Bioproduction

Support consistent and higher plasmid DNA yields across auxotrophic *E. coli* strains with a chemically defined medium and feed platform

Introduction

As demand for viral vectors and mRNA vaccines accelerates, the mission of biomanufacturers to increase production of plasmid DNA (pDNA) has become critical. The challenges for production teams are clear—improve lot-to-lot consistency and achieve higher yields with the diverse spectrum of *E. coli* strains used for pDNA production.

Using chemically defined (CD) and animal origin–free (AOF) components in media and feeds can support reduced variability and uncertainty. To improve yields, studies have shown that fed-batch fermentation with nutrient feeds can deliver much higher volumetric yields compared with a simple fed-batch process [1]. Combined, these methods can help production teams meet their goals to achieve consistent performance and higher pDNA yields.

With these performance goals in mind and to support a broader spectrum of *E. coli* strains, the Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM) Plus and Bacto™ CD Supreme Feed (2X) platform was developed. The formulations were developed using a Phenotype MicroArray™ panel (Biolog Inc.) to screen ~800 different molecules across diverse auxotrophic *E. coli* strains (Figure 1). Principal component analysis (PCA) defined the distinct target nutritional components. Based on design of experiment (DOE) methods, the target components were tested in various formulations, and a robust CD and AOF media and feed platform was developed to support the needed enhanced and consistent yields across auxotrophic strains commonly used for pDNA production.

Evaluations of the Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed platform were conducted by an external service provider and the Jenner Institute (Oxford) with NEB™ Stable strains (New England Biolabs) producing plasmids. The platform was shown to achieve higher cell densities and enhanced pDNA yields. Additionally, the medium showed reliable, consistent performance across lots, in both liquid and dehydrated powder medium (DPM) formats, and relative to using Terrific Broth from various vendors.

Enable predictable enhanced production

- Up to 120% higher pDNA yields
- No auxotroph-specific supplementation required
- Chemically defined and animal origin–free formulations help ease regulatory approval
- Reliable, consistent performance across lots and formats

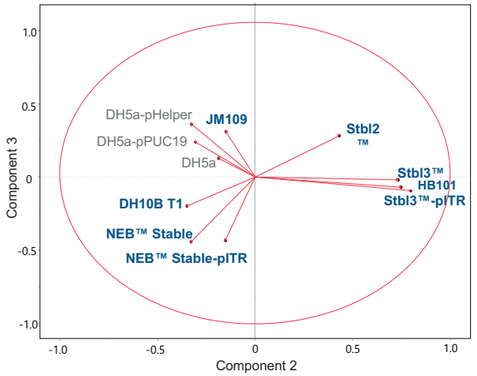

Figure 1. Example of development PCA results. After using a microbial Phenotype MicroArray panel to screen a range of auxotrophic *E. coli* strains, PCA revealed the key nutritional component targets across the strains. DOE-based testing of the target components in various formulations identified the optimized formulations. Strains supported by Gibco™ Bacto™ CD Supreme FPM are shown in gray, with additional strains supported by Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed (2X) shown in blue.

Benchmark performance

An external service provider tested NEB Stable cells for growth, pAAV2-GFP-ITR plasmid production, and acetate accumulation in 250 mL mini bioreactors with Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed (2X) compared to using Terrific Broth (TB) with yeast extract feed or a lab-prepared CD simple feed (CDSF) using an exponential feeding strategy. Further test details are shown in the methods section.

When using the Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed platform, the test results showed up to 151% higher cell growth (Figure 2), 120% higher productivity (Figure 3), and 92% less acetate accumulation (data not shown).

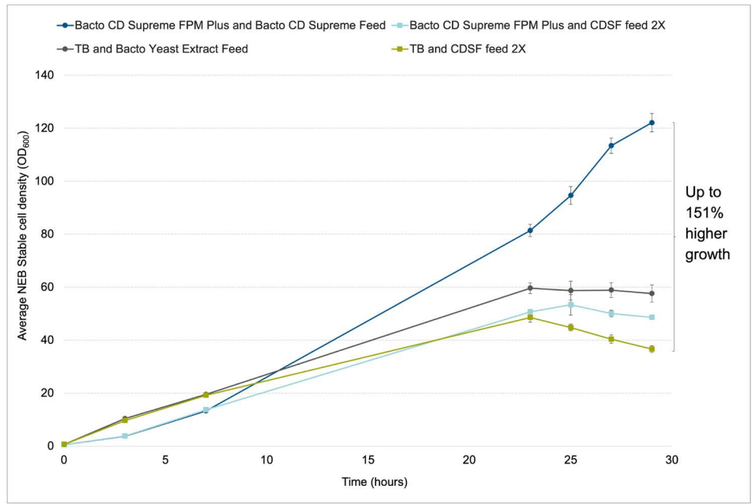

Figure 2. Cell density. NEB Stable cells using Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed with an exponential feed strategy supported up to an average of 151% higher cell density than TB with a lab-prepared CDSF feed, and 129% higher density compared to using Bacto CD Supreme FPM Plus with CDSF feed (n = 2–3 vessels per condition).

External service provider with NEB Stable cells producing pAAV2-GFP-ITR

Mini bioreactor system at 250 mL volume

Bacto CD platform compared to TB and yeast extract feed or lab-prepared CDSF feed

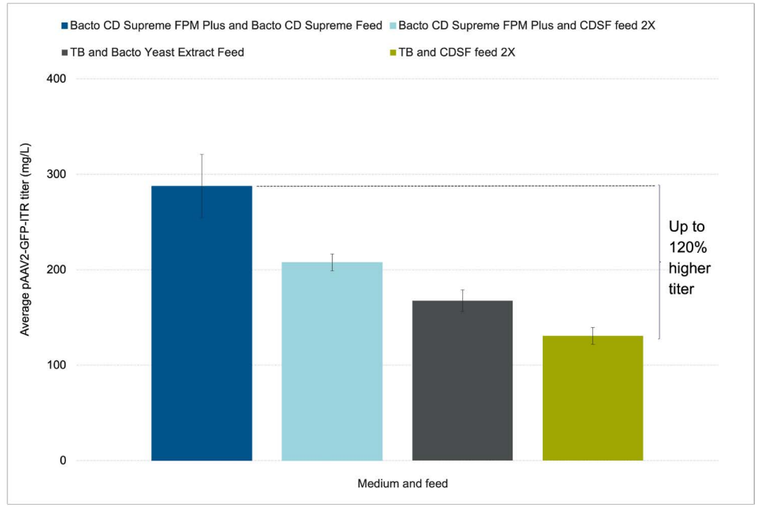

Figure 3. Productivity. Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed helped deliver up to 120% higher average pAAV2-GFP-ITR titer than TB with CDSF feed, and a 38% increase compared with Bacto CD Supreme FPM Plus paired with CDSF feed.

The Jenner Institute (Oxford) evaluated NEB Stable cell growth and pUC19-derived plasmid production using Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed with an exponential feeding strategy in a 10 L stirred glass bioreactor at a 4.4 L volume. The Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed platform was compared to their lab-prepared CD basal medium, requiring isoleucine and leucine supplementation, with their CD feed. For pUC19 plasmid expression, a temperature shift to 42°C was conducted upon reaching an OD >60. Further test details are shown in the methods section.

Their evaluations with Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed showed 16% higher cell growth (Figure 4) with 59% higher volumetric yields (Figure 5) and 36% higher specific yield (data not shown). The Jenner Institute also noted the potential for a total yield of approximately 4 g at the full 10 L bioreactor volume, a yield typically requiring 10–20x larger bioreactors.

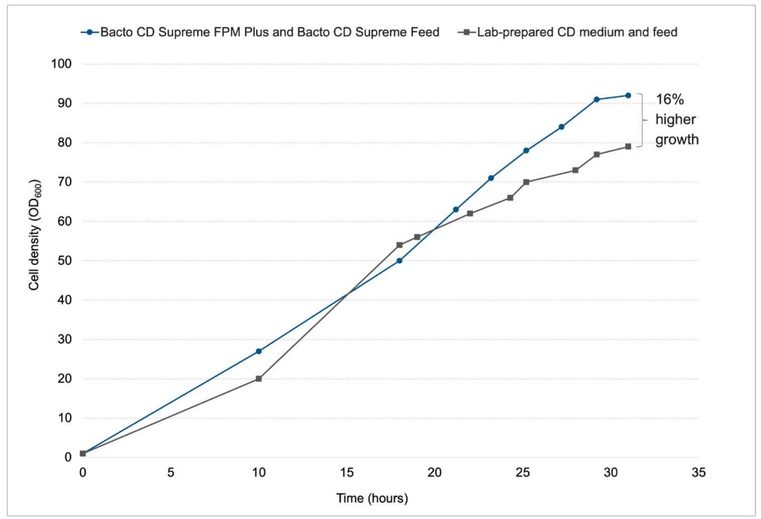

Figure 4. Cell density. The Jenner Institute evaluation of Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed with NEB Stable cells found 16% higher cell density compared to their lab-prepared CD medium and feed. A temperature shift to 42°C was conducted at OD >60 for pUC19 plasmid expression (n = 1 per condition).

The Jenner Institute with NEB Stable cells producing a pUC19-derived plasmid

10 L stirred glass bioreactor at 4.4 L volume

Bacto CD platform compared to lab-prepared CD medium and feed

Performance consistency

Bacto CD Supreme FPM Plus in both liquid and DPM formats was evaluated with NEB Stable cells producing pAAV2-GFP-ITR pDNA using a simple fed-batch mode in shake flasks. Additionally, TB from various vendors was evaluated in parallel using the same methods. Further test details are shown in the methods section.

The results showed consistent average cell densities and pAAV2-GFP-ITR pDNA productivity with CV of ≤5% between lots and the liquid and DPM formats (Figure 6). Enhanced plasmid production consistency was also shown relative to using TB sourced from various vendors.

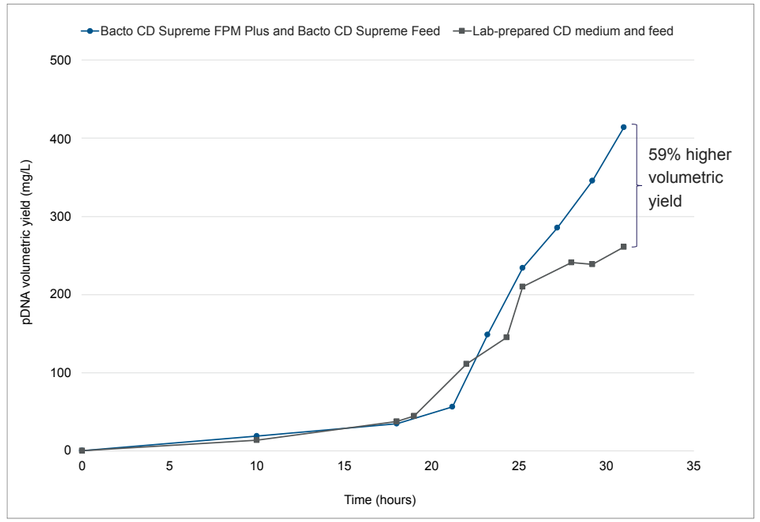

Figure 5. Productivity. With Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed, the Jenner Institute showed 59% higher volumetric pDNA yield.

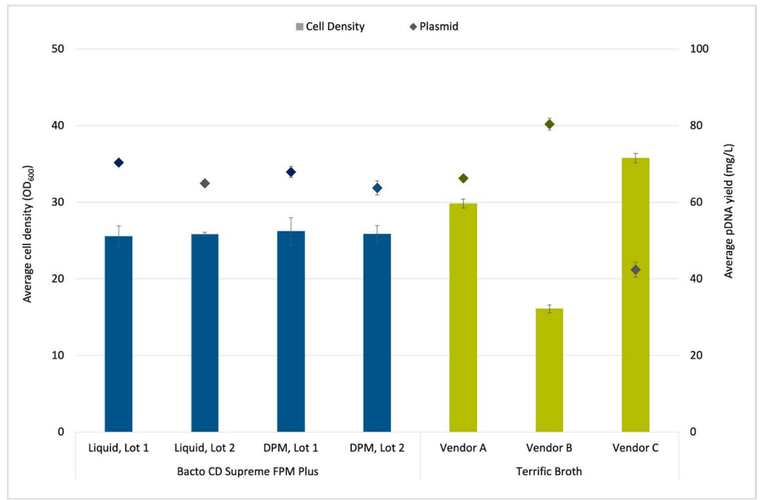

Figure 6. Growth and productivity. Evaluation of NEB Stable performance with Bacto CD Supreme FPM Plus showed improved consistency in cell density and pAAV2-GFP-ITR pDNA yield between lots and formats (CVs ≤ 5%), and greater consistency in plasmid yield when compared to TB sourced from different vendors (n = 2 vessels per condition).

Conclusion

Overall, results from an external service provider and the Jenner Institute evaluations showed the Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed (2X) platform with NEB Stable strains consistently supports higher cell growth and plasmid yields. The Jenner Institute also noted the ~4 g potential total yield at the full 10 L bioreactor volume—typically this yield requires 10–20x larger bioreactors. Additionally, enhancement between lot and format performance consistency was shown with the CD medium, as well as when compared to the less-defined TB from different vendors.

Additional benefits:

- CD and AOF for reduced risk of inadvertent contaminants and to help ease regulatory approvals
- Liquid and DPM formats with flexibility to autoclave or filter-sterilize the DPM
- Multiple bioprocessing packaging options
- Supply assurance with global redundant and equivalent manufacturing facilities
- Access to highly experienced and knowledgeable field application scientists and R&D scientists for consultation and guidance

Methods: details of each study

Study	Benchmark performance		Performance consistency
Testing provider	External service provider	The Jenner Institute (Oxford, England)	R&D teams
Cell line	NEB Stable cells (New England Biolabs)		
Molecule produced	pAAV2-GFP-ITR	pUC19-derived plasmid	pAAV2-GFP-ITR
Culture vessels and volumes	Culture Nexxys 250 system (Culture Biosciences), 250 mL maximum volume	Biostat B system (Sartorius) with 10 L glass vessel, 4.4 L maximum volume	Shake flasks
Test condition 1	Bacto CD Supreme FPM Plus with 10 mL/L glycerol and Bacto CD Supreme Feed (1X), supplemented with 400 mL/L glycerol (filtered)	Bacto CD Supreme FPM Plus with 15 g/L glycerol and Bacto CD Supreme Feed (2X), supplemented with 600 g/L glycerol (filtered)	Multiple lots of liquid and DPM Bacto CD Supreme FPM Plus supplemented with 15 mL/L glycerol (filtered)
Test condition 2	Bacto CD Supreme FPM Plus and lab-prepared CDSF feed, supplemented with 400 mL/L glycerol (filtered)	–	TB from multiple vendors, supplemented with 15 mL/L glycerol (autoclaved)
Control condition 1	TB and yeast extract feed, supplemented with 400 mL/L glycerol (autoclaved)	Lab-prepared CD medium, supplemented with 15 g/L glycerol and 1 g/L each of isoleucine and leucine, and lab-prepared CD feed, supplemented with 600 g/L glycerol (filtered)	–
Control condition 2	TB (autoclaved) and lab-prepared CDSF feed (filtered)	–	–
Feeding strategy and key culture conditions	Exponential fed-batch*	Exponential fed-batch** with temperature shift to 42°C when OD_{600} >60	Simple fed-batch
Cell density quantification	Cedex Bio Analyzer (Roche), OD at 583 nm	UV-1600PC Spectrophotometer (VWR), OD at 600 nm	Thermo Scientific™ GENESYS™ 50 UV-Visible Light Spectrophotometer, OD at 600 nm
Plasmid extraction and isolation	Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit	QIAprep™ Spin Miniprep Kit (Qiagen)	Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit
Plasmid quantification	Thermo Scientific™ NanoDrop™ Spectrophotometer		

* The exponential feeding profile started when a dissolved oxygen (DO) spike occurred or there was a drop in the stir speed, typically 10–14 hours after fermentation start. The feed rate (pump rate) is programmed to increase exponentially based on the equation: $F = C1 \times e^{C2t}$, where F = flow rate (g/min), C1 = feed rate constant = 0.00256 g/min, C2 = growth rate constant (μ) = 0.13 (adjusted per strain growth rate), and t = EFT (hours). The C1 coefficient is adjusted according to vessel working volume, and C2 is based on experimental strain growth rate testing and carbon requirements.

** After the depletion of glycerol in the medium was detected by the rapid increase of dissolved oxygen (DO spike), the feeding was initiated. The exponential feed rate was set to maintain the growth rate μ = 0.11 over approximately 20 hours. The temperature was kept constant at 30°C during the biomass accumulation phase. After reaching OD_{600} >60, the temperature was raised to 42°C for 8–9 hours to induce plasmid replication, then decreased to 26°C for 2 hours to promote plasmid reparation processes and decrease open circular forms of plasmids.

Reference

1. Bello Roufai MB, Hromyak JT, White CG, Vasconcelles D, Concepcion D (2024) Developing fed-batch strategy to optimize plasmid DNA production in *Escherichia coli* DH5α for cost-effective manufacturing. Cytotherapy 26(6): S134.

Ordering information

Product	Format	Packaging size	Cat. No.
Bacto CD Supreme Fermentation Production Medium Plus	Liquid	1,000 mL bottle	A9378401
Bacto CD Supreme Feed (2X)		500 mL bottle	A9378201
Bacto CD Supreme Fermentation Production Medium Plus	DPM	500 g	A9378501
		10 kg	A9378502
Bacto CD Supreme Feed (2X)		250 g	A9378301
		5 kg	A9378302

Learn more at **thermofisher.com/bacto-plus**