Optimizing of Bioreactor-Based Plasmid DNA Production in *E.coli*

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Introduction

Bulk quantities of double strand DNA (dsDNA) are required to manufacture a myriad of biomaterial precursors, such as hydrogel scaffolds. To meet this demand, we need access to gram scale quantities of DNA. Plasmid DNA (pDNA) is circular dsDNA that can be isolated from bacteria, but traditional flask culture methods produce low yields. To address this issue, our lab has established a simple and scalable method for the bulk production and purification of pDNA using a benchtop bioreactor and modified alkaline lysis coupled with anion exchange chromatography (**Fig. 1**).[2] With a current method in place, variables such as choice of plasmid, temperature, feed, pH, and oxygen saturation can be changed to maximize dsDNA produced by bacteria.

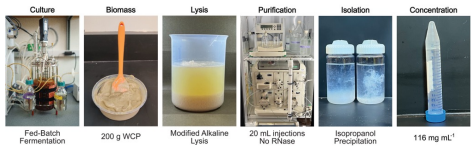

Figure 1. *Process overview for bulk DNA production and purification.*[2]

pDNA Production and Analysis

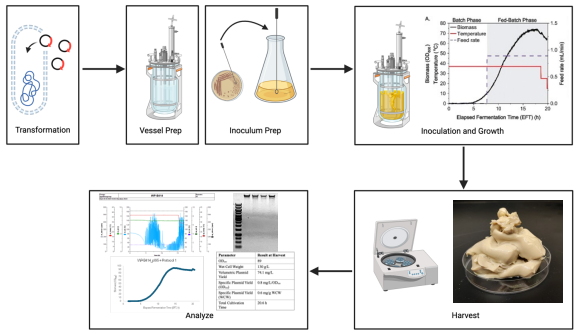

Figure 2. *Process schemata for bulk dsDNA production and extraction.*

Our current methods (**Fig. 2**) employ a simple fed-batch fermentation protocol where cells are grown at a constant temperature. After an initial batch phase, cells are provided nutrients at a constant rate for the remainder of the culture. These methods have produced volumetric plasmid yields as high as 208 mg/mL. This method has produced replicable results but up to 2.7 g/L has been reported in literature using more complex methods.

Bioreactor Operation: pEYFP vs pI95

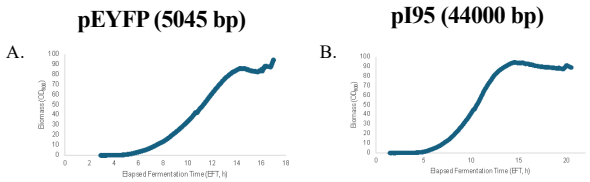

Figure 3. *Biomass accumulation of DH5α cell containing pEYFP (A) or pI95 (B) during culture growth monitored using BugLab probe.*

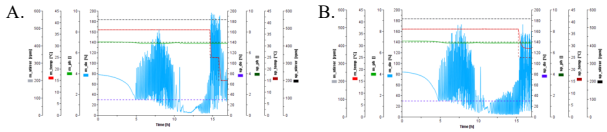

Figure 4. *Changes in stirrer, temp, pH and DO data monitored during the run for pEYFP (A) and pI95 (B).*

Table 1. Yield analysis for pEYFP (A) and pI95 (B). pEYFP run and the pI95 run had theoretical yields of 680 mg and 309 mg, respectively.

A.

Parameter	Result at Harvest
OD_{600}	88.9
Wet Cell Weight	163 g/L
Volumetric Plasmid Yield	136 mg/L
Specific Plasmid Yield (OD_{600})	1.5 mg/L/OD_{600}
Specific Plasmid Yield (WCW)	0.85 mg/g WCW
Total Cultivation Time	17 h

B.

Parameter	Result at Harvest
OD_{600}	89
Wet Cell Weight	130 g/L
Volumetric Plasmid Yield	74.1 mg/L
Specific Plasmid Yield (OD_{600})	0.8 mg/L/OD_{600}
Specific Plasmid Yield (WCW)	0.6 mg/g WCW
Total Cultivation Time	20.6 h

Despite similar growth profiles, and changes in DO/pH, yields for the culture containing pEYFP was high. This is likely due to the significant difference in size between the two plasmids. Larger plasmids are metabolically more challenging for cells to make leading to lower yields.

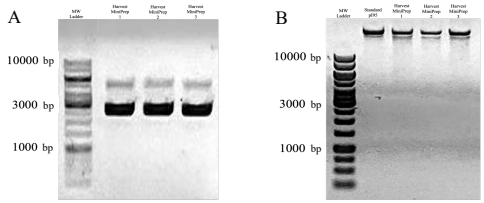

Figure 6. *Gel electrophoresis of mini prep samples taken at harvest for pEYFP (A) and pI95 (B). Electrophoresis is used to assess extent of supercoiled (SC) and open circle (OC) isoforms present at the end of cultivation. On average, pEYFP samples were 84% SC and 16% OC while pI95 samples were 20% SC and 80% OC. This will be compared to samples purified in-house to determine if our method significantly impacts SC/OC content.*

Future Work

Next steps will focus on alternate protocols with more complex methods to maximize pDNA yields.

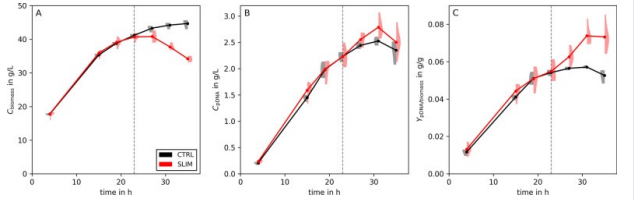

Figure 7. *Limiting sulfate during the growth has decreased biomass, but increased yields of pDNA.*[3]

Reduction of magnesium sulfate in the feed medium, could possibly increase current DNA yields by 10 fold.[3]

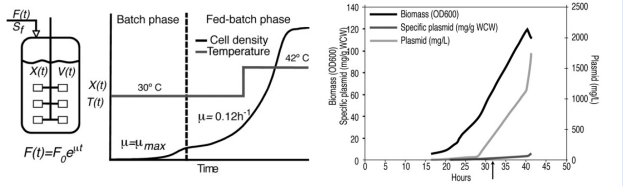

Figure 8. *An alternate protocol that utilizes a temperature shift and exponential federate can be implemented with pUC origin vectors to increase yields as high as 2.7 g/L when grown with proprietary media.*[1]

For our next run we will test out a more complex protocol that uses a temperature shift in combination with an exponential federate to drastically increase pDNA yields. This approach is only compatible with pUC origin vectors which we have a variety of in our lab. Literature reports yields as high as 2.7 g/L but this requires access to their proprietary media. This process will likely still result in higher yields but will require more optimization.

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