Efficient bacterial RNA extraction using the Omni Bead Ruptor Elite bead mill homogenizer.

Introduction

Efficient, high-quality RNA extraction is crucial for modern nucleic acid applications such as qPCR, RNA sequencing, and transcriptomic analysis. Traditional bacterial lysis methods, such as enzymatic digestion with lysozyme or proteinase K, are time-consuming and can compromise RNA integrity due to prolonged exposure to degrading conditions. The **Omni Bead Ruptor Elite™ bead mill homogenizer** offers a rapid and efficient alternative, utilizing mechanical disruption to achieve complete cell lysis while preserving RNA quality. When paired with a commercially available total RNA extraction kit, this workflow ensures high-purity RNA with excellent RNA Integrity Number (RIN) values for reliable downstream analysis.

This technical note aims to address the misconception that bead-based homogenization is excessively harsh and leads to less desirable outcomes for RNA quality metrics when evaluating bacteria and other microorganisms. Our findings demonstrate that the Omni Bead Ruptor Elite bead mill homogenizer enables efficient bacterial lysis in a fraction of time, while maintaining RNA integrity, providing a robust and reproducible solution for high-quality RNA extraction in applications such as microbiome research, pathogen detection, and gene expression analysis.

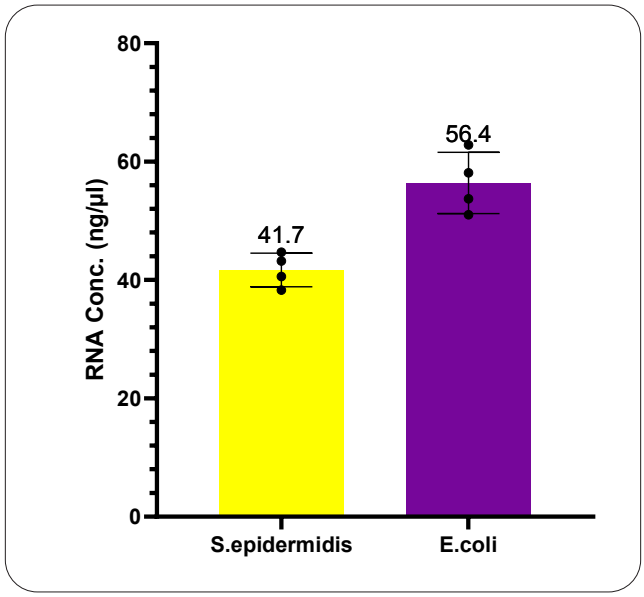

Figure 1: RNA extracted from bacterial samples using Omni Bead Ruptor Elite, followed by purification with the total RNA extraction kit. The bars represent the spread of RNA concentrations across the samples, with the average concentration indicated at the top.

Table 1: Average RNA yield and RIN value for each organism processed on the Omni Bead Ruptor Elite.

Row Labels	Average RNA Conc. (ng/ul)	Average of RIN	Average of $A_{260/280}$
E.coli	56.4	8.3	1.9
S.epidermidis	41.7	8.5	2.0

Methods

One mL of cultured *Escherichia coli* and *Staphylococcus epidermidis* (n=4) was pelleted and resuspended in a lysis buffer and proteinase K mixture provided in the RNA extraction kit. Homogenization was performed on the Omni Bead Ruptor Elite bead mill homogenizer (Revvity, Cat # 19-042E) using the Omni Bead Ruptor Elite 2 mL tube carriage (Revvity, Cat # 19-373) and Universal Microbial Homogenizing Mix 0.1 mm Ceramic (2 mL Tubes) (Revvity, Cat # 19-632). The samples were run at 5 m/s for three cycles of one minute each, with a one-minute dwell on ice between cycles. RNA extraction followed the instructions provided in the kit manual, with all steps conducted on ice or 4°C. Mixing steps were performed using a multichannel pipette instead of vortexing. RNA was eluted into 60 µL of elution buffer and quantified using the NanoDrop 2000, followed by RNA Integrity analysis on the Agilent® Bioanalyzer® 2100.

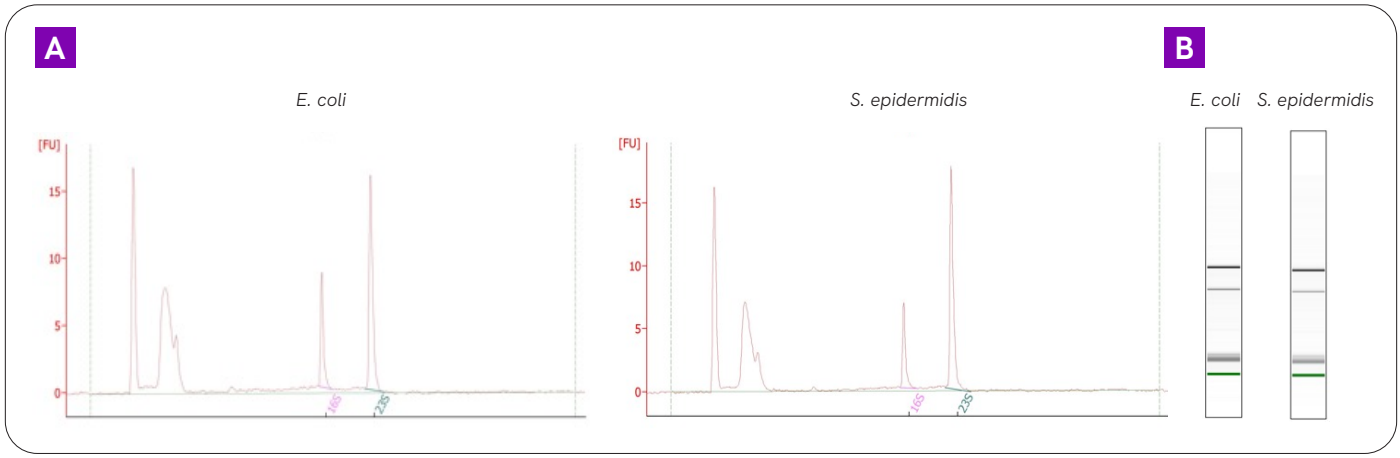

Figure 2. RNA integrity analysis of bacterial samples homogenized using the Omni Bead Ruptor Elite. RNA was extracted from *E. coli* and *S. epidermidis* using a commercially available total RNA extraction kit and analyzed on the Agilent® 2100 Bioanalyzer®. The figure displays representative results from one *E. coli* and one *S. epidermidis* sample. **(a)** Electropherograms display distinct 5S, 16S, and 23S rRNA peaks, indicating high RNA integrity. **(b)** Gel-like images confirm the presence of well-defined 16S and 23S bands, demonstrating successful RNA extraction and preservation of ribosomal RNA quality.

Results

The average RNA concentrations recovered from cultured *S. epidermidis*, and *E. coli* were determined to be 41.7 ng/µL and 56.4 ng/µL, respectively. Figure 1 illustrates the concentrations along with the distribution across each of the samples. The corresponding average RNA integrity numbers (RINs) for these samples were 8.3 and 8.5, both of which exceeded the benchmark threshold of 7, indicating high RNA quality and integrity. Based on gel analysis, the RNA demonstrated good quality with prominent 23S and 16S bands (Figure 2). 5S RNA peak is also visible, further supporting the quality of the extracted RNA. The UV absorbance ratio and the RINs were found to be above the accepted benchmark values as shown in Figure 3. No shearing was visible in the electrophoretograms, confirming that the RNA was intact and suitable for further downstream analyses.

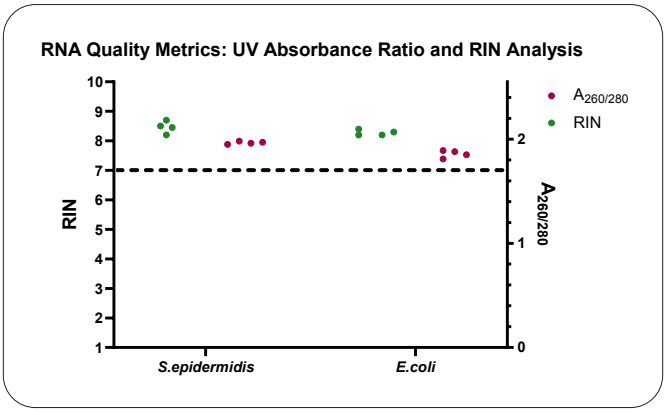

Figure 3: RNA quality metrics assessment. The left y-axis represents the RIN (green), while the right y-axis corresponds to the absorbance ratio (magenta). Each dot represents a replicate of *S. epidermidis* and *E. coli* analyzed. The black dotted lines indicate the minimum accepted quality control (QC) thresholds (RIN = 7[1,2,3] and absorbance ratio = 1.7).

Conclusions

The Omni Bead Ruptor Elite bead mill homogenizer successfully lysed bacterial cells, overcoming the challenges posed by their protective cell walls to achieve complete disruption. The rapid lysis facilitated an RNA extraction that was completed in less than 2 hours, beginning with cultured cells and ending with eluted nucleic acid. Importantly, the integrity of the RNA was preserved throughout the process. The resulting RNA met all required quality metrics, including concentration, absorbance ratios, and RIN, confirming its suitability for downstream applications such as gene expression analysis and RNA sequencing. These results highlight the Omni Bead Ruptor Elite bead mill homogenizer as an effective and dependable tool for isolating high-quality RNA from bacterial samples.

References

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