A Scalable, Two-Step Purification Process for Plasmid DNA

with Simon Åberg

Plasmid DNA (pDNA) plays a critical role in biopharmaceutical manufacturing — e.g., by providing a template for mRNA synthesis, delivering genes of interest to production cell lines and viral vectors, and even serving as a basis for DNA vaccines. Although plasmid production in microbial hosts is a well-characterized process, downstream purification of the needed supercoiled (sc) forms can be time-consuming, resource-intensive, and difficult to scale. The latter concern is especially problematic considering how many applications require pDNA — sometimes large quantities of it. In a November 2023 Ask the Expert presentation, Simon Åberg (senior research engineer for viral-vector applications in the Genomic Medicine R&D division of Cytiva) described his company's efforts to improve pDNA purification. Leveraging upstream adjustments and membrane chromatography capsules, Cytiva scientists have established a scalable, two-step purification process that can be performed with single-use (SU) technology and reusable membrane capsules.

Åberg's Presentation

Process Requirements: Cytiva's legacy pDNA-manufacturing process began with fermentation of *Escherichia coli* hosts, followed by steps for cell concentration and for lysis and flocculation. After depth, tangential-flow, and normal-flow filtration (TFF, NFF) of lysed material, plasmids underwent three purification steps: size-exclusion chromatography (SEC) using Sepharose 6 Fast Flow resin, removal of open-circular (oc) plasmid forms with PlasmidSelect Xtra resin, and anion-exchange (AEX) chromatography using Source 30Q resin (all resins from Cytiva). Purified material was processed through another TFF step and then formulated for bulk fill–finish.

Although the legacy process performed robustly and fulfilled regulatory expectations, purification required considerable time, did not scale easily, and was incompatible with Cytiva's prepacked ReadyToProcess chromatography columns. Because processing involved large quantities of ammonium-sulfate buffer, the workflow also posed sustainability concerns. Cytiva scientists sought to establish a two-step purification process that yielded clinical-grade plasmids while reducing processing times, improving scalability, increasing SU compatibility, and minimizing ammonium sulfate consumption. To meet internal expectations for clinical-grade products, the updated process also needed to yield sc pDNA of >95% purity, with host cell protein (HCP), host cell DNA and RNA (hcDNA, hcRNA), and endotoxin levels well below values set forth in regulatory guidances.

Upstream Adjustments for Downstream Success: Before addressing purification improvements, Åberg underscored the importance of evaluating the entire pDNA-manufacturing process. "Optimizing upstream and clarification steps can give you advantages later in downstream," he explained. For instance, excessive antifoam concentrations can influence ratios between oc and sc plasmids, as can glycerol accumulation from ineffective feed strategies. Optimizing fermentation parameters can help to minimize generation of oc forms, reducing contaminants to be removed during subsequent purification steps.

Åberg also highlighted considerations for cell concentration and lysis. In the former case, development scientists can leverage standard centrifugation formats for laboratory-scale work; large batches could undergo microfiltration by TFF or continuous centrifugation in an SU format. Lysis steps are critical, he continued, because they must be rigorous enough to release pDNA from host cells but not so harsh that they generate considerable impurities. Performing calcium-chloride precipitation after cell lysis can be a quick, simple, and cost-effective way to separate RNA impurities before processing material downstream.

An Improved Purification Process: Cytiva scientists shortened the purification workflow from three to two chromatography steps. Lysed and filtered material is processed through a Mustang Q XT membrane adsorber capsule for capture chromatography. Then, plasmids are loaded onto Capto PlasmidSelect resin for oc pDNA removal. Compared with the legacy process, the updated workflow features higher capacity and improved pressure-flow properties. Such gains enabled elimination of the Source 30Q polishing step.

Mustang Q XT technology comes in different capsule sizes to accommodate small and large processing needs. Åberg demonstrated the adsorber's capabilities with chromatograms from pDNA purification using Mustang Q

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XT5 (5 mL) and XT140 (140 mL) capsules for laboratory and production scales, respectively. The XT5 capsule was loaded with 12 mg of pDNA per membrane volume (MV) and the XT140 capsule with 9 mg/MV. Run at a flow rate of 5 MV/min, both formats had a capacity of ~16 mg/MV. They also exhibited high throughput, processing 25 mL of material per minute at laboratory scale and 700 mL/min at production scale. After an elution step with 3 M sodium chloride, the XT5 membrane process provided a step yield of 58%, and the XT140 counterpart generated a yield of 67%. Åberg noted that because pDNA binds strongly to AEX media, high yields can be difficult to achieve. Implementing multiple elution cycles can help to maximize plasmid recovery.

Åberg also presented data about Capto PlasmidSelect enrichment of sc plasmid forms. In one case, a laboratory-scale (18 mL) column loaded with 2.8 mg pDNA/mL resin was processed at a rate of 220 cm/h, then eluted using 11 column volumes (CV) of water at 120 cm/h. Column capacity was ~3 mg plasmid/mL resin with a step yield of 77%. The production-scale process leveraged a 1-L ReadyToProcess SU column prepacked with Capto PlasmidSelect resin. A load of 0.9 mg/mL was processed at 179 cm/h, then eluted at a gradient, also with 11 CV of water, at 90 cm/h. The column had a similar binding capacity and achieved a step yield of 72%. Users can apply an isocratic solution during elution, although doing so necessitates optimization of buffer conductivity.

Purification based on Mustang Q XT membrane adsorbers and Capto PlasmidSelect resin raises several technical and operational advantages. Compared with the legacy process, the improved workflow reduces processing time by 60% and requires 90% less ammonium-sulfate solution, presenting significant sustainability gains. Meanwhile, pDNA resulting from the new process continues to meet all regulatory and internal acceptance criteria for quality attributes such as sc pDNA titer (100% at production scale), hcDNA levels (0.6 µg genomic DNA/mg pDNA), HCP levels (below the limit of quantitation, LoQ), and endotoxin levels (also below the LoQ).

Associated Analytics: In addition to presenting pDNA-product data, Åberg explored analytical methods for testing in-process material. He described using agarose-gel electrophoresis (AGE) to measure hcRNA levels in samples from different downstream stages. Results demonstrated that calcium-chloride precipitation of postlysis material reduced hcRNA significantly, facilitating subsequent removal of such impurities. Åberg also showed data from chromatographic analyses of pDNA concentrations using Sepharose High Performance resin. Industry analysts often assess pDNA concentrations by microvolume spectrophotometry. But as Åberg explained, the latter method tends to overestimate plasmid titers at early process stages, when the greatest amount of hcRNA remains in sampled material. Because the Sepharose High Performance method separates such impurities from plasmids, it provides accurate titer measurement independent of sample type or stage.

Similarly, Cytiva has leveraged Capto PlasmidSelect resin for rapid screening of sc and oc plasmid concentrations. The method generates results comparable with those obtained by capillary gel electrophoresis (CGE) but can be performed in about 15 minutes. Both methods can be performed easily using an Äkta pure 25 chromatography system, facilitating data collection and saving valuable time during process development.

Questions and Answers

Why must sc pDNA isoforms be enriched during processing? Linear and oc plasmids usually are generated by "nicks" in a DNA strand. Enriching sc isoforms provides quality assurance, helping to minimize potential for mutated plasmids in final products.

Have regulators established homogeneity requirements for pDNA used to produce mRNA-based vaccines and therapeutics? This discussion is ongoing. Regulatory agencies have yet to establish clear guidelines for pDNA when it is used as a process intermediate. Perhaps such guidance will develop as mRNA-based candidates reach the biologics license application (BLA) stage. We know, however, that good manufacturing practice (GMP) in the United States requires pDNA products to have sc isoform concentrations of >95%. That requirement is unlikely to change in the near term.

Does the updated purification process eliminate steps for prechromatography buffer exchange and TFF? Cytiva highly recommends performing those steps before transferring material to chromatography. Postlysis calcium-chloride precipitation and TFF remove significant amounts of contaminants, including hcRNA, and buffer exchange is required to optimize conductivity levels for binding with Mustang Q XT membrane technology.

What column size is needed for the Capto PlasmidSelect analytical method? The method involves a 1-mL HiTrap column (from Cytiva) loaded with ~500 µL of sample.

How can the linear gradient be replaced with an isocratic elution step during purification with the Capto PlasmidSelect resin? First, the process conductivity must be optimized for a given plasmid. The key is to find conductivities that will provide a good window of separation between oc isoforms (which will flow through) and sc plasmids (which will come as eluate).

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