APPLICATION NOTE #201413

Total Genomic DNA and Protein Extraction from Escherichia coli K12 Cells on the Bead Ruptor 4

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Introduction

Escherichia coli (E. coli) is frequently used as a model organism in microbiology and molecular biology studies. E.coli was one of the first organisms to have its genome completely sequenced which has allowed it to be a major contributor to recombinant DNA technology. The process of gene cloning in E.coli involves a series of steps including the isolation of target DNA or protein. Analyte extraction is typically done through sonication or bead milling. Sonication is the most popular technique for extracting proteins. With the sonication process, the cells are lysed via cavitation produced by a high frequency oscillating probe to disrupt cellular membranes releasing the internal cellular components. There are a number of drawbacks to the use of sonicators including excessive heat generation, variations in yield and sonicators are only able to process one sample per cycle. Ultrasonic homogenizers also shear DNA making them unsuitable for many DNA applications.

As an alternative to ultrasonic homogenizers, bead mills are higher throughput homogenizers that are able to process multiple samples at once through the rapid oscillation of tubes containing high speed projectiles that impact and dissociate cells. The efficiency of the bead milling process allows it to quickly extract cellular compounds from cultured cells such as E. coli while maintaining their molecular integrity. The Omni International Mini Bead Ruptor 4 (BR 4) is designed as a personal use bead mill to facilitate efficient lysis of tissues and cells.

The BR4 is capable of homogenizing up to four 0.5 mL, 1.5 mL, 2mL or 7 mL samples using bead media to assist in sample disruption process.

Here, we demonstrate the extraction of DNA and protein from E. coli K12 cells using the Bead Ruptor 4. Extraction efficiency and analyte integrity was evaluated.

Materials & Methods

Equipment

- **Bead Ruptor 4** (Cat #25-010)
- **2 mL Tough Mico-Organism Lysing Mix** (Cat #19-622)
- **Zymo Research ZR Fungal/Bacteria DNA Miniprep** (Cat #D6005)

Sample Temperature

0.7 g of 0.5 mm glass beads was added to five 2 mL screw cap polypropylene tubes with 1 mL of DDH2O water. Each tube was processed in the Bead Ruptor 4 at all available speeds (1-5) for 30, 45, 60 and 75 seconds and the intratube temperature was recorded.

DNA Extraction and Separation

5 mL of E. coli K12 stock culture (Carolina Biological) was divided into three 1.6 mL aliquots in 2 ml polypropylene reinforced tubes (Cat# 19-647). Each aliquot was centrifuged at 11,000 x g for 10 minutes to pellet cells. The supernatant was removed and each cell pellet was re-suspended in 750 µl of lysis solution provided from the ZR Fungal/Bacterial DNA Miniprep kit (ZYMO Research).

Each cell suspension was added to 2 mL tubes containing 0.5 mm glass beads (Cat# 19-622). The suspensions were then homogenized in the Bead Ruptor 4 (Cat# 25-010) at speeds of 3, 4 and 5 for 45 seconds. After processing, the lysates were transferred to clean 2 mL tubes and centrifuged at 10,000 x g for 1 minute to pellet any cell debris. DNA extraction was carried out per the manufacturer's instructions using the ZYMO Research Miniprep kit. 1 µl of the DNA elution was quantified on a NanoDrop Spectrophotomer (Thermo Fisher) to determine DNA yields.

10 ul of each sample was mixed with 10ul of sample buffer (Bio-Rad). The DNA was separated on a 5% TBE agarose gel (Bio-Rad) at 150 V for 45 minutes and stained in ethidium bromide for 15 minutes. The gel was washed for 10 minutes in DD H2O and visualized on a GelDoc EZ System (Bio-Rad).

Protein Extraction and Separation

A 15 mL nutrient broth culture of E.coli K12 was grown up overnight at room temperature. The cells were harvested by centrifuging the culture at 3,000 x g for 10 min. The cells were washed twice and resuspended in 1 ml Tris-HCL (pH 7.6). The suspension was added to a 2 mL tube containing 0.7 g of 0.5 mm glass beads and processed on the Bead Ruptor 4 for 60 seconds at a speed of 5. The lysate was then cooled on ice for 5 minutes then centrifuged at 11,000 x g for 5 minutes to pellet cell debris. The supernatant was removed and cooled on ice for another 5 minutes. 1 µl of the supernatant was analyzed at 280 nm on a NanoDrop spectrophotometer to determine protein

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Protein Extraction and Separation (cont.)

concentrations. 10 µl of each sample was added to 10 ul of Laemmli sample buffer (Bio-Rad) and heated at 95°C for 5 minutes. The proteins were then separated by electrophoresis on a 4-20% Tris-Glycine SDS polyacrylamide gel (Bio-Rad) at 150 V for 45 minutes in Tris-Glycine SDS running buffer (Bio-Rad). The gel was silver stained using the ProteoSilver Stain kit (Sigma-Aldrich) per the manufacturer's instructions and visualized on a GelDoc EZ System.

Results

In this study we determined the Bead Ruptor 4's capability of extracting DNA and proteins from E. coli K12 cells. The isolation of these compounds from E. coli cells is critical because E. coli is used extensively as a host for introduction of DNA and protein sequences and is a critical scientific research tool.

As a first step, the intratube sample temperature was monitored over time and as a function of BR4 speed setting to determine the extent of temperature increase due to the homogenization process. The purpose of this study was to evaluate temperature as a function of processing speed and time. It was observed to even at the highest speed setting and processing time of 75 seconds the intratube temperature did not exceed 34°C (Figure 1). By precooling the tubes to 4°C prior to processing, extended processing can be performed at the highest speed of five without temperatures exceeding 20°C for up to 75 seconds. This makes the BR 4 ideal for extraction of heat sensitive compounds such as RNA from very tough tissues.

DNA and protein yields were quantified by spectrophotometry (Table 1). Genomic DNA yields averaged 23 ng/µl and were independent of speed setting. Genomic DNA was fully liberated from the E. coli cells at speed exceed 3 with no increase in DNA yield observed at higher speeds. Proteins were extracted at a concentration of 1.08 mg/ml. Gel electrophoresis indicated that the extracted genomic DNA was of good quality with no shearing observed as evident from the single bands in lanes two through four (Figure 2).

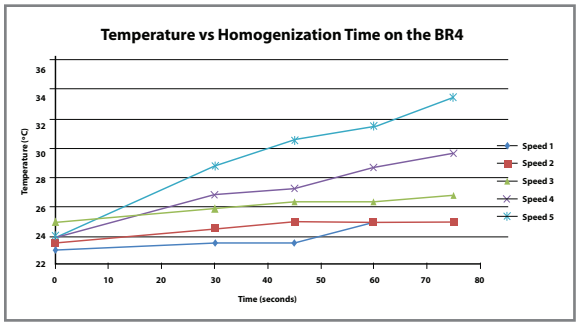

Figure 1
Intratube temperature as a function of homogenization speed and processing time. It was observed that at speeds below three the temperature increase was neglible even after processing for 75 seconds.

Bead Ruptor 4 Speed Setting (Time = 60 sec)	Average DNA Concentration (ng/ul)	Average Protein Concentration (mg/ml)
3	21.07	NA
4	25.03	NA
5	22.63	1.08

Table 1 DNA and protein concentrations measured post homogenization.

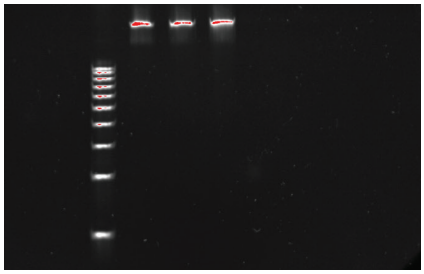

Figure 2 DNA agarose gel electrophoresis of E.coli K12 lysate. Lane 1: 100bp ladder. Lane 2: Speed 3 DNA. Lane 3 DNA: Speed 4 DNA. Lane 4: Speed 5 DNA.

The extraction procedure was further evaluated by protein gel electrophoresis (Figure 3). Figure four indicates that proteins with a broad molecular weight range were extracted from the E. coli cells following homogenization on the Bead Ruptor 4.

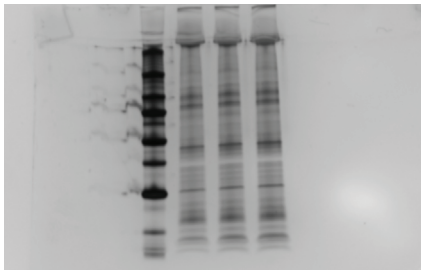

Figure 3 Protein polyacrylamide gel electrophoresis of E.coli K12 lysate. Lane 1: Protein Ladder. Lane 2-4: E. coli K12 protein extracts

Conclusion

The Bead Ruptor 4 is capable of disrupting E. coli K12 cells up to a speed of 5 quickly and with very little heat generated. The DNA extraction was achieved at speeds in excess of three with no detectable shearing. Protein yields were significant and the extraction procedure liberated proteins with a broad molecular weight range within forty seconds of processing.

Bead Ruptor 4: 25-010

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