Optimized Methodology for Sequential Extraction of RNA and Protein from Small Human Biopsies

Skin Tissue

CASE STUDY

Berglund S.R. ; Schwietert C.W. ; Jones A.A. ; Stern R.L. ; Lehmann J. ; Goldberg Z.

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Introduction

Skin tissue, although easily accessible, is difficult to process owing to its natural resistance to mechanical shearing and high levels of RNase and proteases. Currently, these complications result in degraded RNA samples with variable yield. We have developed a method for sequential extraction of high quality RNA and protein from a single 3 mm full thickness skin punch biopsy.

Two extraction techniques were used to disrupt the biopsy samples, homogenization and bead beating.

Overview

- **Keywords:** Tissue biopsy, clinical samples, RNA extraction, protein extraction
- **Aim of the study:** Optimization of RNA and protein extraction from skin tissue
- **Application:** Western blot & RNA quality analysis
- **Sample name:** Tissue biopsy
- **Sample type:** Human skin biopsies from a 3 mm punch
- **Material:** FastPrep® instrument, Lysing Matrix D tubes
- **Buffer:** Guanidine Thiocyanate lysis buffer

Protocol and Parameters

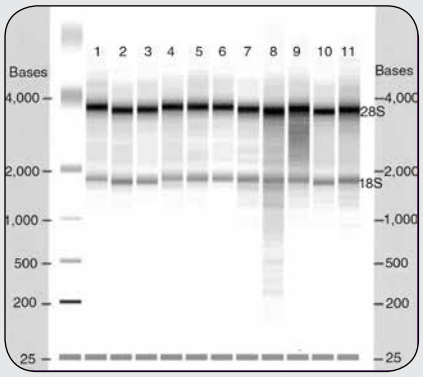
1. Add the 19 mg of skin sample to a Lysing Matrix D tube.
2. Add 1mL of a guanidine thiocyanate lysis buffer (5.1 M guanidine thiocyanate, 50 mM sodium citrate, 50 mM EDTA, 0.5% β-mercaptoethanol).
3. Homogenize in the FastPrep instrument for 3 x 40 seconds at a speed setting of 6.0 m/s. Place the tubes on ice for 5 minutes between each run.
4. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.
5. Proceed with the RNA and protein extraction protocol.

CASE STUDY

Results

High Quality RNA Isolation with FastPrep instrument
RNA 2100 Bioanalyzer analysis of FastPrep samples

The RNA was run on an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA) using the RNA 6000 Pico LabChip kit to determine the quality of the samples. The 28S and 18S ribosomal bands show a greater than 2:1 ratio and the calculated RNA ribosomal integrity numbers of the samples ranged from 8.4 to 8.9, verifying a high quality RNA.



Gel image for 11 RNA samples.

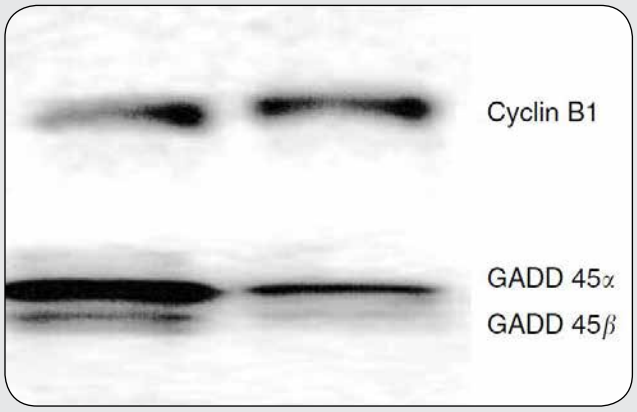
Higher RNA & protein yield obtained with FastPrep instrument
RNA and protein quantification

For each method of tissue disruption, the quantity and quality of RNA (as an OD 260/280 ratio), and the quality of protein is shown. The RNA was quantified using a Nanodrop spectrometer and the protein content was determined using a Bradford-based assay. For RNA, an OD 260/280 of 2.0 is optimal.

	RNA average quantity per biopsy (µg)	RNA average 260/280 ratio	Protein average quantity per biopsy (µg)
FastPrep® bead-beater	1.4 (± 0.4 µg)	2.0 (± 0.05)	170 (± 50 µg)
Polytron Homogenizer	0.8 (± 0.4 µg)	1.8 (± 0.11)	90 (± 40 µg)

Quality assessment of extracted protein
Western blots using biopsy sample protein

Approximately 10-15 mg of protein from two different biopsy samples processed with the FastPrep instrument (Qbiogene, Irvine, CA)were used to determine the quality of western blotting. The top panel was probed with mouse anti-GADD 45. The GADD 45 used (Santa Cruz Biotechnology Inc., Santa Cruz, CA) recognizes both the alpha and beta subunits of the protein.



Conclusion

Sample variability and exposure to exogenous contamination were reduced using the FastPrep bead beating instrument, which allows processing up to 24 samples very quickly. This method yields 1-2 µg of RNA and 150 mg of protein, which is usable in many sensitive downstream applications including microarray, quantitative real-time PCR, two-dimensional gel electrophoresis, and western blot analysis.