High Throughput Lysis of E. coli for Multiple Analytes Using the Bead Ruptor 96

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APPLICATION NOTE

High Throughput Lysis of E. coli for Multiple Analytes Using the Bead Ruptor 96

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Introduction

Bacterial research is an ongoing and ever-changing realm of science. In the past, researchers have been limited to testing bacteria in culture environments. Now, with newer technology, researchers rely less on older methods of plating or growing, and more on the latest genomic and proteomic methods for their research such as qPCR and mass spectrophotometry. To support these research needs, sample prep methods and products need to be developed so that bacteria can be processed for multiple types of downstream analysis. The 96 well plate format allows the researcher to conduct multiple types of analyses in one plate without the hassle of multiple samples in separate tubes . Here, we evaluated the lysis and recovery of DNA, RNA, and proteins from E. coli using the Omni Bead Ruptor 96 and an Omni 96 well plate with 0.1mm ceramic bead media. All three analytes were evaluated for purity and concentration.

Materials and Methods

- **Omni Bead Ruptor 96 (Cat. No. 27-0001)**
- **Omni 96 well plate with 0.1mm ceramic bead media (Cat. No. 27-6006)**
- **Omni Bacterial DNA Kit (26-008B)**
- **Omni Bacterial RNA Kit (26-011B)**

Bead Ruptor 96 Cat#27-0001

Sample Preparation and Separation

Escherichia coli MM294 cultures were grown in 6mL of Tryptic Soy Broth (TSB) for 16 hours at 37°C. Three different cultures of E. coli were centrifuged at 8000 x g for 5 min minutes to form a cell pellet in each tube. These pellets were resuspended in separate buffers that were specialized for recovery of specific analytes. For DNA recovery, the pellet was resuspended in 700µL of DNA lysis buffer (DLB) and 10 uL Antifoam provided in the Omni DNA extraction kit. The pellet that was chosen for RNA extraction was resuspended in 700µL prechilled RNA lysis buffer (RLB)and 10 uL Antifoam, and the pellet that was chosen for proteins was resuspended in 900µL of phosphate buffered saline pH. 7.2 (PBS). All 3 of the resuspended cultures were transferred into duplicate wells in one Omni 96 well plate with 0.1mm ceramic beads. The 96 well plate was placed in the Omni Bead Ruptor 96, and the processing parameters were set to 30 hertz for 2 minutes. Once homogenization of the cells was completed each different extract from the 96 well plate was removed and analyzed with different protocols.

DNA extraction

The homogenate from the 96 well plate was transferred to 2 microcentrifuge tubes and centrifuged at 10,000g for 5 mins. The supernatant was then used as the protocol required in step 8 of the Omni Bacterial DNA Kit, and the rest of the extraction was completed by following the remaining steps provided in the DNA extraction kit. A second set of extractions were preformed using the provided lysozyme in the Bacterial DNA Extraction Kit to compare eluted DNA quality between extraction methods. The enzymatic digestion of the bacteria was carried out according to the extraction kit's instructions. Eluted DNA was quantified on a Nanodrop 2000 and imaged for genomic quality using 150µg of genomic DNA and a 1% agarose gel stained with ethidium bromide.

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RNA extraction

Immediately after processing on the Bead Rutor 96, the homogenate was pipetted from the 96 well plate into microcentrifuge tubes that were kept on ice to preserve RNA integrity. After icing the sample, the Omni Bacterial RNA Extraction Kit instructions were followed starting at step 7. The sample remained on ice during the extraction to prevent the RNA from degrading. All centrifuge steps were performed at 4°C. Eluted RNA was examined for quality and quantity using an Agilent Bioanalyzer 2100 and an RNA Nano 6000 chip (Cat. No. 5067-1511)

Protein extraction

The homogenate from the 96 well plate was analyzed alongside the extract from a 1% Triton X100 sample for total protein content. Total sample proteins were quantified spectrophotometrically using a Nanodrop 2000. Then, equal amounts of proteins were loaded into a Bio-Rad 4-20% TGX gel (Cat. No. 4561091), and proteins were separated by size with electrophoresis. The TGX gel was stained with Coomassie Brilliant Blue kit (Thermo Fisher Cat. No. LC6060) followed by de-staining. The image captured on a Bio-Rad Gel Doc (Cat. No. 1708270EDU).

Results

DNA recovered from the use of the Omni Bead Ruptor 96 and a 96 well plate with 0.1mm ceramic bead media was able to recover a consistent and pure sample of DNA (Figure 1). DNA purity was determined by dividing the absorbance of the sample at 260nm by the absorbance of the sample at 280nm. Samples that report a ratio of 1.8 to 2 are considered pure DNA. The DNA sheared after 1 minute of bead beating as seen in Figure 2. However, the yield of DNA was comparable to the enzymatic digestion and was completed in 2 minutes rather than with an hour of incubation in lysozyme. This data suggest that high quality DNA can be isolated from cells using the Omni Bead Ruptor 96.

RNA was recovered from the homogenate (as mentioned above) using an Omni Bacterial RNA Purification Kit. An average concentration between the two samples of 28.9ug/ul as seen in Figure 4. The RIN score generated by the Agilent 2100 software for both samples reported RIN scores of 9.9 or greater. RIN values are an algorithmically generated score that calculates the integrity of the RNA sample on a scale from 1-10 with 1 being the most degraded and 10 being the least degraded. These results suggest that the Omni Bead Ruptor 96 can be used to successfully isolate high quality RNA.

Proteins was isolated from the sample and higher concentration were observed using the Omni Bead Ruptor 96 than when using Triton x-100 alone (Figure 5). Similar band patterning was observed in both lanes which suggest that the Omni Bead Ruptor 96 can be used to isolate protein, at a potentially higher concentration than detergent alone, and proteins are similar in detectability (Figure 6.)

Conclusions

The Bead Ruptor 96 in combination with the Omni 96 well plate (0.1mm ceramic bead media) is capable of lysing bacteria comparable to methods of lysing which require a detergent. DNA from bead beating does become more sheared than that of enzymatically lysed cells, but the quantity and quality of DNA recovered from bead beating is higher due to a higher lysis efficiency. More lysis efficiency is also seen in the protein extraction where bead beating was compared to lysis with a detergent. The RNA extracted proved to be very pure and intact as seen in the RIN values provided by the bioanalyzer. Analytes released by bead beating in this plate can be used in a variety of downstream analysis such as PCR, RT-PCR, or western blotting. This plate can be integrated with other 96 well plate format systems that require high throughput sample preparation which need to liberate a maximum amount of the desired analyte.

Nanodrop Averages		
	DNA yeild (ng/uL)	260/280
Bead Mill 1	7.5	1.95
Bead Mill 2	5.7	2
Enzymatic	6.2	1.9

Figure 1. DNA quantities from duplicate samples.

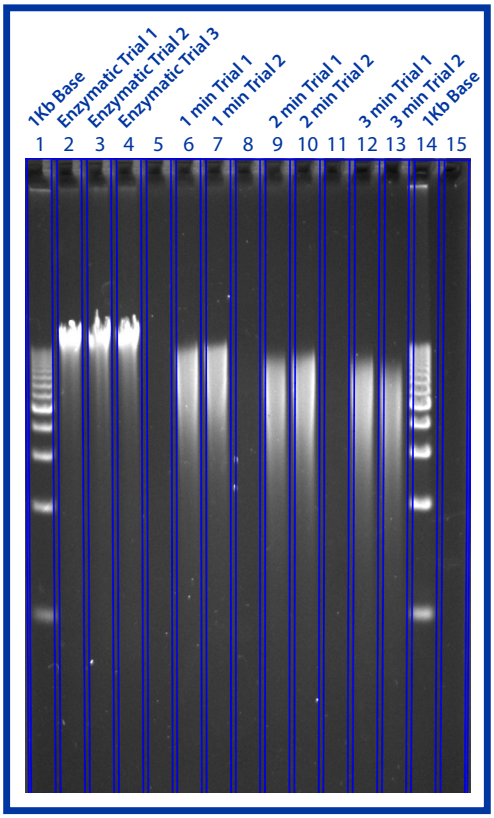

Figure 2. DNA gel from eluted DNA of both Enzymatic and Bead Beating lysis.

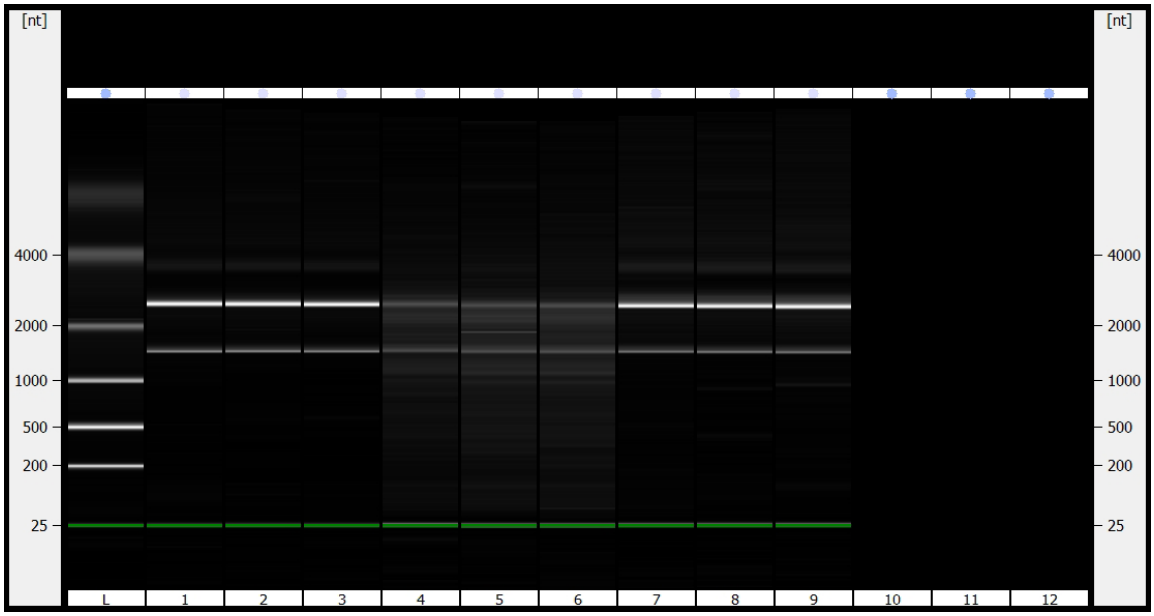

Figure 3. Gel like image from Agilent 2100 Bioanalyzer showing the quality of the duplicate extractions in lanes 1-3(Sample 1) and 7-9 (Sample 2).

Average yields from bioanalyzer		
	Concentration (ng/uL)	RIN score
Sample 1	35.5	10
Sample 2	22.3	9.85

Figure 4. Bacterial RNA average recovery from *E. coli*.

Total Protein Average Concentration	
	Concentration (ng/uL)
Bead Beating	17.3
Triton-x-100	4.8

Figure 5. Protein concentrations from both bead milling and Triton x100 lysis.

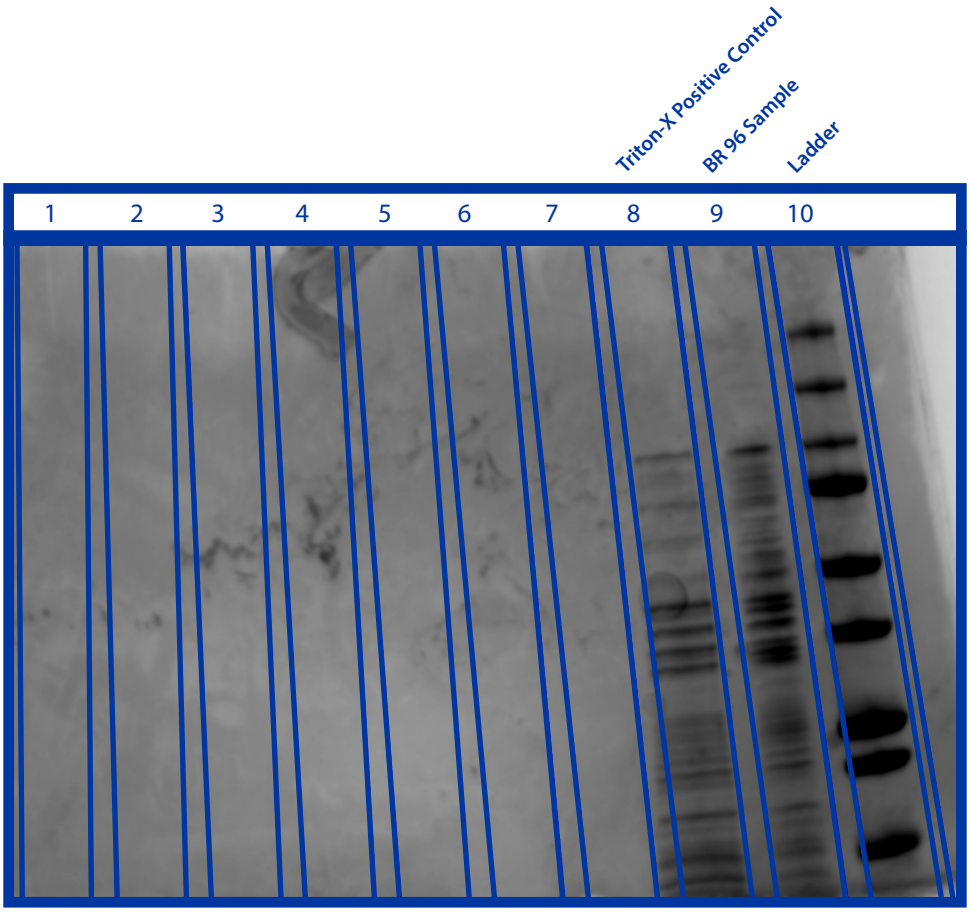

Figure 6. Protein repertoire of the lysed samples as shown with Coomassie Blue.

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