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Tech Note

Tangential Flow Filtration (UF/DF) of Plasmid DNA

Recommendations

Pellicon® 2 cassette with Biomax® 100 kDa C-screen/ V-screen can be used for concentration and diafiltration with high loading and yield. The V-screen configuration is recommended for high concentration or high viscosity feed streams.

Table 1. Recommended filter options for UF/DF step.

Options	UF/DF Membrane
Option 1	Pellicon® 2 cassette with Biomax® 100 kDa C-screen
Option 2 (high concentration/viscosity)	Pellicon® 2 cassette with Biomax® 100 kDa V-screen

Overview

Attributes

Precipitated plasmid is separated, concentrated, washed and then re-suspended in the appropriate buffer. This is typically accomplished 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 molecular weight cut-off (MWCO) to retain this 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, the DNA can be shear-sensitive and tends to increase with plasmid size[2]. The result can be degradation and reduction of the overall yield.

Technical Data

The selected molecular weight cutoff (MWCO) depends on the pDNA structure and can range from 30 kDa to 300 kDa. 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.

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

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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 kDa and larger MWCO[3]. 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 LMH. The plasmid is usually completely retained at low filtrate flux and sieving can be observed at higher fluxes[4].

The feed flux chosen for the concentration and diafiltration typically ranges between 4 and 6 LMM to reduce shear 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) and open channel or 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.

Table 2. Operating parameters for MF-TFF.

Parameters	Value
Device	Pellicon® 2 with Biomax® 100 kDa C-screen
Volumetric loading	70–140 L/m²
Feed flux	4–6 LMM
Permeate average flux	20–50 LMH
TMP	≤10 psi
Volumetric concentration factor (VCF)	3–50 (V-screen for high concentration)
Diafiltration volume (DF)	3–10

References

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