PROTOCOL

Nucleic acid extraction and quantitative PCR from formalin-fixed umbilical cord and placenta tissue.

Authors

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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 any genetic analysis methodologies is sample preparation, which is traditionally conducted manually, chemically or a combination of the two. Bead beating homogenization using the Omni 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 Omni Bead Ruptor Elite bead mill homogenizer for downstream nucleic acid extraction and proof-of-concept qPCR amplification.

Omni Bead Ruptor Elite™ bead mill homogenizer

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

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor 2 mL Tube Carriage (Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL) (Cat # 19-628)

Sample acquisition

De-identified paraformaldehyde-fixed human umbilical cord and placenta samples were obtained from Emory University Hospital, Tissue Procurement Lab under standard IRB protocols.

Umbilical cord and placenta sample preparation and DNA extraction

For sample preparation, 200 mg of tissue was added to a 2 mL Hard Tissue Homogenizing Mix tube (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 Omni Bead Ruptor Elite bead mill homogenizer (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 commercially available tissue DNA lysis buffer 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. DNA extraction was then carried out using a commercially available tissue DNA extraction kit following manufacturer's instructions.

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 (Bio-Rad, Cat. # 1855201) and amplified for 45 cycles. Amplicons were visualized on 2 % agarose gel stained with ethidium bromide (Bio-Rad, Cat # 161-0433) for product visualization.

Table 1: Umbilical cord and placenta Bead Ruptor Elite bead mill homogenization summary.

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

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).

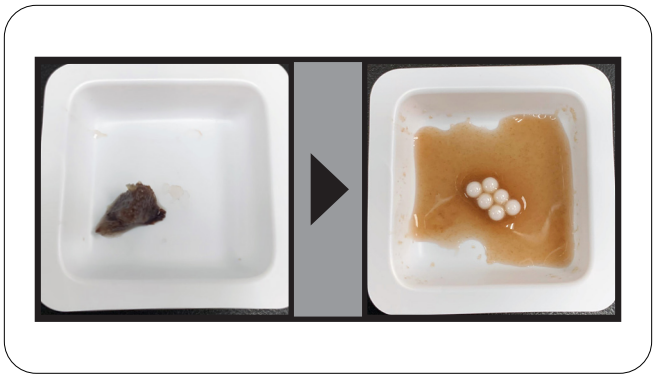

Figure 1: Pre- and post-homogenization photos of 200 mg placenta.

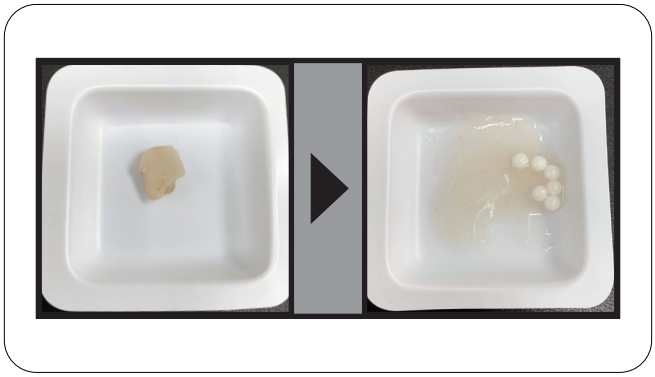

Figure 2: Pre- and post-homogenization photos of 200 mg umbilical cord.

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.

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

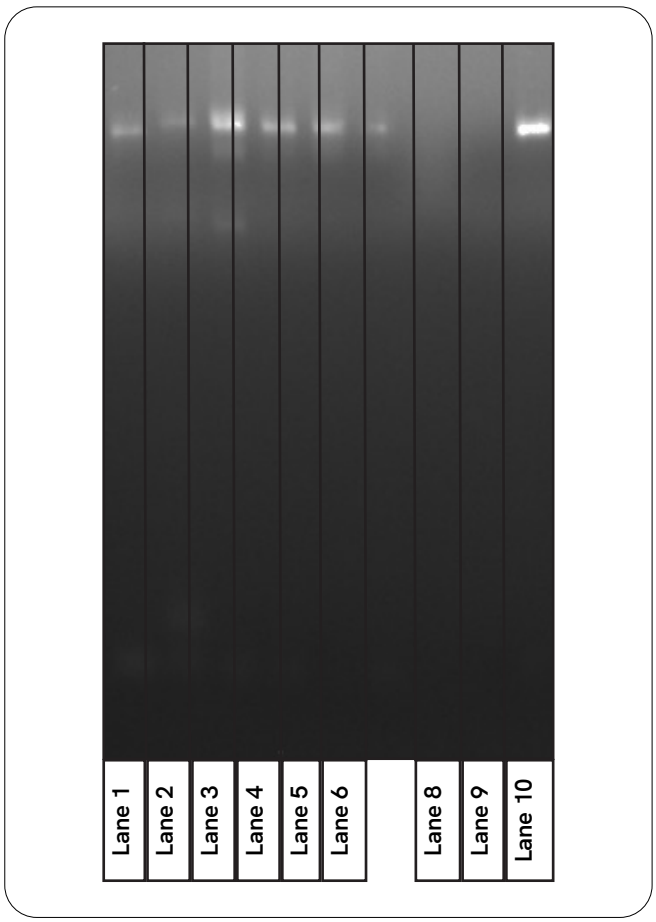

Figure 3: Amplicon gel showing 18S product in placenta samples (Lanes 1-3) and cord samples (Lanes 4-6) at 676 bp, when compared to the molecular ruler (Lane 10). The positive control (Lane 9) showed 18S product, while the negative control (Lane 8) had no product visualization.

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 sample preparation using the Bead Ruptor Elite™ bead mill homogenizer.

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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