# Integrating design of experiments to improve Lentiviral vectors purification

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## INTRODUCTION

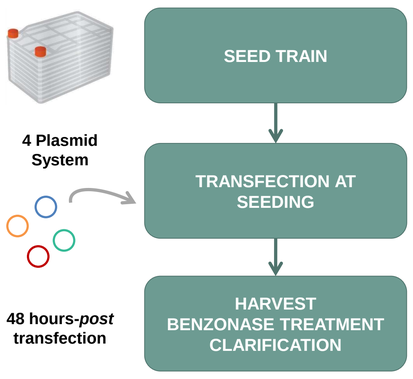
### BACKGROUND

Lentiviral vectors (LVVs) have rapidly become a powerful tool for gene and cell therapy. Currently, the low stability of the virus, mostly due to the fragility of the membrane envelope, as well as the low production titters, are hampering the clinical-to-market transition of gene and cell-based therapies. Therefore, there is the need to produce high volumes and consequently use operation units that allow an efficient virus recovery and concentration. Anionic exchange chromatography (AEX) is one of the most critical steps that could lead to the virus self-inactivation. For that reason, the major focus of this work is the improvement of the capture step using the Design of experiments (DoE) by tuning the more suitable operation conditions.

### AIM & STRATEGY

**IMPROVING THE CAPTURE STEP DURING LVVs PURIFICATION USING DoE**

**LVVs PRODUCTION**




**DESIGN OF EXPERIMENTS**

1 ESTABLISHING THE DESIGN SPACE

2 IDENTIFICATION OF CRITICAL PROCESS PARAMETERS (CPP)

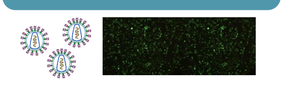
These experiments were performed using HTS 96 well plates

3 ANALYSIS OF THE PROCESS PARAMETRIC STUDY

These experiments were performed in small scale devices

**ANALYTIC CONTROL**

INFECTIOUS PARTICLES (IP)



p24 CAPSID PROTEIN (PP)

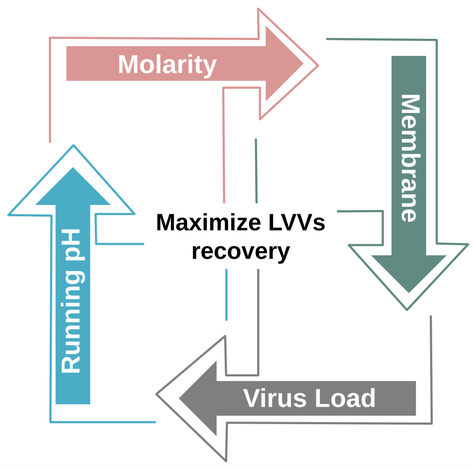
## RESULTS

### 1 ESTABLISHING THE DESIGN SPACE

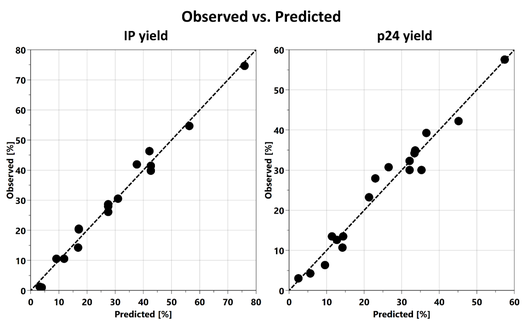
| FACTOR | RANGE |
|---|---|
| Virus Load | Low - High |
| Running pH | 7 – 8 |
| Elution Molarity | 0.7 – 1.3 M NaCl |
| Membrane | Mustang Q/ Competitor |

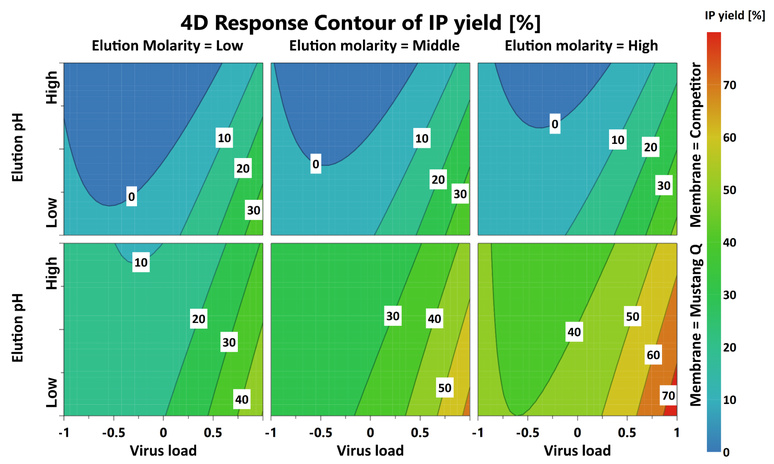
Responses used to establish the model were:

- **Infectious particles (IP)** titration
- **p24 protein (PP)** quantification

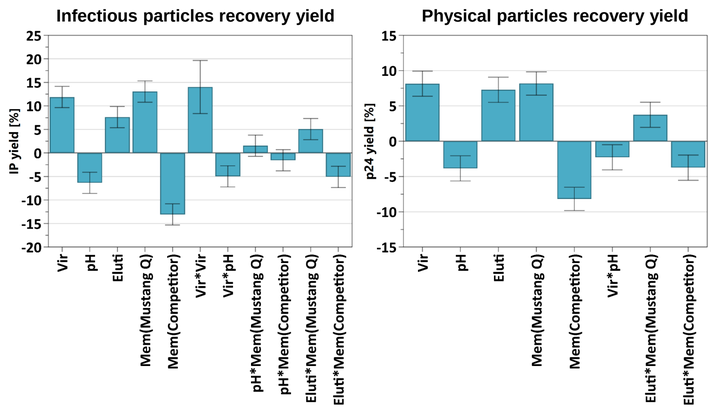



### 2 IDENTIFICATION OF CRITICAL PROCESS PARAMETERS (CPP)




**Coefficients (scaled and centered)**

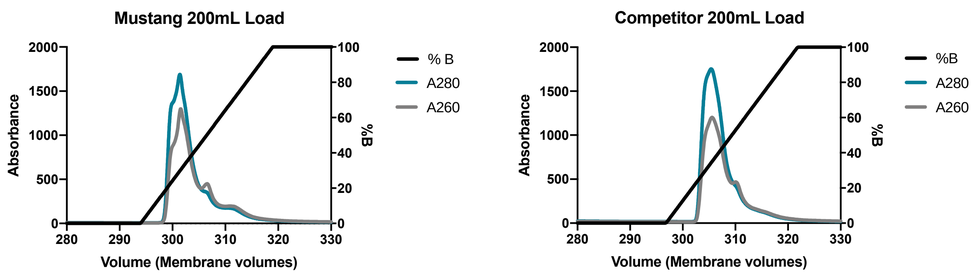



**The critical process parameters that had a positive impact in LVV yield were:**

- High virus load
- Low pH
- Mustang Q membrane
- High salt concentration

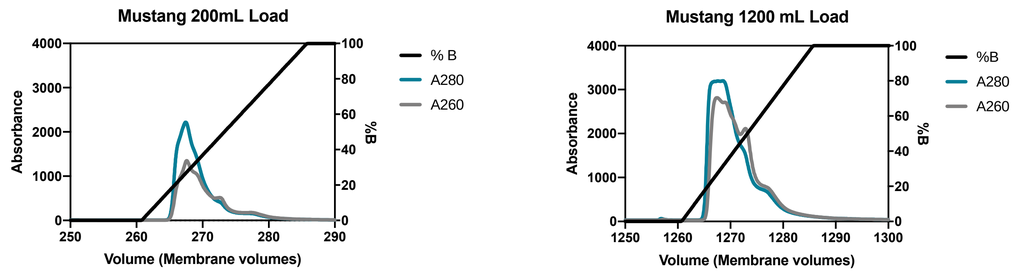
### 3 ANALYSIS OF THE PROCESS PARAMETRIC STUDY

**Mustang Q and Competitor were evaluated using small scale devices**




| MEMBRANE | IP recovery yields (%) | P24 recovery yields (%) | DNA removal (%) | Protein removal (%) |
|---|---|---|---|---|
| Mustang Q | 91 - 95 | 38-41 | 81 | > 97 |
| Competitor | 33-58 | 23 | 77 | > 97 |

**The Mustang Q performance was evaluated with 6x higher virus loading**




- The **Mustang Q** membrane showed a **better performance** in comparison with the **Competitor** membrane used in this study.
- A preliminary **Mustang Q capacity** value was determined based on the loading studies (1.5E+12 PP/mL).

## FINAL REMARKS

- It was determined the membrane with the **higher** recovery yield – **Mustang Q;**
- DoE experiments indicated that CPPs with the most impact on LVVs recovery in AEX were: **virus load, pH, membrane and molarity;**
- Small scale devices confirmed the results obtained in the **HTS 96 well plates** which corroborate the **robustness** and **scalability** of these membrane supports;
- **Protein** and **DNA** impurities were efficiently removed during the chromatographic step.

## REFERENCES

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## ACKNOWLEGMENTS

iNOVA4Health Research Unit (LISB.OA-01-0145-FEDER-007344), which is cofunded by Fundação para a Ciência e Tecnologia / Ministério da Ciência e do Ensino Superior (FCT/MCES), through national funds, and by FEDER under the PT2020 Partnership Agreement, is acknowledged. ASM acknowledge FCT/MCES for the PhD fellowship PD/BD/135501/2018. The authors, TQF and JGO, thank funding from FCT/MCES through the project PTDC/EQU-EPQ/29306/2017. Pall Life Sciences is acknowledged for funding and scientific support.