

Improving Consistency and Scale-Up of Plasmid DNA Manufacturing with a Chemically Defined Media Platform

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INTRODUCTION

In response to growing demand for gene therapies, viral vectors, and mRNA-based vaccines and therapeutics, plasmid DNA (pDNA) manufacturing continues to expand with the global market estimated at approximately USD 2.6 billion in 2025. *Escherichia coli* is widely used for producing pDNA however, yield, quality, and scalability can vary depending on strain genetics and fermentation media. Traditional complex media can support microbial growth but may introduce variability and contamination risk, which can complicate process scale-up.

To address these challenges, a nutritionally informed, chemically defined (CD), animal origin-free (AOF) *E. coli* platform medium and feed were developed to support consistent and scalable pDNA production across multiple commonly used *E. coli* strains and strain-plasmid combinations.

RESULTS

Optimum nutritional strategy for broad strain coverage

Commonly used *E. coli* strains for gene therapy plasmid production were evaluated for their nutritional diversity. Distinct nutritional groups were identified, as illustrated in Figure 1. Principle component analysis (PCA) defined the distinct target nutritional components.

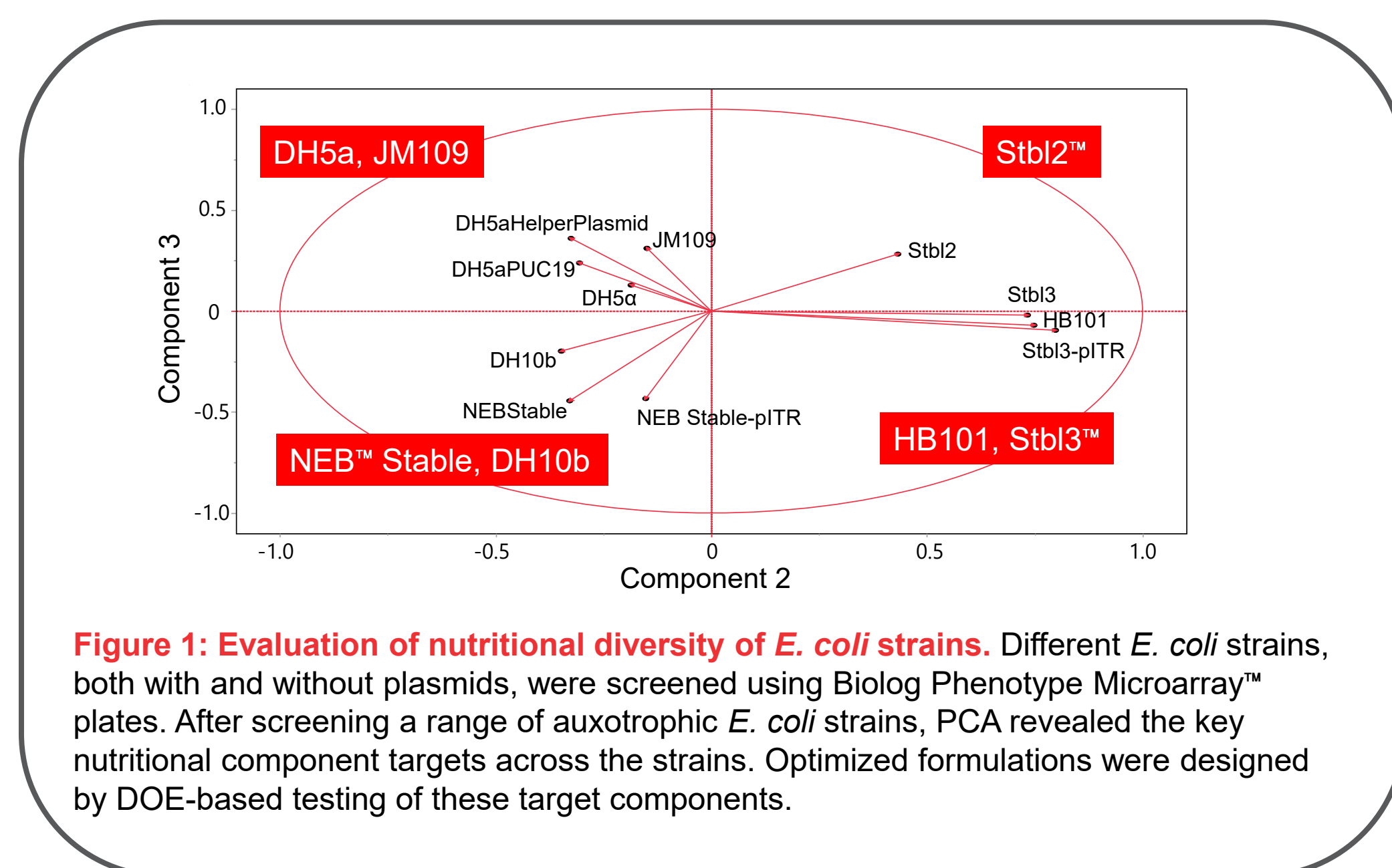


Figure 1: Evaluation of nutritional diversity of *E. coli* strains. Different *E. coli* strains, both with and without plasmids, were screened using Biolog Phenotype Microarray™ plates. After screening a range of auxotrophic *E. coli* strains, PCA revealed the key nutritional component targets across the strains. Optimized formulations were designed by DOE-based testing of these target components.

Using design of experiments (DOE), key components were systematically tested, leading to the development of a robust CD, AOF media and feed platform—Bacto CD Supreme FPM Plus and Bacto CD Feed—designed to deliver enhanced performance and broad coverage across auxotrophic strains used in pDNA production (Figure 2).

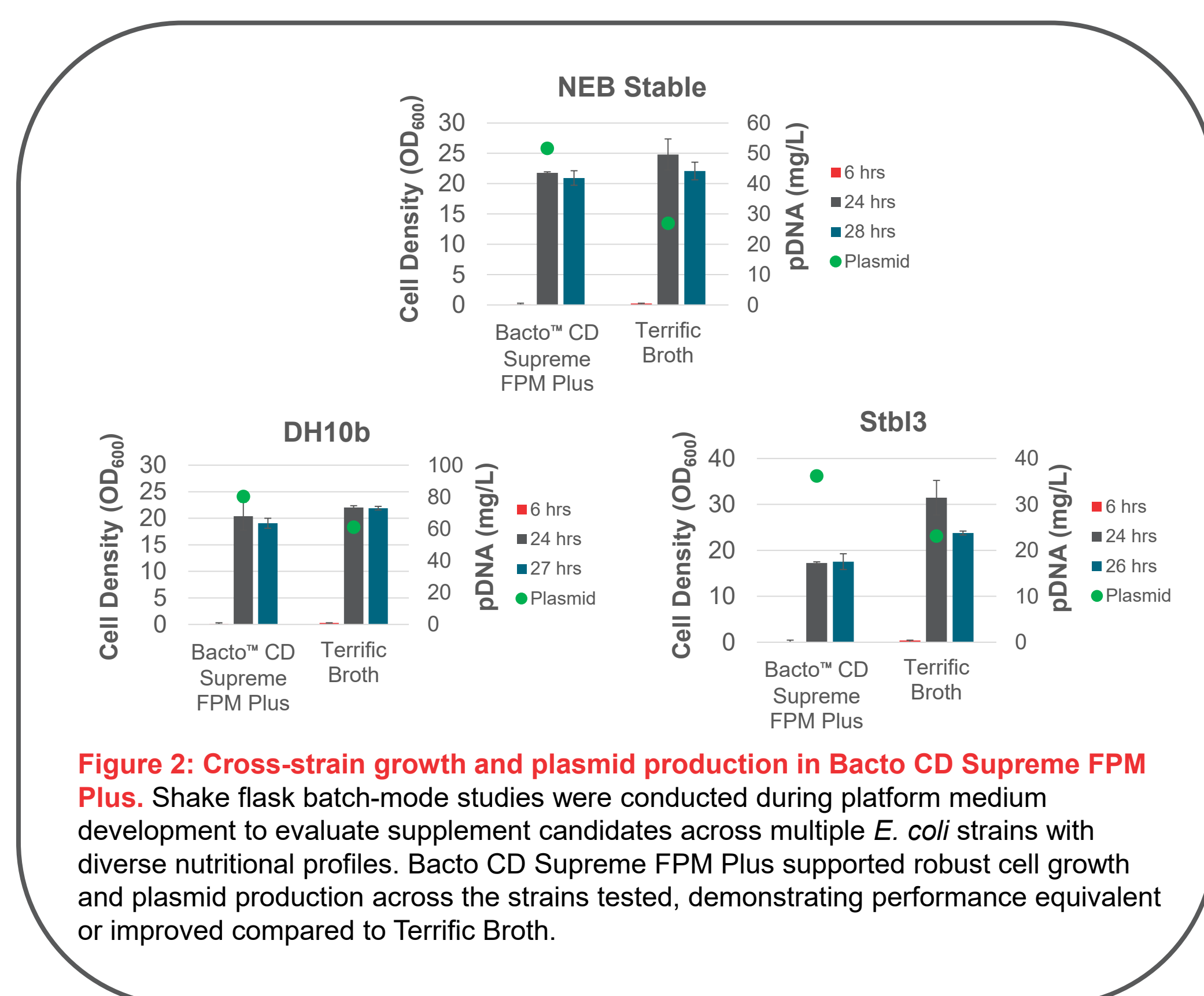


Figure 2: Cross-strain growth and plasmid production in Bacto CD Supreme FPM Plus. Shake flask batch-mode studies were conducted during platform medium development to evaluate supplement candidates across multiple *E. coli* strains with diverse nutritional profiles. Bacto CD Supreme FPM Plus supported robust cell growth and plasmid production across the strains tested, demonstrating performance equivalent or improved compared to Terrific Broth.

CONCLUSIONS

Bacto CD Supreme FPM Plus and Bacto CD Feed together provide a CD, AOF medium and feed platform for scalable *E. coli* plasmid manufacturing. The system supports robust growth and high plasmid yields across multiple strains and constructs relevant to gene therapy

Nutritional diversity across plasmid-bearing and plasmid-free strains was evaluated using Phenotype MicroArray™ screening and principal component analysis to guide formulation design. The resulting Gibco™ Bacto™ CD Supreme FPM Plus medium and Gibco™ Bacto™ CD Supreme Feed (2X) demonstrated improved growth performance, enhanced plasmid yields, reduced scale-dependent variability, and reliable performance from development through pilot scales. The platform is compatible with the Thermo Scientific™ HyPerforma™ Enhanced Single-Use Fermenter (eS.U.F.) systems, enabling integration into GMP manufacturing workflows.

Robust, lot-to-lot consistent performance with significant gains in plasmid yield

Bacto CD Supreme FPM Plus demonstrated consistent performance across multiple lots when compared to Terrific Broth (TB) from various vendors (Figure 3A). In mini-bioreactor studies, use of the platform medium and feed with NEB Stable strain 1 resulted in up to 105% higher cell growth and 72% higher pDNA yield compared to TB with a yeast extract feed (Figures 3B, 3C).

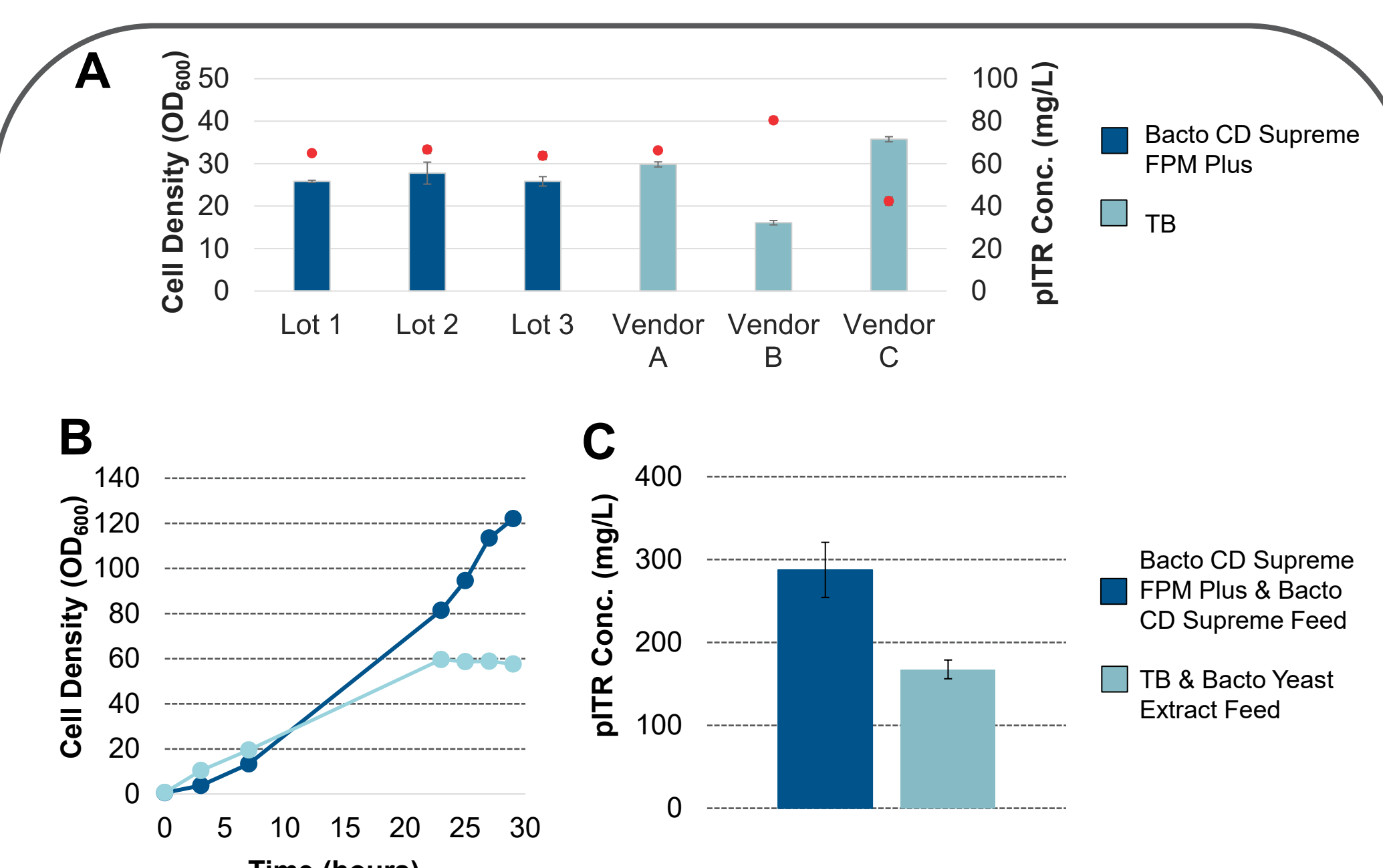


Figure 3: Cell density, productivity, and performance consistency. (A) Shake flask: NEB Stable strain 1 in Bacto CD Supreme FPM Plus showed more consistent cell density and pDNA yield (CVs ≤5%) than TB from multiple vendors. (B-C) Mini-bioreactors: Using Bacto CD Supreme FPM Plus with exponential feeding increased cell growth (B) by up to 105% and plasmid yield (C) by 72% compared to TB with yeast extract feed. All conditions were evaluated using glycerol.

In 10 L bioreactor studies, evaluation with NEB Stable strain 2 by The Jenner Institute (Oxford, <https://www.jenner.ac.uk/>) showed 16% higher cell growth, 59% higher volumetric yield, and 36% higher specific yield relative to in-house, chemically defined media (Figure 4). Overall, Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed provides a robust and scalable alternative to traditional or in-house CD media and feed for enhanced cell growth and pDNA productivity.

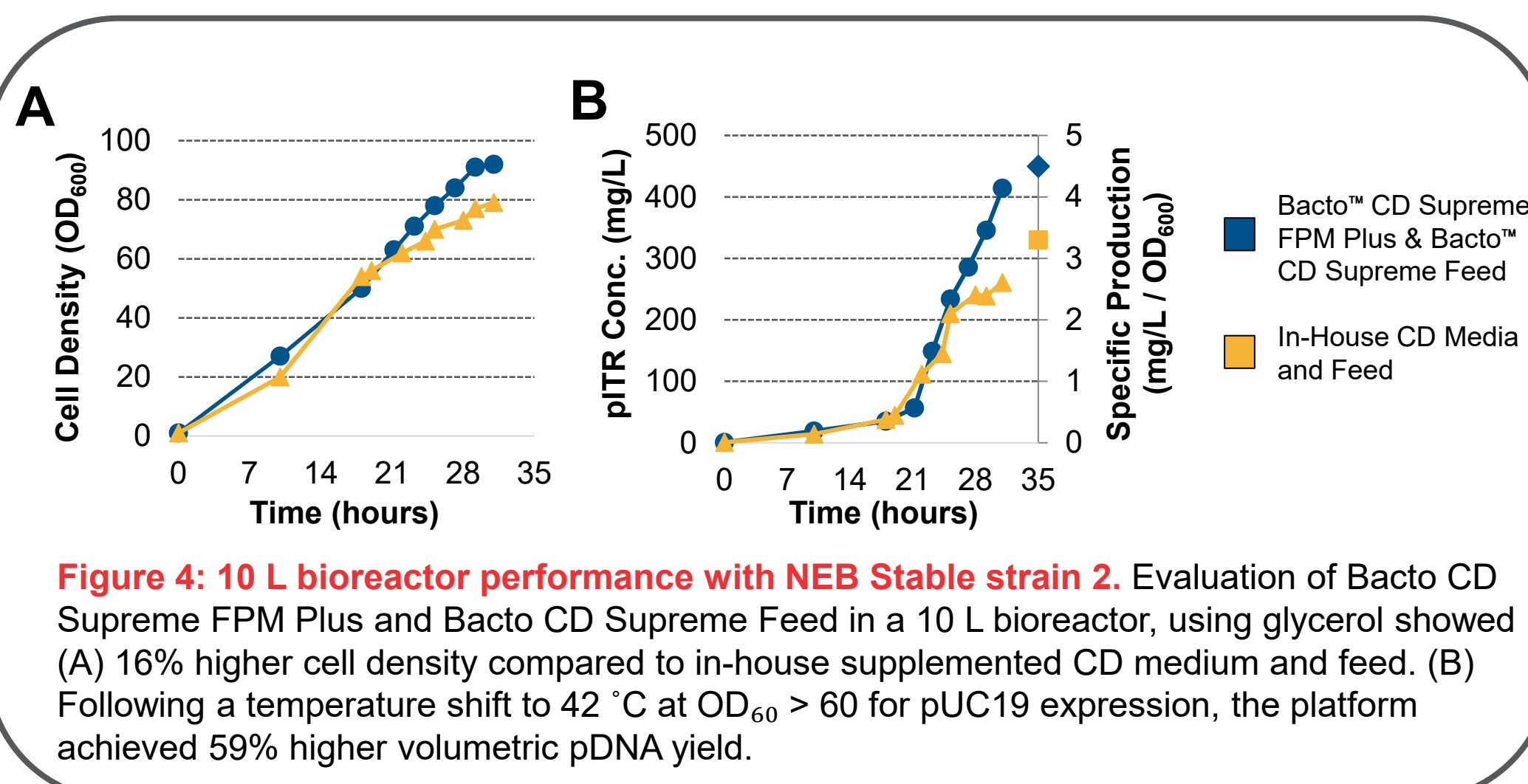


Figure 4: 10 L bioreactor performance with NEB Stable strain 2. Evaluation of Bacto CD Supreme FPM Plus and Bacto CD Supreme Feed in a 10 L bioreactor, using glycerol showed (A) 16% higher cell density compared to in-house supplemented CD medium and feed. (B) Following a temperature shift to 42 °C at OD₆₀₀ > 60 for pUC19 expression, the platform achieved 59% higher volumetric pDNA yield.

and mRNA applications, helping reduce process risk, minimize media development efforts, and lower overall production costs while simplifying workflows.

MATERIALS AND METHODS

Strains used: One Shot™ Stbl3™, One Shot™ MAX Efficiency™ DH10B T1, DH5α (Thermo Fisher Scientific), JM109 (ATCC 53323™), HB101 (ATCC 33694™), NEB™Stable (New England Biolabs). Bacterial growth was determined using the Roche Cedex™ Bio Analyzer. The metabolic profile screening were performed using Biolog Phenotype Microarrays™ using Bacto™ CD Supreme Fermentation Production Medium (FPM). Plasmid quantification was performed using GeneJET™ Plasmid Miniprep Kits and a Genesys™ spectrophotometer (Thermo Fisher Scientific). Bacterial culture was performed in 125 mL baffled shake flasks for *Escherichia coli* at 35 °C, 300 rpm, with glycerol (10-15 mL/L). Batch and fed batch bioreactor studies were performed in Eppendorf BioFlo™ 320 and in DASbox™ Mini Bioreactor. The typical process was at 35 °C, at a DO setpoint of 30%, at a pH of 7.0, using an exponential feeding strategy, and using 400 g/L glucose or glycerol.

Enabling consistent manufacturing scale-up

The platform was evaluated using a widely used *E. coli* DH10B strain engineered to produce a plasmid for in vitro mRNA transcription (pIVT). The first step was to identify an appropriate feeding strategy to supply the strain with the required nutrients without accumulation. Feeding strategies and rates were evaluated at the 250 mL scale. In Figure 5, an exponential feed equation with a small exponential constant was selected.

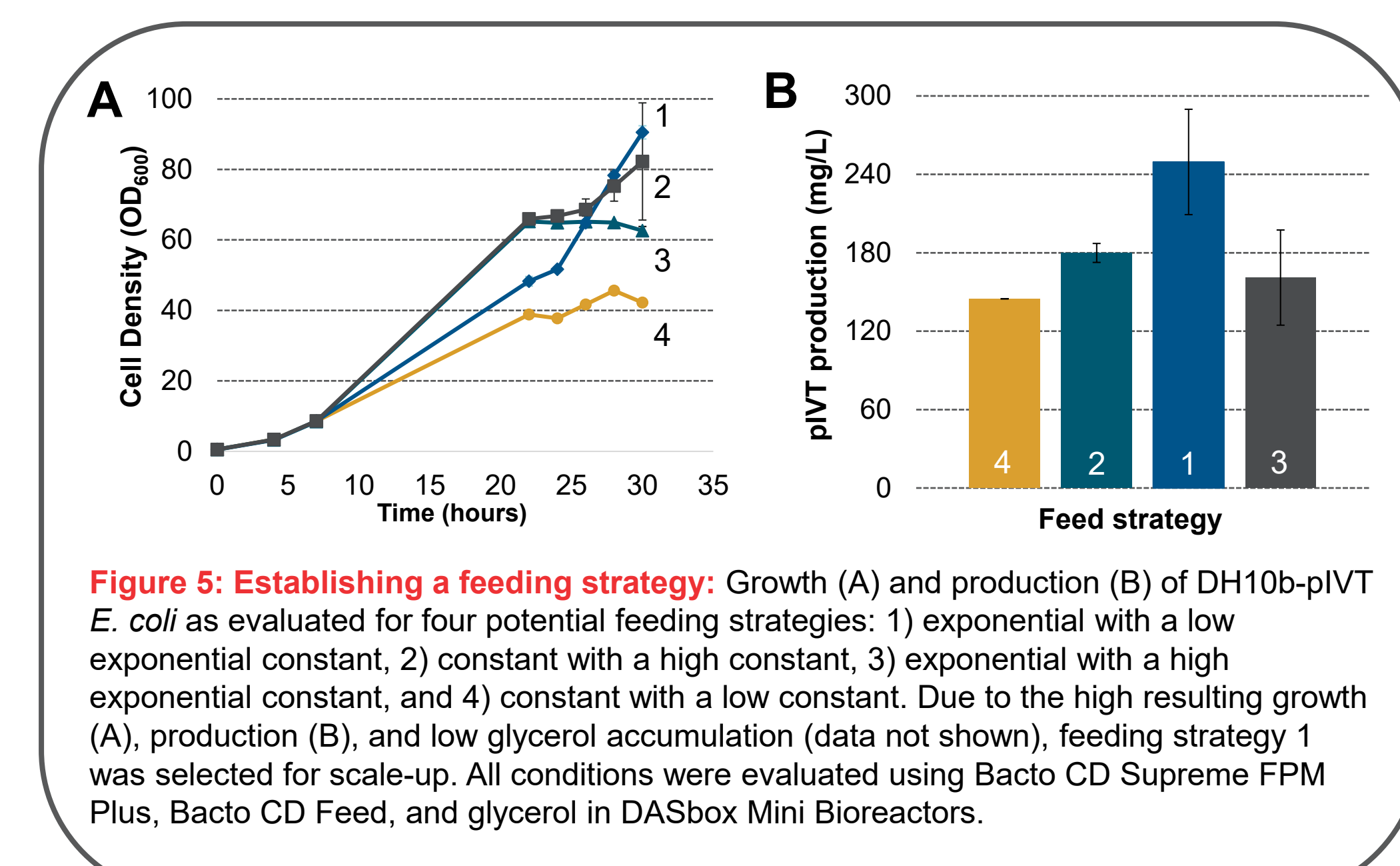


Figure 5: Establishing a feeding strategy: Growth (A) and production (B) of DH10b-pIVT *E. coli* as evaluated for four potential feeding strategies: 1) exponential with a low exponential constant, 2) constant with a high constant, 3) exponential with a high exponential constant, and 4) constant with a low constant. Due to the high resulting growth (A), production (B), and low glycerol accumulation (data not shown), feeding strategy 1 was selected for scale-up. All conditions were evaluated using Bacto CD Supreme FPM Plus, Bacto CD Feed, and glycerol in DASbox Mini Bioreactors.

After developing a feeding strategy at a 250 mL scale, scalability was evaluated from a 3 L benchtop to a 30 L pilot-scale in a single-use fermentation system. The results demonstrated that predictable, scalable, high-performance cell growth and plasmid production are achievable with the right platform medium and feed system.

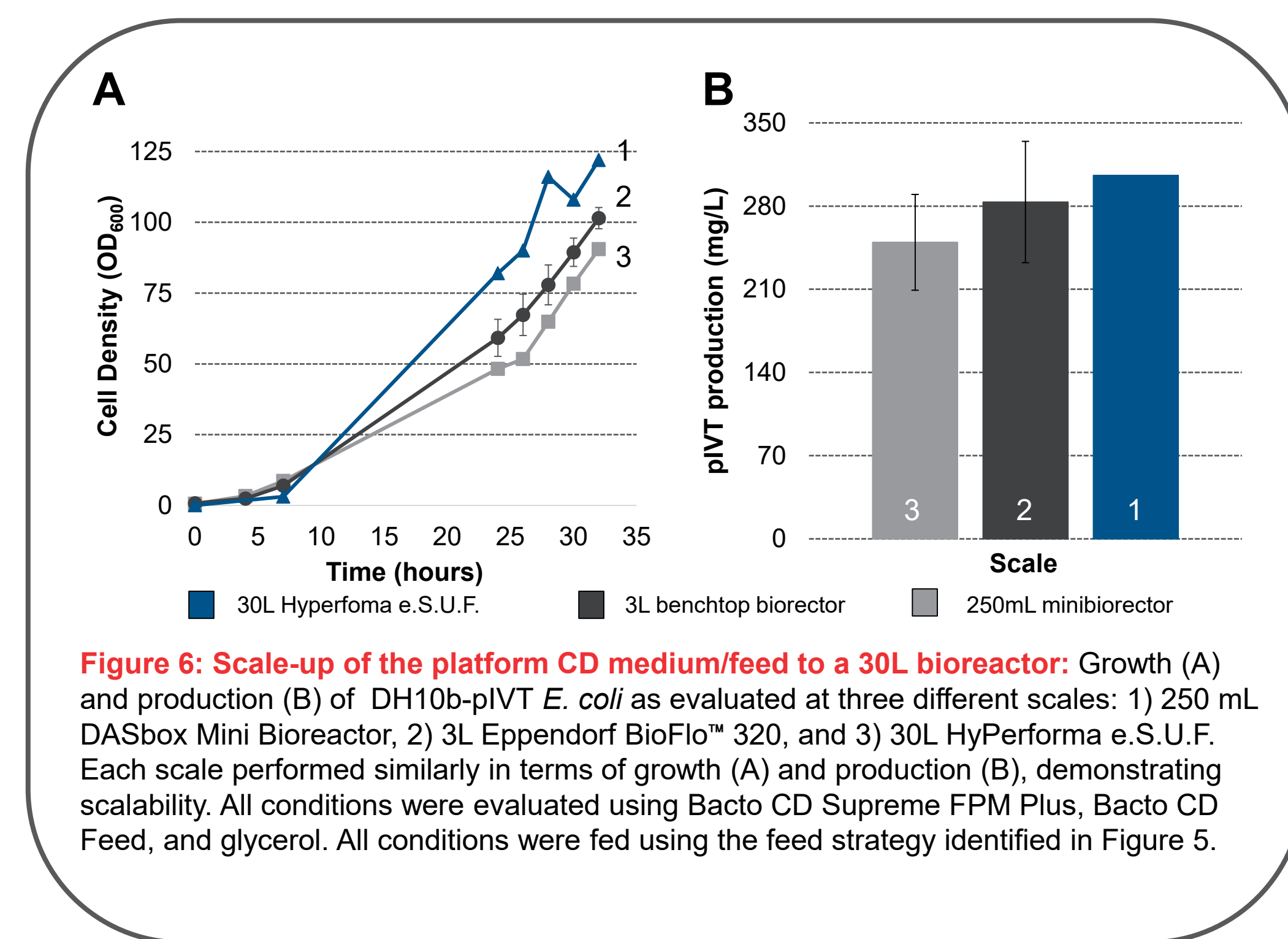


Figure 6: Scale-up of the platform CD medium/feed to a 30L bioreactor: Growth (A) and production (B) of DH10b-pIVT *E. coli* as evaluated at three different scales: 1) 250 mL DASbox Mini Bioreactor, 2) 3L Eppendorf BioFlo™ 320, and 3) 30L HyPerforma e.S.U.F. Each scale performed similarly in terms of growth (A) and production (B), demonstrating scalability. All conditions were evaluated using Bacto CD Supreme FPM Plus, Bacto CD Feed, and glycerol. All conditions were fed using the feed strategy identified in Figure 5.

TRADEMARKS/LICENSING

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