

# Manufacturing pDNA for Therapeutic Use

## Case Study: West Nile Virus Vaccine

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**&**

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## Challenges in Manufacturing pDNA

**Yield: Biomass is inherently poor**

**Maintaining stability at high OD growth**

**Volume Vs. Efficiency Vs. Viscosity**

**Volume reduction/liquid handling**

**Lack of suitable chromatographic media types (changing)**

**RNA reduction without RNase A creates a greater process burden**

**Progression toward GRAS agents can limit use of some methods/facilities**



## **Manufacture of Research Grade Material**

**Use of RNase A greatly facilitates production (standards changing)**

**Flexibility in methods**

**Release specifications still quite liberal (standards changing)**

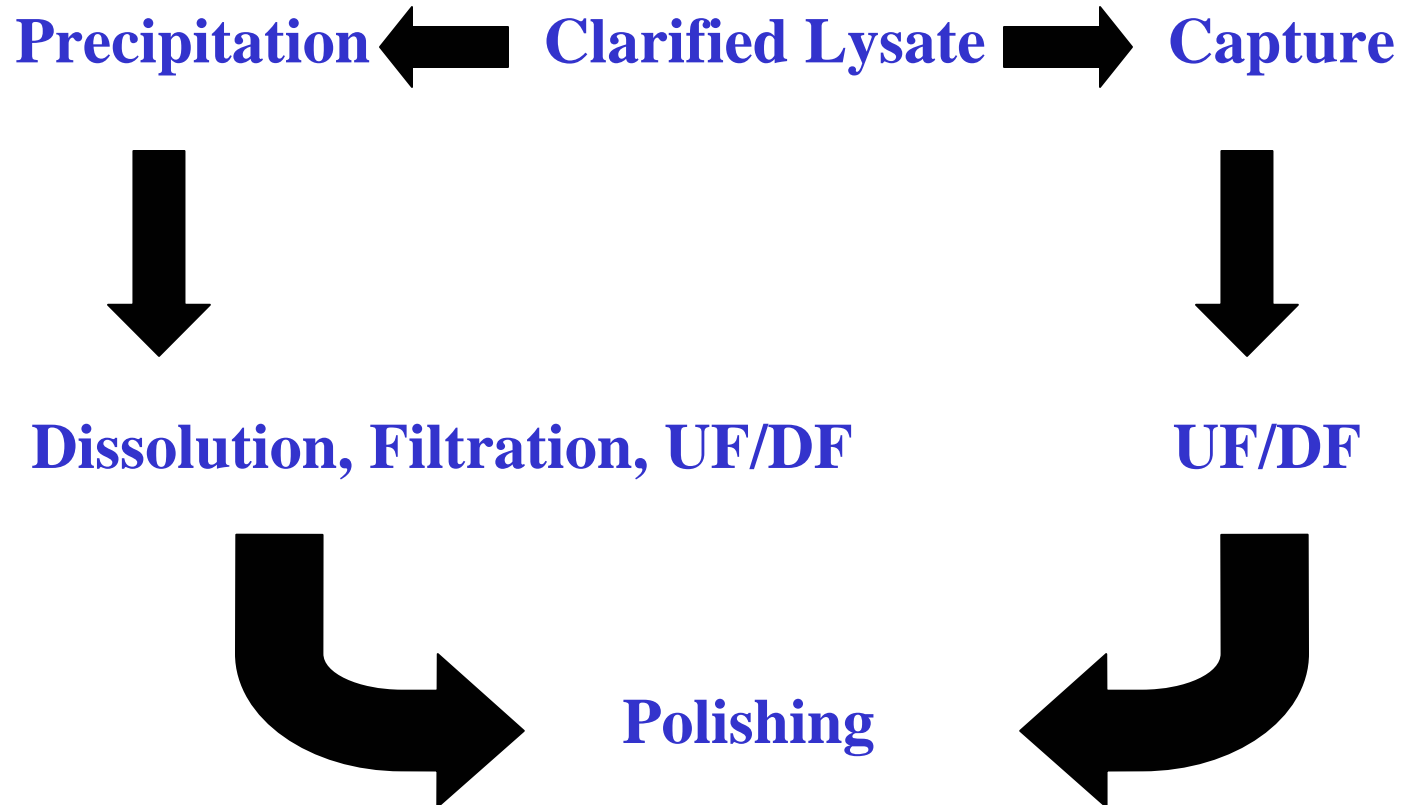
**Client host cell/temperature/media seldom optimized**

**Unstable and/or multimeric plasmids are common**

**High turnover prevents much plasmid yield optimization**



## Scale and Yield Determines Methodology



## **Production of pVAX-WN-1**

**Aldevron was contacted by the CDC in October 2002 for rush production of 200 mg of the experimental pDNA (pVAX-WN-1) for use as a genetic vaccine in Condors.**

**Aldevron was concurrently working with ProMetic Bio-Sciences to develop the resin Perfluorosorb<sup>®</sup> S for use as a polishing step in plasmid purification.**

**Perfluorosorb<sup>®</sup> S was used as the sole chromatographic step in the purification of the initial 200 mg and then as the final step of a two column process to purify a further 400 mg.**



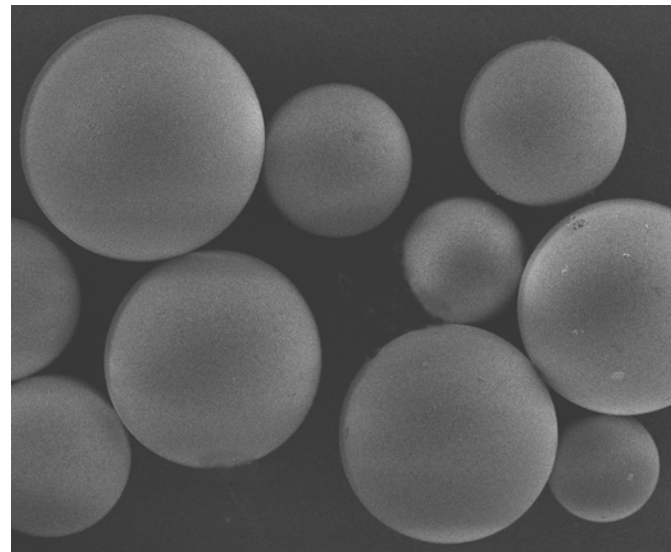
# Perfluorosorb<sup>®</sup> S Resin

## Perfluorosorb<sup>®</sup> S Properties

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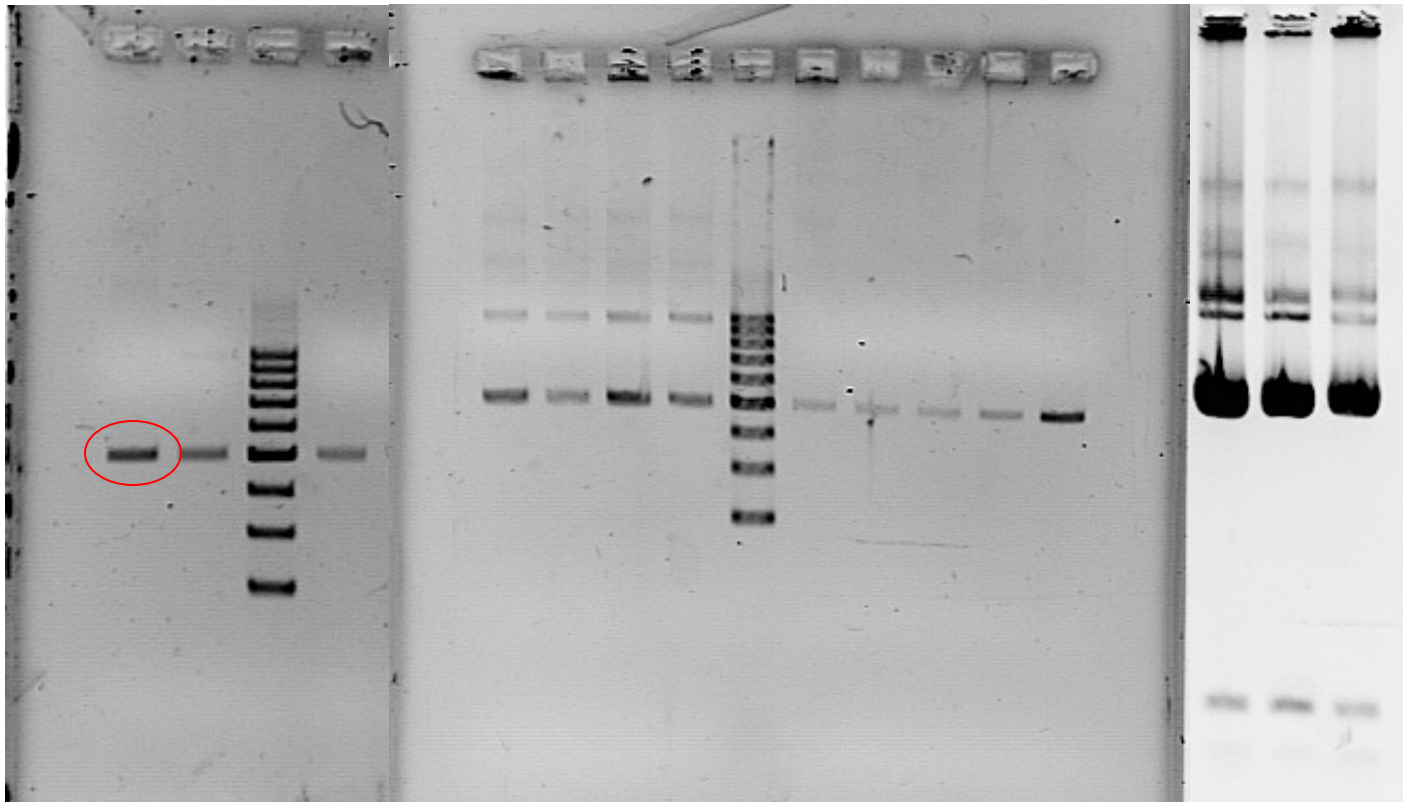
<b>Particle Shape</b>	<b>Spherical</b>
<b>Particle Size</b>	<b>45 <math>\mu\text{m}</math></b>
<b>Particle Surface Area</b>	<b>300 <math>\text{m}^2 \text{g}^{-1}</math></b>
<b>Particle Pore Size</b>	<b>1000 <math>\text{\AA}</math></b>
<b>Particle Chemistry</b>	<b>Fluorine-containing polymeric surface</b>

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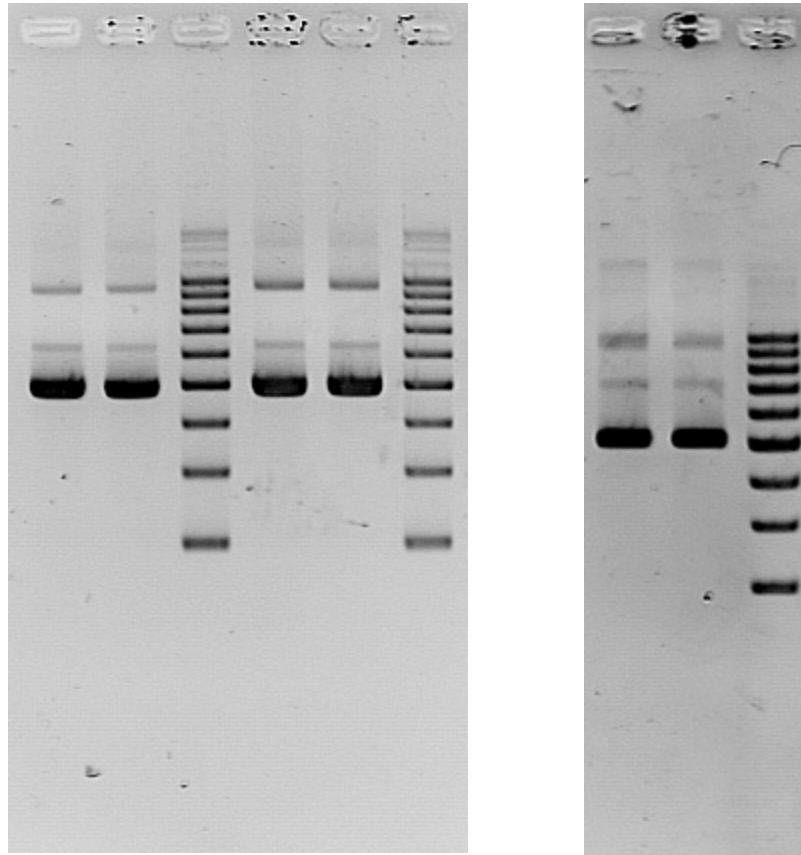
# pVAX-WN-1 Production (Lot 1)

## Screening (host, temperature and media)

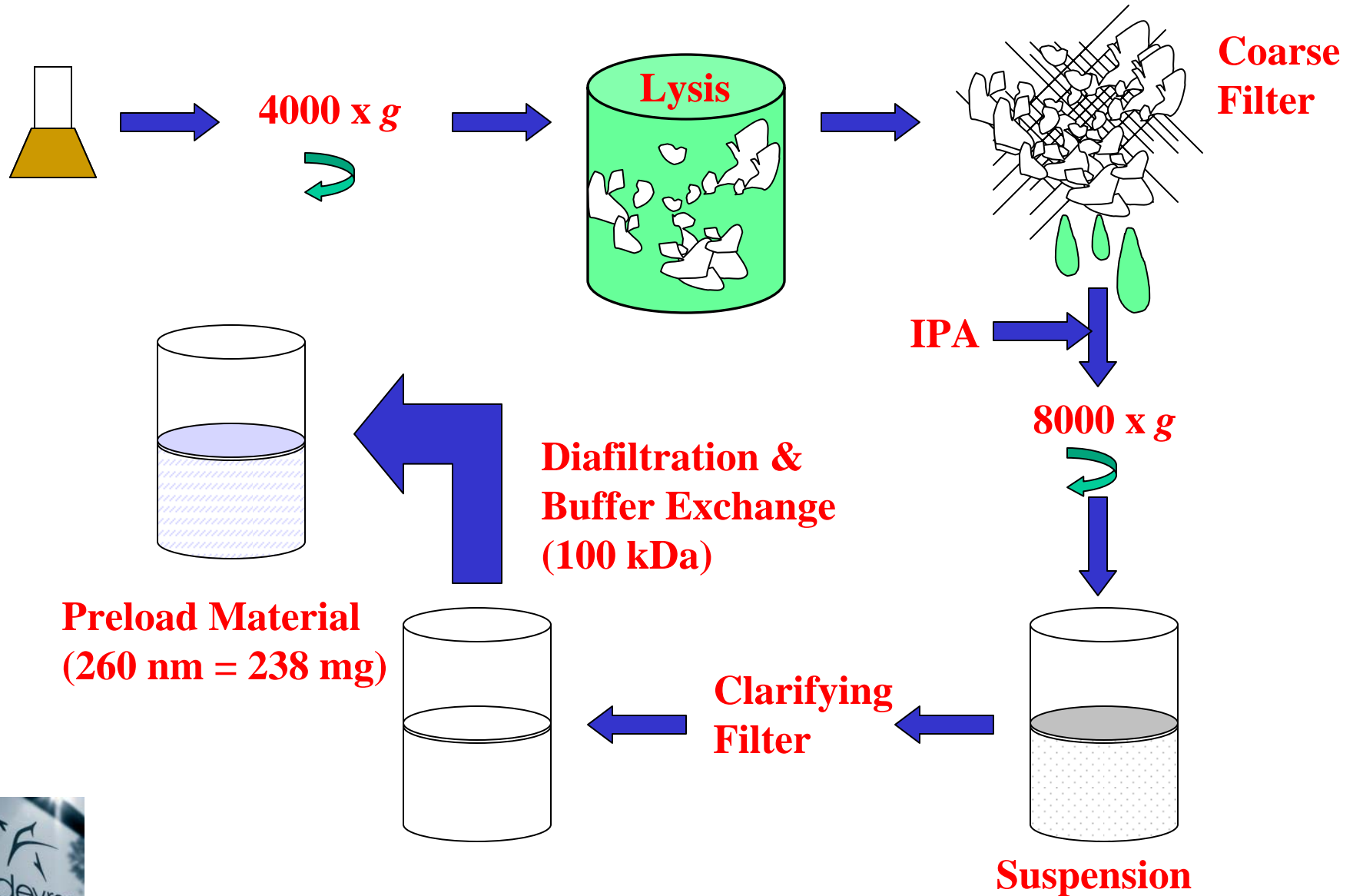


## pVAX-WN-1 Production (Lot 1)

Unstable in most hosts and at  $OD > 8$  and  $T > 34^{\circ}\text{C}$   
XL1-Blue, XL10-Gold and STBL4 ( $30^{\circ}\text{C}$ ) were acceptable  
Yield was better in XL1-Blue



# pVAX-WN-1 Production (Lot 1)



## **pVAX-WN-1 Production (Lot 1)**

### **Chromatography**

**VL 44 containing 200 cc Perfluorosorb<sup>®</sup> S**

**Loaded at 0.4 mg cc<sup>-1</sup> based on preload 260 nm reading**

**Flow rate maintained at 90 cm h<sup>-1</sup> (23 ml min<sup>-1</sup>)**

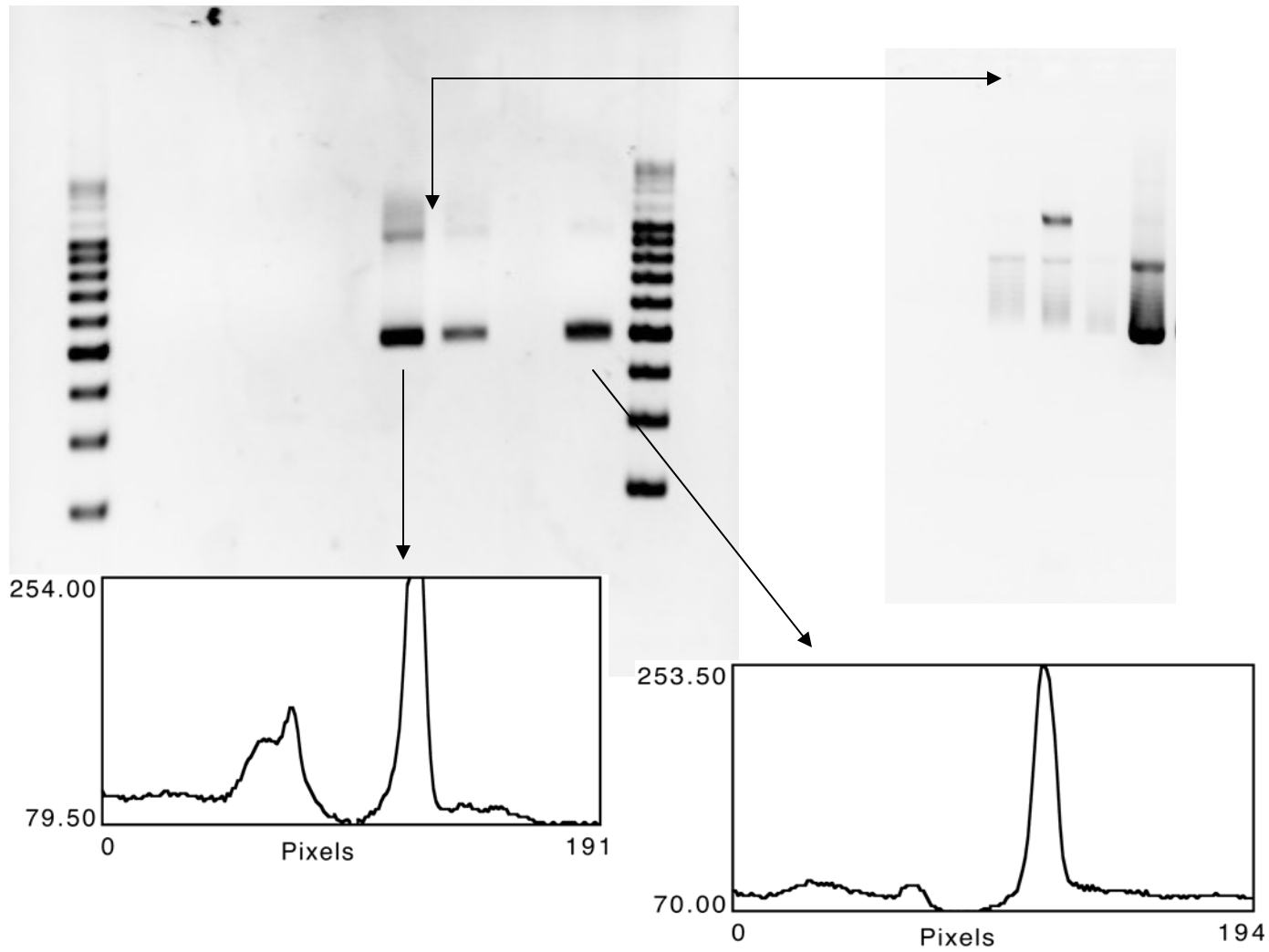
**Loading buffer contained TEAA (40 mM)**

**Series of buffers containing NaOOCCH<sub>3</sub> and EtOH were run as steps**

**Sanitized with 0.5 M NaOH and repeated two more times**



# pVAX-WN-1 Production (Lot 1)



## pVAX-WN-1 Production (Lot 1)

### Lot release specifications for pVAX-WN-1 produced with non-chromatographic capture and reverse phase polishing

Property	Limit/Range	Result/(Method)
Supercoiled DNA	> 90 %	> 95 % (AGE)
Protein	< 2 %	< 2 % (BCA)
Endotoxin	< 10 EU/mg	<< 100 EU/mg* (LAL)
RNA	< 2 %	Undetectable (AGE)
Host cell DNA	< 2 %	< 2 % (AGE)
260/280	1.8-2.0	1.89 (Spectrophotometer)

\*Original release specifications only called for < 100 EU/mg.

The samples were tested with a semi-quantal clot assay.



## **pVAX-WN-1 Production (Lot 2)**

**400 mg scale production**

**Fermentation (60 L) 34 °C, DO = 30 %, AF = 0.5-1 Volumes min<sup>-1</sup>**

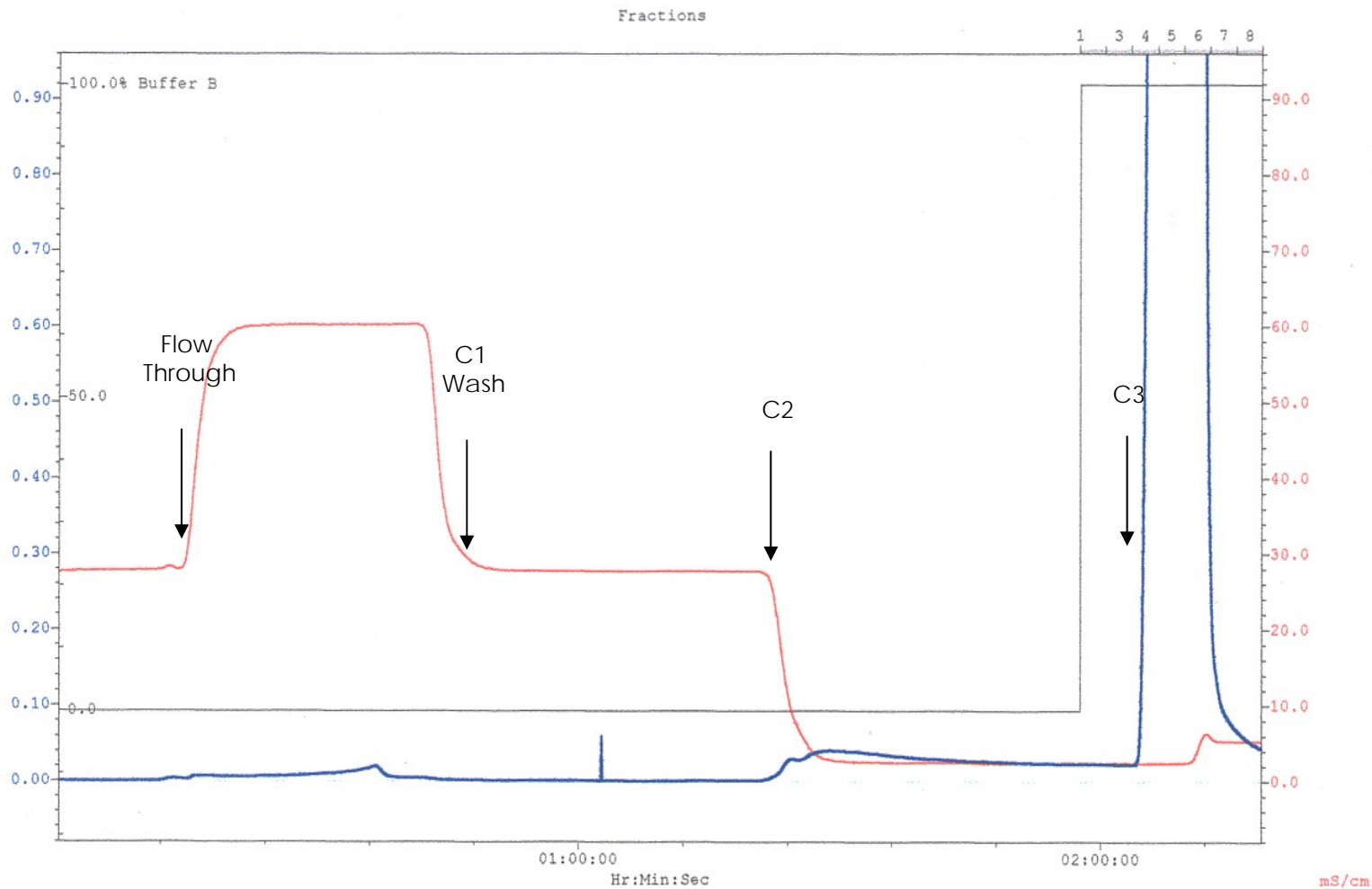
**Lysate earth filtered to < 0.8 µm clarity**

**Anion Exchange Capture (Fractogel<sup>®</sup> DMAE)**

**AX effluent was diafiltered (260 nm = 534 mg)**



# Perfluorosorb<sup>®</sup> S Loading Adjusted AX Effluent

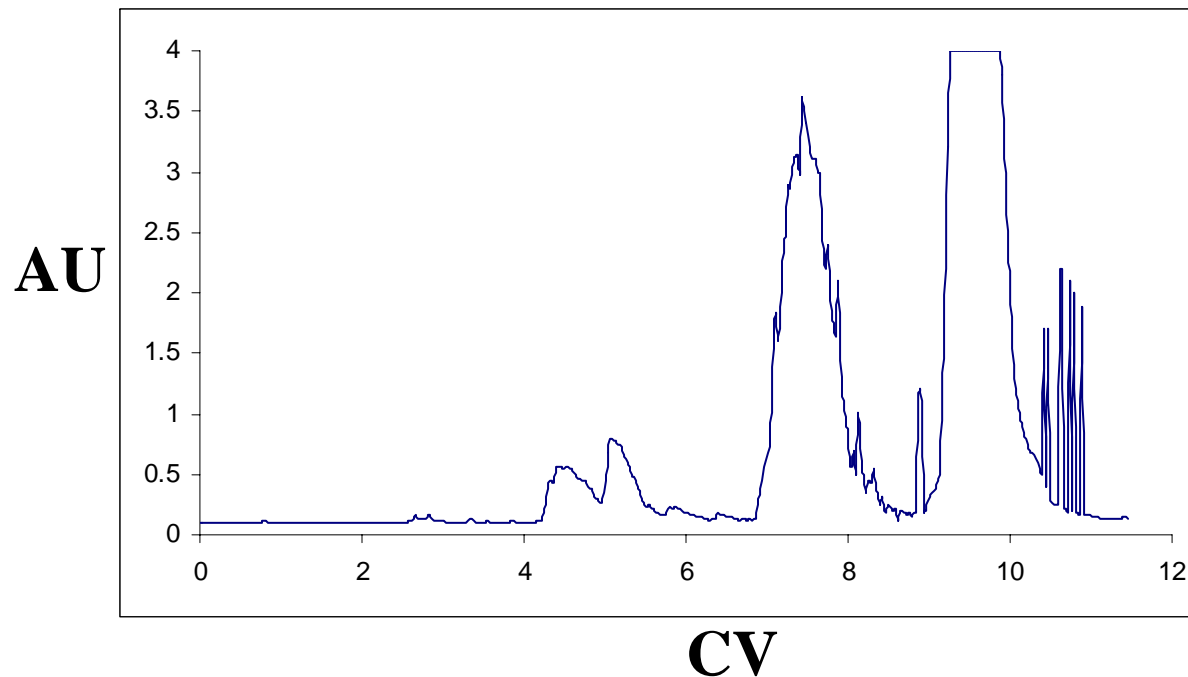


## pVAX-WN-1 Production (Lot 2)

900 cc (9.5 cm id) Perfluorosorb<sup>®</sup> S column for polishing

TBAP used as ion-pairing agent (4 mM)

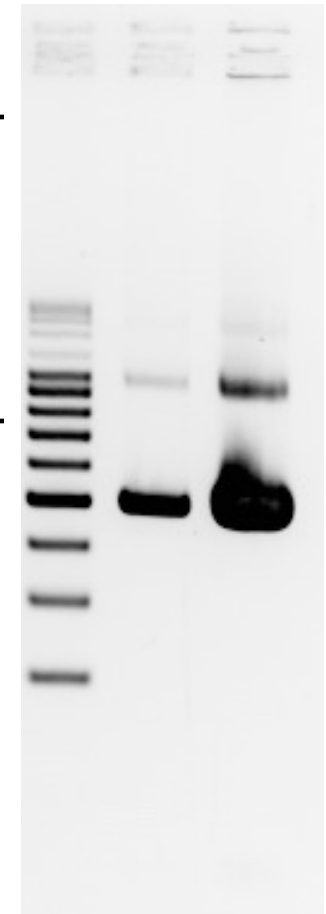
LFR 90 cm h<sup>-1</sup> (lowered to 70 cm h<sup>-1</sup> upon elution)



## pVAX-WN-1 Production (Lot 2)

**Lot release specifications for pVAX-WN-1 produced with anion- exchange capture and reverse phase polishing**

<b>Property</b>	<b>Limit/Range</b>	<b>Result/(Method)</b>
Supercoiled DNA	> 90 %	> 95 % (AGE)
Protein	< 2 %	< 2 % (BCA)
Endotoxin	< 10 EU/mg	< 10 EU/mg (LAL)
RNA	< 2 %	Undetectable (AGE)
Host cell DNA	< 2 %	< 2 % (AGE)
260/280	1.8-2.0	1.86 (Spectrophotometer)



## Conclusions

Either isolation method followed by RPC produced material of extremely high quality

Significant CCC enrichment was achieved

Upstream RNase A free method(s) of isolation would allow clinical manufacture

Man hours  $\text{mg}^{-1}$  were lower with AX capture

