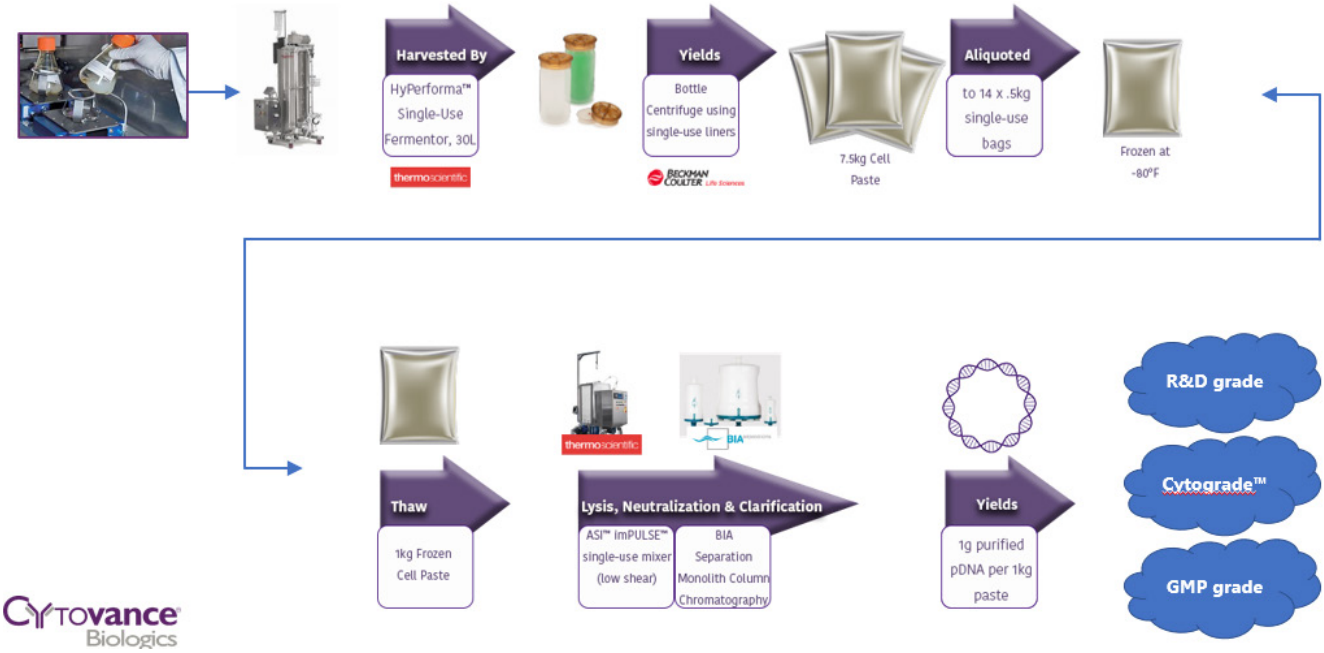
Cytovance Biologics White Paper #2

Cytograde™ pDNA: Manufacture pDNA Without Sacrificing Quality Or Budget

David Schmidt, Cytovance Biologics

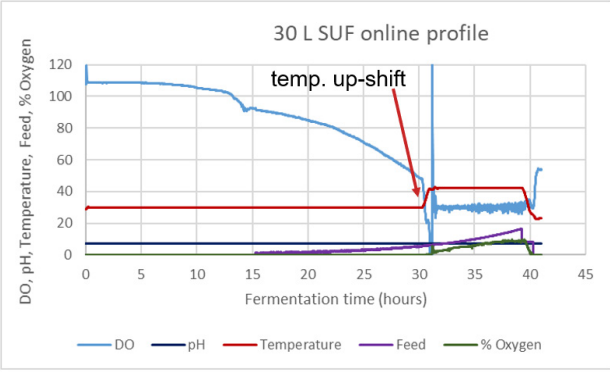
Many pharmaceutical manufacturers require clarity on quality standards for the use of plasmid DNA (pDNA) as a reagent, or as the final active pharmaceutical ingredient (API). Organizations working on viral vector-based gene therapies use pDNA as an integral part of their production workflow. However, as pDNA can also be the final API in therapeutics such as DNA vaccines – and subsequently be in direct contact with patients – establishing efficient, cost-effective quality oversight is crucial to minimize risks while staying on budget.

At Cytovance, a CDMO specializing in rapid development and manufacture of large molecule APIs, many of our clients engage us to create a "GMP light" development framework that reduces overhead, while guaranteeing quality assurance. Although "GMP light" does not exist, the team at Cytovance has developed a compelling new service offering – **Cytograde™ pDNA** – to help our clients produce **high-quality, affordable pDNA** for any process and at any developmental stage. Cytovance Biologics integrates world-class processes and scalable single- or multi-use equipment to efficiently produce superior plasmids.

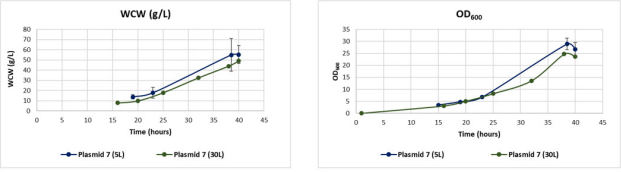


CYTOGRADE™ PRODUCTION

Cytovance offers several scales of single- and multi-use fermenters to meet the needs of your pDNA project. We take steps to carefully manage temperature shift control, a key parameter associated with pDNA production. Our systems utilize fixed agitation (~600 rpm), a fixed total gas flow rate (1 vv), O2 supplementation, and exponential feeding to keep temperatures stable throughout fermentation.



Our processes and equipment – combined with precision tracking of biomass yields specific to different-sized plasmids – help ensure comparable plasmid production at different scales for a range of plasmid sizes.



Wet cell weight (WCD) and OD_{600} obtained from both scales (30 L SUF and 5 L Glass Fermenters) showed similar growth at both scales.

CYTOGRADE™ PURIFICATION

Our integrated equipment includes pre-packed chromatographic monolith, which provides low-shear purification.

To ensure optimal purification of our plasmids, we utilize a diethyl aminoethyl (DEAE) capture step that separates pDNA from host cell proteins (HCP) and RNA. Hydrophobic interaction chromatography (HIC) then polishes the pDNA based on hydrophobicity to remove endotoxins and separate supercoiled (sc) structures.

Purification is divided into three stages:

PRIMARY RECOVERY

- Neutralization removes most of genomic DNA (gDNA), proteins and some endotoxin
- Calcium chloride removes most high-molecular weight RNA

CAPTURE

- Diethyl aminoethyl (DEAE)
- DNA capture step based on charge
- Separates RNA and HCP from pDNA

POLISH

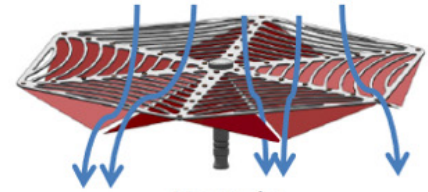
- Hydrophobic interaction chromatography (HIC)
- Polishing step based on hydrophobicity
- Removes endotoxins
- Separates supercoiled pDNA from open circular and gDNA

Our specialized mixing equipment effectively folds molecules and gDNA while minimizing fragmentation. By avoiding exposure to high-shear forces, we foster even mixing intensity and propagation. Our integrated single-use equipment facilitates:

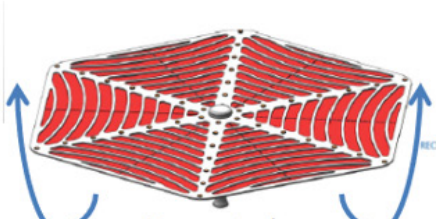
- High-flow velocities
- High-binding capacity
- Low shear in channels
- Large-plasmid purification (2 µm and 6 µm pores)
- Higher recovery of sc plasmid
- Lower buffer consumption
- Reduced purification time
- Good resolution
- CGMP grade material (if required)

Cell Lysis

Thermo Scientific™
inPULSE™ S.U.M.



Upstroke



Downstroke

CYTOGRADE™ QUALITY

As part of our initial pilot program to develop **Cytograde™ pDNA**, we evaluated multiple plasmids, ranging from 5.4 kb to 13.5 kb, at 5 L, 10 L, and 30 L fermenters.

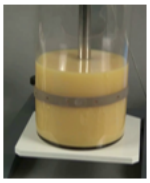
Harvest OD600 for the plasmids was approximately 29 to 107 and wet cell weight was approximately 50 to 270 g/L. Titer was 0.9 to 3.8 mg/g paste. Low-shear mixing and low-shear hollow fiber tangential flow filtration (TFF) facilitated highly efficient chromatographic separation, delivering impressive final products. Typical yield is 1 mg/g of cell paste that retains biological activity, as validated through adeno-associated virus (AAV) testing.

CQA	FINAL PRODUCT	INDUSTRY TARGET
pDNA PURITY (A260/A280)	1.9	≥1.8
HOMOGENEITY % SC	>85%	>80%
ENDOTOXINS	<0.05 EU/mg	<40 EU/mg plasmid
HOST CELL PROTEINS	<1%	<1%
gDNA	ND	<1%
RNA	<1% Not visible on the gel	<1% Not visible on the gel

With **Cytograde™ pDNA**, Cytovance is fully capable of offering customers an **innovative, efficient, and cost-effective platform** to purify pharmaceutical-grade pDNA at dynamic scales.

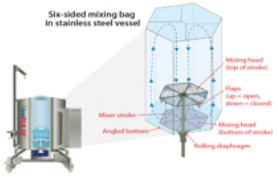
CYTOGRADE™ TESTING

Following plasmid production, Cytovance uses in-process control (IPC) testing to analyze pDNA products and ensure key specifications are met. In addition to our Cytograde™ pDNA we offer R&D and CGMP grade testing to assess identity, purity, process residuals, and safety. With our scalable technology, we can maintain consistent specifications across each quality grade.



Harvest

pDNA Titer Analysis by Plasmid Mini-Prep



Lysis and RNA Precipitation

pDNA Titer Analysis, RNA Detection

Clarification

pDNA Titer Analysis, Homogeneity by Electrophoresis

Concentration and buffer exchange

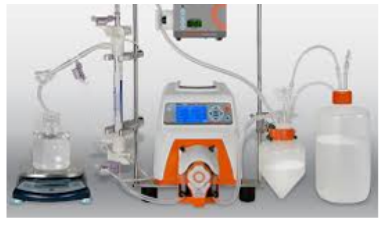
pDNA Titer Analysis, Homogeneity by Electrophoresis

Column 1

A260/A280 Content
Electrophoresis
Residual HCP
Residual DNA
Residual RNA

Column 2

A260/A280 Content
Electrophoresis
Residual HCP
Residual DNA
Residual RNA



Final Formulation

Full Release Testing

	Assay	Method	Cytovance's Target Specification	Grade		
				R&D	Cytograde™	GMP
Content	Plasmid Concentration	UV Spectrophotometry (A260 nm)	Product Specific	X	X	X
Quality	Appearance Testing	Visual Inspection	Product Specific	X	X	X
	pH Testing	USP <791>	Product Specific			X
	Osmolality Testing	USP <785>	Product Specific			X
Identity	Identity by Gel Migration	EtBr Stained Agarose Gel Electrophoresis	Migration consistent with Reference Standard or size confirmed by supercoiled ladder	X	X	X
	Restriction Enzyme Digestion	Plasmid digested with product-specific enzymes and EtBr Stained Agarose Gel Electrophoresis	Number and migration of digestion fragments consistent with Ref Std	X	X	X
	Plasmid Sequencing	Outsourced	Sequence Confirmed			X
Purity	Purity Analysis by 260/280 nm Absorbance Ratio	UV Spectrophotometry (A260/A280 nm)	1.8 - 2.0	X	X	X
	DNA Homogeneity	EtBr Stained Agarose Gel Electrophoresis with Densitometry Analysis	>90% Supercoiled	X	X	X
Process Residuals	Residual Host Cell Proteins	Micro BCA	<1%		X	X
	Residual Host Cell DNA	Quantitative PCR	<1%		X	X
	Residual Host Cell RNA	SYBR green Stained Agarose Gel Electrophoresis with densitometry analysis	<1%		X	X
	Residual Kanamycin	ELISA	Product Specific		X	X
Safety	Endotoxin	Chromogenic Kinetic Analysis	Product Specific		X	X
	Mycoplasma by qPCR (Optional)	quantitative PCR (21 Day PTC for GMP Grade)	Negative for Mycoplasma Detection		X	X
	Sterility	Direct Inoculation USP <71>	Negative for Growth		X	X
	Sterility (Bacteriostasis/Fungistasis)	Direct Inoculation USP <71>	Negative for Bacteriostasis or Fungistasis		X	X

IMPROVE QUALITY AND MANAGE COSTS WITH CYTOGRADE™ pDNA

In today's increasingly competitive market, successfully commercializing a product with pDNA requires a strategic and cost-conscious approach at every stage of development. Fortunately, Cytovance Biologics is ready and able to handle pDNA production at several different scales, delivering superior product and superior value.

REACH OUT TO THE pDNA EXPERTS AT CYTOVANCE BIOLOGICS TODAY.

AUTHOR CONTACT

dschmidt@cytovance.com

ABOUT CYTOVANCE BIOLOGICS

Cytovance Biologics is a leading biopharmaceutical contract development and manufacturing organization (CDMO) that excels in the rapid and cost-effective development and manufacture of large-molecule active pharmaceutical ingredients (APIs) from both mammalian cell culture and microbial fermentation such as monoclonal antibodies, fragment antibodies, bispecifics, enzymes, fusion proteins, vaccines, and other biological products, including plasmid DNA and cell-based therapeutics. In addition to our clinical and commercial CGMP API manufacturing services, Cytovance offers well-integrated development services supporting the entire product life cycle, including cell line development, cell banking, microbial strain development, process and analytical development, and process characterization. A centralized, responsive program management team coordinates all critical chemistry, manufacturing, and controls (CMC) activities for each client program around raw materials management, QC testing, ICH stability studies, and regulatory support. Our 140,000 sq. ft., state-of-the-art facilities in Oklahoma City are designed to meet U.S., EU, and other global regulatory standards.

Cytovance offers deep industry expertise and unique customized services for the scale-up and cGMP manufacture of protein-based therapeutics from early-stage preclinical development to commercial production, for both mammalian and microbial projects. Further information can be found at ***www.cytovance.com.***