related impurities of LV batches



At the end of the entire process, 20 to 30 mL of sterile purified and filtered drug product are obtained *(one point = one batch)*.

LV product quality is monitored based on:

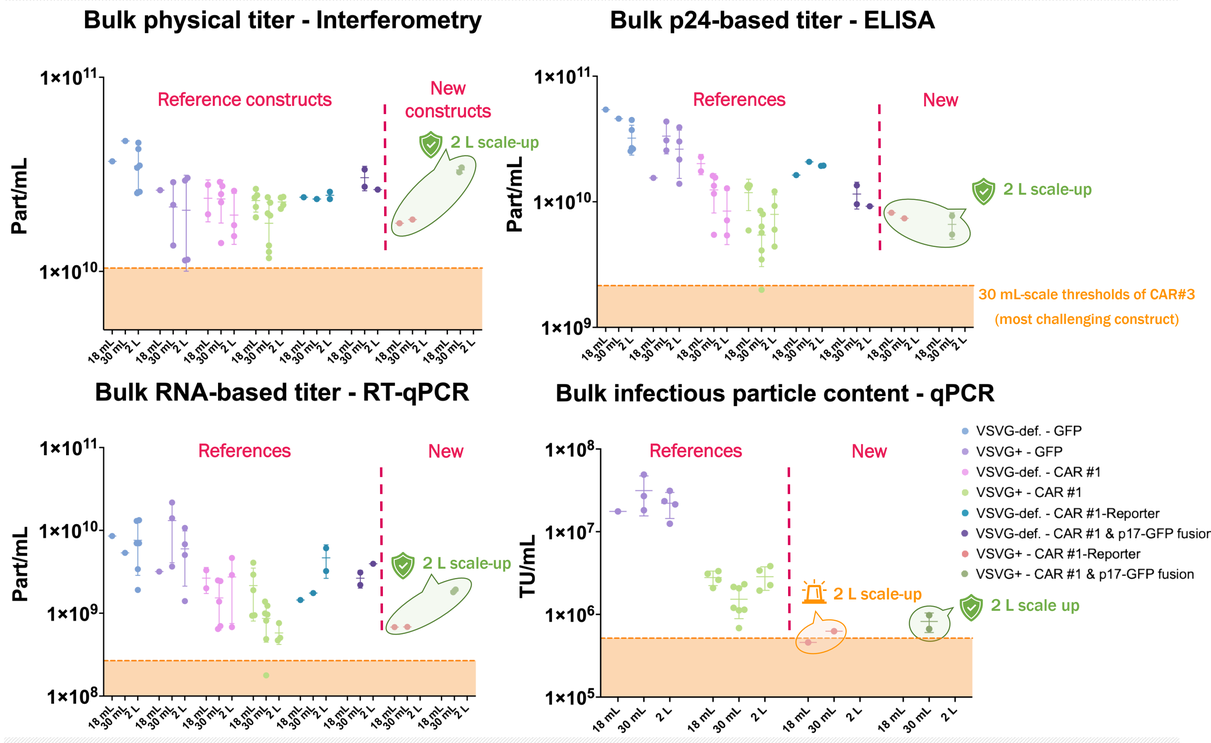
- ✓ Viral particle concentrations (physical, p24-based and RNA-based titers)
- ✓ Infectious titers determined by qPCR on Jurkat cells (3 days post-transduction)
- ✓ Process-related impurities contents (Host Cell Proteins and residual DNA)

Downscaling for the evaluation of new constructs

USP process performance from 18 mL to 2 L

USP process parameters routinely operated for the manufacturing of LV DPs for our own developments were tested and transferred to the 30 mL- then the 18 mL- scale to develop a screening tool for new LV variants based on their manufacturability at the 2 L scale and their functionality.

- ❑ Physical titers were measured by interferometry, anti-p24 ELISA and RT-qPCR
- ❑ Functionality was assessed for VSV-G+ vectors by transduction of Jurkat cells



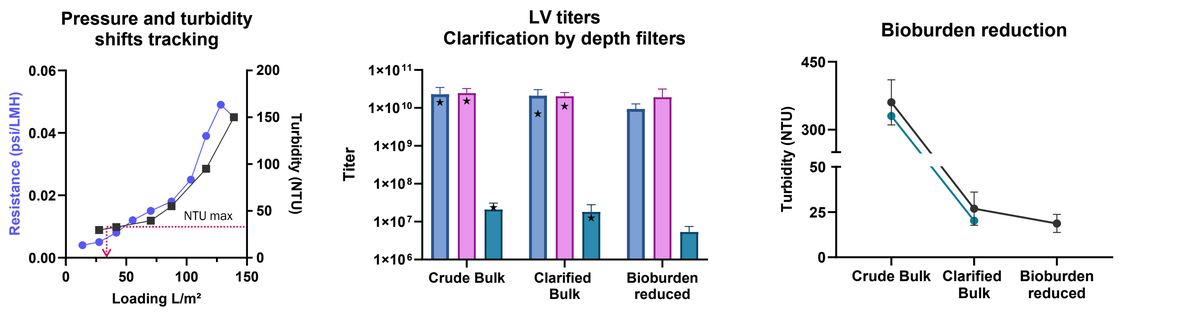
- ❑ Data shown here highlight the robustness of the USP process with VSV-G+ and VSV-G-deficient CD19-CAR & GFP-encoding, non-purified and clarified bulk LVs *(one bulk = one point)*
- ❑ Variants of the CAR construct (such as CD19-CAR-P2A-Reporter gene, CD19-CAR & p17-GFP fusion) were engineered and the feasibility of their bioproduction was evaluated. Consistent titers from the 18 mL- up to 2 L-scale validated the power of the downscaling screening approach
- ✓ **The predictability of yields at the 18 mL-scale now allows us to evaluate, at lower cost and in a shorter timeframe, new genetic constructs provided by potential partners**

Downscaling to improve yields and scalability of DSP process units

- ❑ Challenge: Low recovery yields and high batch-to-batch variability during clarification and concentration-diafiltration steps
- ❑ Identified potential root cause: oversized surface area

→ Small Scale Filtration Systems Models to lower material consumption, increase throughput of tested conditions and rapidly identify most efficient scale

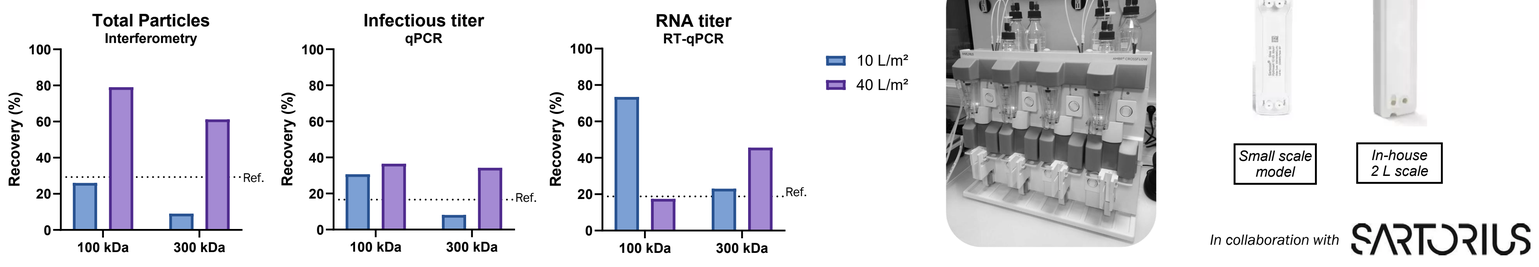
Case study #1: Clarification optimization by depth filters sizing model



The downscaling of the clarification step has been validated by **3 independent studies** based on process parameters (pressure, turbidity) and LV integrity (RNA-and p24-based titers) and functionality (infectious titer).

- ✓ P_{max}™,V_{max}™ and T_{max}™ sizing methods were tested and identified **2-fold smaller filtration areas** with comparable performances
- ✓ Best small-scale parameters have been successfully validated with our current 2 L-scale bioprocess, thereby confirming the scalability of such optimization
- ✓ An additional bioburden reduction filter has been implemented to tightly control the quality of filtrates
- ✓ A turbidity extrapolated-model was established to anticipate any rise of Bulk impurities and size the most suitable depth filter train compatible with scale-up

Case study #2: TFF optimization by cut-off comparison with Ambr® Crossflow



A TFF scale-down model was conducted with our current 2 L-scale bioprocess parameters (C/D/C mode, pressures, diavolumes and diafiltration buffer) and analytical read-outs. Two loading volumes were tested and revealed different performances between 100 and 300 kDa 50 cm^2 membranes:

- ✓ Oversizing of current cassette area (300 kDa - 0.14 m^2) was detrimental for particle recovery due to LV entrapment in pore mesh (at 10 L/m^2 - our reference parameter) despite a reduced process time
- ✓ Small scale model revealed that a **2-fold reduction of surface area** may translate into a **2-fold gain in recovery yields**
- ✓ Two options now available: 10 L/m^2 & 100 kDa membrane with wash step **= recovery x4** *vs* 40 L/m^2 & 300 kDa membrane no wash **= recovery x3**
- ❑ After Filtration area optimization, loading pressure is under evaluation

Cross-functional analytical tools

Complementary Quality Control methods are used for in-process control of the USP/DSP process performance and the evaluation of the quality of the vector as final drug product or payload for TNPs.

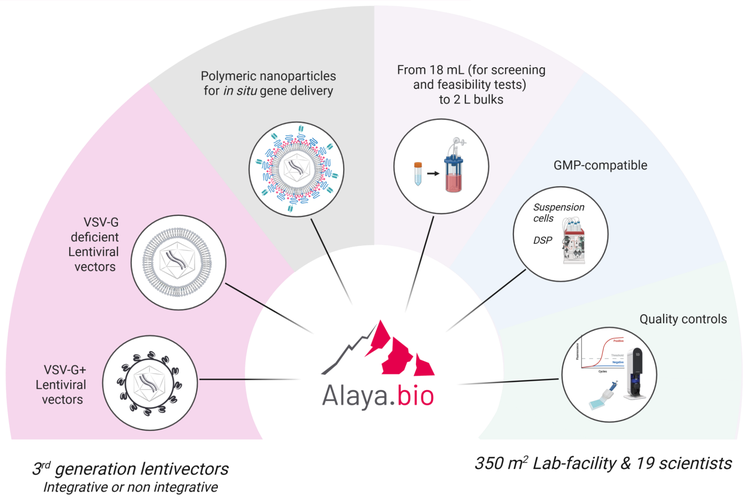
Quality attributes of LV payloads

- ❑ Viral p24 capsid protein quantification (ELISA)
- ❑ Proviral viral RNA copies quantification (RT-qPCR)
- ❑ Particle concentration, size and aggregation (NTA, interferometry)
- ❑ Infectious titers (qPCR post-transduction) & GOI functionality (cytometry)
- ❑ Zeta potential and viral particle diameter (DLS)

Process-related impurities

- ❑ Host Cell Proteins quantification (ELISA)
- ❑ Residual dsDNA quantification (Quant-IT™ PicoGreen™)

Alaya.bio platform highlights



- ✓ A universal and GMP-compatible bioprocess developed for the production of high-quality LV batches for *ex vivo* or *in situ* applications
- ✓ Cost- and time-effective evaluation of the manufacturability of new constructions for internal or third-parties is achieved with our small-scale production platform
- ✓ Our bioproduction platform is amenable to non-integrative LV alternatives when looking for transient expression of therapeutically relevant GOIs

Got questions or just curious?
We'd love to hear from you - **hello@alaya.bio**