

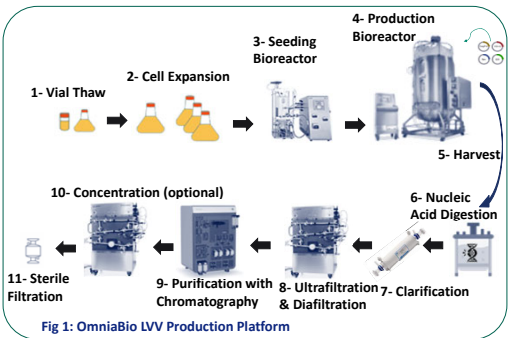
Increased Yields and Purity for Lentivirus Production: OmniaBio's Manufacturing Approach

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Introduction

- OmniaBio offers substantial expertise in process development and GMP manufacturing of potent lentiviral vectors (LVV).
- We offer two suspension serum-free HEK293 cell lines—available in cGMP and research grade that yield high LVV production
 - Transient transfection cell line
 - Stable inducible packaging cell line
- Here, we showcase how our refined platform has been further optimized using:
 - Design of Experiment (DoE) approaches in Upstream development
 - Collaborations with Cytiva and CCRM to substantially increase product purity

Methods



Analytics

Potency/Identity	
Infectious Virus Titre: Flow Cytometry or Digital Droplet PCR (ddPCR)	Physical Titre Determination: P24 Elisa
Impurity	
Residual Host Cell DNA analysis: PicoGreen Assay	Residual Host Cell Protein Analysis: HCP Elisa
Safety	
Endotoxin Testing	Mycoplasma Testing
Sterility Testing	RCL Testing

Results

Optimizing CAR Construct – 2X Increase

- A series of three iterative transfection experiments were conducted at small-scale (6-well plates or 125 mL shake flasks (SF)) to optimize the expression of CAR-LVV.
- A 2X increase in titer was observed.
- The top performing condition identified in the DoE was confirmed in stirred-tank bioreactors (STR) with **no loss in titer**, resulting in a robust/reproducible process.

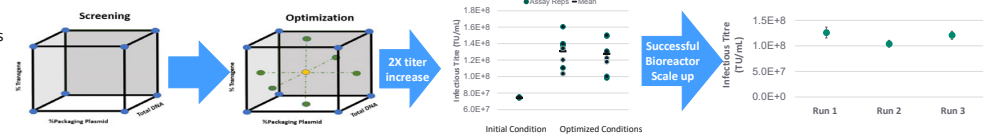


Fig 2: Series of DOEs to optimize the expression of CAR-LVV

Robust Productivity and Scalability

- Upscaled the optimized process into the 3 L BioFlo™ and Cytiva's Xcellerex™ XDR50 single-use bioreactor and determined the culture parameter set points.
- Two proof-of-concept runs conducted, with each run involving an XDR-50 and a 3 L BioFlo™ setup running in parallel.
- The final step of cell scale-up (n-1) was carried out using the XDR-50 system.
- Titers obtained from both XDR-50 runs exhibited remarkable similarity,
- No significant difference observed between the BioFlo™ runs and the XDR-50 runs.

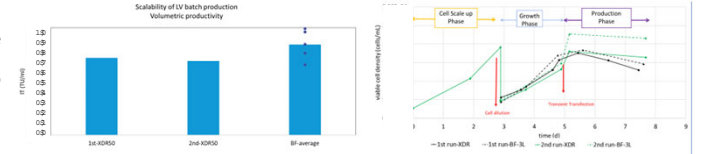


Fig 3: Robust Scalability Confirmed in Two Proof-of-Concept Runs

DSP Unit Operations Performance - High recovery and GFP/CAR Equivalence

- Established a fast, scalable, and robust downstream protocol for LVV:
 - Benzonase treatment: degraded the majority of DNA below 200 bp, to meet the regulatory requirement of no open reading frames (ORF).
 - Clarification: identified scalable and compatible consumables and fine-tuned this step to maximize virus recovery.
 - UF/DF: achieved good recovery and clearance of both protein and DNA from host cells.
 - Chromatography: Identified high-throughput technology and a suitable scale-down model for process development; developed a multimodal chromatography (MMC) step that combines size exclusion and anion exchange properties.
 - Final sterile filtration at 0.22 µm: achieved maximized recovery by optimizing filter material, layers, and other factors.
 - Notably, while this step has been known to reduce vector infectivity, Recovery>80% were observed in our process.
- The overall infectious vector recoveries of ~30% was confirmed for two CAR constructs as well (Fig 4).

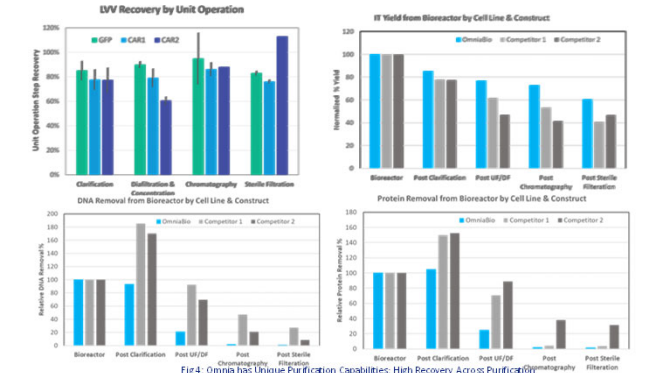


Fig 4: OmniaBio's Unique Purification Capabilities; High Recovery Across Purification

Conclusions

Upstream Process

- We've finely tuned CAR construct transfection parameters, achieving not only high titer but also scalability, making it easily adaptable for client-specific processes.

Downstream Process

- We've achieved an impressive 20-30% overall recovery rate while maintaining high purity, scalability, and customization for client-specific needs.

In collaboration with



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