Scalable Ultrafiltration-Diafiltration Process of Clarified Plasmid DNA (pDNA) Using Pall's T-Series Cassettes with Omega™ Membrane

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INTRODUCTION

Plasmid DNA (pDNA) manufacturing has its own unique challenges to generate high yield downstream processes and products with high titer and purity. The design of a suitable separation and concentration strategy depends on many variables including the nature of the pDNA and its interaction with the filtration module. The objective of these development studies was to assure an ultrafiltration / diafiltration (UF/DF) processing strategy that minimizes product loss at the TFF step following clarification. As a result of the characteristics of the pDNA product, more retentive UF membranes than traditionally recommended were required to achieve high pDNA yield over the post-clarification step. Figures 1 and 2 outline a generic pDNA upstream and downstream process along with technical solutions from the Cytiva and Pall portfolio.

Figure 1
Plasmid DNA upstream process

Figure 2
Plasmid DNA downstream process

♦ *Products from Cytiva, a fellow Danaher Operating Company*

PLASMIDS

Plasmids are small dexyribonucleic acid (DNA) molecules that are physically separated from chromosomal DNA. They are small, circular, double-stranded DNA molecules that replicate independently in bacteria.

- pDNA is an important genetic engineering tool used to clone, amplify, or express genes in genetic and biotechnology labs.
- Good manufacturing practices (GMP)-grade pDNA are used for *ex vivo* applications, DNA vaccines, gene therapy applications, recombinant proteins production, mRNA, and viral vector manufacturing.

ACKNOWLEDGMENTS

- *Pall Corporation: Jon Petrone, Senior Director, Global Technical Consultancy*
- *Akron Bio: Sebastian Almeida, Senior Director of Manufacturing and CMC, Biologics*

Pall

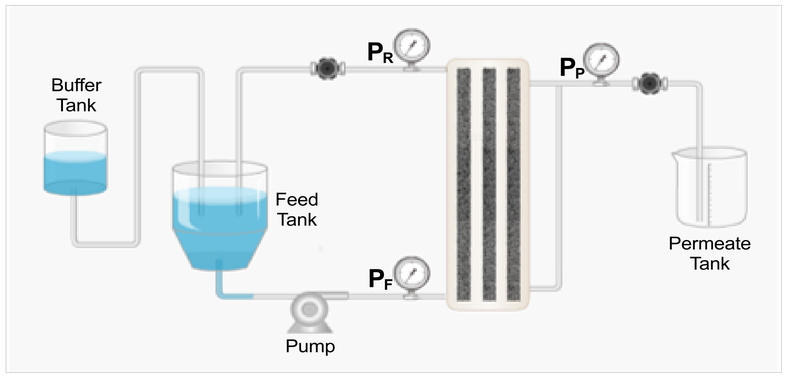
Akron Bio

PLASMIDS: Materials and Methods

Tangential Flow Filtration (TFF)

TFF is a method used to concentrate and buffer exchange biomolecules. Processes for concentration and desalting of pDNA solutions have operating volumes in the range of 10 mL to thousands of liters. These studies describe one of many challenges and the corresponding process solution while using TFF for pDNA process development applications.

Figure 3
Flow path through a TFF system



Fluid is pumped from the feed tank into the feed port of the TFF cassette, across the membrane surface (crossflow), out the retentate port, and returned into the feed tank. The crossflow sweeps the membrane surface to reduce the amount of larger molecules and aggregates that are retained at the membrane surface. Transmembrane pressure (TMP):

$$TMP = (P_{FEED} + P_{RETENTATE}) /2 - P_{PERMEATE}$$

is the force that drives liquid through the membrane. Liquid that flows through the membrane (permeate) carries molecules smaller than the membrane pores through the filter. For the diafiltration (DF) step, a buffer tank is connected to the feed tank and DF buffer is added to the feed tank at the same rate the permeate is removed, resulting in a constant volume operation. The retentate is recirculated to the feed tank and the permeate is collected in the permeate tank.

PLASMID DNA PURIFICATION CHALLENGES

The production of pDNA products requires many purification steps designed to create a pDNA product that is effective, meets regulatory guidelines, and meets internal quality standards.

Materials and Methods

Nucleic acid molecules in solution have unique biochemical properties. Table 1 provides a guide to select the appropriate nominal molecular weight cut-off (MWCO) for DNA and RNA applications.

Table 1
Selection guide for nucleic acid applications

MWCO (kDa)	Base Pairs (Bp)	Bases (Bs)
1	5 – 16	9 – 32
3	16 – 32	32 – 65
5	25 – 50	50 – 95
10	50 – 145	95 – 285
30	145 – 285	285 – 570
50	240 – 475	475 – 950
100	475 – 1450	950 – 2900
300	1450 – 2900	2900 – 5700

Post-Clarification TFF Studies

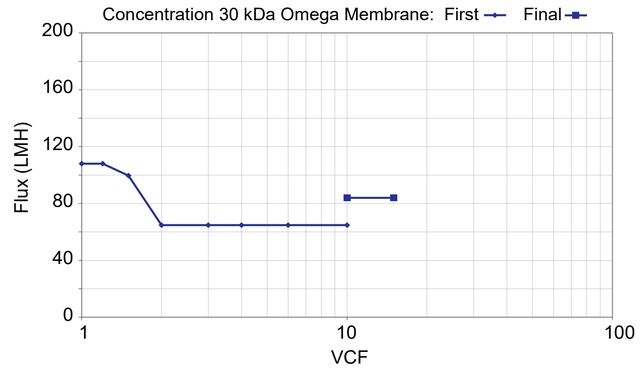
According to Table 1, a membrane with a 300 kDa MWCO is recommended to process a pDNA with 1450–2900 Bp. This TFF study was performed using an Akron Bio pDNA product with ~5000 Bp. With the objective of achieving high recovery of this pDNA, membranes with MWCOs of 100 and 30 kDa were evaluated.

RESULTS AND CONCLUSIONS

Permeate Flux as a Function of the Volume Concentration Factor (VCF)

The concentration of the pDNA increased in direct proportion to the decrease in the feed solution volume. During the diafiltration process, the pDNA was washed to remove media components. The step is described in Figure 4.

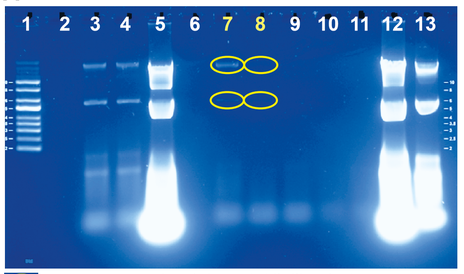
Figure 4
Permeate flux as a function of volume concentration factor (30 kDa)



The initial concentration step was performed at a TMP of 12.5 psig (0.86 barg) for the cassette with Omega 30 kDa membrane. The initial permeate flux was 108 liters/m²/hour (LMH). The flux decreased by ~40% to 65 LMH as the concentration reached a VCF of ~2X. The permeate flux then remained constant at 65 LMH until the desired VCF of 10X was achieved. Following the diafiltration, an overconcentration to a VCF of ~15X was obtained. The product was recovered, and the product concentration was confirmed with no presence of pDNA in the permeate. The average permeate fluxes during the first and final concentration steps for the 30 kDa membrane were 80 LMH and 85 LMH, respectively.

Figure 5
Annotated gels description and takeaways

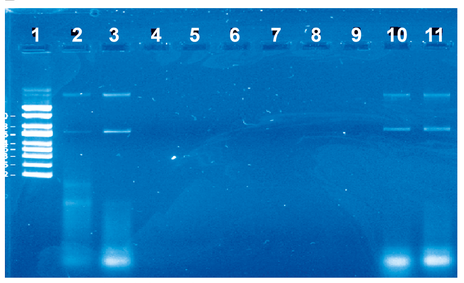
A



pDNA permeate breakthrough (diafiltration)

Lane 1 : Supercoiled (SC) ladder
Lane 2 : (Empty)
Lane 3 : Post-depth filtration (3125 mL)
Lane 4 : Post-0.2 µm filtration (4000 mL)
Lane 5 : Post-UF retentate (250 mL)
Lane 6 : Post-UF permeate (3750 mL)
Lane 7 : Post-DF #1 permeate (400 mL)
Lane 8 : Post-DF #2 permeate (400 mL)
Lane 9 : Post-DF #3 permeate (400 mL)
Lane 10: Post-DF #4 permeate (400 mL)
Lane 11 : Post-DF #5 permeate (400 mL)
Lane 12 : Post-recirculation (pre-flush, 250 mL)
Lane 13 : Recovery flush (152 mL)

B



No permeate breakthrough

Lane 1 : Supercoiled (SC) ladder
Lane 2 : Neutralized lysate
Lane 3 : Post-clarification (3000 mL)
Lane 4 : Post-UF permeate (2700 mL)
Lane 5 : Post-DF #1 permeate (300 mL)
Lane 6 : Post-DF #2 permeate (300 mL)
Lane 7 : Post-DF #3 permeate (300 mL)
Lane 8 : Post-DF #4 permeate (300 mL)
Lane 9 : Post-DF #5 permeate (300 mL)
Lane 10: Final feed / retentate (195 mL)
Lane 11 : Post-recovery flush (100 mL)

The use of Omega membrane with a 100 kDa MWCO resulted in permeate breakthrough during diafiltration (Figure 5A, lanes 7 and 8). However, no pDNA bands were observed in permeate while testing with the Omega membrane with a 30 kDa MWCO. The pDNA concentration corresponded with the volumetric concentration factor. Refer to Figure 5B for details.

Pall Centramate™ and Centrasette™ cassettes with Omega 30 kDa polyethersulfone (PES) membrane are an effective technology for the concentration and diafiltration of pDNA (~5 kbp) when buffer characteristics promote plasmid compaction.

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