Designing a Plasmid DNA Downstream Purification Process
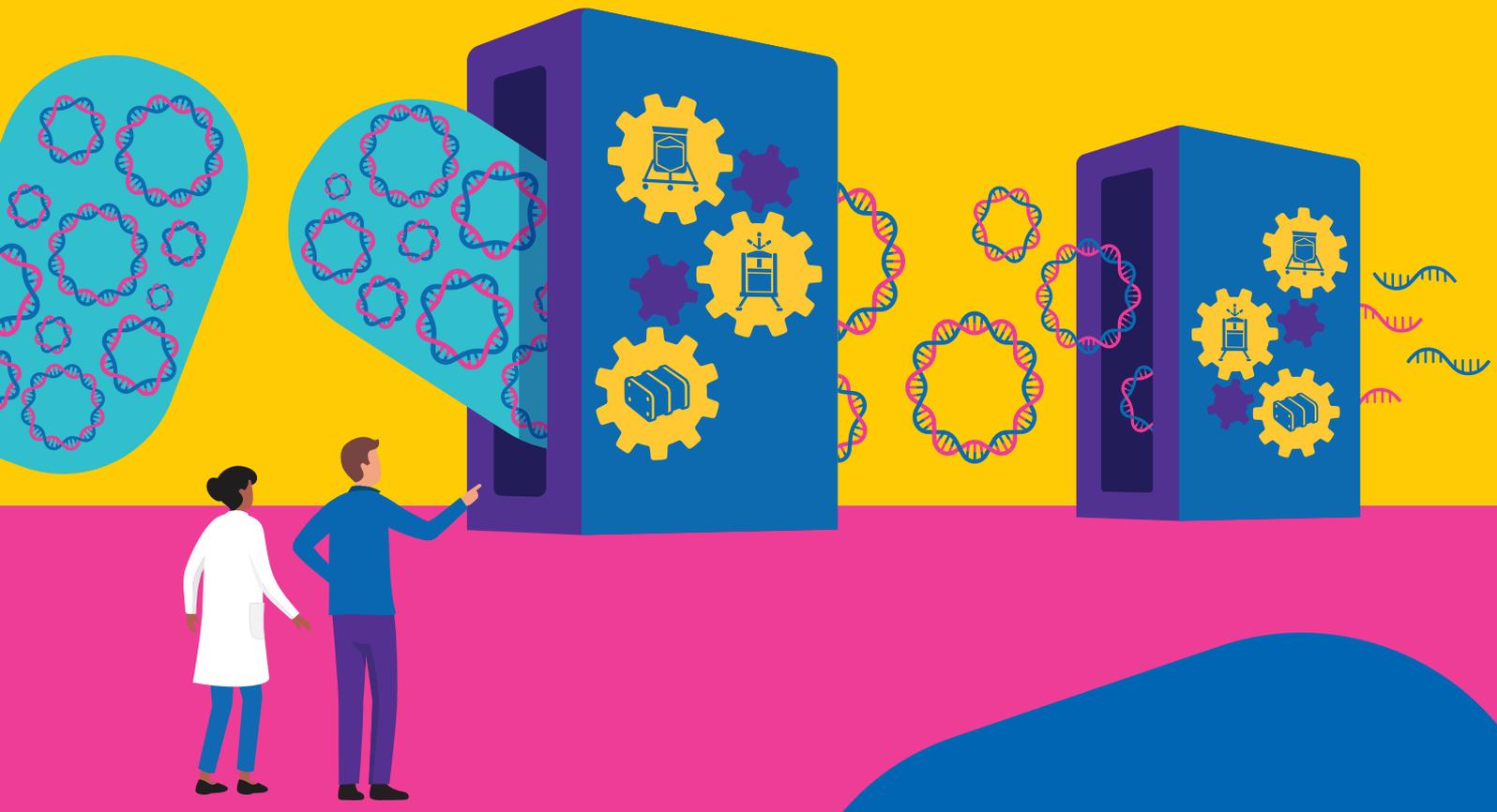
For mRNA, plasmid-based DNA Vaccines, and Viral Vector applications

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Introduction

Our Mission

mRNA plasmid-based DNA vaccines, and advanced modalities using viral vectors, are at the forefront of the fight against cancer, cardiovascular, immunological, and infectious diseases. To reach their full potential and benefit as many patients as possible, however, workflows used for their manufacture must achieve greater efficiency. A critical step in the process is production of plasmid DNA (pDNA), which traditionally, delivers low yields and requires complex purification schemes.

Plasmid DNA (pDNA) is an essential component of, and key technology for production of viral vector, mRNA, and vaccine therapies. The far-reaching potential of novel therapeutic modalities and vaccines that rely on pDNA is driving increased demand and the need for improved production strategies.

To meet this growing demand, upstream productivity must be increased to achieve yield and efficiency goals, ensure robust impurity removal and maximize downstream recovery, all while ensuring patient safety.

pDNA manufacturing presents several challenges. Production suffers from low productivity of microbial fermentation and the purification process is complicated by the fact that plasmids are quite large and possess a highly negative charge. The bacterial lysate contains contaminants with properties similar to pDNA leading to low resolution separation, in addition, the bacterial lysate can be highly viscous. A low flow rate is needed for chromatography, and it can be difficult to achieve the desired concentrations at the final tangential flow filtration (TFF) step.

Additionally, pDNA is sensitive to mechanical damage, which can lead to changes in its topological form. Plasmid isoforms include supercoiled (fully intact and wound around itself), open circular (one strand is broken and the molecule relaxes) and linear (both strands are broken with free ends). Supercoiled plasmids are recognized as the most therapeutically relevant and regulatory agencies set expectations for the supercoiled percentage in final drug substance for DNA vaccines.

The manufacturing schemes for pDNA were first developed in the mid-1980s and have since relied on well-established traditional production processes, typically fermentation using a microbial source, usually *E. coli*.

This process development book provides you with guidance for your plasmid DNA downstream process development, in addition to representative data, as we explore Cell Harvest, Lysis, Neutralization and Clarification; Chromatographic Purification; Tangential Flow Filtration (TFF); and Sterile Filtration unit operations.

This guide also includes data from our collaboration with a biotechnology company focused on the design and development of RNA-based therapeutics and products. Look for case study highlights throughout the guide.

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Plasmid Production Overview

This eBook describes all the steps of a complete platform for pDNA manufacturing (**Figure 1**). Each of these steps is explored below, along with strategies to optimize and streamline the purification workflow. It also includes a comprehensive case study with results, for each of the downstream operating units.

The purification of pDNA can be challenging as the starting material, typically clarified lysate from alkaline lysis of bacterial cells after neutralization, has a complex composition with no more than 3% of the content being pDNA while the remaining 97% represent impurities. Most of the critical impurities are negatively charged (RNA, genomic DNA, endotoxins), similar in size (open circular pDNA, genomic DNA, high molecular weight RNA), and hydrophobic (endotoxins).

Purification requires a combination of unit operations including TFF, chromatography, and sterile filtration. Chromatographic methods can include a range of different modalities, either singly or combined. The final bulk pDNA must meet quality specifications set by regulatory agencies and should be free from host cell impurities

pDNA Production Platform

Fermentation → Cell Harvest → Cell Lysis → Clarification → Chromatography (1-2 steps) → UF / DF → Final Sterile Filtration

Figure 1: General process flow for pDNA manufacturing

Cell Harvest, Lysis, Neutralization and Clarification of Plasmid DNA

1. Recommendations

Plasmid DNA (pDNA) is typically produced via fermentation using a microbial source. Following *E. coli* fermentation, the primary downstream purification begins with harvesting of the cells, lysis, and clarification. During cell harvest, cells are concentrated, and the fermentation broth is removed via centrifugation or microfiltration tangential flow filtration (MF-TFF).

For MF-TFF, open-channel, flat-sheet TFF devices such as Prostak™ modules with Durapore® 0.1 or 0.2 μm microfiltration membranes or Pellicon® 2 cassettes with Ultracel® or Biomax® 1,000 kD ultrafiltration (UF) membranes and V-screen are recommended.

The harvested *E. coli* cells are then disrupted to release the plasmid DNA. Lysis is most often performed via an alkaline method. Alkaline lysis with 0.1–0.5 N NaOH with 0.1–0.2% SDS is commonly used. Lysis time and mixing should be optimized.

Precipitation/flocculation is the first step to separate the supercoiled pDNA by selectively precipitating and removing impurities (high molecular weight RNA and genomic DNA, proteins, and endotoxins) typically by use of 0.7–3 M potassium acetate with or without $CaCl_2$ (0.8–1.5%), pH range 5.0–7.5.

Lysate can be clarified using depth filtration, such as Clarisolve® filter, Millistak+® HC, and Millistak+® HC Pro filters, to achieve high filtration capacity and yield. These filters are available in a wide range of formats with sizes from 0.014 m^2 to 1.1 m^2. Preclarification/pretreatment significantly affects the capacity of the depth filter and process development should therefore be carefully considered for optimization of the step. Yield from the clarification step is generally >90%.

Table 1 summarizes the recommended filters for clarification.

Option	Primary	Secondary (if needed)	Bioburden
Option 1	Clarisolve® 60HX filter	Milligard® PES 1.2/0.45 µm filter	Millipore Express® SHC 0.5/0.2 µm filter
Option 2	Millistak+® HC D0HC filter		
Option 3	Millistak+® CE20 filter	Millistak+® CE50 or Polysep™ II 1.0/0.5 µm filter	

Table 1: Summary of filters used for clarification.

Note – Filter selection and capacity depend on whether feed is pretreated/untreated. Pretreatment will have a significant impact on performance

2. Overview

When harvesting pDNA, using MF-TFF and normal flow filtration (NFF) attributes, parameters, and considerations outlined in **Table 2** are important.

Attributes	Parameters	Key Considerations
Filtration capacity	Filter selection (chemistry and pore sizing)	Viscosity of pDNA solution
Filtration flux	Filtration endpoint	
pDNA yield	Driving force	High pH of lysis - near denaturization point of pDNA
Impurity reduction (gDNA, protein, and RNA)	Feed treatments	High solids content from fermenter
Bioburden protection and reduction	Mixing formulation (pH, conductivity, buffer components)	

Table 2: Overview of MF-TFF and NFF step.

2.1. Cell harvest

2.1.1. Attributes

Bacterial cells containing the plasmid of interest are typically harvested by either centrifugation or tangential flow filtration (TFF). Centrifugation is often more cost-effective for the harvest step when smaller batch volumes (<10 L) or larger batch volumes (>1,000 L) need to be processed.

2.1.2. Parameters

Bacterial cells containing the plasmid of interest are typically harvested based on OD600nm. Harvest OD600 depends on the type of media used in fermentation and the type of fermentation. OD-based harvest parameters are outlined in **Table 3**.

Fermentation media	Harvest OD600
LB media	3-5
Super broth media	Up to 8
Super broth media with glycerol	25-35

Table 3: Harvest OD values for different types of fermentation media.
(Reference: based on Input from Industry)

High cell density fermentation techniques for culturing *E. coli* have been developed to improve productivity and obtain high cell density[2].

The goal of fermentation is to maximize cell density of dry cell weight at approximately 40–60 g/L and pDNA titers of approximately 1 g/L. It was possible to reach 2.2 g/L with use of optimized vectors and optimization of the fermentation process.

TFF devices used in a harvest step include Prostak™ modules with MF membranes such as Durapore® (PVDF) 0.1, 0.22 or 0.45 µm, or Pellicon® 2 cassettes with V-screen (suspended screen) and open grade UF membranes such as Biomax® (PES) or Ultracel® (Composite Regenerated Cellulose) 1,000 kD. When using membrane cut-offs such as these, it is important to utilize a two-pump (permeate-controlled) TFF system[3]. The TFF harvest step typically involves a 2–5X volumetric concentration followed by a 3–5 volume diafiltration for washing out spent media components and extracellular impurities prior to further downstream purification. TFF harvest is typically operated at low transmembrane pressure (TMP; 3–5 psi) and ΔP (<7 psi) with a control on the permeate flux (**Table 4**).

Parameters	Value
Device	Prostak™ modules with 0.1, 0.22 or 0.45 µm Durapore® membranes or Pellicon® 2 cassettes with 1,000 kD Biomax® or Ultracel® membrane and V-screen.
Volumetric loading	10-60 L/m²
Feed flow	7-9 L/min/m²
TMP	<0.5 bar
Average flux	20-30 L/m²/h (LMH)
Volumetric concentration factor	2 to 5
Diafiltration volume	3 to 5

Table 4: Operating parameters for MF-TFF.
(Reference: Internal data)

E. coli cells could be harvested into a pellet by batch centrifugation using 4,500–6,000 g for ~15–20 min (at room temperature or ~4 ºC). Other types of centrifuges such as continuous-feed, intermittent solids-discharge, disc-stack, batch-discharge or solid bowl could also be used on the harvest step.

2.2. Cell lysis

2.2.1. Attributes

The methods used for cell disruption can be divided into two main categories – chemical (alkali, detergents, enzymes, osmotic shock) and physio mechanical (heat, shear, agitation, ultra-sonification, and freeze-thawing) lysis.

Alkaline lysis (NaOH at pH ~12) accompanied by detergents such as sodium dodecyl sulfate (SDS) is the most common approach. The detergent solubilizes the cell walls, and the alkaline environment denatures genomic DNA. It is important to optimize the lysis incubation time as it directly impacts the quality and quantity of plasmid DNA. Longer incubation time could lead to irreversible denaturation of plasmid DNA and shear degradation of genomic DNA. It is critical to have efficient but not too aggressive mixing employed on the alkaline lysis step to ensure there are no pH extremes causing irreversible denaturing of the plasmids or degrading it due to excessive shear.

A completely different method for cell lysis involves the use of newly developed autolytic *E. coli* strains. The pDNA is recovered by autolytic extraction under slightly acidic, low-salt buffer conditions and treatment with a low concentration of nonionic detergent. Genomic DNA remains associated with the insoluble cell debris and is removed by solid-liquid separation using a thermal flocculation followed by coarse filtration.[1]

2.2.2. Parameters

During the alkaline lysis method, cells are treated at specific, narrow range of pH (typically around pH 12) at which the genomic DNA will be irreversibly denatured, while the pDNA double chain remains intact (pH range of 12.0 to 12.5). The optimum pH value varies depending on the type of plasmid and host strain. A deviation of more than 0.1 pH unit from the optimum value may affect the yield and it is therefore critical to maintain a tight control of the pH range during alkaline lysis; at a pH >12.5, pDNA becomes irreversibly denatured and if the pH is too low, genomic DNA won't be completely denatured and could complicate further downstream purification process.

The incubation time for a standard alkaline lysis is short and the step is usually completed typically within 5 minutes. The degree of lysis could be controlled by measuring viscosity/residence time in a vessel.

In a laboratory setting, mixing is often performed gently by hand, which is not feasible at larger scales.

For achieving complete but gentle mixing of large lysis volumes, batch mixing in a mechanically agitated vessel (specialized vessel design with utilizing baffles, low power number impellers, feed lines) and/or continuous flow-through devices/in-line static mixers have been used, taking into consideration viscous non-Newtonian properties of the lysate. Mobius® single-use mixers can be very effective for batch lysis.

2.3. Precipitation/flocculation

2.3.1. Attributes

Precipitation/flocculation is the first step in removing host cell contaminants in a pDNA manufacturing process. Neutralization can be done using a high concentration of sodium or potassium acetate with or without surfactant, RNase, or $CaCl_2$. This step causes precipitation of detergent solubilized proteins including high molecular weight genomic DNA. Smaller, covalently closed circular pDNA renatures into double stranded molecules and remains in a soluble state. RNase can be added into the neutralization buffer for degradation of high molecular weight RNA impurities (RNA could be present at least 20X amount of pDNA). Some chaotropic salts, such as lithium chloride, ammonium acetate, and calcium chloride have the additional advantage of precipitating high molecular weight RNA together with the proteins. Polyethylene glycol (PEG) and polyethylenimine (PEI) can also be used for precipitation of genomic DNA.

2.3.2. Parameters

Rapid neutralization occurs with high-salt buffer (such as sodium or potassium acetate at concentration of 0.7 M–3.0 M and pH ~5–7.5, with/without 0.8–1.5% $CaCl_2$) in the presence of a detergent (1% SDS).

A low-cut off PEG precipitation (at 4% w/v) can also be used for precipitation of genomic DNA with up to 20% (w/v) of the precipitate formed during the step. Homogenous mixing during neutralization and precipitation is critical to maintain pDNA quality.

Based on our internal data, impurities such as high molecular weight RNA and genomic DNA, proteins and endotoxins can be selectively precipitated using high salt buffer, PEG and PEI. Proper optimization is recommended.

To separate the precipitated solids, typical clarification methods such as settling with decanting, depth filtration and centrifugation are used. Product loss has been observed occasionally with filtration, and therefore filters with low adsorption are preferred.

2.4. Clarification

2.4.1. Attributes

Clarification unit operations for pDNA processes should enable removal of solid content from the feed stream. Feed streams can either be untreated, pretreated or preclarified. Post chemical lysis and neutralization with sodium or potassium acetate leads to development of large floccules/precipitates.

Pretreatment has a major impact on the clarification filter capacity and must be selected carefully along with a consideration of the scalability of the process. Pretreatment options include use of gravity settling and separation, PEG, PEI, bag filters, stainless steel screen filters, paper filters, and centrifugation.

2.4.2. Parameters

To achieve the desired attributes, clarification operations should ensure proper filter selection to handle the solid load of the lysate. Depth filters are ideal, as capacity can be high and adsorptive interactions are masked by the high salt concentration of lysate feed allowing high yield. Feed flux and filtration endpoints can be optimized to ensure minimal filter area is used and high yield of pDNA is achieved. Additionally, product recovery operation such as blow down and buffer flushing should be considered.

3. Technical Data

3.1. Harvest

Biomax® or Ultracel® 1,000 kD V-screen membranes or Durapore® MF TFF membranes are used for harvest at low TMP and permeate control. Normalized water permeability (NWP) recovery post use is >90%. The load challenge reported for Biomax® 1,000 kD membrane ranges from 10–60 L/m^2 with an optimum permeate flux around 10–30 LMH.

Centrifugation is one of the preferred methods for harvesting at lab scale; at large scale, centrifugation process can be cumbersome and provide low yield. Disk stack centrifuges operating at high speed with intermittent ejection gave supercoiled plasmid yields as low as 40% because of shear damage during discharge.[4]

case study

Cell Harvest by Tangential Flow Filtration (TFF)

Objective: Retain, concentrate, and wash the *E. coli* cells, which are approximately 2 x 0.5 µm

Materials and Methods

Device

- 0.1 m² Pellicon® 2 cassette with 1000 kD Biomax® (polyethersulfone) membrane, V-screen for high viscosity & particles

Membrane preparation

- Water flush: 20 L/m²
- Clean-in-place recirculation: 0.2 N NaOH, 20 L/m² single pass
- Buffer flush: 20 L/m² 10 mM Tris, 1 mM EDTA, pH 8 (TE)

Critical flux testing

- Adjust permeate flow rate (pump 2) and monitor TMP
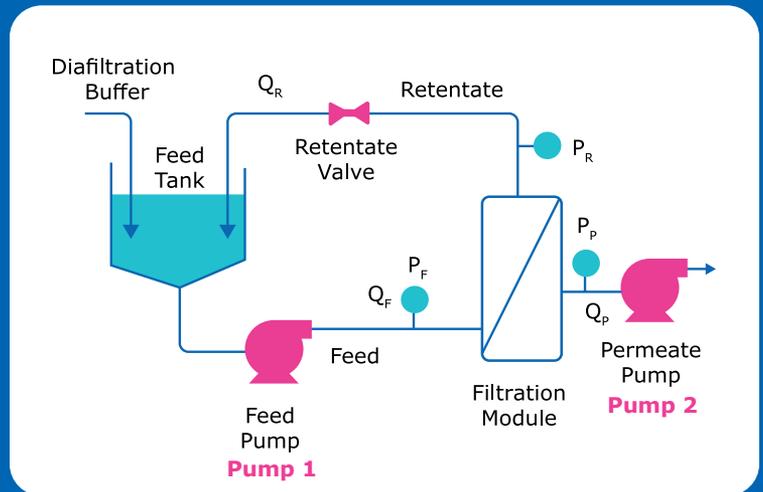
Cell concentration

- Tank volume reduction with permeate to waste; cells are retained
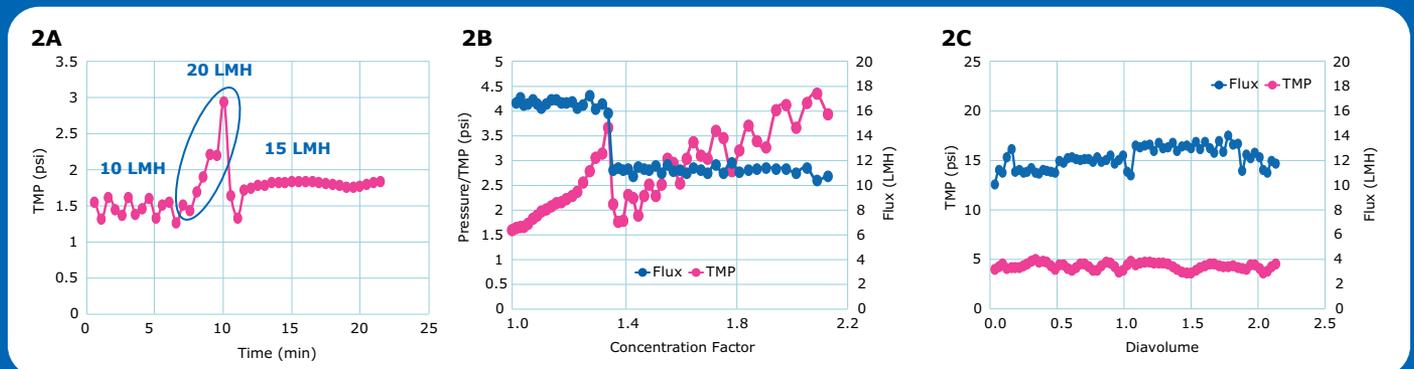
Cell wash

- Constant volume diafiltration adds TE buffer at same rate as permeate removal

Cell Recovery

- Drain tank and flush retentate line and flush tank

Results

Critical flux testing was performed to identify the optimal flow rate for operation. Permeate flux was ramped up until instability in the transmembrane pressure (TMP) was observed (**Figure 2A**). While TMP was stable at a low flux of about 10 LMH, instability of pressure was observed at the 20 LMH flux range indicating that the process should be operated below this value. TMP of about 15 LMH was used for the operational flux.

Over the course of cell concentration, viscosity of the solution increased with a resulting exponential increase in TMP, indicating instability. To accommodate this, flux was reduced to 11 or 12 LMH. While a slight increase in TMP was still observed at this reduced flux, it was more manageable from an operational perspective. Using this approach, a 2.1-fold concentration factor of cells was achieved (**Figure 2B**).

The cell wash step was performed over the course of two diavolumes to exchange into Tris-EDTA (TE) buffer and was performed at an average flux of 12 LMH (**Figure 2C**) to remove impurities including media components, HCPs (host cell proteins), and nucleic acids. The process was stable as shown by the TMP, which remained low (~5 psi) during the wash step.

3.2. Lysis and neutralization

Cell lysis is typically carried out at pH 12–12.5 with 0.2% SDS, followed by neutralization using potassium acetate (0.7–3 M). Typically, neutralization is carried out at a pH of approximately 5.0. But it has been reported at pH 6.0 and pH 7.5. Use of $CaCl_2$ is common for RNA precipitation during neutralization.

Floccules generated during the neutralization step after undisturbed incubation commonly float on top of the liquid.

Pre-clarification methods reported in our internal database show pretreatment by use of a range of approaches were used approximately 75% of the time; in 25% of the studies, no treatment or prefiltration was used (**Figure 3**).
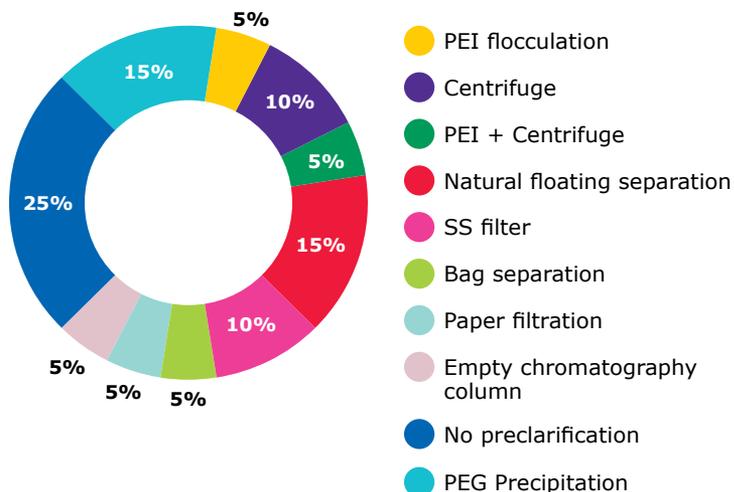
Preclarification method

Figure 3. Various reported pretreatment/prefiltration conditions.
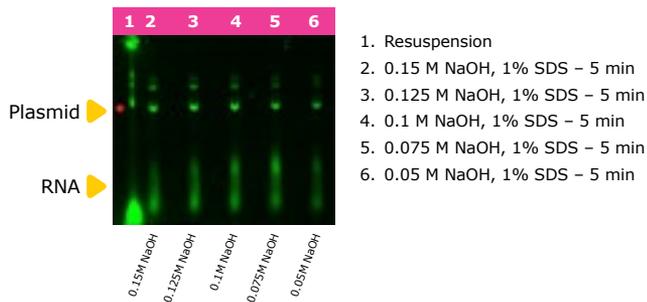
case study

Cell Alkaline Lysis

Objective: Identify optimal conditions for *E. coli* lysis to release pDNA product and neutralize the resulting solution.
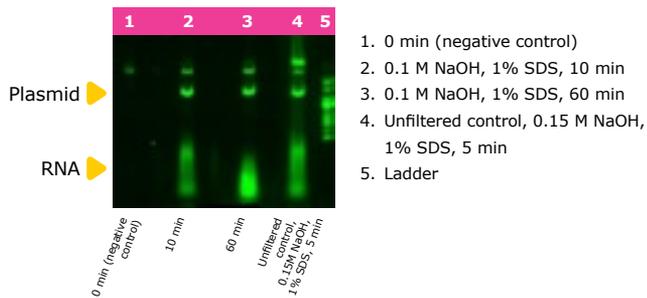
Materials and Methods

- 125 g wet weight/L of cells were resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- Experimental lysis buffers containing 1% SDS and variable NaOH concentrations (ranging from 0.05 M to 0.15 M NaOH) were added to different pools of resuspended cells in a 1:1 volume ratio. Lysis was evaluated over variable timepoints ranging from 1 - 60 minutes.
- Neutralization buffer (3 M Potassium Acetate, pH 5.5) was added in a 1:1 volume ratio to quench the reaction.
- The mixture was centrifuged at 12 kg for 30 minutes and filtered (0.45 μm)
- PAGE or fluorescent dye was used to evaluate assay nucleic acid content in the final solution
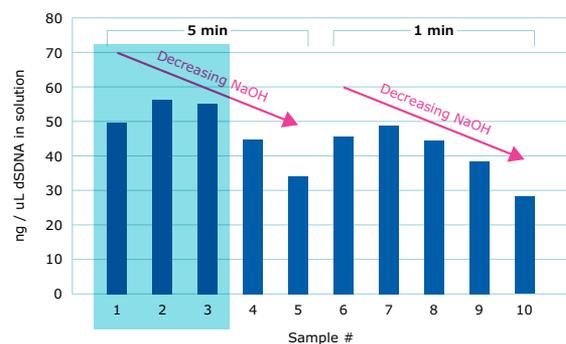
Results

4A 5-minute lysis, 1% SDS, variable NaOH

1. Resuspension
2. 0.15 M NaOH, 1% SDS – 5 min
3. 0.125 M NaOH, 1% SDS – 5 min
4. 0.1 M NaOH, 1% SDS – 5 min
5. 0.075 M NaOH, 1% SDS – 5 min
6. 0.05 M NaOH, 1% SDS – 5 min

4B 0.1M NaOH, 1% SDS, variable time

1. 0 min (negative control)
2. 0.1 M NaOH, 1% SDS, 10 min
3. 0.1 M NaOH, 1% SDS, 60 min
4. Unfiltered control, 0.15 M NaOH, 1% SDS, 5 min
5. Ladder

4C Confirmation by Quant-iT dsDNA assay

1. 0.150 M NaOH, 1% SDS – 5 min
2. 0.125 M NaOH, 1% SDS – 5 min
3. 0.100 M NaOH, 1% SDS – 5 min
4. 0.075 M NaOH, 1% SDS – 5 min
5. 0.050 M NaOH, 1% SDS – 5 min
6. 0.150 M NaOH, 1% SDS – 1 min
7. 0.125 M NaOH, 1% SDS – 1 min
8. 0.100 M NaOH, 1% SDS – 1 min
9. 0.075 M NaOH, 1% SDS – 1 min
10. 0.050 M NaOH, 1% SDS – 1 min

Operating setpoints for lysis

- 5-10 min
- 0.1-0.15 M NaOH
- 1% SDS

Lysis results for different buffer conditions and time points are shown in **Figure 4A** for a constant 5-minute lysis time and variable sodium hydroxide concentrations. The smear at the bottom of the gel indicates RNA impurity, while the bands toward the top of the gel represent pDNA product. As the sodium hydroxide concentration was reduced, there was a decreased intensity of the plasmid DNA in solution, indicating that a higher concentration of sodium hydroxide was preferred for lysis.

The bottom gel (**Figure 4B**) represents 0.1 M NaOH and variable time points. Lane 1 is a negative control with no incubation time, lane 2 is a 10-minute incubation time, and lane 3 is 60 minutes. At the 60-minute time point, there is plasmid DNA and a very intense smear at the bottom of the gel, possibly indicating degradation of plasmid DNA product. In contrast, the 10-minute time point looks ideal.

Orthogonal assays were run using the Quant-iT double-stranded DNA assay to determine the concentration of DNA for the different samples (**Figure 4C**). The 5-minute time point and highest concentration of NaOH give the highest concentration of double-stranded DNA, which confirms the data shown in the gels. **As a result, operating setpoints for the lysis step were determined to be between 5 and 10 minutes, with 0.1 M to 0.15 M sodium hydroxide, and 1% SDS.**

3.3. Clarification

A review of internal data for clarification filtration of post lysis and neutralization feeds showed that filtration capacity varies significantly based on whether the feed is pretreated or untreated.

Feed quality impacts the NFF operation. Our internal database shows two kinds of feed, either pretreated (feed turbidity 20 to <500 NTU) or untreated feed (feed turbidity >1,000 NTU).

The pretreatment condition reported in most studies in our database was gravity separation of floccules and solution; solutions were carefully filtered without disturbing floccules/sediments and a product loss of approximately 20% was reported in floccules.

Another pretreatment method includes use of stainless-steel filter, bag filter, empty column, paper filter, centrifuge, PEI flocculation and centrifugation, and use of Polygard® CR100 µm/Polygard® CR50 µm filters. Capacity of the Polygard® CR filters were in range of 0.55–8 L/inch.

Filters commonly used for pretreated or untreated feed are listed in **Table 5**. Average capacity of the filters is shown in **Figure 5**.

Filter	Media	Pretreated		Untreated	
		Operating flux (LMH)	Avg. capacity range (L/m²)	Operating flux (LMH)	Avg. capacity range (L/m²)
Clarisolve® 60HX filter	Polypropylene	100–150	150–300	100–150	50–300
Clarisolve® 40MS filter	Polypropylene + cellulosic + inorganic filter aid	100–150	190–460	100–150	50–250
Millistak+® D0HC filter	Diatomaceous earth and cellulose	90–150	115–200	90–150	25–100
Millistak+® C0HC filter	Diatomaceous earth and cellulose	100–150	85–300	100–150	30–100
Millistak+® CE20 filter	Cellulose	60–200	100–400	50–100	50–100
Millistak+® CE50 filter	Cellulose	60–200	100–285		
Millistak+® HC Pro-D0SP filter	Polyacrylic + Silica	100–150	100–275	100–150	150–200

Table 5. Recommended filters, conditions and capacity expected capacity ranges.
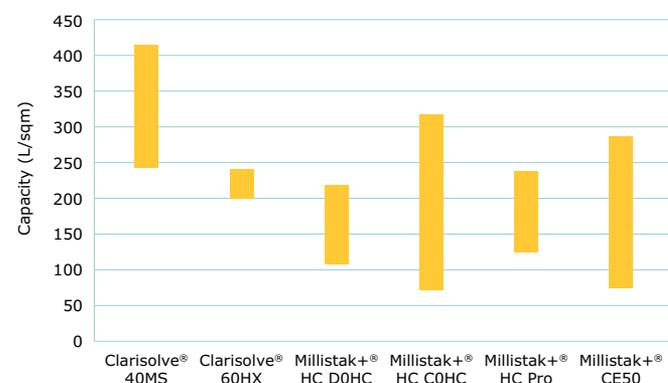
5a. Untreated
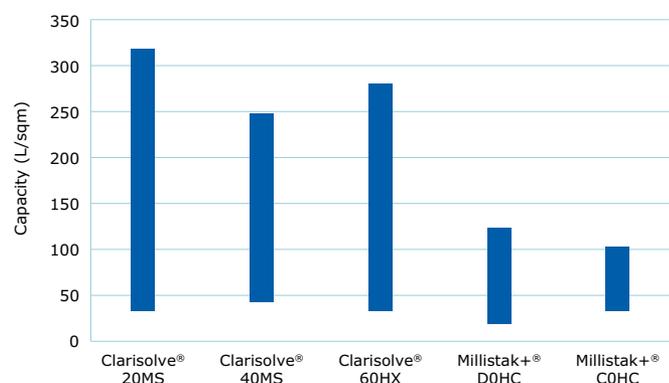
5b. Pretreated

Figure 5a/b. Average capacity range of Clarisolve®, Millistak+® HC, and Millistak+® HC Pro filters for pretreated and untreated feed.

Milligard® PES 1.2/0.45 μm filter can be used as a secondary filter for the Clarisolve® filter. Reported capacity for the Milligard® PES 1.2/0.45 μm filter after Clarisolve® filter is >150 L/m². A secondary filter such as Millistak+® XOHC and Millistak+® HC Pro X0SP filters, can also be evaluated if required but recovery needs to be monitored.

Millistak+® CE 50 filter is generally reported to be used as primary or secondary filter based on feed conditions.

A combination of Millistak+® CE20 or CE30 or CE40 filter as primary filter with Millistak+® CE50 filter as secondary filter can also be evaluated. The reported capacity for Millistak+® CE20 filter is >300 L/m²; Millistak+® CE30 filter is >150 L/m², Millistak+® CE40 filter is >100 L/m²; whereas for Millistak+® CE50 filter reported capacity ranged from 80–320 L/m².

Recovery of >90% is reported with Clarisolve® and Millistak+® filters. Clarification recovery for Millistak+® filters can be increased using a chase with salt containing buffer.

It is observed that the clarification unit operation is run at low flux considering viscosity of feed. Typical operation flow was in range of 60–150 LMH.

Use of Millipore Express® SHC filter has been reported as a bioburden reduction filter after clarification with average capacity range of 400–650 L/m² based on feed quality

4. References

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4. Kong S, et al. Large-scale plasmid DNA processing: evidence that cell harvesting, and storage methods affect yield of supercoiled plasmid DNA. Biotechnol Appl Biochem (2008) 51(1):43–51.

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case study

Clarification

Objective

Lysate clarification using a series of depth and sterile filtration.

Materials and Methods

Following alkaline lysis and subsequent neutralization with 3M potassium acetate, impurity species (genomic DNA and some host cell proteins) flocculate and float to the top of the solution. Plasmid DNA product re-anneals upon neutralization and remains in the bottom layer. The bottom product layer is clarified by depth filtration, followed by sterile filtration. Several options were evaluated for clarifying filtration:

Single stage and dual-stage depth filtration options

- Clarisolve® 60HX – open pore size filter made of low binding polypropylene + cellulose materials
- Millistak+® C0HC – tighter pore size filter made diatomaceous earth, which is positively charged and therefore, can impact the yield of negatively charged pDNA

Sterile filtration

- Millipore Express® SHC – Cast PES membrane, sterilizing grade, single-use

Filter Train 1 consisted of Clarisolve® 60HX, followed by Express® SHC. Filter Train 2 utilized both Clarisolve® 60HX and Millistak® C0HC depth filters, followed by Millipore Express® SHC.

Results

Filter Train 1
- Clarisolve® 60HX
- Millipore Express® SHC

Filter Train 2
- Clarisolve® 60HX
- Millistak+® C0HC
- Millipore Express® SHC

Depth filter sizing data showed that Filter Train 1 resulted in very low pressure drop over the course of this experiment; turbidity breakthrough was observed, however, between 200 and 300 liters per meter squared of loading. For the tighter Filter Train 2, higher pressure was observed, as would be expected from a tighter depth filter, but there was no turbidity breakthrough (**Figure 6A**). The lower turbidity material from Filter Train 2 resulted in a higher sterile filter throughput compared to Filter Train 1, as seen by the flux decay data (**Figure 6B**). Filter Train 1 resulted in much higher yield at 98% versus Filter Train 2 which had 82% product yield. This was due to the diatomaceous earth in the C0HC filter which does have some charge which contributes to product yield loss. **Based on these results, the process incorporated Filter Train 1 to maximize yield for this step.**

6A

Depth Filter Sizing
Constant flow test (Pmax™, Tmax)
Monitor pressure rise & turbidity

Legend: Filter Train 1 Pressure — Filter Train 2 Pressure — Filter Train 1 Turbidity ● Filter Train 2 Turbidity ●
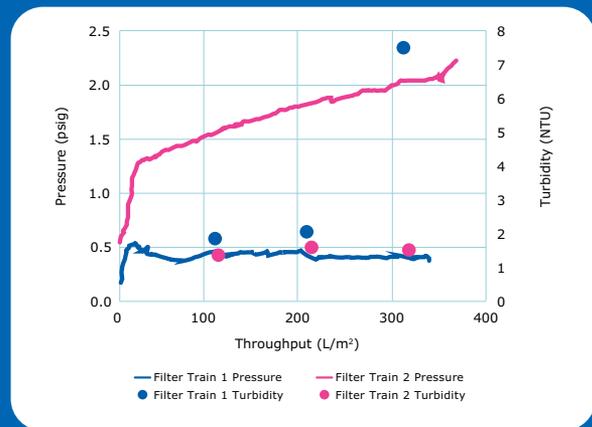
6B

Sterile Filter Sizing
Constant pressure test (Vmax™)
Watch flux decay

Legend: Filter Train 1 Pressure ◆ Filter Train 2 Pressure ▲
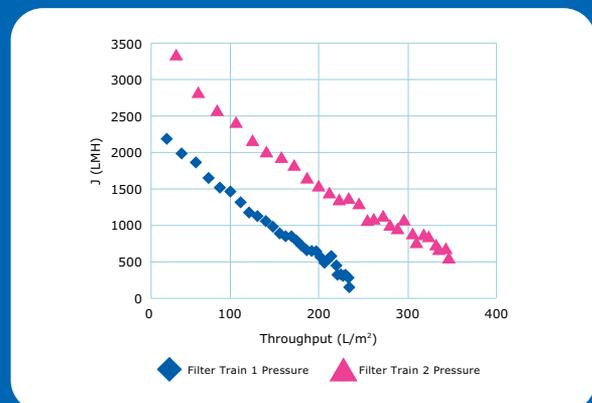
Chromatographic Purification of Plasmid DNA

1. Challenges

Widespread application of pDNA in vaccines and gene therapy is driving increased demand and as a response, plasmid manufacturing must become more efficient with improved productivity. Intensification of chromatographic steps can help address this demand.

The purification of pDNA is difficult because many of the critical impurities are negatively charged, similar in size and possess similar levels of hydrophobicity. The final bulk pDNA must meet quality specifications set by regulatory agencies for purity and must contain greater than 90% pDNA in a supercoiled isoform.

The most commonly used techniques for plasmid purification are anion exchange chromatography (AEC) and hydrophobic interaction chromatography (HIC). Both techniques have been implemented for capture or intermediate purification/polishing and are often combined[1,4]. Size exclusion chromatography (SEC) is sometimes included as part of the downstream scheme; it is typically chosen as the last step due to its disadvantages of low throughput and slow kinetics.[1,3] HIC is able to separate the native supercoiled pDNA from pDNA isoforms, from more hydrophobic nucleic acid impurities (RNA, genomic DNA, denatured pDNA), and from endotoxins.[3] AEC achieves the removal of proteins, low molecular weight RNA, and endotoxins but its efficiency is highly dependent on sample composition. A high salt concentration in the load should be applied to maximize pDNA capture.

For conventional chromatography resins pDNA purification is a challenge because it is much larger than the proteins for which resins were originally designed. pDNA's size prevents it from entering the pore structure of resin beads causing low binding capacity and slow mass transfer. Additionally, the increase in size increases pressure drop and processing times due to feed viscosity, low resolution of isoforms, and potential fouling. Despite the drawbacks most existing large-scale process utilize chromatography resins because of their comparatively high resolution. However, pDNA manufacturers are undertaking an intensification of chromatography steps through the use of convective media (i.e. membranes, monoliths, and fiber based technologies) in an effort to improve productivity and increase overall output.

2. High-throughput capture using anion exchange chromatography

2.1. Natrix® Q chromatography membrane

Natrix® Q chromatography membrane devices provide significant performance improvement compared to traditional resins. Productivity (g/L/hr) increases dramatically because convective channels created by the macroporous polymer structure have a high-density of quaternary amine binding sites with rapid mass transfer. These binding sites are accessible to even very large target molecules, like pDNA, at fast flow rates while maintaining purification targets required for GMP manufacturing.

With a primary capture binding capacity of ~10 mg/mL from a clarified lysate supplemented with NaCl, Natrix® Q chromatography membrane can remove greater than 95% of the initial large RNA. In one example, Natrix® Q chromatography membrane achieved ≥80% pure plasmid DNA, with ~10% residual RNA (A260 based), and a yield of ~80% with only 30 minutes of runtime.

The simple-to-install, scalable, single-use capsule design also reduces set-up time and facility footprint to enable convenient operations and quick implementation in any manufacturing environment. Without the need for extensive packing, validation, or storage, Natrix® device operation is straightforward, meaning easy campaign set-up and changeover. Overall, the productivity increase and operational simplicity make Natrix® Q chromatography membrane a compelling solution for pDNA manufacturers.

3. Recommendations

3.1. Salt supplemented lysate as feed for anion exchange purification

The standard feed used as starting material for purification runs was original *E. coli* lysate[7], clarified by centrifugation and subsequent depth filtration, and directly supplemented with NaCl.

Salt supplementation of the clarified feed prior to anion exchange capture chromatography is a particularly useful approach for removing RNA impurities without requiring RNase. The RNA species bind less strongly to anion exchange media as compared to the more strongly charged pDNA. Therefore, salt supplementation to an optimal conductivity will allow RNA impurities to flow through the AEX adsorbent unbound, while pDNA binds and is subsequently eluted with high purity.

The optimal salt concentration for supplementation must be pre-determined prior to the purification runs for each resin/membrane adsorber. This is done by measuring plasmid binding capacity at increasing sodium chloride concentrations. The principle is demonstrated with the examples of Fractogel® EMD DEAE (M) and Fractogel® EMD DMAE (M) resins in **Figure 7**.
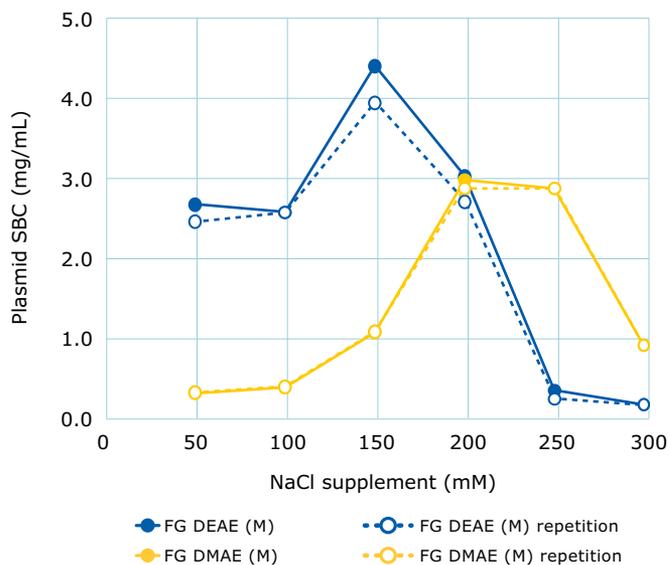

Figure 7. Batch assay for determination of optimal NaCl concentration for lysate supplementation. Static binding capacities (SBC) were measured in 96-well filter plates (1 mL per well). Plasmid feed was original clarified lysate (pH 5.0, 67 mS/cm) supplemented with increasing NaCl concentrations. **FG = Fractogel® EMD resin**.

case study

Anion exchange (AEX) membrane capture chromatography
A. AEX load optimization

Objective: Optimize NaCl concentration in the load material to promote capture of plasmid DNA product while RNA impurities are removed in the load flowthrough.

Materials and Methods

Device - Natrix® Q Micro 0.2 mL Membrane Volume (MV). A new Natrix® Q device was used for each test.

Clarified lysate conditions

- 6.5 kbp pDNA, 24 µg/mL titer. 1.5 M K-acetate buffer, pH 5.3, 86.9 ms/cm
- Nucleic acid content: 3.8% pDNA, 96.2% RNA. Endotoxin content: 380,000 EU/mg pDNA

- The clarified lysate was loaded to 11 mg pDNA/mL of membrane at three different load conductivities. In the first test, no additional salt was added. In the second test, 35 mM NaCl was supplemented into the clarified lysate, and in the third test 75mM NaCl was supplemented into the clarified lysate.

Load conductivity adjustments

Step	Mobile Phase	Membrane Volumes	Flowrate
Equil	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)	50 MV	10 MV/min
Load	Clarified, sterile filtered lysate	11 mg pDNA/mL membrane	10 MV/min
Wash	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)	20 MV	10 MV/min
Elute	100 mM Tris, pH 9 + 1 M NaCl	50 MV	5 MV/min
CIP	1 M NaOH + 2 M NaCl	20 MV	10 MV/min

Test 1 — Native

Test 2 — 35 mM NaCl Addition

Test 3 — 75 mM NaCl Addition
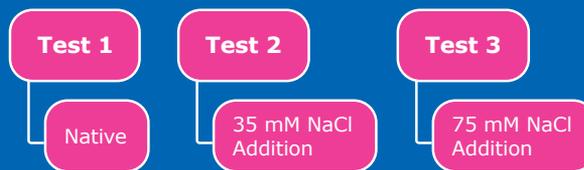
Capture pDNA while impurities (RNA) flowthrough.

Analytics

- DNA and RNA content assessed by HPLC (Tosoh DNA-NPR method)
- Endotoxin content assessed by Charles River Endosafe assay
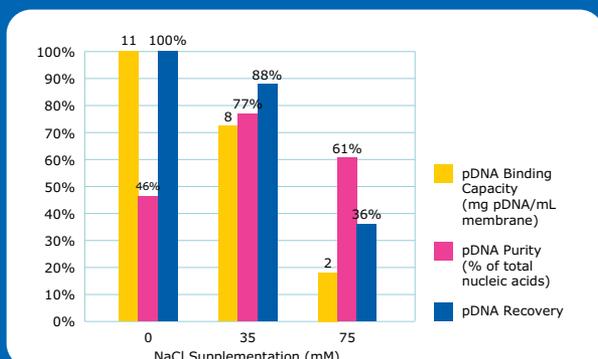
Results

Figure 8. Impact of salt supplementation on capacity, purity, recovery.

A high binding capacity at 11 mg of plasmid per mL of membrane was achieved when no sodium chloride was added into the feed. However, the eluate plasmid DNA purity was quite low for this condition – just 46% of eluate nucleic acid was pDNA, with the remainder being RNA. This indicates that a high percentage of RNA bound to the membrane under these load conditions.

When the salt concentration was increased to 35 mM, there was a slight reduction in binding capacity to 8 mg plasmid per mL of membrane. However, most of the RNA impurity flowed through the membrane at this elevated conductivity, resulting in improved purity (77% of eluate nucleic acid was pDNA). High yield was also observed in the elution pool at 88%. Endotoxin clearance was also achieved with this strategy; the elution pool has 3,100 endotoxin units per mg of plasmid.

When the NaCl concentration was further increased to 75 mM, a significant drop-off in binding capacity was observed. At this elevated concentration neither the RNA nor the plasmid DNA product are able to sufficiently bind to the anion exchange membrane (**Figure 8**). It was therefore determined that lysate supplementation with 35 mM NaCl offered the best balance of capacity, purity, and recovery on Natrix® Q membrane.

B. AEX wash optimization

Objective: Assess the ability of detergent and EDTA wash buffers to further improve Natrix® Q RNA and endotoxin clearance.

Materials and Methods

Device - Natrix® Q Micro 0.2 mL Membrane Volume (MV). A new Natrix® Q device was used for each test.

Feed - Clarified and sterile filtered pDNA lysate, supplemented with 35 mM NaCl

Control Wash

Step	Mobile Phase
Equil	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Load	Clarified, sterile filtered lysate
Wash	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Elute	100 mM Tris, pH 9 + 1 M NaCl
CIP	1 M NaOH + 2 M NaCl

Detergent Wash

Step	Mobile Phase
Equil	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Load	Clarified, sterile filtered lysate
Wash	1 M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Detergent Wash	0.1 M Tris, 10 mM NaCl, + 0.5% detergent, pH 7.5
EDTA Wash	0.1 M Tris, 10 mM NaCl, + 2 mM EDTA, pH 7.5
Elute w/ EDTA	100 mM Tris, 1 M NaCl + 2 mM EDTA, pH 9
CIP	1 M NaOH + 2 M NaCl

Results

	Nucleic Acid Content	Endotoxin Content	Cycle Time
Feed	4% DNA, 96% RNA	380,000 EU/mg	
Control wash (measured from eluate pool)	77% DNA, 23% RNA	3,100 EU/mg	55 min
Detergent wash (measured from eluate pool)	95% DNA, 5% RNA	500 EU/mg	65 min

To optimize the wash strategy for the anion exchange capture step, control wash conditions were compared to an experimental detergent wash strategy where a low concentration of detergent was added to the wash as well as 2 mM EDTA to a wash and elution buffer. This approach improved the nucleic acid purity from 77% to 95% and reduced the endotoxin concentration to 500 endotoxin units per milligram of plasmid.

3.2. Performance overview of anion exchange products

Recommended Process Step	Resin/Membrane Absorber	Dynamic Binding Capacity (mg/mL)	Residence Time, 10 cm BH (min)	CV/min	RNA Removal	Yield ccc-form	Purity (A260 based)
High throughput Capture	Natrix® Q Chromatography Membrane	~10	0.1–0.03	10–33	>95%	≥80%	>80% pDNA
	Eshmuno® Q Resin	~2.5	3–0.3	0.3–3.3	>95%	~75%	>95% pDNA
Intermediate Purification/ Polishing	Fractogel® EMD DEAE resin	~2.5	4–2	0.25–0.5	>95%	≥80%	>95% pDNA
	Fractogel® EMD DMAE resin	~3	4–2	0.25–0.5	>95%	≥95%	>95% pDNA

Table 6. Performance overview of anion exchange resins and membrane adsorber for purification of plasmid DNA.

case study (continued)

C. AEX productivity comparison, resin vs. membrane

Objective: Compare the observed productivity of Natrix® membrane chromatogaphy against historical reference data for a chromatography resin.

Materials and Methods

- **Adsorbers:** Performance of Natrix® Q chromatography membrane compared to historical reference data for Fractogel® DMAE resin data (see to **Table 6** for resin chromatography reference data)

- **Feed basis:** Consider a 300 L batch of clarified lysate containing 7.1g pDNA

Membrane Chromatography

Batch Size	300	L
pDNA mass	7.1	g
Titer	0.024	g/L
Binding Capacity	8	g/L
Minimum Membrane Volume	885	mL
Volume per cycle	442.5	mL
Recommended Device	460	mL
Cycle Load	7.7	g/L
Flow Rate	4.6	L/min
Total Step Time (2 cycles)	2.12	hr
Productivity	**7.26**	**g pDNA/L/hr**

Resin Chromatography

Batch Size	300	L
pDNA mass	7.1	g
Titer	0.024	g/L
Binding Capacity	3	g/L
Minimum Resin Volume	2.36	L
Volume per cycle	150	L
Recommended CV	2.36	L
Cycle Load	3	g/L
Flow Rate	0.59	L/min
Total Step Time (1 cycle)	9.9	hr
Productivity	**0.30**	**g pDNA/L/hr**

Results

Use of Natrix® Q Chromatography Membrane increased productivity of the chromatography step 24x, from 0.30 g pDNA/L/hr to 7.3 g pDNA/L/hr. These gains can be attributed to two factors:

1. The binding capacity of pDNA for the membrane is 8 g/L compared to just 3 g/L for the traditional resin; and

2. Membrane chromatography offers very fast flowrates due to its open pore structure, enabling a cycle time of just over 1 hour while the slower resin process can require nearly 10 hours per cycle. This can be leveraged to run several cycles with membrane chromatography in a rapid cycling approach

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Ultrafiltration/Diafiltration (UF/DF) of Plasmid DNA

4.1 Recommendations

Pellicon® 2 cassettes with Biomax® or Ultracel® membranes and C-screen or V-screen can be used for concentration and diafiltration of pDNA with high loading and yield. The V-screen configuration is specifically recommended for high concentration or high viscosity feed streams.

The selected molecular weight cut-off (MWCO) depends on the pDNA structure and can range from 30 kD to 300 kD. The standard rule of thumb is to use a membrane cutoff that is 3–5 X tighter in pore diameter than the diameter of the product of interest. For common plasmid sizes of 5–20 kbp, 100 kD is often selected.

4.2 Overview

Attributes

Precipitated plasmid is separated, concentrated, washed and then resuspended in the appropriate buffer. This is typically accomplished by using tangential flow filtration (TFF) as this technique is easily scalable, highly selective and cost-effective.

Because the starting concentration of plasmids is generally much lower than that of a typical antibody or recombinant protein feed stream, use of TFF prior to chromatography also functions as a concentration step to further improve downstream purification.

This membrane-based separation and concentration step needs to be optimized to achieve high performance without compromising the plasmid integrity. TFF relies on the size difference between pDNA and contaminants present in the lysate such as linear DNA, RNA and endotoxins. Therefore, the TFF membrane must have an appropriate MWCO to retain the pDNA and allow sieving of contaminants and the initial buffer. In addition to these retention and purification capabilities, TFF should be able to manage the increased viscosity throughout the process step and have a high capacity to enable an acceptable footprint at scale.

Parameters

The performance of a TFF step depends on the feed conditions, MWCO, feed and filtrate/permeate flux and system pressure. The desired plasmid purity, formulation, and concentration specification without product damage can be achieved through optimization of these hydraulic parameters.

Challenges

Due to their structure, plasmids can sometimes pass through pores that are smaller than their apparent molecular weight. This sieving can be more predominant with flux increase. The sieving coefficient also increases at higher ionic strength due to reduction in the effective plasmid size observed in these conditions[1].

Additionally, air introduction needs to be avoided at all times, since air-liquid interphases can impact the integrity of the plasmid.

4.3 Technical Data

Loss of the pDNA in the permeate can potentially be addressed by polarizing the membrane (using full recirculation mode with permeate diverted into the feed tank) prior to starting the TFF run with the permeate line directed to exhaust. This will create a stable polarization layer that will improve the retention.

Additionally, base buffer salt concentration, concentration of pDNA, presence of RNA, transmembrane pressures (TMP) and delta P should be optimized for effective retention of the product. Higher salt concentration has been shown to reduce the plasmid radius[1]. In these conditions, the plasmid structure seems to be more tightly twisted, exhibiting a condensed effective size.

In terms of parameters, a lower TMP is favored. Use of a two-pump, permeate-controlled system is preferred for 100 kD and larger MWCO[2]. Depending on the specific configuration of the membrane used, the step is typically operated at TMP ≤10 psi for a permeate flux of ~20-50 L/m²/h (LMH). The plasmid is usually completely retained at low filtrate flux and sieving can be observed at higher fluxes[3].

The feed flux chosen for the concentration and diafiltration typically ranges between 4 and 6 L/min/m² (LMM) to reduce mechanical stress that can ultimately lead to DNA degradation. High loading in the range of 70 to 140 L/m² can be achieved if these pressure and flux parameters are well optimized with the correct membrane.

As viscosity also increases, particularly at concentrations approaching and exceeding 10 mg/mL, tight screens are not recommended. Coarse C-screen or suspended V-screen TFF device configurations should be applied for medium (5–10 X) to higher concentration (30–50 X) activities; TFF process optimization is required, however.

These typical operating parameters are summarized in **Table 7**.

Parameters	Value
Membrane	100 or 300 kD Biomax® or Ultracel® membranes
Volumetric loading	70–140 L/m²
Feed flux	4–6 L/min/m²
Permeate average flux	20–50 L/m²/h
TMP	≤10 psi
Volumetric concentration factor (VCF)	3–50
Diafiltration volume (DV)	3–10

Table 7. Typical operating parameters for UF/DF of pDNA.

References

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case study

Ultrafiltration/Diafiltration (UF/DF) of plasmid DNA
D. Buffer Exchange

Objective: Buffer exchange using tangential flow filtration (TFF).

Materials

Device - Pellicon® cassette, size 88 cm^2

Membrane - 300 kD Ultracel® (composite regenerated cellulose) membrane; retains ~10 kD DNA while allowing water, ionic and low molecular weight impurities to pass through

Feed Screen was selected for moderate viscosities

Membrane preparation
- Water flush: 20 L/m^2
- Clean-in-place recirculation: 0.2 N NaOH, 20 L/m^2 single pass
- Buffer flush: 20 L/m^2 10 mM Tris, 1 mM EDTA, pH 8 (TE)

Concentration step
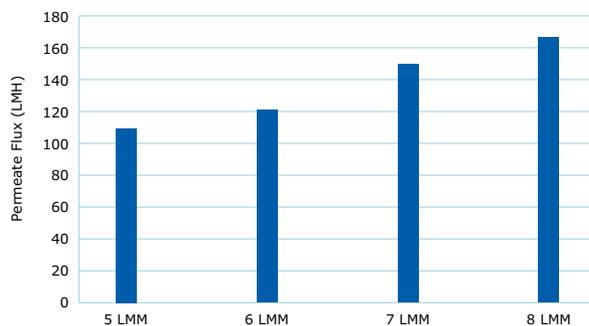- Tank volume reduction with permeate to waste

Diafiltration step
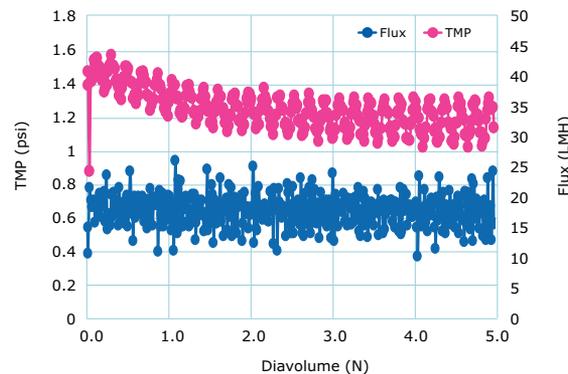- Constant volume diafiltration: add TE buffer at same rate as permeate removal

Recovery step
- Drain tank, then flush retentate line and tank

Results

Critical Flux

Diafiltration

The diafiltration buffer exchange was performed using a 300 kD Ultracel® membrane to retain the pDNA and pass water and low molecular weight impurities. A critical flux experiment at a 4 LMM crossflow was performed to show high 80-125 LMH fluxes were acceptable without fouling. Constant volume diafiltration at 4 LMM for 5 DV into a TE buffer showed declining TMP (no fouling). A retentate flush showed a 96% yield.

Sterilizing Grade Filtration Unit Operations for Plasmid DNA Processes

5.1 Recommendations

Millipore Express® SHC can be used to achieve high filtration capacity, flux, and yield for Plasmid DNA (pDNA) filtration in a variety of formats including pre-sterile capsules with sizes ranging from 0.014 m^2 to 3.0 m^2. Capacity and yield of the unit operation can vary significantly, especially with larger plasmids (~10 kbp and greater), and as such, process development should be carefully considered for optimization of the step.

5.2 Overview

Attributes

Sterilizing Grade Filtration unit operations for pDNA processes should include:

- A membrane with the ability to remove bioburden from the feed stream
- A device and filtration system that can prevent the introduction of bioburden
- A membrane that can reduce particulates, provides high capacity and high flux, and allows pDNA to flow through

Parameters

A Sterilizing Grade Filtration unit operation for pDNA processing can be optimized by changing the:

- Membrane used for sterile filtration
- Filtration device and system used
- Driving force (flowrate or pressure)
- Formulation of the pDNA solution
- Purity of the pDNA solution
- Conformation of the pDNA (supercoiled, linear, open-circular, etc.)
- Endpoint of the filtration

Key Considerations

The large size of pDNA can present a challenge for sterile filtration unit operations, as the product can be retained by the filters, leading to yield loss and low filtration capacity. Viscosity must also be considered as flow rates for sterile filtration steps can be low due to viscous material. Finally, a sterile filter must be proven to retain bacteria, which can be problematic for pDNA vaccines containing adjuvants. Key considerations are summarized in **Table 8**.

Attributes	Parameters	Parameters
Sterility assurance	Membrane pore size	Large size of pDNA
Particulate reduction	Membrane chemistry	
Filtration capacity and flux	Driving force	Viscosity of pDNA solution
pDNA yield	Formulation	Bacterial retention for adjuvanted pDNA solutions
	Filtration endpoint	

Table 8. Key considerations for sterile filtration of pDNA solutions.

5.3 Technical Data

Process parameters should be optimized to achieve highest sterilizing grade filtration performance. While some plasmids present unique filtration challenges, for many smaller plasmids of less than 10 kbp, development of a robust unit operation could be as simple as confirming filter sizing using Vmax™ or Pmax™ methodology.[1]

A review of internal data for sterilizing grade filtration of pDNA feeds showed that filtration capacity, flux and yield can vary significantly, depending on the size of the plasmid, with larger plasmids presenting the greatest filtration challenge. Other researchers have also shown that filtration performance declines as plasmid size increases. The most significant filtration challenge occurs with pDNA of 20 kbp and larger – although 10–20 kbp pDNA often also cause filtration issues[2,3]. **Table 9** summarizes the review of internal data and published studies.

Plasmid DNA Size (kbp)	Expected Sterilizing Grade Filtration Yield (%)	Expected Sterilizing Grade Filtration Capacity (L/m²)
<10	>90	>50
10–20	>80	Variable
>20	<80	<20

Table 9. Expected performance for sterilizing grade filtration of purified pDNA based on internal studies and literature search.

While the size of pDNA impacts sterilizing grade filtration performance, internal data and published studies both show that buffer composition can alter the plasmid conformation and subsequent radius of gyration. Specifically, salt concentrations have been shown to directly impact both the radius of gyration and diffusion coefficient of pDNA (**Table 10**).[4,5,6]

NaCl Concentration[a] (mM)	RS[b] (nM)	D[c] (m²/s)
10	6.9	4.0×10^{-12}
40	5.8	5.2×10^{-12}
100–300	4.5	5.5×10^{-12}

Table 10. Plasmid DNA properties.

[a] In TE buffer. [b] From Hammermann et al. (1998) for 2.69 kbp plasmid. [c] From Nguyen and Elimelech (2007) for 3.0 kbp plasmid with values adjusted to account for TE species in buffer solution (refer to text for details).

Changing the salt concentration has empirically demonstrated a greater than 2× increase in sterilizing grade filtration capacity and yield in internal studies and published studies[3].

Using membranes for ultrafiltration, a study demonstrated a significant change in the sieving of pDNA with a change in salt concentration, providing further evidence that salt concentration heavily influences membrane filtration of pDNA[7].

In addition to impact of pDNA size, studies have shown that supercoiled plasmid gives better filtration performance than open-circular; the purity of supercoiled pDNA can thus significantly impact unit operation outcomes of a sterilizing grade filtration step. One study cited an increase of approximately 10× in filtration capacity going from 90% to 95% supercoiled content.[2]

The filtration endpoint has been found to be significant in internal studies. Under constant pressure, plasmid concentration in the filtrate decreases at high flux decay, while constant flowrate operation has shown yield decline when pressure drop increases above a threshold. While both findings suggest that plasmid yield correlates with membrane fouling, detailed studies are needed to investigate the mechanism of action.

Both PVDF and PES membranes have shown success in filtering pDNA solutions. PES is preferred as it tends to have both higher capacity and flux versus PVDF and can be less damaging to larger plasmids[3]. Internal studies have shown higher yield for PES filters, although more detailed studies are needed to confirm this finding.

Data from internal and published studies suggest that altering the pDNA concentration can affect yield and capacity. Some published data have shown increased mass throughput with increased pDNA concentration.[2] Internal data suggest; however, this may not always be true; increased concentration may cause some self-association of pDNA molecules depending on the background buffer and purity, resulting in lower filtration capacity and yield. While concentration of pDNA is certainly a critical operating parameter, specific approaches for optimizing performance via dilution or concentration need to be better defined.

A review of sterilizing grade filtration operation conditions showed that feed flux or pressure has little to no impact on filtration capacity or yield (**Table 11**). It is possible, however, that high driving force could compromise plasmid integrity due to mechanical stress, especially for larger plasmids[3].

Optimization Parameter	Yield	Capacity	Product Integrity
Salt concentration	X	X	
Supercoiled pDNA content (purity)	X	X	
Filtration endpoint	X		
Membrane type – PVDF or PES	X – PES	X – PES	
pDNA concentration	X	X	
Feed flux or pressure			X

Table 11. Critical parameters for optimizing plasmid DNA sterilizing grade filtration unit operations.

After a thorough review of published and internal data, critical parameters have been defined and can be applied to process development activities. Critical quality attributes of yield, capacity, and product integrity can be optimized through various parameters.

- Yield can be optimized by increasing salt concentration, increasing pDNA purity, defining the filtration endpoint to avoid extreme fouling, screening membranes, and exploring various pDNA concentrations.

- Capacity can be optimized through increasing salt concentration, increasing pDNA purity, or testing different pDNA concentrations.

- Product integrity through sterilizing grade filtration can be impacted by membrane type and feed flux or pressure.

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Conclusion

pDNA is a new, rapidly growing therapeutic modality for use in viral vector manufacturing, mRNA manufacturing, and *in vivo* gene therapy. Its recent application in vaccines has been enabled by encapsulation formulation technology that prevents enzymatic degradation and increases its half-life.

In this guide, the feasibility of a lab-scale pDNA manufacturing process was demonstrated. This process includes MF-TFF, alkaline lysis, depth filter clarification, membrane chromatography, and ultrafiltration formulation.

Incorporation of single use processing provides the foundation for robust and easy scale up. Manufacturing implementation at larger scales will also require studies to verify performance of workflow operations including lysis, mixing and flocculation, and membrane module manifolding. As needed, additional purification steps may be investigated to enhance performance (purity, robustness, cost, yield, etc.) using coarse flocculate filtration, $CaCl_2$ addition, isoform separation, and final product concentration

Acknowledgements

Learn more
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