APPLICATION NOTE

Nucleic Acid Extraction and Quantitative PCR from Formalin-Fixed Umbilical Cord and Placenta Tissue

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Bead Ruptor Elite™
Bead Mill Homogenizer

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

Summary

Genetic analysis of the newborn is often conducted via dried blood spot gDNA sequencing to study epidemiological data [1]; however, placenta and umbilical cord samples also contain nucleic acids and are a sample matrix of interest to researchers. Similarly, genetic analysis of placenta and umbilical cord within the scope of environmental drug research is of particular interest to discover how chemical exposures during pregnancy affect both disease progression and carcinogenic mutation potential of newborn genetic material [2].

Upstream of genetic analysis methodologies is sample preparation, which is traditionally conducted manually, chemically or a combination of the two. Bead beating homogenization using the Bead Ruptor Elite™ Bead Mill Homogenizer is a quick and efficient way to overcome the harsh and time-consuming nature of alternative chemical or manual digestion methods, while still producing a homogenate suitable for downstream analysis.

Herein, we outline sample preparation of fixed placenta and umbilical cord tissues on the Bead Ruptor Elite™ Bead Mill Homogenizer for downstream nucleic acid extraction and proof-of-concept qPCR amplification.

Sample Aquisition

De-identified paraformaldehyde-fixed human umbilical cord and placenta samples were obtained from Emory University Hospital, Tissue Procurement Lab under standard IRB protocols. potential of newborn genetic material [2].

Umbilical Cord and Placenta Sample Preparation & DNA Extraction

For sample preparation, 200 mg of tissue was added to a 2 mL Hard Tissue Homogenizing Mix tube (OMNI International, Cat # 19-628) along with 600 µl phosphate buffered saline (PBS), pH 7.2 (Gibco, Cat # 20012027). Tissue samples were weighed out with a tolerance ± 5 mg. The tissue samples were homogenized on the Bead Ruptor Elite™ Bead Mill Homogenizer (OMNI International, Cat # 19-042E) at 5.0 m/s for 3 cycles of 30 seconds with a 10 second dwell (Table 1). After the homogenization step, 600 µl DLB was added to the 2 mL tube and vortexed for 10 seconds. The tissue homogenate was then incubated at 95°C for 30 minutes to de-crosslink paraformaldehyde. After 30 minutes elapsed, the de-crosslinked homogenate was centrifuged at 10,000 xg for 5 minutes to pellet cell debris. The remainder of the DNA extraction was conducted following from Step 7 of the OMNI Tissue DNA extraction kit (OMNI International, Cat # 26-007) manual.

Quantitative PCR

Five microliters of eluted DNA was added to qPCR mix (New England Biolabs, Cat # M3003S) along with 18S primers. All volumes of reaction mix, primers, and nuclease-free water were prepared according to manufacturer's instructions as laid out for a 20 µl final reaction volume. 18S was targeted with forward primer 5′ - CAG CAG CCG CGG TAA TTC C – 3′, reverse primer 5′ - CCC GTG TTG AGT CAA ATT AAG C – 3′ yielding a product size of 676 bp. An 18S-positive DNA extract was used as the positive control along with PCR-grade water for the negative control. Reactions were loaded into the BioRad CFX Connect Real Time Instrument (BioRad Cat. # 1855201) and amplified for 45 cycles. Amplicons were visualized on 2% agarose gel stained with ethidium bromide (BioRad, Cat # 161-0433) for product visualization.

Results

A complete homogenate was obtained from placenta and umbilical cord samples after 90 seconds of homogenization on the Bead Ruptor Elite™ Bead Mill Homogenizer (Figures 1-2). Quantitative PCR performed on eluted DNA from placenta and umbilical cord showed presence of eukaryotic ribosomal 18S gene in both placenta and cord samples, when compared to controls, indicating that de-crosslinking and DNA extraction were successful in purifying PCR-ready nucleic acid (Table 2). A 2% agarose gel separation of qPCR products showed a hyperdense band at 676 bp for all samples containing either amplified 18S DNA from placenta or umbilical cord, or 18S-positive extract while the negative control did not contain any separated nucleic acid (Figure 3).

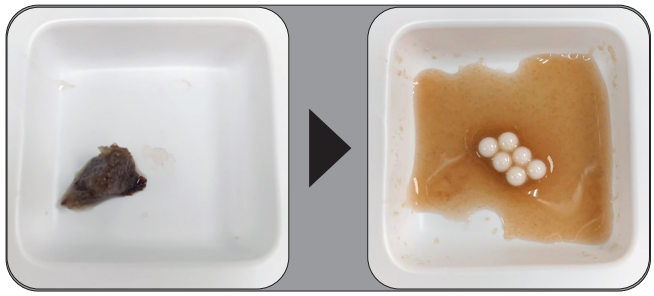

Figure 1. Pre and post homogenization photos of 200 mg placenta

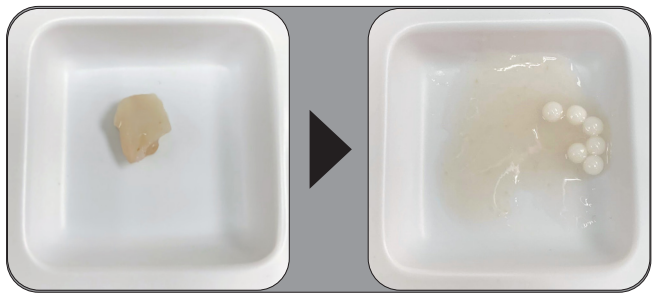

Figure 2. Pre and post homogenization photos of 200 mg umbilical cord

Sample Type	Sample Weight (mg)	Speed (m/s)	Time (sec)	Cycles	Dwell Time (sec)
Umbilical Cord	200	5	30	3	10
Placenta	200	5	30	3	10

Table 1. Umbilical Cord and Placenta Bead Ruptor Elite™ Bead Mill Homogenization Summary

Figure 3. Amplicon gel showing 18S product in placenta samples (Lanes 1-3) and cord samples (Lanes 4-6) at 676 bp, when compared to the molecular ruler (Lane 10). The positive control (Lane 9) showed 18S product, while the negative control (Lane 8) had no product visualization.

Results

In this study, the Bead Ruptor Elite™ Homogenizer's ability to process a variety of soft tissue in order to extract proteins was evaluated. High concentrations of protein from all soft tissue samples were detected post homogenization. Concentrations were determined by spectrophotometry as shown in Table 2. The highest concentration was from spleen at an average of 57.468 mg/mL and the lowest concentration was from brain at 10.687 mg/mL. SDS-PAGE analysis indicating a broad protein repertoire was obtained from each sample with abundant bands observed across the entire molecular weight range (Figure 1).

Sample Name	Cq Value
Placenta 1	34.06
Placenta 2	33.40
Placenta 3	31.56
Umbilical Cord 1	33.11
Umbilical Cord 2	32.91
Umbilical Cord 3	36.06
18S Positive Control	29.80
Negative Control	N/A

Table 2. Cq values obtained from qPCR amplifying 18S gene. For placenta and umbilical cord samples, Cq data was reported as an average of triplicate samples.

Conclusions

When using the Bead Ruptor Elite™ Bead Mill Homogenizer to process placenta and umbilical cord tissue, front-end sample preparation of tough sample matrices is simplified, yielding a homogenate suitable for nucleic acid extraction and PCR amplification. Providing a PCR target proving eukaryotic origin in the outlined experiments, it was shown that PCR-suitable nucleic acid is the product of the Bead Ruptor Elite™ Bead Mill Homogenizer & OMNI Tissue DNA kit sample preparation solution.

References

[1] Rajatileka, Shavanthi et al. "Isolation of human genomic DNA for genetic analysis from premature neonates: a comparison between newborn dried blood spots, whole blood and umbilical cord tissue." BMC genetics vol. 14 105. 29 Oct. 2013, doi:10.1186/1471-2156-14-105

[2] Hansen, C et al. "Detection of carcinogen-DNA adducts in human fetal tissues by the 32P-postlabeling procedure." Environmental health perspectives vol. 99 (1993): 229-31. doi:10.1289/ehp.9399229

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