FlexiPro™ Chrom

VERDOT
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EDITING LIFE

FlexiPro Chrom Features:

- **Multi-scale downstream workflows with a single system**
- **One instrument, four Flow Kits covering 0.6 – 560 LPH range**
- **Flow Kit installation in <15 minutes**
- **High precision gradient and dilution**
- **Wide conductivity range 0-500 mS/cm**
- **Dual regulation of flow rate and pressure**
- **21 CFR Part 11 and USP VI Compliant**

Versatile GMP manufacturing for plasmids and viral vectors

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Recent years have seen remarkable progress in the field of cell and gene therapy, driven by genetic engineering advances in autologous and allogeneic therapies, CAR-T cell therapy (chimeric antigen receptor T-cell therapy), and stem cell research. In general, cell therapy involves the transplantation of patient or donor cells that have been genetically modified back into the patient to repair or replace damaged cells (or tissue) to restore function or to fight the disease. The most promising aspect of cell therapy is that it aims to address the root cause of disease at the cellular level, offering the potential for long-lasting or even curative outcomes for diseases once considered incurable.

As cell and gene therapies continue to advance, manufacturing processes are also evolving. One of the major obstacles in manufacturing has been the need for ultra small and medium scale GMP processing with single use capabilities while maintaining flexibility for future process improvements.

In this study, Cellectis evaluated the versatile VERDOT® FlexiPro™ Chrom system for multi-scale GMP manufacturing. By purifying both plasmids and lentiviral vectors on the same single-use system, valuable cleanroom space could be conserved while remaining agile for evolving manufacturing processes.

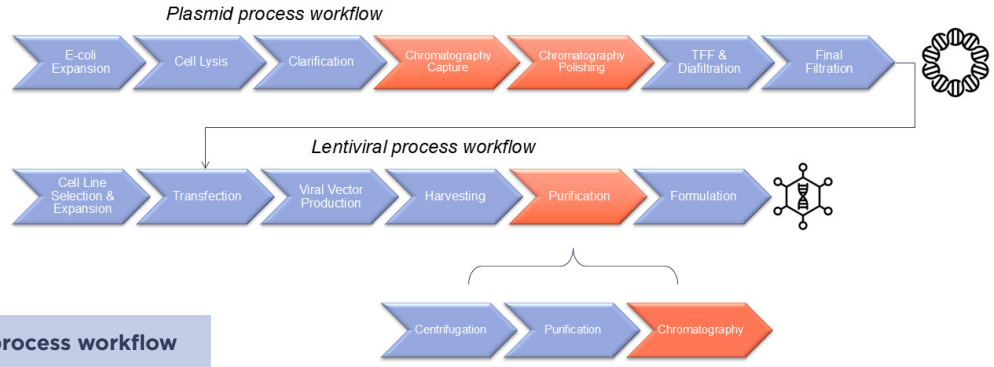

Figure 1. Cellectis process workflow

Case Study

Cellectis Overview

Cellectis is a clinical-stage biotechnology company that uses its pioneering TALEN® gene-editing platform to develop innovative therapies for the treatment of serious and rare diseases such as Acute Lymphoblastic Leukemia and Non-Hodgkin's Lymphoma, for which clinical trials are underway. These therapies are based on modified allogeneic T cells derived from healthy donor cells. The donor-derived T cells are transduced using a lentiviral vector to express a chimeric antigen receptor (CAR) and are further genetically modified using TALEN® gene-editing technology.

The manufacturing of clinical batches for these cell therapies requires the prior production of critical starting biological components — namely, lentiviral vectors (rLV), and messenger RNA (mRNA) — both of which also require the production of DNA plasmids (pDNA), as described in Figure 1.

In general, plasmids are critical and costly intermediates in the manufacturing of cell and gene therapies (CGT) and mRNA-based therapies, where they can account for up to 30% of the production costs. In addition, lentiviral vectors can also represent up to 50% of the production costs in CGT.

In order to quickly move a conceptual genomic design for a multi-engineered cellular product (a "smart cell") into the clinic, Cellectis developed a strategy to internalize the entire manufacturing chain from DNA, mRNA, viral vectors, electroporation buffers, all the way to final cellular product. This approach also enables the ability to rapidly perform versioning of these product candidates with various genetic attributes.

This requires a flexible and versatile GMP platform that can operate at multiple process scales.

The VERDOT FlexiPro Chrom system includes four different flow kit sizes in a single compact unit, including one of the lowest flow rates available in a single-use GMP chromatography system. Cellectis evaluated the FlexiPro Chrom system for manufacturing pDNA and viral vectors.

VERDOT FlexiPro Chrom System

The FlexiPro Chrom system (Figure 2) provides a flow rate range of 10 mL/min to 560 L/hr using interchangeable pump heads with paired single-use flow kits, delivering optimal purification performance across all chromatography steps, from capture to polishing. With this design, a single system can cover multiple process scales.

Other design features include:

- Very low flow ranges for small scale GMP processing for advanced therapeutics
  - Ultra Low Flow Kit (10-110 ml/min) with 1/16" ID tubing for columns as small as 8 mL volume
  - Very Low Flow Kit (1-30 L/hr) with 1/8" ID tubing for columns ranging from 0.5-1.0 L volume
- Concurrent control of flow and pressure to provide the desired flow rate while ensuring that maximum pressure is not exceeded
  - This feature is particularly beneficial for monolithic columns, which often generate significant backpressure
- High precision instrumentation for very shallow and precise linear gradients
- Conductivity probe with a wide measurement range (0-500 mS/cm), which is particularly useful for plasmid purification
- Dual wavelength UV (260 nm and 280 nm) for fractionation based on the ratio of nucleic acids to proteins
- Scalable from process development to commercial manufacturing with the widest processing range in a single GMP system

Figure 2. FlexiPro Chrom offers single-use flexibility with four Flow Kit options

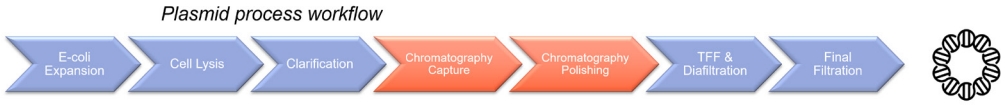

Figure 3. Cellectis plasmid process workflow

Plasmid Purification

Plasmids are critical in the manufacturing of CGT as well as mRNA-based vaccines and therapies. Biopharmaceutical companies like Cellectis predominantly use bacterial production with *E. coli*. These well-characterized bacteria can biosynthesize plasmids with high productivity and reliability since plasmids are naturally occurring molecules in *E. coli*.

However, this approach requires multiple filtration and purification steps to remove all by-products from the fermentation process. Plasmids must be of exceptional purity, as even trace levels of specific contaminants can jeopardize subsequent manufacturing steps for the target cell therapies.

Generally, plasmid purification involves at least two process steps to eliminate critical impurities such as:

- Host Cell Proteins (HCPs)
- Genomic DNA
- RNA from the host cells
- Endotoxins
- Unwanted plasmid forms
- Residual antibiotics
- Other cellular debris

Purification Strategy

Cellectis uses a charged membrane and a resin column for these steps (Figure 3), leveraging the strong negative charge of plasmids due to the presence of phosphate groups in the DNA backbone.

- First chromatography step: charged membrane eliminates *E. coli* contaminants (e.g., endotoxins, HCP, etc.)

- Second chromatography step: resin chromatography for separation of the correct plasmids from their unwanted isoforms

The use of membranes is well-suited for large molecules like plasmids as they offer much easier access to ligands. Unlike porous bead chromatography resins that require plasmids to diffuse into the pores — thereby limiting mass transfer or even blocking ligand access when the pores are too small — membranes provide a direct path to the ligands.

The use of resin comes with additional challenges, most notably a significant backpressure which requires operating above 3 bars. Membranes allow operation at lower pressures but present another issue with a large dead volume, which dilutes the eluted product.

First Chromatography Step

The first chromatography step is a strong anion-exchange (AEX) membrane adsorber that operates in "bind & elute" mode. Plasmids are bound to the membrane while contaminants such as endotoxins and HCP flow through. The membrane has a nominal dead volume of 200 mL.

The purification method has five steps, all at a flow rate of 13.5 L/hr (Figure 4).

1. Sanitization: Sodium hydroxide
2. Equilibration: Slightly basic pH and mild ionic strength
    a. UV is zeroed at the end of this step.
3. Load: Raw product is loaded onto the membrane
4. Wash: Same as equilibration buffer
5. Elution: High-salt buffer

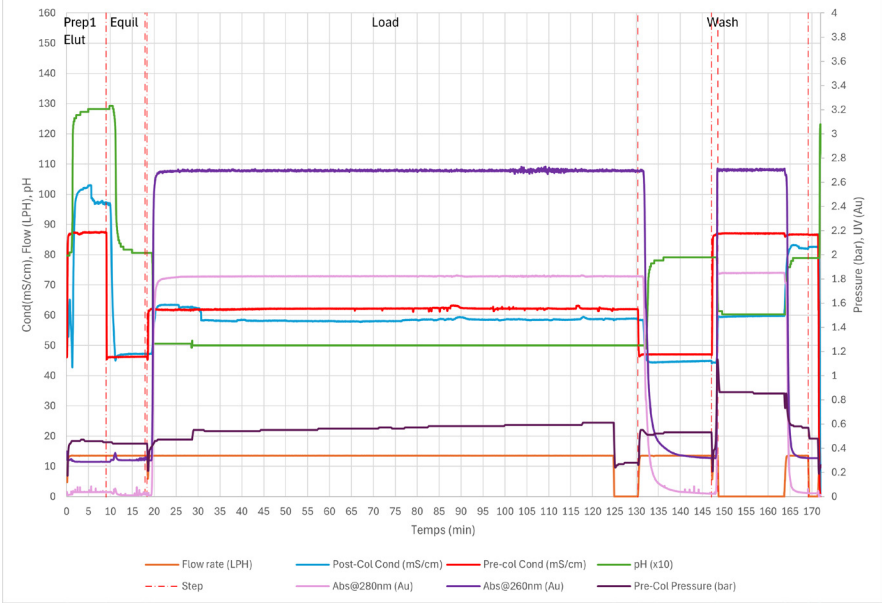

Figure 4. Chromatogram for the first chromatography step of plasmid purification

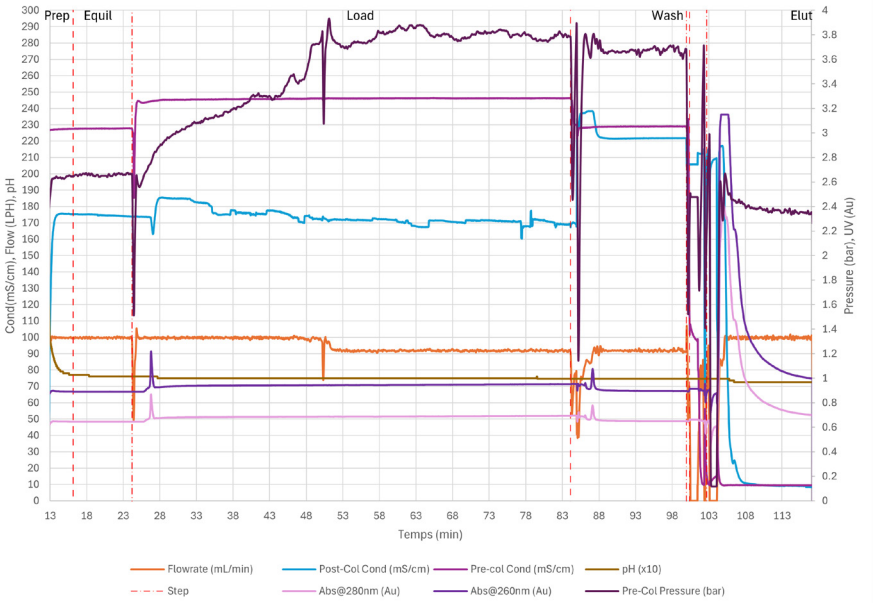

Figure 5. Chromatogram for the second chromatography step of plasmid purification

The load step has a relatively high salt concentration, which is noteworthy in the context of ion exchange chromatography. Plasmids are highly negatively charged due to their high phosphate group content and bind strongly to an anion exchanger at neutral pH. However, contaminants such as HCPs and endotoxins, which also carry negative charges (i.e., carboxylate groups in proteins and phosphate-like groups in endotoxins), can bind weakly to the anion exchanger.

Using high salt during the load step helps to shield the negative charges of the contaminants, reducing their interaction with the membrane. At the same time, the charge density of plasmids remains sufficient for strong binding to the positively charged membrane, enabling selective retention of the plasmids.

Elution uses a higher salt concentration buffer, as is standard for ion exchange processes. The eluate is then further purified using the second chromatography step.

Second Chromatography Step

The second chromatography step also operates in "bind & elute" mode, using a 200 mL Thiophilic Adsorption Chromatography (TAC) resin column. A specific form of plasmid binds to the resin while unwanted isoforms (i.e., open circular plasmids, linearized plasmids, and *E. coli* genomic DNA) flow through.

One key advantage of TAC resins is that the eluate from the previous chromatography step can be directly diluted into the ammonium sulfate equilibration buffer without the need for diafiltration, streamlining the workflow.

This purification process has five steps, all performed at a flow rate of 100 mL/min (Figure 5):

1. Sanitization: Sodium hydroxide
2. Equilibration: Ammonium sulfate buffer at slightly basic pH and high ionic strength
    a. The UV is zeroed at the end of this step
3. Load: Eluate from first chromatography step diluted with ammonium sulfate equilibration buffer
4. Wash: Same as equilibration buffer to remove unbound impurities
5. Elution: The bound plasmid is eluted with a low salt buffer

Thiophilic Adsorption Chromatography (TAC) works on the principle that some proteins in high salt can bind to an immobilized ligand that contains a sulfone group in proximity to a thioether group. The bound proteins are then eluted in decreasing salt concentrations. Supercoiled plasmids, which are compact with a limited surface area exposed to the solvent, are able to bind preferentially compared to other DNA forms (i.e., open circular plasmids, linearized plasmids or genomic DNA). This step effectively separates the desired plasmid isoform to exclude undesired contaminants and ensures a high purity final product.

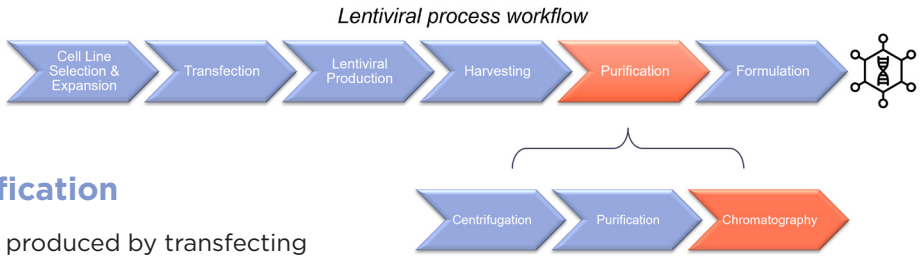

Figure 6. Cellectis lentiviral vector process workflow

Lentiviral Vector Purification

Lentiviral vector particles are produced by transfecting cultured cells with four plasmids completing an incubation period and harvesting culture supernatant. The subsequent purification step removes HCPs, residual DNA (both genomic and plasmid), cellular debris, and process-related impurities, as shown in Figure 6.

A monolithic column is particularly suitable for lentiviral vector purification due to its unique structure of interconnected channels. These channels allow efficient mass transfer for large biomolecules like lentiviral vectors while minimizing shear stress, which is critical for preserving the structural and functional integrity of the viral particles.

Cellectis uses an anion exchange monolithic column for lentiviral vector purification. At slightly basic pH, lentiviral particles are negatively charged, allowing separation from impurities based on charge density differences.

A buffer with mild ionic strength is used during loading. This enables the lentiviral particles, which have a higher charge density, to bind strongly to the positively charged column. Impurities such as HCPs, which are less negatively charged, are effectively washed away at this stage.

After sanitization in sodium hydroxide, the purification method has 4 steps (Figure 7):

1. Equilibration: Mild ionic strength buffer
   a. The UV is zeroed at the end of this step
2. Load: The product is loaded using flow control to maintain a target flow rate of 18 L/hr with a maximum pressure of 3 bars
3. Wash: Same as equilibration buffer to remove unbound impurities
4. Elution: Elute lentiviral particles with a high salt buffer (~80 mS/cm)

In this application, it is essential to balance the optimal flow range for the monolith's performance — preferably in the higher values — with the pressure limitations of the disposable flow kits, which are restricted to a maximum of 4 bars. This balance is particularly challenging because the pressure drop across the column can vary during the product loading phase and between batches, even at the same flow rate.

The FlexiPro Chrom system's capability for dual flow regulation is especially advantageous in this application.

FlexiPro Chrom provides control of both flow rate and pressure, maintaining the desired flow rate unless the maximum pressure limit is approached. If the pressure exceeds the set threshold, the system automatically prioritizes pressure regulation by reducing the flow rate. This functionality is clearly demonstrated in the chromatogram during the loading phase, as shown in Figure 7: the flow rate (orange line) begins at 18 L/hr but transitions to pressure-based regulation once the pressure (black line) exceeds the 3 bar limit for pump regulation. Consequently, the flow rate progressively decreases from 18 to 11.5 L/hr by the end of the loading phase, while the pressure is precisely maintained at 3 bars.

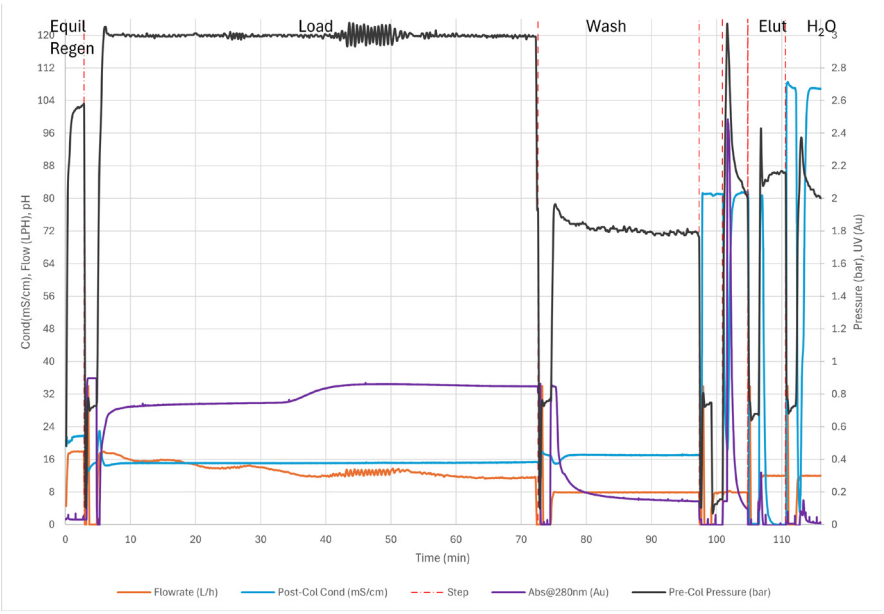

Figure 7. Chromatogram for the rLV monolithic column step

Purification Workflow Challenges and Solutions

The FlexiPro Chrom system enables Cellectis to overcome several challenges in their manufacturing process that includes a charged membrane, a resin column, and a monolithic column. The purification workflow includes a wide range of salt concentrations and requires accurate flow rates with multiple pressure constraints.

Flowmeter Precision:
The FlexiPro Chrom system employs an ultrasonic flowmeter for precise flow rate regulation in a closed loop. This technology offers high precision and low sensitivity to viscosity compared to turbine flow sensors.

Given the wide range of ionic strengths involved in this purification process, the ultrasonic flowmeter is automatically recalibrated at the start of each step where ionic strength changes significantly. This automated recalibration ensures precise flow measurement throughout the entire process.

Conductivity Measurement:
The wide range of ionic strengths involved in this purification (from the µS/cm range up to 500 mS/cm) exceeds the capabilities of most single-use conductivity probes.

However, the FlexiPro Chrom system integrates a Conducell 4USF VP120 high-precision 4-electrode sensor from Hamilton®, originally designed for multi-use applications.

VERDOT selected this conductivity probe for single-use kits due to its wide range (0-500 mS/cm) and designed a small-volume measurement cell to reduce the dead volume below 4 mL, ensuring accurate conductivity measurements across a broad range.

The FlexiPro Chrom is the only single-use chromatography system available with this range.

Dual regulation of flow and pressure:
Monolithic columns often produce significant backpressure and can limit flow rates in disposable flow kits due to pressure limitations.

The FlexiPro Chrom allows simultaneous flow and pressure control to maximize processing efficiency.

Conclusion

The Cellectis purification process ensures consistent production of a plasmid vector with purity necessary for the delivery of the gene of interest, supporting the high standards required for DNA and viral vector production used in cell and gene therapy applications.

In this study, Cellectis used the VERDOT FlexiPro Chrom system for purifying both plasmids and lentiviral vectors on the same single-use system.

The FlexiPro Chrom system allows versatile GMP manufacturing across multiple process scales because of its wide range of flow rates.

The FlexiPro Chrom is particularly useful for cell and gene therapy applications, for the following reasons:

- Versatility: Capable of operating at low flow rates
  - Ultra Low Flow Kit (ULFK, 10-110 ml/min) with 1/16" ID tubing
  - Very Low Flow Kit (VLFK, 1-30 L/hr) with 1/8" ID tubing
  - Designed for minimal hold-up volumes and improved flow rate accuracy
- Specialized Instrumentation: Conductivity probe with a wide measurement range (0-500 mS/cm) and UV measurement down to 0.001 AU.
- Continuing Innovation: Features such as automatic flowmeter recalibration during the process and simultaneous flow and pressure regulation, tailored to meet the specific needs of Cellectis.

The close collaboration between Cellectis and VERDOT has improved the current performance, ease of use, and GMP suitability of Cellectis' manufacturing process and established a foundation for ongoing advancements. This continuous progress ensures that the system will keep evolving, becoming even more efficient and better suited to meet future challenges, further contributing to Cellectis' success in therapeutic innovation.