Developing Fed–Batch Strategy to Optimize Plasmid DNA Production in *E. coli* DH5α for Cost–Effective Manufacturing

Jacob Hromyak, Carson White, Dylan J. Vasconcelles, Dennis O. Concepcion, and Mahajoub Bello-Roufai
Akron Bio, 600 Tallevast Road, Suite 201 & 202, Sarasota, FL 34243

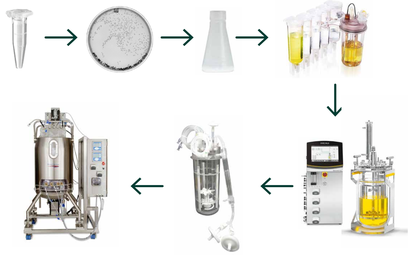
ABSTRACT

The biotechnology industry has seen a rise in the demand for plasmid DNA (pDNA) due to its applications in gene therapies and vaccines. However, meeting this demand poses challenges due to the yield and productivity of pDNA, which are affected by various factors such as host strain, plasmid type, growth medium, and growth conditions. To develop a high-yield and scalable process for pDNA production in *Escherichia coli* DH5α transformed cells, a three-level single-factor experimental design was employed. The focus was on changing the culture media composition. Recent improvements in small and large-scale pDNA yield and productivity were achieved by selecting the right media and optimizing the process. The results showed that using Media B in conjunction with a nutrient-dense feed supported high cell density cultures of up to an OD_{600} of 114, resulting in a volumetric yield of 946 mg/L and a specific yield of 8.93 mg/g. These yields and productivities were 7- to 9-fold times higher than conventional exponential fed-batch cultures in TB media.

BACKGROUND

- Akron Bio is an agile manufacturing partner to the cell and gene therapy industry, leveraging its portfolio of cytokines, media supplements, and plasmid and endonuclease manufacturing services to provide advanced therapy developers the scale, compliance, and regulatory support necessary to drive novel treatments from discovery to commercialization.
- Evaluate growth and kinetics of plasmid production and dependence on cell media composition and culture parameters
- Develop a robust protocol to support high cell density cultures and offer high yield, research–grade pDNA to meet the needs of research stage programs.

Plasmid	Size (kbp)
PLA01	9.1
PLA02	9.2
PLA03	11.3



MATERIALS & METHODS

- RCB generation from transformed clonal stock
- Media screening for growth kinetics and plasmid productivity
- AMBR 250M for conformation of media selection and to measure the effect of controlled DO on growth and plasmid
- Roche Cedex Bioanalyzer for offline measurement of optical density
- Qiagen Miniprep kit for purification and quantification of supercoiled pDNA
- Define phases of fermentation; Batch, Fed–Batch, Cool Down
- Optimization of temperature shift and cool down phase using AMBR 250M DoE to further increase plasmid yield
- Use of Biostat B Twin DCU, MFCS program, and 5L glass Univessel for execution of final developed process
- Custom 5.7L SUF scaled down version of Xcellerex XDR-50 bag powered by magnetic stir table

RESULTS

SHAKE FLASK

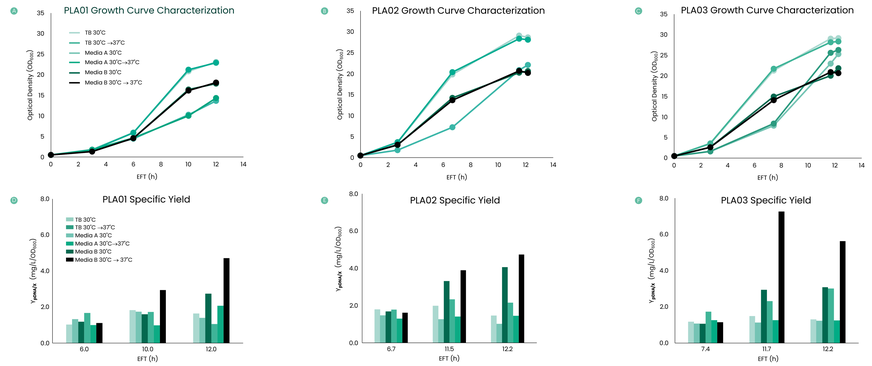


Figure 1: Growth curve characterization for media and temperature screening performed in 125 mL Thomson Ultra Yield shake flask for PLA01 (A), PLA02 (B) and PLA03 (C). Growth proceeded until stationary phase indicated by a decline in mu and/or optical density, Temperature shift occurred between EFT 6 – 8. Specific plasmid yields from screening study for PLA01 (D), PLA02 (E) and PLA03 (F). Specific plasmid yields from screening study. Only sample points with an optical density greater than 4.0 were submitted for analysis.

AMBR 250M

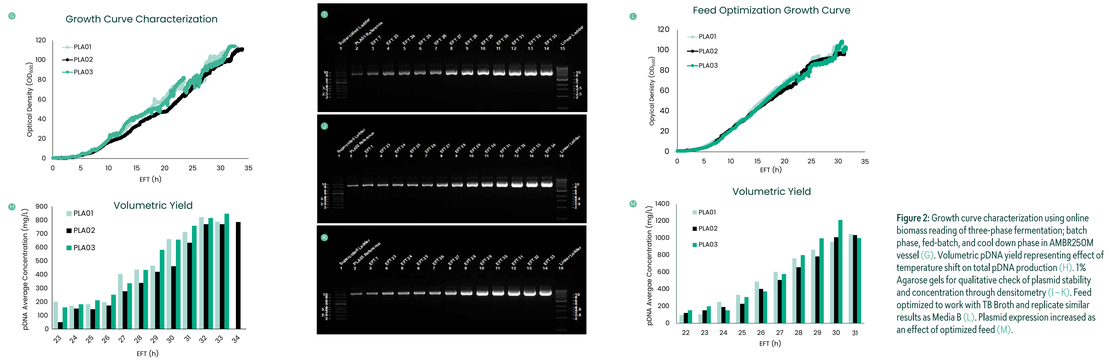


Figure 2: Growth curve characterization using online biomass reading of three-phase fermentation; batch phase, fed-batch, and cool down phase in AMBR250M vessel (G). Volumetric pDNA yield representing effect of temperature shift on total pDNA production (H). 1% Agarose gels for qualitative check of plasmid stability and concentration through densitometry (I – K). Feed optimized to work with TB Broth and replicate similar results as Media B (L). Plasmid expression increased as an effect of optimized feed (M).

5L BIOSTAT B/CERCELL

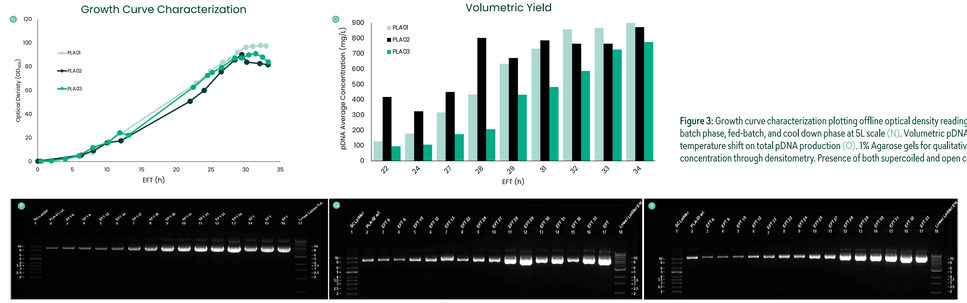


Figure 3: Growth curve characterization plotting offline optical density reading of three-phase fermentation batch phase, fed-batch, and cool down phase at 5L scale (N). Volumetric pDNA yield representing effect of temperature shift on total pDNA production (O). 1% Agarose gels for qualitative check of plasmid stability and concentration through densitometry. Presence of both supercoiled and open circular plasmid (P-R).

DISCUSSION

Shake Flask

- Shake flask media screening supports the use of Media B
- TB and Media A tend to support higher cell densities (Graphs A – C) in shake flasks, however, there is minimal plasmid production in comparison to other cell culture media
- Temperature shift has no effect on cell density; however, plasmid productivity increases exponentially (Graphs D – F)
- Media and temperature to achieve higher plasmid yield with lower cell biomass
- Cost and time savings for scale up purposes when evaluating GMP process at large scale

AMBR 250M

- Development of three–phase fermentation process; batch phase, fed–batch phase, cool down phase
- Necessity for fed–batch to reach high cell densities and cool down phase for plasmid isoform preservation
- Batch phase governed by depletion of glycerol determined by observation of DO spike and/or offline glycerol reading
- Feed rates based off exponential equation factoring in μ, biomass at start of feed phase, and specific carbon source consumption
- Cool down phase optimized for 2 hours; longer durations presented degradation of supercoiled plasmid
- Fed – Batch supports high cell densities for all plasmid models (Graph G)
- Supports previous findings of increased plasmid productivity from increasing temperature (Graph H)
- Agarose imaging and densitometry confirms increase in plasmid production (Images I – K)

Feed Optimization

- Optimized feed process was executed on TB Batch media measure effect of fed – batch TB cultures on cell density and plasmid growth (Graph L)
- Use of same protocol performed with Media B
- Cell density of TB culture were comparable to Media B cell cultures
- Presence of fed–batch with TB increased plasmid productivity of cells 9 to 10-fold (Graph M)

BIOSTAT B/CERCELL

- Execution of final developed process from AMBR 250M scale
- Comparable cell density growth to AMBR 250M, limitation due to adjustment of P.I.D. control (Graph N)
- pDNA yields representative of scale down model (Graph O)
- Process successfully transferred to Cercell SUF for PD engineering run of scaled down, 50L Xcellerex in manufacturing

CONCLUSION

- Development and optimization have led to 8 to 10x fold increase in pDNA titers from Akron's historical plasmid production process
- Process has served as the foundation for future development work with NEB Stable, Stbl3, and NEB5
- Further optimization work with other cell lines will explore increasing feed concentration to shorten length of fermentation while maintaining plasmid quality and concentration levels

REFERENCES/ACKNOWLEDGEMENTS

The whole Akron Bio team has been instrumental in the production of this work.