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Improved endotoxin removal using ecofriendly detergents

Intensified plasmid DNA capture

Juan Amor

DCVMN International 2024

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Agenda

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1- Plasmid Production overview

2- High productivity direct plasmid capture step

3- Endotoxin clearance strategy for plasmid capture

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Plasmid Production Overview

01

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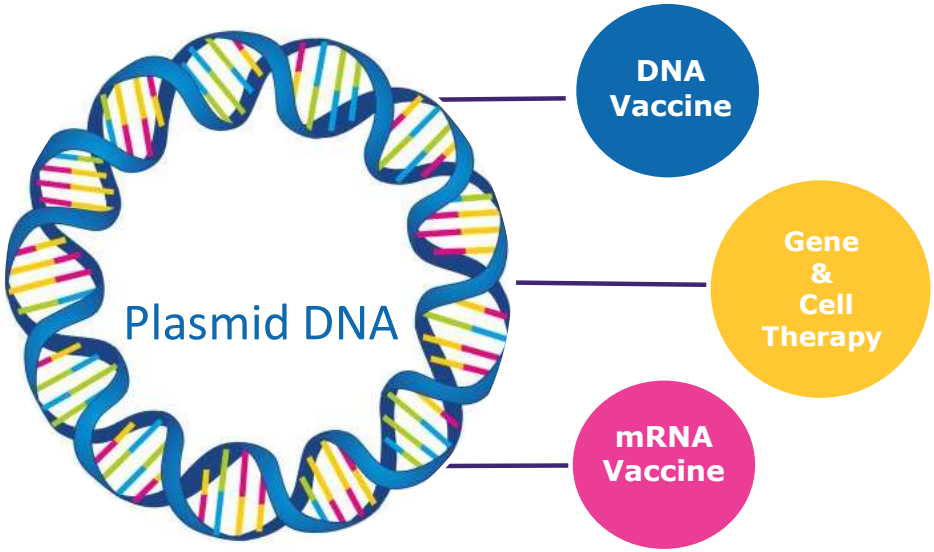
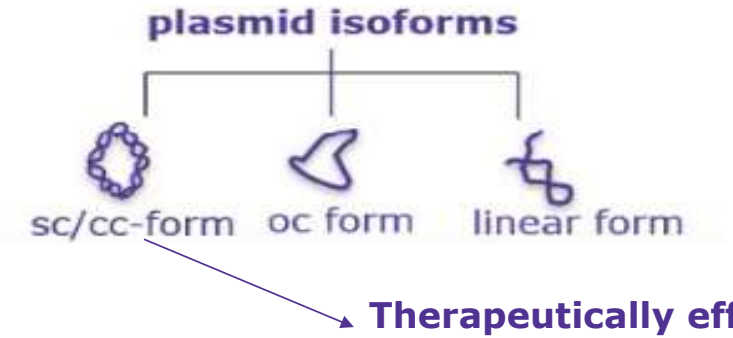
Background
What is plasmid and why do we care?



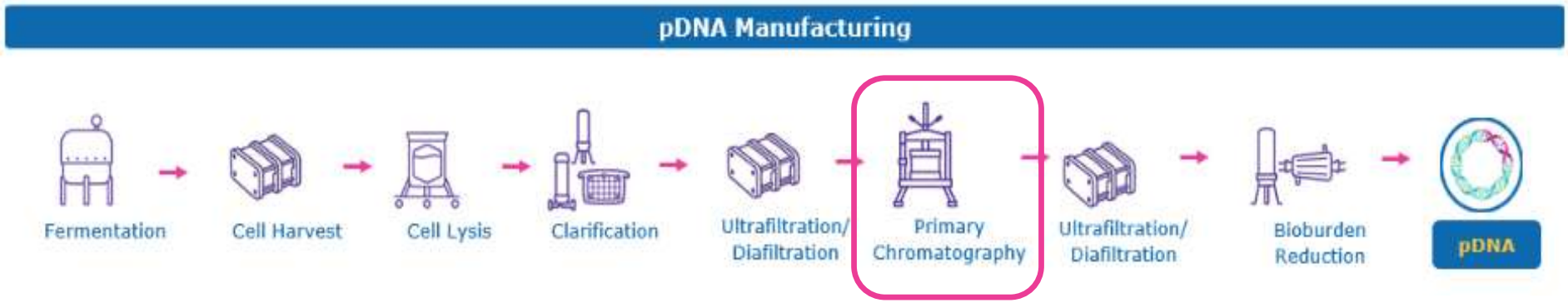
Characteristic

- **Extrachromosomal circular DNA**
- Commonly in **bacterial organisms**
- Sensitive to mechanical stress



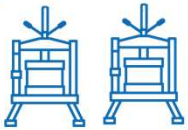
Solutions to improve plasmid production are needed!

Process Overview
Plasmid Manufacturing



Chromatography is a current bottleneck

Purification
Capture, Polishing



1-2 Chromatography steps

AEX -> HIC
HIC -> AEX

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High productivity direct plasmid capture step

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Natrix® Q Chromatography Membrane in Single-use Format
Improved performance enabling high-throughput capture

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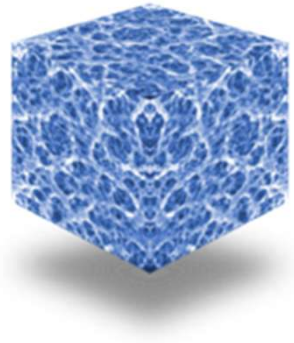
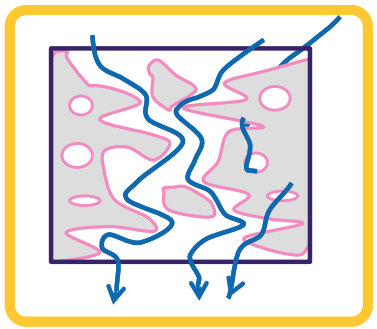
Material Properties

- Composite membrane, high mechanical strength
- Macroporous hydrogel, nominal pore size: 0.4 µm
- Large accessible surface for plasmids and other macromolecules





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Plasmid DNA Case Study

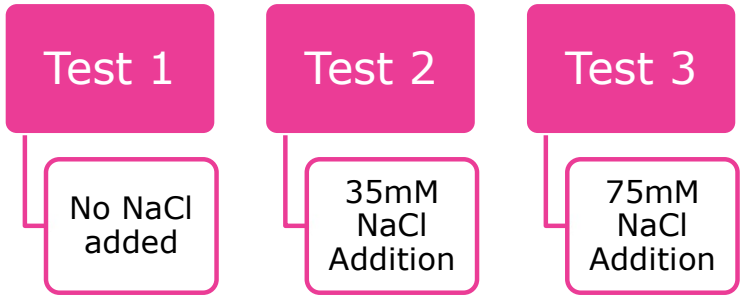
AEX Capture Chromatography - Test Overview and Methods

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:

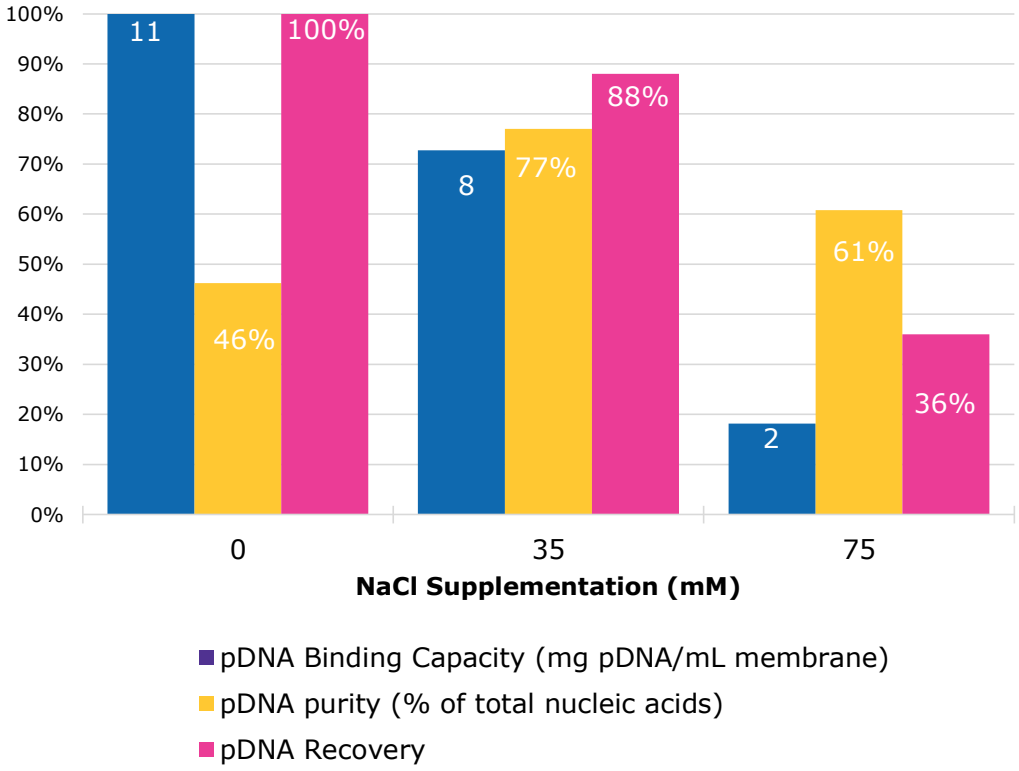
- DNA and RNA content assessed by HPLC (Tosoh DNA-NPR method)[1]
- Endotoxin content assessed by Charles River Endosafe assay

[1] Urthaler 2005

Plasmid DNA Case Study

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

? Can RNA and endotoxin purity be further improved with an alternative wash strategy?

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Endotoxin clearance strategy for plasmid capture

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Endotoxin
Molecule characteristics

- **Structure**
- Unique group of Lipopolysaccharides (LPS)
- Occur naturally in the cell wall of Gram-negative bacteria
- **Physico-Chemical Properties**
- Amphiphilic character: negatively charged core region <-> hydrophobic lipid A part
- Self-aggregating properties; formation of supramolecular structures (micells)

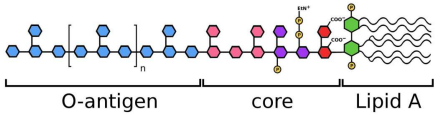

Fig 1. LPS molecular structure[1]

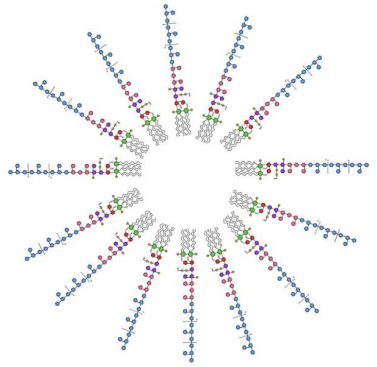

Fig 2. Micellar LPS structure

[1] Peri F, Pure Appl. Chem. 2012; 84(1): 97–106

Due to their pyrogenic effects, endotoxins are a critical impurity to remove from therapeutics.

Detergent Use in Bioprocessing
Solution to improve endotoxin clearance

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- Some plasmid feeds may result in high endotoxin content in the AEX eluate
  - Dependent on cell culture characteristics, lysis conditions, AEX material, etc.
- **Triton™ X-100** has been used by the biopharmaceutical industry for effective endotoxin clearance
- **Triton™ X-100** has a broad range of uses:
  - Virus inactivation for blood-derived therapeutics, recombinant proteins
  - Cell lysis in viral vector production
  - Protein solubilization

However:

- As of January 2021, **TRITON™ X-100** has been prohibited in the EU unless authorization is granted
- This decision affects European manufacturers and downstream users

There is a need to identify eco-friendly TRITON™ X-100 alternatives

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Detergents Use in Bioprocessing

Considerations when choosing a detergent

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Process Requirements

- Effective endotoxin removal
- Does not adversely affect yield or quality of the product
- Low or no interference with product related analytical assays
- Does not interfere with subsequent purification
- Can be sufficiently removed from the product by the capture and subsequent DSP steps

Material Attributes

- Environmentally compatible
- High quality, purity
- Low toxicity potential

User Requirements

- Supply availability
- Suitability for different applications

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Plasmid DNA Case Study

AEX Capture Chromatography- Wash Strategy

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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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Plasmid DNA Case Study

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

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Conclusions

1 **Intensified plasmid capture solution: Membrane adsorber and optimal salt supplementation approach offers 3x plasmid binding capacity at 10x higher throughput.**

2 **Identified eco-friendly detergent for endotoxin removal that meet process, material, and user requirements**

3 **Identified eco-friendly detergent protocol for robust and high endotoxin clearance**

- Integration into wash buffer preferred
- Low residual detergent levels
- No impact on plasmid binding capacity, yield, and purity
- **Deviron™ C16** detergent: clearance >500x more than without detergent

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Thank you