APPLICATION NOTE

# High-throughput lysis of *E. coli* for multiple analytes using the Omni Bead Ruptor 96 bead mill homogenizer.


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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 bead mill homogenizer and a 96-well plate with 0.1 mm ceramic bead media. All three analytes were evaluated for molecular integrity and concentration.

## Materials and methods

- **Omni Bead Ruptor 96 bead mill homogenizer (Cat # 27-0001)**
- **0.1 mm Ceramic Beads Pre-Filled 2 mL 96 Deep Well Plates (Cat # 27-6006)**
- **Bead Ruptor 96 Well Plate Adapters (Cat # 27-101)**

**Omni Bead Ruptor 96 bead mill homogenizer**

For research use only. Not for use in diagnostic procedures.

**Sample preparation and separation**

*Escherichia coli* MM294 cultures were grown in 6 mL 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 700 µL of commercially available tissue DNA lysis buffer. The pellet that was designated for RNA extraction was resuspended in 700 µL prechilled commercially available tissue RNA lysis buffer, and the pellet that was chosen for proteins was resuspended in 900 µL of phosphate buffered saline (PBS) pH. 7.2. All 3 of the resuspended cultures were transferred into duplicate wells in one 96-well plate pre-filled with 0.1 mm ceramic beads. The 96-well plate was placed in the Omni Bead Ruptor 96 bead mill homogenizer, and the processing parameters were set to 30 Hz 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 10,000 x g for 5 mins. The supernatant was then used as the protocol required per commercially available bacterial DNA extraction kit. A second set of extractions were preformed following the normal protocol for commercially available bacterial DNA extraction kit with the addition of a lysozyme digestion step prior to loading the lysate onto the spin-column in the initial instance. Eluted DNA was quantified on a Nanodrop 2000 and imaged for genomic integrity using 150 µg of genomic DNA and a 1 % agarose gel stained with ethidium bromide.

**RNA extraction**

Immediately after processing on the Omni Bead Ruptor 96 bead mill homogenizer, the homogenate was pipetted from the 96-well plate into microcentrifuge tubes that were kept on ice to preserve RNA integrity. A commercially available bacterial RNA extraction kit protocol was followed hereafter according to manufacturer's instructions. 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 integrity and quantity using an Agilent Bioanalyzer 2100 and an RNA Nano 6000 chip

**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 4-20 % TGX gel (Bio-Rad, Cat #4561091), and proteins were separated by size with electrophoresis. The TGX gel was stained with Coomassie Brilliant Blue kit (Thermo Fisher Scientific) followed by de-staining. The image captured on a Bio-Rad Gel Doc Imager.

## Results

DNA recovered from the use of the Omni Bead Ruptor 96 bead mill homogenizer well plate homogenizer and a 96 well plate with 0.1 mm ceramic bead media was able to recover a consistent and pure sample of DNA (Figure 1). DNA integrity was determined by dividing the absorbance of the sample at 260 mm by the absorbance of the sample at 280 nm. 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 DNA can be isolated from cells using the Omni Bead Ruptor 96 bead mill homogenizer.

An average RNA concentration between the two samples of 28.9 µg/µL 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 bead mill homogenizer can be used to successfully isolate RNA.

Proteins was isolated from the sample and higher concentration were observed using the Omni Bead Ruptor 96 bead mill homogenizer 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 bead mill homogenizer can be used to isolate protein, at a potentially higher concentration than detergent alone, and proteins are similar in detectability (Figure 6.)

## Conclusions

The Omni Bead Ruptor 96 bead mill homogenizer in combination with the 96-well plate (0.1 mm 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 integrity 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 yield (ng/µL) | $A_{260}/A_{280}$ |
| 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.

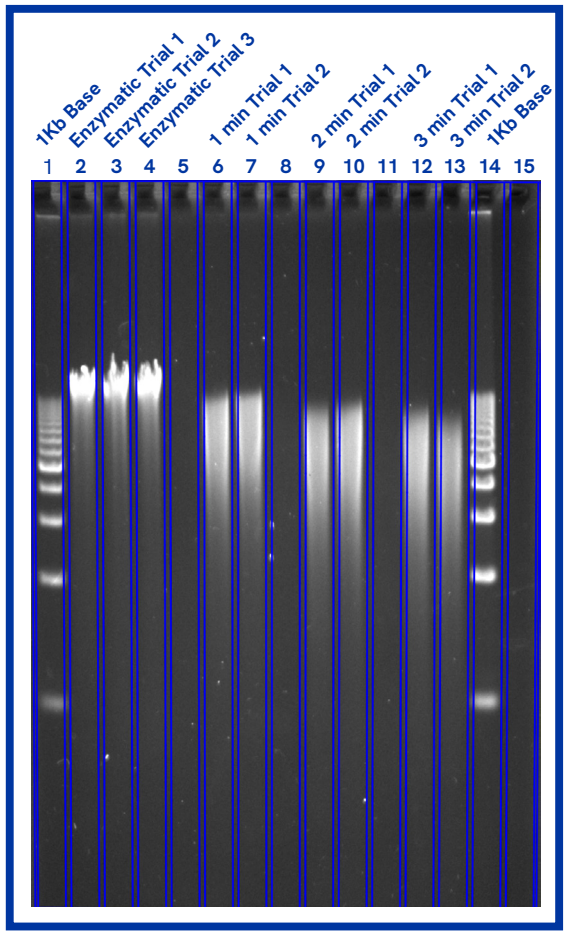


Figure 2: DNA gel from eluted DNA of both enzymatic and bead-beating lysis.

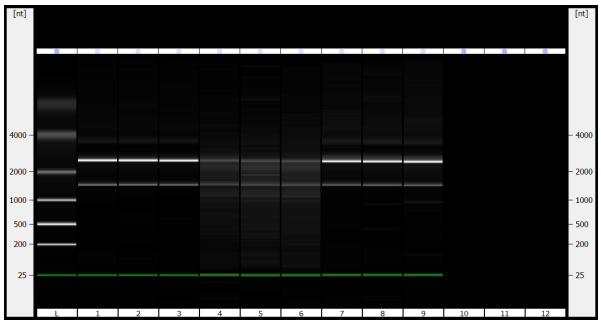


Figure 3: Gel-like image from Agilent 2100 Bioanalyzer showing the integrity of the duplicate extractions in lanes 1-3 (Sample 1) and 7-9 (Sample 2).

| Average yields from bioanalyzer | | |
|---|---|---|
| | Concentration (ng/µL) | 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/µL) |
| Bead beating | 17.3 |
| Triton X-100 | 4.8 |

Figure 5: Protein concentrations from both bead-milling and Triton X-100 lysis.

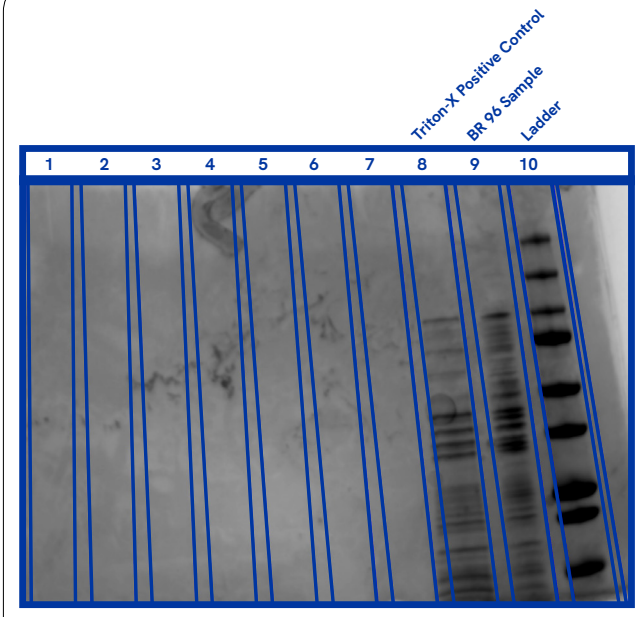


Figure 6: Protein repertoire of the lysed samples as shown with Coomassie Blue.

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