Chromatographic purification of plasmid DNA

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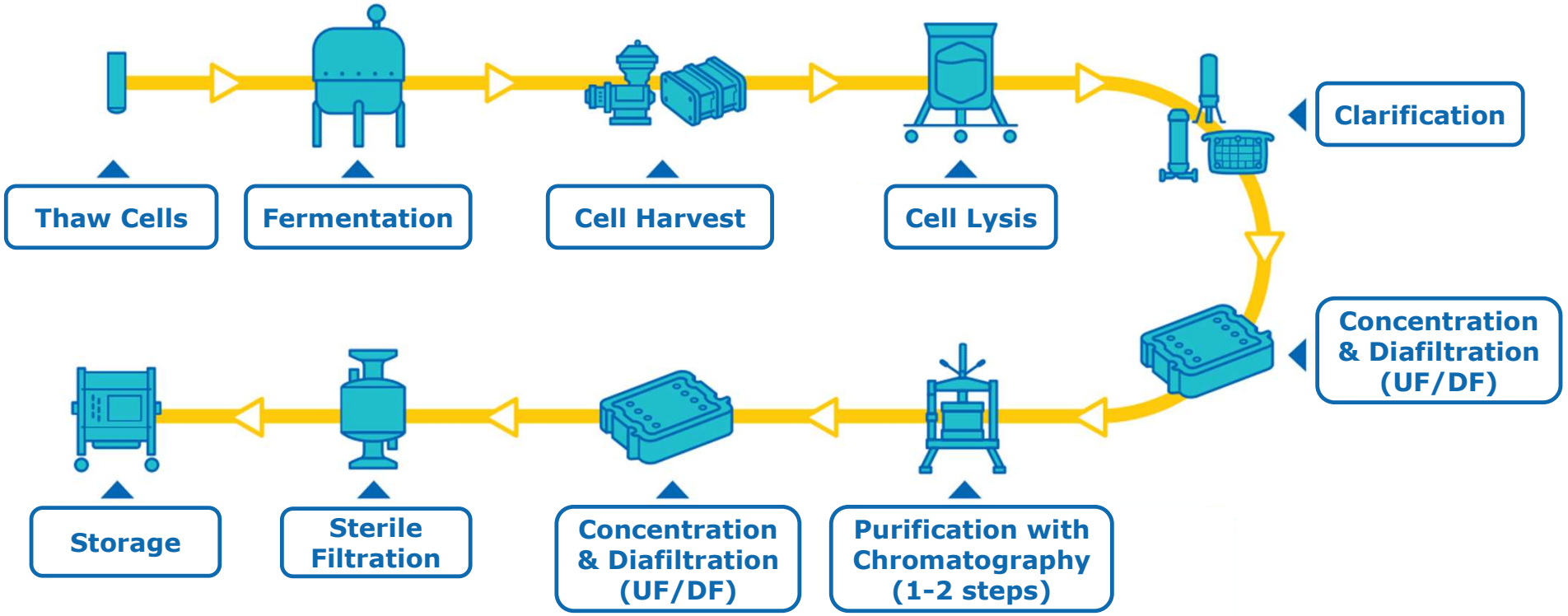
pDNA purification challenges & considerations

Typical pDNA process flow



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pDNA purification challenges & considerations

Unique challenges of pDNA purification

- Similarity of product and contaminants (genomic DNA (gDNA), endotoxin, RNA, plasmid isoforms) leads to **low resolution separation**.
- Feed often **highly viscous**, complicating downstream processing.
- **Shear sensitivity**
- Lack of platform process and integrated solutions

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pDNA purification challenges & considerations

Chromatography - Common approaches

- **Goal:** Separate supercoiled (ccc) plasmid from oc-/linear isoforms and residual impurities (HCP, nucleic acid, endotoxin) by charge, size or hydrophobicity
- Combination of **Anion exchange** and **Hydrophobic interaction**

1 Anion Exchange Chromatography (AEX)

- Applicable for **capture, intermediate and polishing**
- **Weak AEX resins** give highest recovery and selective impurity removal
- **Separate plasmid from proteins, RNA and gDNA and removing endotoxin**
- Separation of plasmid isoforms difficult

2 Hydrophobic Interaction Chromatography (HIC)

- Works by salt promoted binding (≈ 2.5 M NH_4SO_4)
- **Separate isoforms**: Supercoiled pDNA is less hydrophobic than RNA, oc- and linear- plasmid forms and denatured gDNA

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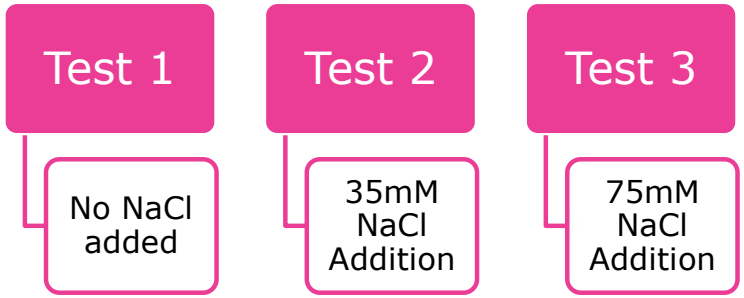
AEX Capture Chromatography - Test Overview and Methods

Clarified lysate conditions:

- 6.5 kbp pDNA, 24 µg/mL titer. 1.5M 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

Step	Mobile Phase	Membrane Volumes	Flowrate
Equil	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)	50 MV	10 MV/min
Load	Clarified, sterile filtered lysate pH 5.2	11 mg pDNA/mL membrane	10 MV/min
Wash	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)	20 MV	10 MV/min
Elute	100 mM Tris, pH 9 + 1M NaCl	50 MV	5 MV/min
CIP	1M NaOH + 2 M NaCl	20 MV	10 MV/min

Impact of NaCl supplementation on RNA clearance



Capture pDNA while impurities (RNA) flowthrough

Analytics:

- DNA and RNA content assessed by HPLC (Tosoh DNA-NPR method)[1]
- Endotoxin content assessed by Charles River Endosafe assay

[1] Urthaler 2005

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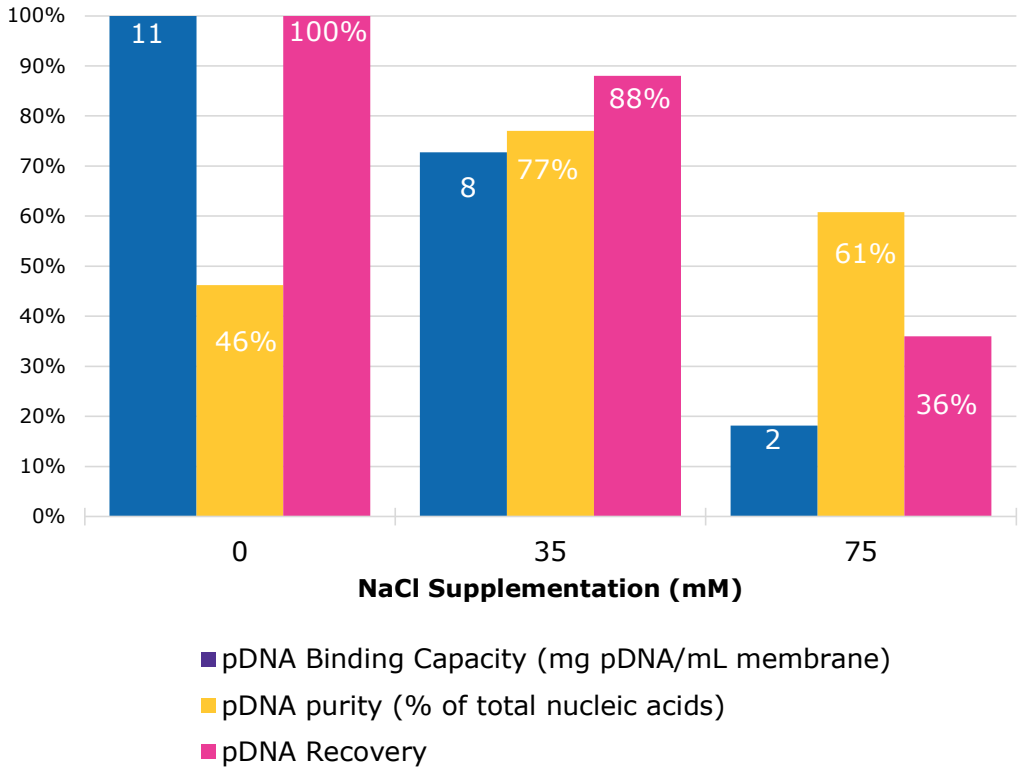
AEX Capture Chromatography - Results

Impact of Salt Supplementation on Capacity, Purity, Recovery



35 mM NaCl supplementation offers best balance of capacity, purity, recovery:

- Capacity = 8 mg pDNA/mL membrane
- Nucleic acid purity = 77% pDNA
- pDNA recovery = 88%
- Endotoxin content = 3,100 EU/mg pDNA

AEX Capture Chromatography- Wash Strategy

Control Wash

Step	Mobile Phase
Equilibration	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Load	Clarified, sterile filtered lysate pH 5.2 + 35mM NaCl
Wash	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Elute	100 mM Tris, pH 9 + 1M NaCl
CIP	1M NaOH + 2 M NaCl

Detergent Wash

Step	Mobile Phase
Equilibration	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Load	Clarified, sterile filtered lysate pH 5.2 + 35mM NaCl
Wash	1M K-Acetate + 150 mM NaCl, pH 5.0 (75 mS/cm)
Detergent Wash	0.1M Tris, 10mM NaCl, + **0.5% detergent**, pH 7.5
EDTA Wash	0.1M Tris, 10mM NaCl, + **2mM EDTA**, pH 7.5
Elute w/EDTA	100 mM Tris, 1M NaCl + **2mM EDTA**, pH 9
CIP	1M NaOH + 2 M NaCl

Results

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

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AEX Capture Chromatography- Membrane Vs Resin

100 L batch of clarified lysate, 3.6 g pDNA

Membrane Chromatography				Resin Chromatography		
Binding Capacity	8	g/L	⟷	Binding Capacity	3	g/L
Membrane Volume	0.46	L		Resin Volume	1.18	L
Flow Rate	4.6	LPM		Flow Rate	0.3	LPM
Step Time	1.1	hr	⟷	Step Time	9.9	hr
Cycles	1	cycle		Cycles	1	cycle
Productivity	**7.3**	**g pDNA/L/hr**		**Productivity**	**0.3**	**g pDNA/L/hr**

Natrix® Q membrane is 24x more productive than resin

Thank You!