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Application Note:
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Optimization of Plasmid DNA Production in *Escherichia coli* Utilizing the Thermo Scientific MaxQ 8000 Incubated Stackable Shakers

Robert Reames, Production Manager, Aldevron
Meagan Gelinske, Microbiologist, Aldevron
Shyam Thapa, R&D Scientist, Aldevron

KEY WORDS

- ***E. coli* cultures**
- **Plasmid DNA (pDNA)**
- **Plasmid growth optimization**
- **MaxQ 8000 orbital shaker**

Summary

This application note describes an experiment used to optimize plasmid DNA yield in *E. coli* shake flask cultures using Thermo Scientific MaxQ 8000 incubated shakers. We were able to determine the effect that agitation speed and medium type had on cell density in shake flask cultures, and develop an optimized protocol for the production of plasmid DNA. This optimization resulted in increased plasmid DNA yields up to 16-fold higher than traditional growth protocols.

Introduction

Escherichia coli are commonly used for plasmid DNA (pDNA) production. There are many laboratory strains of *E. coli* that have been developed for this purpose, each with specific mutations designed to optimize plasmid yield and quality, depending on variations in each plasmid of interest. The importance of producing pDNA in *E. coli* is steadily increasing in the biotechnology industry due to its many uses, including DNA vaccine development, gene therapy research, diagnostic kits, recombinant protein expression, and other applications. While there has been extensive research focused on developing growth strategies for pDNA production in fermentors, the majority of research applications do not warrant this approach due to the time and costs associated with optimizing fermentation strategies. Therefore, this facilitates the need for developing new and improved growth protocols in order to optimize pDNA production in shake flasks. When shake flask growth is optimized, it can greatly decrease growth volumes required, which in turn decreases overall costs and increases throughput. This application focuses on *E. coli* shake flask growth optimization using the MaxQ™ 8000 incubated stackable shakers.

Maximizing *E.coli* Cell Density in Shake Flask

The yield of pDNA is typically expressed in milligrams of pDNA per Liter of culture (mg/L). There are many sequence dependent factors that can affect the yield of a plasmid DNA vector including gene toxicity, origin of replication, plasmid size, and structural motifs. Because these factors are inherently dependent on the sequence of the plasmid they typically are not able to be changed or optimized. Therefore, this application focuses on optimizing the cell density of *E. coli* shake flask cultures to increase plasmid yield.

Increasing cell density in shake flasks has been addressed by many different strategies. Most have involved development of an enriched medium with additional buffering capacity to maintain pH, typically in the form of phosphate, and additional carbon source, typically in the form of glycerol or glucose, to supply cells with the energy source needed. These enriched media are typically not fully utilized without considering the dissolved oxygen (DO) needed to support high cell densities. Bacterial cultures need to have adequate DO available for optimal cell growth and pDNA production. As cell density increases during growth, the DO requirements will also increase. The relative amount of DO generated by agitation is proportional to the shaking speed of the flask as measured in revolutions per minute (RPM). Generally 200-250 RPM is the standard speed utilized for most shake flask cultures. Baffles can further enhance aeration by providing an additional mechanism for medium agitation.

This application note describes one of many experiments used to examine the effects of agitation on cell density and pDNA yield in *E. coli* shake flask cultures. MaxQ 8000 incubated shakers were selected due to the ability to be stacked three high and shake at 400 RPM.

Procedure

Three commonly used expression plasmids, pVAX1©, pcDNA™ 3.1, and pUC18 were each transformed into DH5α™ *E. coli* cells. Each transformation was plated on Luria Bertani (LB) agar plates with the appropriate antibiotic and incubated at 37° C overnight.

The following day one 10 mL LB broth starter culture with the appropriate antibiotic was made from each transformation plate using a single colony. After approximately 6 hours of incubation at 37° C, 1 mL of each log phase starter culture was used to inoculate 0.5L cultures of LB medium and RG medium (proprietary in-house enriched medium) as summarized in Table 1. All 400 RPM growths also received 0.25 mL of sterile 1:10 antifoam diluted in sterile water to control excessive foaming. Each growth was performed in a 2.5 L Ultra Yield™ baffled flask (Thomson

Medium Type	Growth Volume (mL)	Shaker Speed (RPM)	Incubation Period (Hours)
LB	500	200	16
RG	500	200	16
RG	500	400	16

Table 1. Growth Conditions

Instrument Company, Oceanside, California). All flasks were placed in MaxQ 8000 incubated stackable shakers set to the appropriate shake speeds and incubated at 37° C. A 12-hour growth curve analysis was also performed for pcDNA™ 3.1 by taking OD_{600} readings hourly starting at 5 hours postinoculation.

At 16 hours postinoculation 1.0 mL samples were taken from each LB medium culture and 0.2 mL samples were taken from each RG medium culture for determination of plasmid yield. These samples were harvested by centrifugation (5 minutes at 13,000 RPM), the medium was decanted, and the resulting harvested cell biomass from each culture was processed using a QIAprep® Spin Miniprep Kit in the QIAcube™ automated processing system. Yield was determined using a Thermo Scientific NanoDrop ND-1000 spectrophotometer. The remainder of each culture was harvested by centrifugation (15 minutes at 5,000 RPM) for determination of wet cell biomass weight. Purified pDNA fractions were analyzed for purity and homogeneity by agarose gel electrophoresis (AGE).

Results

Under this design, agitation was shown to have a critically important role in increasing cell density of *E.coli* shake flask cultures when using an enriched medium. On average, the OD_{600} and biomass of cultures grown at 400 RPM in RG medium was shown to be nearly twice that of identical cultures grown at 200 RPM (Figures 1 and 2).

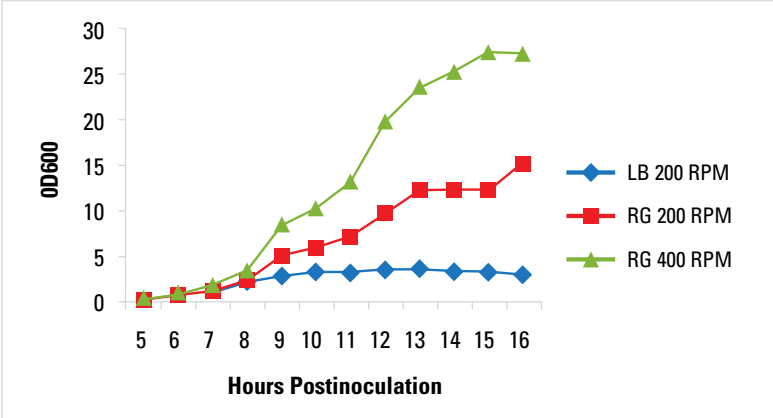


Figure 1. pcDNA™ 3.1 Growth Curve

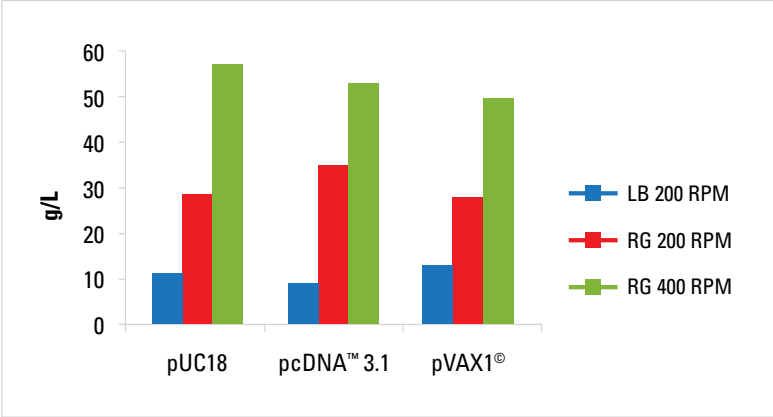


Figure 2. Cellular Biomass

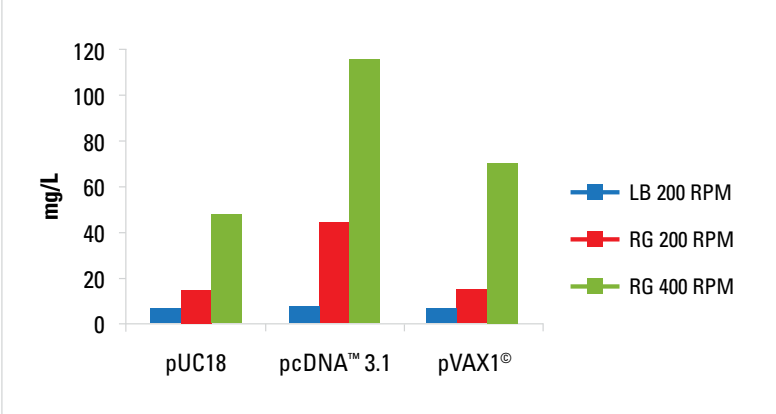
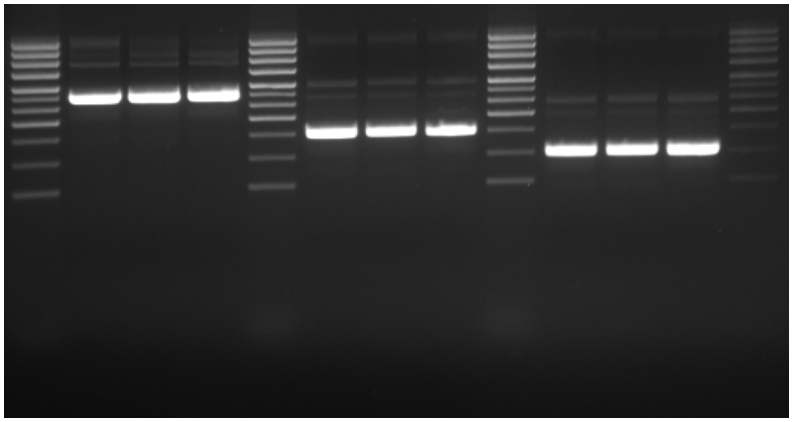


Figure 3. pDNA Yield



Lane 1: Invitrogen™ Supercoiled DNA Ladder
Lane 2: pcDNA™ 3.1 – LB 200 RPM
Lane 3: pcDNA™ 3.1 – RG 200 RPM
Lane 4: pcDNA™ 3.1 – RG 400 RPM
Lane 5: Invitrogen™ Supercoiled DNA Ladder
Lane 6: pUC18 – LB 200 RPM
Lane 7: pUC18 – RG 200 RPM
Lane 8: pUC18 – RG 400 RPM
Lane 9: Invitrogen™ Supercoiled DNA Ladder
Lane 10: pVAX1© – LB 200 RPM
Lane 11: pVAX1© – RG 200 RPM
Lane 12: pVAX1© – RG 400 RPM
Lane 13: Invitrogen™ Supercoiled DNA Ladder

Figure 4. AGE Analysis of pDNA. Samples were run on a 1% agarose gel with ethidium bromide and visualized under UV light. Each lane represents 200ng of pDNA.

These results also indicated a significant increase in pDNA yield. On average, the pDNA yield of cultures grown at 400 RPM in RG medium was 3-fold to 5-fold higher than in identical cultures grown at 200 RPM. When compared to the most commonly used bacterial growth medium, LB, pDNA yields were 8-fold to 16-fold higher using a combination of enriched medium and high agitation speeds (Figure 3). Plasmid quality appeared identical for all growth conditions on AGE (Figure 4).

Discussion

This procedure discusses a method for shake flask optimization of pDNA production in *E.coli*. Using the MaxQ 8000 incubated stackable shakers, pDNA-bearing *E.coli* cultures were grown in different medium types at different agitation speeds. The effect that agitation speed and medium type had on cell density in shake flask cultures was used to develop an optimized protocol for the production of pDNA.

Important to the success of this project was the ability to shake cultures at high agitation speeds. MaxQ 8000 incubated stackable shakers have a shake speed range of 25-400 RPM and a space-saving stackable design (can be stacked up to 3 high), making these units ideal for optimized shake flask *E. coli* growth for pDNA production. In addition, the slide-out platforms allow different sized dedicated platforms to be exchanged with ease, replacing the time-consuming task of replacing flask clamps.

Figure 5. Thermo Scientific MaxQ 8000 Incubated Stackable Shakers

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