APPLICATION NOTE #20142

Increased mRNA yield from skin samples using the Bead Ruptor Elite

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Transcriptome research has become increasingly popular to monitor global changes in gene expression[1]. In particular, transcriptome changes between diseases versus healthy states can provide valuable insight into understanding immunoresponse mechanisms in disease states[2]. However, the isolation of high-quality mRNA from tough tissues, such as skin, can represent a significant challenge in research. Herein, we compare two methods for mRNA extraction from two murine skin types with a specific focus on mRNA yields and integrity. Our study demonstrates the efficacy of using bead mill technology as the optimal choice of sample preparation in transcriptomic research.

Materials and Methods

Eight mouse skin samples were obtained from the ear and ventrally. Skin samples were sectioned into 1 cm^2 samples, separated from fat and kept on ice until homogenization. Skin samples were diced into fragments then placed in 1 mL of Trizol and homogenized both manually in a Dounce homogenizer and by bead beating on a Bead Ruptor Elite (Omni International, Cat#19-040E; 19-010-310) in 2 ml bead tubes pre-filled with 2.8 mm RNase/DNase free ceramic beads (Cat# 19-628). Bead beating was performed at 7.45 m/s for 45 seconds.

To evaluate if mRNA yields correlate with starting sample quantity, the homogenization was repeated using 2 x 1 cm^2 ear and ventral skin samples. For the larger samples, bead beating was performed for two cycles at 7.45 m/s for 45 seconds with a 45 sec dwell between cycles.

After homogenization samples were centrifuged to pellet insoluble material. 30 µL of supernatant were subjected to standard mRNA extraction protocols and OD 260/280 readings were taken to determine mRNA concentration and purity (figure 1). Based on determined concentrations, approximately 1 µg was mixed with loading buffer and run on a 1.2% non-denaturing gel following heat treatment at 70°C x 1 min then snap cooling on ice.

The mRNA purity and integrity of each sample was additionally analyzed using an Agilent 2100 BioAnalyzer (figure 2).

Results

Isolation of mRNA from skin samples present numerous challenges including the toughness of skin, RNase abundance and possibility of degradation resulting from the mechanical disruption process. Mechanical disruption through dounce homogenization (glass mortar pestle) has historically been the established protocol for mechanical homogenization, however for tough samples types and large volume sampling, manual dounce homogenization represents a significant bottleneck. Bead milling, a relatively new technology, is a promising method to overcome these issues. Our results indicate bead milling eliminates this obstacle and produces high mRNA yields with exceptional integrity.

280nm absorbance values indicated that mRNA yields ranged from 26 to 99 µg when bead milling was performed compared to 9 – 30 µg achieved through dounce homogenization; representing an average 4 fold increase in mRNA yield (Figure 1). 260/280 nm ratios demonstrate mRNA purity was comparable between both homogenization techniques, and thus bead milling does not affect sample purity.

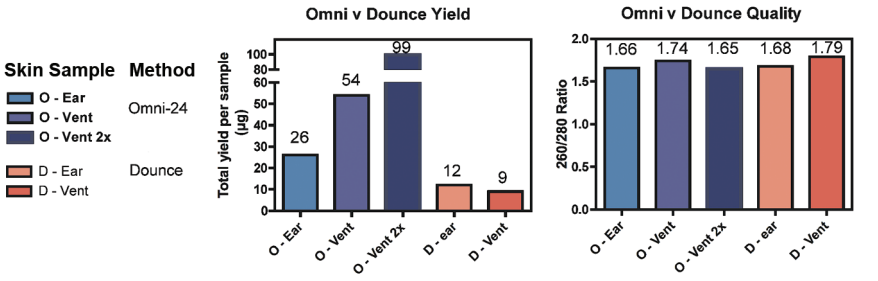

Figure 1 A and B **Total mRNA yields and relative purity:** Absorbance values at 260nm and 280nm were used to determine total mRNA yield and purity following extraction. mRNA yields were on average 4 times higher when bead milling was performed compared to dounce homogenization. Nucleic acid relative purity was comparable between both methods (B).

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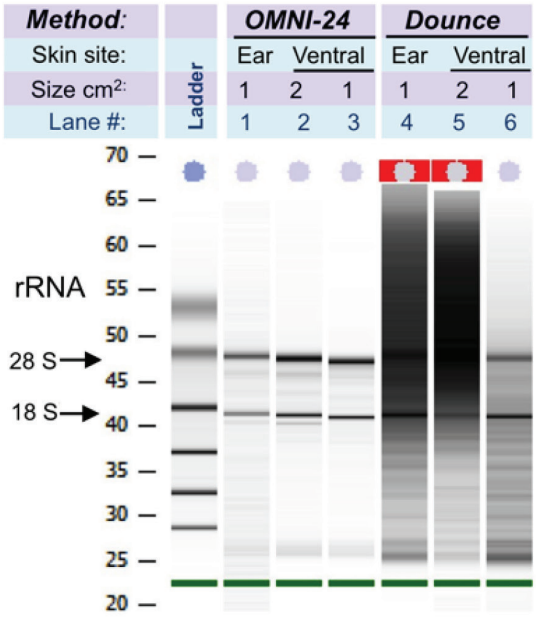
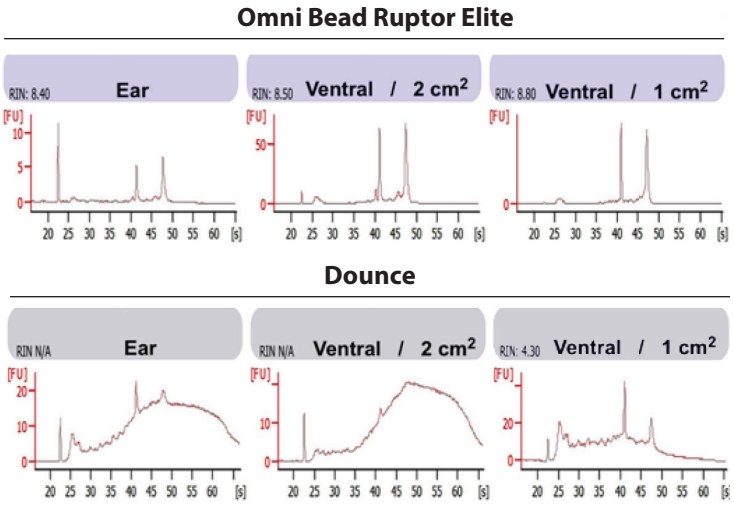
Results (continued)

Electropherograms produced with an Agilent Bioanalyzer 2100 revealed that dounce homogenization produced samples with high molecular weight contaminant DNA and low molecular weight degradation species, while bead milling homogenization produced mRNA of high purity and RNA integrity.





Figures 2 A and B Agilent Bioanalyzer Electropherograms: Electropherograms demonstrate extracted RNA integrity and purity: A synthetic electrophoresis gel (left panel, including RNA 600 ladder) was produced with electropherogram readings of each sample (right panel). Samples homogenized with a Bead Ruptor Elite show well defined 28S and 18S peaks with minimal noise (top right). In contrast, samples produced by Dounce homogenizers indicate the presence of high molecular weight DNA contamination and low molecular weight degraded RNA (bottom right).

Acknowledgements

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Bead Ruptor Elite

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