Efficient and scalable serial extraction of DNA and RNA from frozen tissue samples

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Supplementary Material

Efficient and scalable serial extraction of DNA and RNA from biobanked tissue samples

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Materials & Methods

Detailed procedure for sequential extraction of DNA and RNA from frozen tissues.

Three 10 μm thick OCT embedded frozen tissue sections from breast, colon, spleen, tonsil, or frozen white blood cell pellets (3×10^6 cells) were homogenized in 1 ml Lysis Buffer (7 M guanidine HCl, 50 mM Tris pH 7, 4 U Proteinase K (Roche), 2% Tween-20) by grinding with a 3 mm steel ball for 15 min at 55°C under rotary shaking at 800 rpm. To 200 μL lysate, 0.5 mg of MagPrep Silica HS particles (Merck) was added to capture DNA. Beads were recovered using a magnet for one min and washed 3 times for 1 min in 100 μL Wash Buffer (10mM Tris-HCl pH 6.5). DNA was eluted from the beads by addition of 100 μL Elution Buffer (10mM Tris-HCl pH 9, 1 mM EDTA), mixing 20 times and heating to 65°C for 5 min. To the supernatant post DNA recovery, 0.5 mg Basic MagPrep Silica beads was added, followed by mixing 5 times and incubation for 15 min. Beads were recovered using a magnet for one min and washed in 100 μL ice cold Wash Buffer for 1 min, Wash Buffer with 2U DNase I (Fermentas) for 15 min at 37°C, and in ice cold Wash Buffer for 1 min. Total RNA was eluted from the MagPrep beads by addition of 50 μL Elution Buffer, mixing 20 times and incubation for 5 min at 65°C.

Real time PCR amplification and DNA fragment length assessment.

Real time PCR quantification of the human LINE1 repeat element was carried out using SYBR Green I (Biochemika) detection and forward primer 5'-AAAGCCGCTCAACTACATGG-3' and reverse primer 5'-TGCTTTGAATGCGTCCCAGAG-3' on an ABI 7900 HT instrument (Applied Biosystems). Thermocycling parameters were 94°C for 1 min, followed by 35 cycles of 94°C for 10 seconds, 58°C for 15 seconds and 70°C for 15 seconds.[S1] Assessment of DNA fragment length was performed by electrophoretic separation in a 0.4% agarose gel with a High Range DNA ladder (Fermentas) as size standard.

PCR amplification and sequencing.

PCR amplification and sequencing of exons 1 to 7 of *PRPS1* was carried out using 6 ng of genomic DNA as template. All primers were synthesised by Sigma (see Table S1 for primer sequence). PCR was performed in 5 μL reactions containing 1 × PCR buffer (67 mM Tris-HCl, pH 8.8, 6.7 mM MgCl2, 16.6 mM NH4SO4, 10 mM 2-mercaptoethanol), 1 mM dNTPs, 1 μM forward and 1 μM reverse primers, 6% DMSO, 2 mM ATP, 0.25 U Platinum *Taq*

(Invitrogen) and 3 ng DNA. Reactions were carried out in 96-well ABI 2720 thermocyclers using a touchdown PCR protocol (1 cycle of 96°C for 2 min; 3 cycles of 96°C for 10 sec, 64°C for 10 sec, 70°C for 30 sec; 3 cycles of 96°C for 10 sec, 61°C for 10 sec, 70°C for 30 sec; 3 cycles of 96°C for10 sec, 58°C for 10 sec, 70°C for 30 sec; 41 cycles of 96°C for 10 sec, 57°C for 10 sec, 70°C for 30 sec; 1 cycle of 70°C for 5 min). Templates were purified using AMPure (Agencourt Biosciences) and sequencing carried out with M13 forward primer (5'-GTAAAACGACGGCCAGT-3') and Big Dye Terminator Kit v.3.1 (Applied Biosystems) 1% DMSO was included in sequencing reactions when the GC content of the template exceeded 65%. Dye terminators were removed using the CleanSEQ kit (Agencourt Biosciences) and sequence reactions were delineated on ABI PRISM 3730xl sequencing apparatuses (Applied Biosystems).[S2]

cDNA synthesis and RT PCR.

cDNA was synthesized according to manufacturer's instructions by reverse transcription using AccessQuick™ RT-PCR System (Promega) and used in a touchdown PCR protocol (as described above) with forward and reverse *ACTB* primers 5'-CTGGGACGACATGGAGAAAA-3' and 5'-AAGGAAGGCTGGAAGAGTGC-3' respectively. Control RNA was extracted from blood using QIAamp RNA Blood Mini Kit from Qiagen according to manufacturer's instructions.

Supplementary References

1. T. Sjöblom, S. Jones, L. D. Wood, W. Parsons, J. Lin, T. D. Marber, D. Mandelker, R. J. Leary, J. Ptak, N. Silliman, S. Szabo, P. Buckhaults, C. Farrell, P. Meeh, S. D. Markowitz, J. Willis, D. Dawson, J. Willson, A. Gazdar, J. Hartigan, L. Wu, C. Liu, G. Parmigiani, B. Park, K. E. Bachman, N. Papadopoulos, B. Vogelstein, K. Kinzler and V. Velculescu. *Science,* 2006, **314**, 268-274.
2. C. Rago, D. L. Huso, F. Diehl, B. Karim, G. Liu, N. Papadopoulos, Y. Samuels, V. E. Velculescu, B. Vogelstein, K. W. Kinzler and L. U. Diaz, Jr. *Cancer Res,* 2007, **67,** 9364-9370.

Primer	Sequence
Exon 1 M13F	5'-GTAAAACGACGGCCAGTCGCTTGGTATTGAGTCTGTGG-3'
Exon 1 R	5'-GCTAGTCACAGAGCTGCACCC-3'
Exon 2 M13F	5'-GTAAAACGACGGCCAGTACCTATGGATATGGAGGGCTG-3'
Exon 2 R	5'-ACTCCAGAGGAGTTGGTGCTT-3'
Exon 3 M13F	5'-GTAAAACGACGGCCAGTTGTCTCCTTCTATGAATTTCTGGG-3'
Exon 3 R	5'-CTTCTCTGCAGTCTTCAGCATC-3'
Exon 4 F	5'-TCCCATCAGTTTGAATGTTGC-3'
Exon 4 M13R	5'-GTAAAACGACGGCCAGTCCCATGTGCTAGCTACTTACATCC-3'
Exon 5 F	5'-CCTGACCTTGTGATCCGC-3'
Exon 5 M13R	5'-GTAAAACGACGGCCAGTTCAGCAGGCTGAAGACATTC-3'
Exon 6 F	5'-TGTTGTGGAAGCCTAAGCAGG-3'
Exon 6 M13R	5'-GTAAAACGACGGCCAGTGATGACAAGACTAAATCCTTCAGACC-3'
Exon 7 M13F	5'-GTAAAACGACGGCCAGTCATGACAGGGAAACAGCACAG-3'
Exon 7 R	5'-CGGGTCTTCTGCTGAATTTG-3'

Table S1. Primer sequences used for amplification of exons 1-7 of *PRPS1*.

Extraction	Input RNA (µg)	Input DNA (µg)
1	2	8
2	2	4
3	2	2
4	4	2
5	8	2

Table S2. Experimental conditions for RNA extractions 1 to 5.

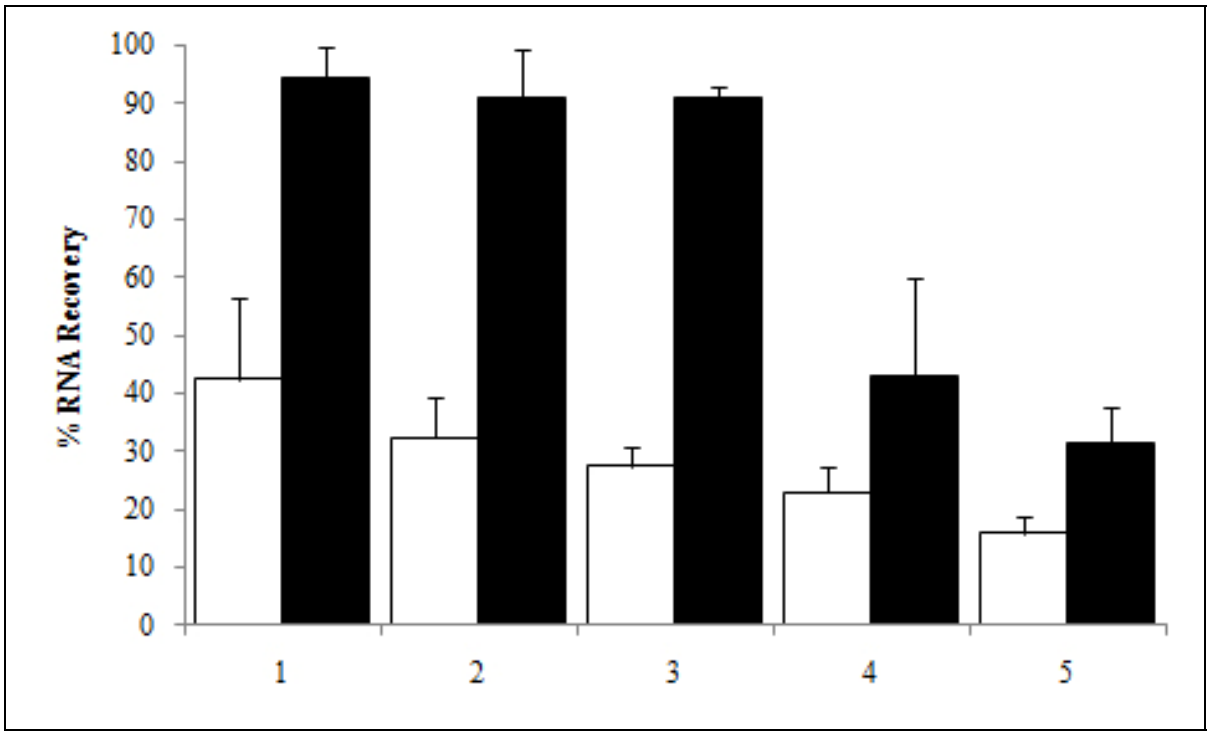

Fig. S1. Prior DNA extraction facilitates higher RNA recovery over a range of DNA:RNA input ratios. RNA was extracted from known mixtures of DNA and RNA (see Table S2) by Basic Silica only (closed bars) and by Silica HS beads followed by Basic Silica (solid bars). RNA concentration was determined using an RNA Nano kit on the Bioanalyzer (Agilent). Percentage recovery is consistently higher upon removal of DNA prior to RNA extraction. RNA recovery is also greater with lower RNA input.

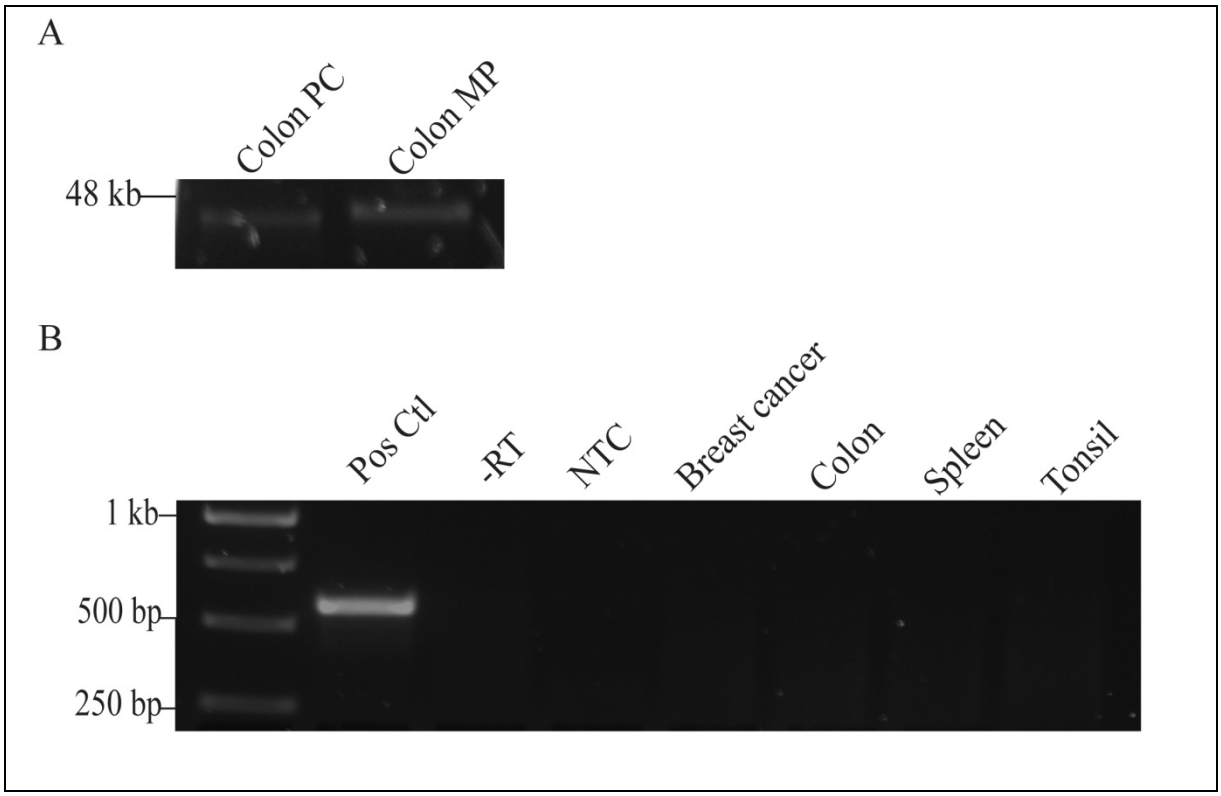

Fig. S2. A: gDNA from colon as extracted by phenol chloroform protocol (PC) and as extracted by protocol outlined in Fig. 1 (MP); B: No RT PCR amplification of *ACTB* using DNA extracted from breast cancer, colon, spleen and tonsil, i.e. no RNA contamination in extracted DNA.

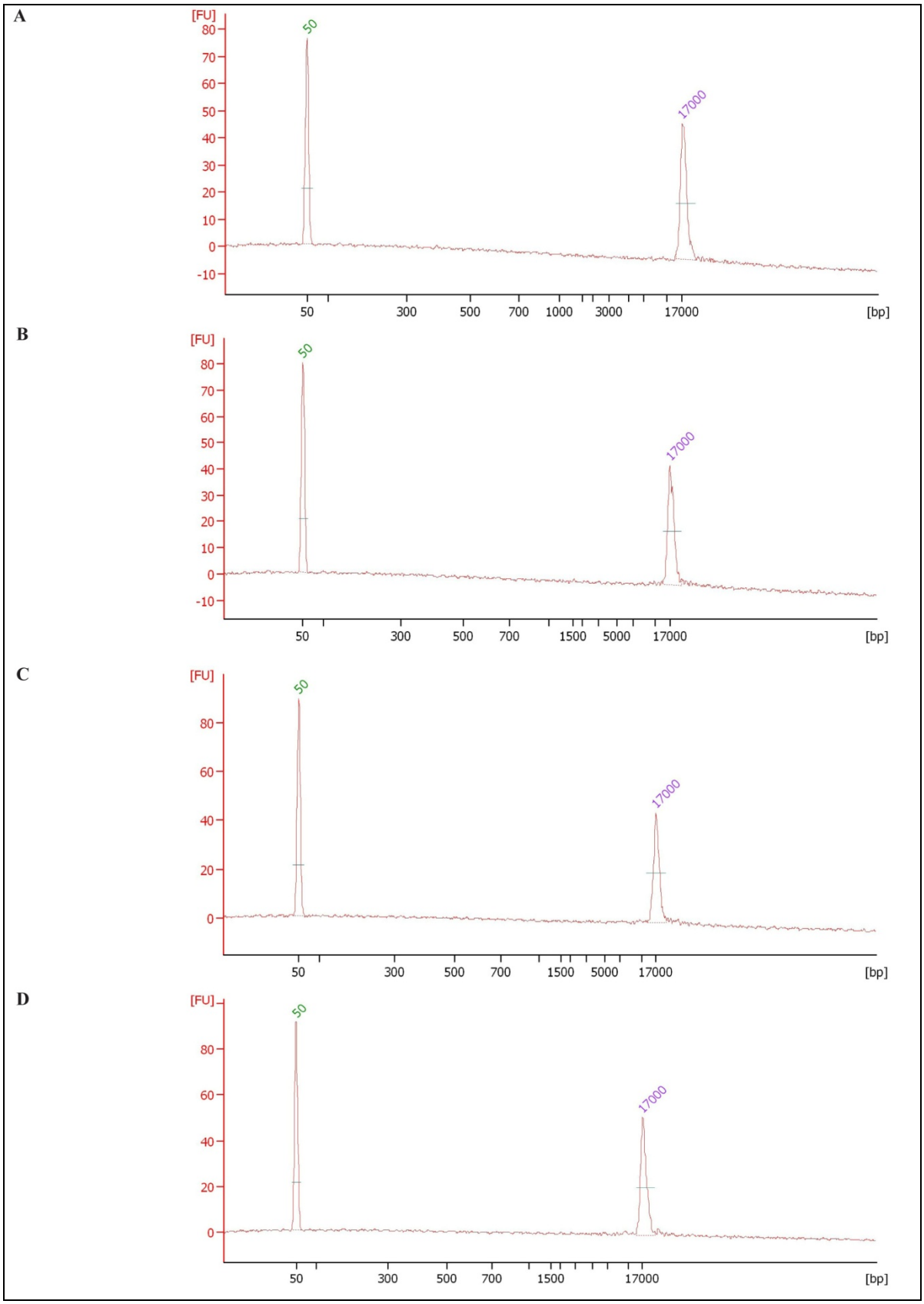

Fig. S3. gDNA from Fig. 2B contains no short fragments between 50 bp and 17 kbp (markers) as illustrated by DNA sizing on an Agilent Bioanalyzer (A: DNA from breast cancer tissue, B: DNA from colon tissue, C: DNA from spleen tissue, D: DNA from tonsil tissue).

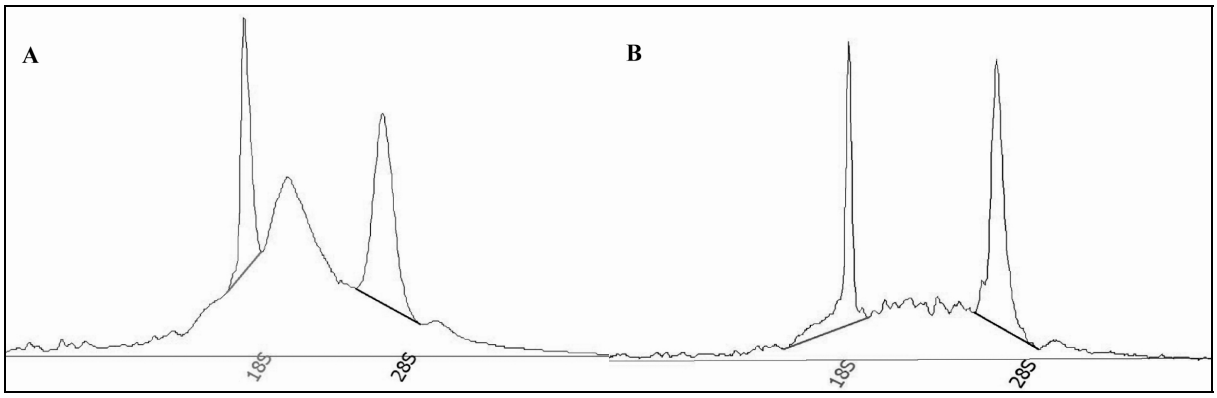

Fig. S4. Electrophoretic separation of a reference RNA sample demonstrating integrity of RNA sample before and after extraction by Basic MagPrep Silica beads [A: RNA input (RIN = 7.1), B: RNA eluate (RIN = 8.7)].