Supplementary Information for

A Novel World-to-Digital-Microfluidic Interface Enabling Extraction and Purification of RNA from Human Whole Blood

Mais J. Jebrail[1], Anupama Sinha[2], Samantha Vellucci[1], Ronald F. Renzi[3], Cesar Ambriz[1], Carmen Gondhalekar[1], Joseph S. Schoeniger[2], Kamlesh D. Patel[3], and Steven S. Branda[1]*

Departments of Biotechnology and Bioengineering[1], Systems Biology[2], Advanced Systems Engineering and Deployment[3], Sandia National Laboratories (Livermore, CA, USA); Stratos Genomics (Seattle, WA, USA)[4]; Stanford University (Stanford, CA, USA)[5]; Oregon State University (Corvallis, OR, USA)[6]

*Corresponding author:

Email: sbranda@sandia.gov

Tel: +1 925 294 6751

Fax: +1 925 294 3020

EXPERIMENTAL SECTION

Reagents and Materials

MagMAX kit Lysis Solution and Wash 1 Solution concentrates were supplemented with isopropanol (50% and 33% v/v, respectively); Wash 2 Solution concentrate with ethanol (80% v/v); RNA Binding Beads concentrate with Lysis Binding Enhancer (50% v/v); and Elution Buffer with Pluronic F127 (0.1% w/v). Molecular biology-grade organic solvents and nuclease-free water were used throughout.

System Assembly

Using a stainless steel rod with a point at one end (Berntsen, Madison WI), 1.55 mm diameter holes were punched into the cap and conical bottom of each of two 500 µL microcentrifuge tubes, which served as the Reaction and Waste Chambers (respectively) of the Extraction Module (Figure 1a). Each cap hole was press-fitted with a 2 cm long Connector Tube, and each bottom hole with a 13 cm long Transfer Tube; each of these were fashioned from Teflon Perfluoroalkoxy tubing (1.5/1.0 mm OD/ID; IDEX-Health and Science, Oak Harbor WA). All chamber/tube junctions were fluidically sealed using optical cement (Norland, Cranbury NJ). The distal end of each Connector Tube was inserted into silicone rubber tubing (6/2 mm OD/ID), which in turn was joined to a miniature peristaltic pump including a double pole, double throw switch to control flow direction (Instech Laboratories, Plymouth Meeting PA) and outfitted with a DC power supply (Fisher Scientific, Fremont CA).

The DMF device that served as the Purification Module was fabricated using a transparent photomask printed at Photo Sciences (Torrance CA). The electrode pads of the bottom DMF plate were masked with polyimide tape (DuPont, Hayward CA) before coating with a 7 µm layer of Parylene-C *via* vapor deposition (Specialty Coating Systems, Indianapolis IN); access for electrical contact was then reestablished by removing the polyimide tape. Finally, a 50 nm layer of Teflon-

AF was added by spin-coating (1% wt/wt in Fluorinert FC-40, 2000 rpm for 60 sec), and the plate was baked at 160°C for 10 min. The top DMF plate was constructed by Delta Technologies Ltd (Stillwater MN).

To couple the Extraction and Purification Modules, an xyz adjustable magnetic stand (Leica Biosystems, Buffalo Grove IL) was used to lower the Extraction Module until the open ends of its Transfer Tubes made contact with the Bridging Electrode at an ~70° angle. The Transfer Tubes were then fitted with a custom-made spacing manifold, to keep them in close proximity to one another (~1 mm) and the Bridging Electrode.

System Operation

Prior to experiments, the inner surfaces of the Extraction and Purification Modules were treated with RNaseZAP solution (Life Technologies, Grand Island NY) and then rinsed with nuclease-free water. Pre-loading of Transfer Tube 1 was accomplished *via* pump-driven aspiration, and pre-loading of the Purification Module *via* pipetting into the air space between the DMF plates while simultaneously applying potential to the recipient actuation electrode. Active mixing of the Lysis Solution and RNA Binding Beads with the blood specimen was accomplished *via* rapid shuttling of the bolus between Transfer Tube 1 and the Reaction Chamber at a flow rate of 15 µL/sec. The beads were collected by immobilizing them upon the surface of the Bridging Electrode using a neodymium magnet (3 mm diameter × 3 mm thick; K&J Magnetics, Jamison PA) positioned directly beneath it while shuttling the bolus between the two Transfer Tubes (*via* the Bridging Droplet) at a flow rate of 7 µL/sec for 3 full passes (Figure 1c). Droplet actuation in the Purification Module was achieved by applying driving potentials of 80-100 V_{rms} generated by amplifying (Digi-Key Corporation, Thief River Falls MN) the output of a function generator (Trek, Medina NY) operating at 18 kHz. Droplet actuation was monitored and recorded by a MVX10 microscope

(Olympus, Center Valley PA) with a high speed QIClick digital camera (Qimaging, Surrey Canada). In the final step of the protocol, the RNA-containing eluate was collected from the Purification Module *via* pipetting after removing the top DMF plate.

RNA Analysis

For qRT-PCR analysis, 25 ng of total RNA (A_{260}/A_{280} of 1.80-1.90) was used as template in each first-strand cDNA synthesis reaction; then the products (2.4-3.8 ng/μL; A_{260}/A_{280} of 1.78-1.81) were diluted 1:10 in water, and 1 μL was used as template in each qPCR reaction. The CFX96 qPCR machine (BioRad, Hercules CA) was programmed to temperature-cycle as follows: 95ºC for 30 sec (1 cycle); then 95ºC for 10 sec followed by 60ºC for 30 sec (40 cycles).

For RNA-Seq, 25 ng of total RNA (A_{260}/A_{280} of 1.80-1.90) was used as template in each cDNA library preparation reaction. Library yields ranged from 3.6 to 5.5 ng/μL.

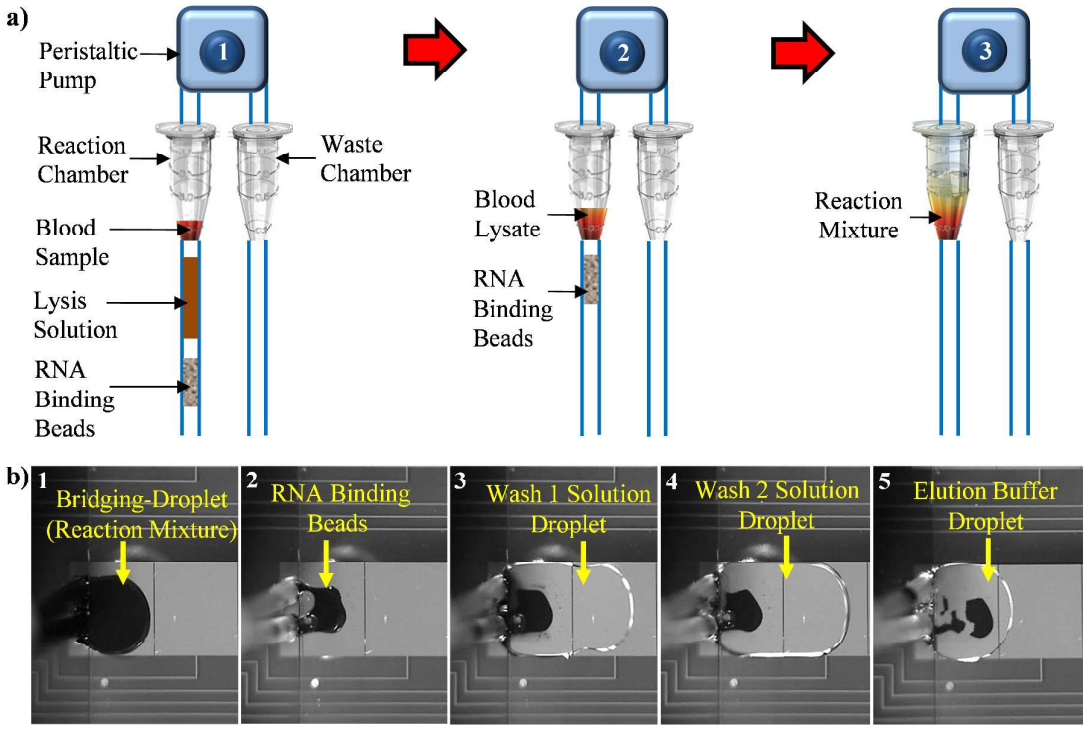

Figure S-1. Extraction and purification of total RNA from human whole blood using the world-to-DMF system. a) Schematic illustrating the initial steps of blood RNA extraction by the Extraction Module: 1) The blood specimen is introduced into the Reaction Chamber, with extraction reagents (Lysis Solution and RNA Binding Beads) pre-loaded in Transfer Tube 1. 2) The Lysis Solution is aspirated into the Reaction Chamber through aspiration driven by the peristaltic pump, and mixing with the blood specimen is accomplished by fluxing between the Reaction Chamber and Transfer Tube 1. 3) The RNA Binding Beads are similarly aspirated into the Reaction Chamber and mixed with the blood lysate. b) Stills from a video (top-view) showing magnetic bead-based steps in blood RNA purification. The reaction mixture, comprised of blood lysate and RNA Binding Beads, is flowed through the Bridging Droplet (frame 1), and the beads immobilized upon the Bridging Electrode surface *via* engagement of the magnet beneath. The supernatant sent to waste (frame 2), and the beads are sequentially washed with 15 µL droplets of Wash 1 Solution (frame 3) and Wash 2 Solution (frame 4) before their introduction into a 10 µL droplet of Elution

Buffer (frame 5). Note that frame 1 shows suspended beads, whereas frames 2-5 show immobilized beads.

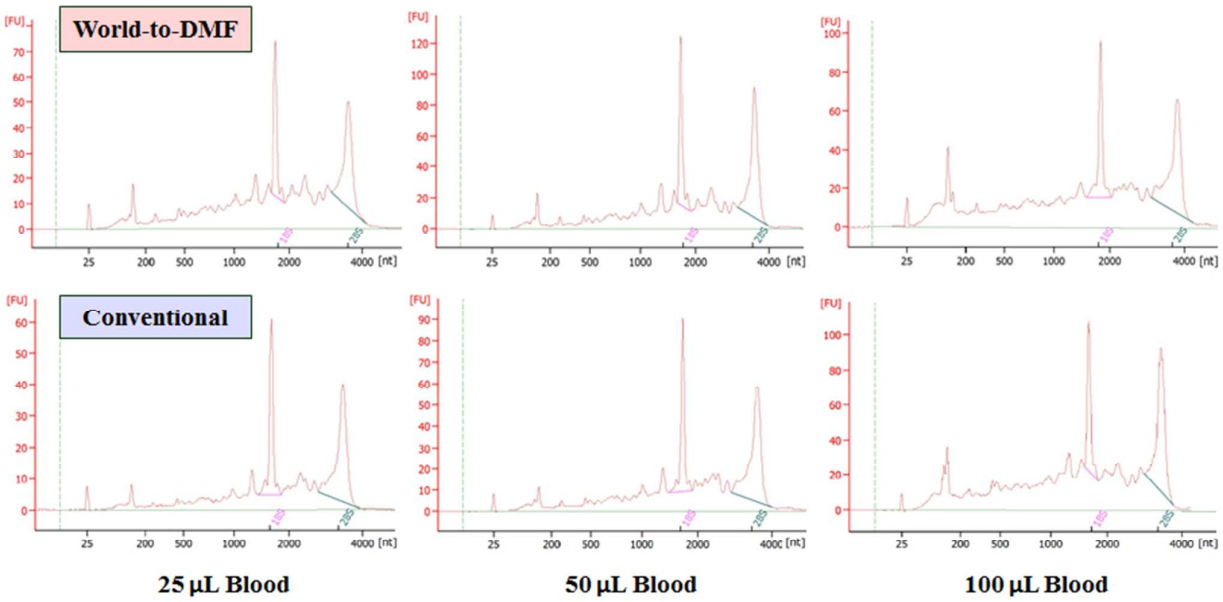

Figure S-2. Characterization of fragment size distribution in total RNA extracted and purified from human whole blood using the world-to-DMF system. Total RNA was prepared from different volumes (25, 50, or 100 µL) of whole blood from three different human donors, using the world-to-DMF system *versus* the conventional approach. The fragment size distribution profile for each RNA sample was generated through Bioanalyzer analysis; the highly-abundant 28S (green) and 18S (pink) rRNA species are indicated.

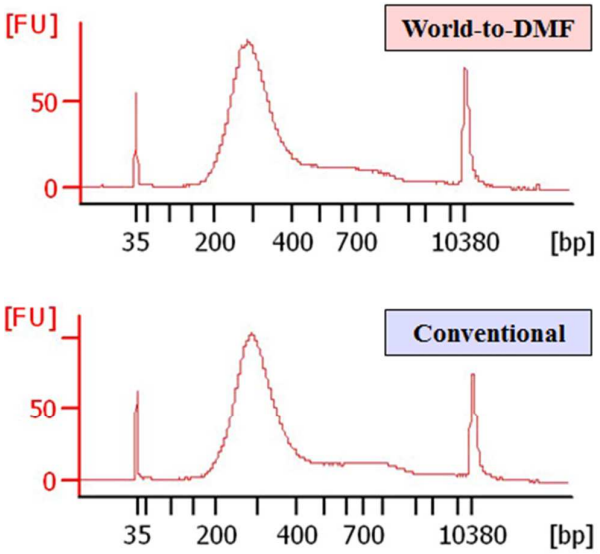

Figure S-3. Characterization of fragment size distribution in RNA-Seq libraries generated from total RNA extracted and purified from human whole blood using the world-to-DMF system. Total RNA was prepared from 50 µL aliquots of whole blood from three different human donors, using the world-to-DMF system *versus* the conventional approach. Each RNA sample served as template in generating a cDNA library for Second Generation Sequencing (SGS). After depleting highly-abundant sequences (primarily rRNA) through molecular normalization, the three libraries generated from RNA prepared by the world-to-DMF system were mixed together in equal ratios, as were the three from conventionally-prepared RNA. The fragment size distribution profile for each three-plexed library was generated through Bioanalyzer analysis. Note that size standards are responsible for the peaks at 35 bp and 10,380 bp.

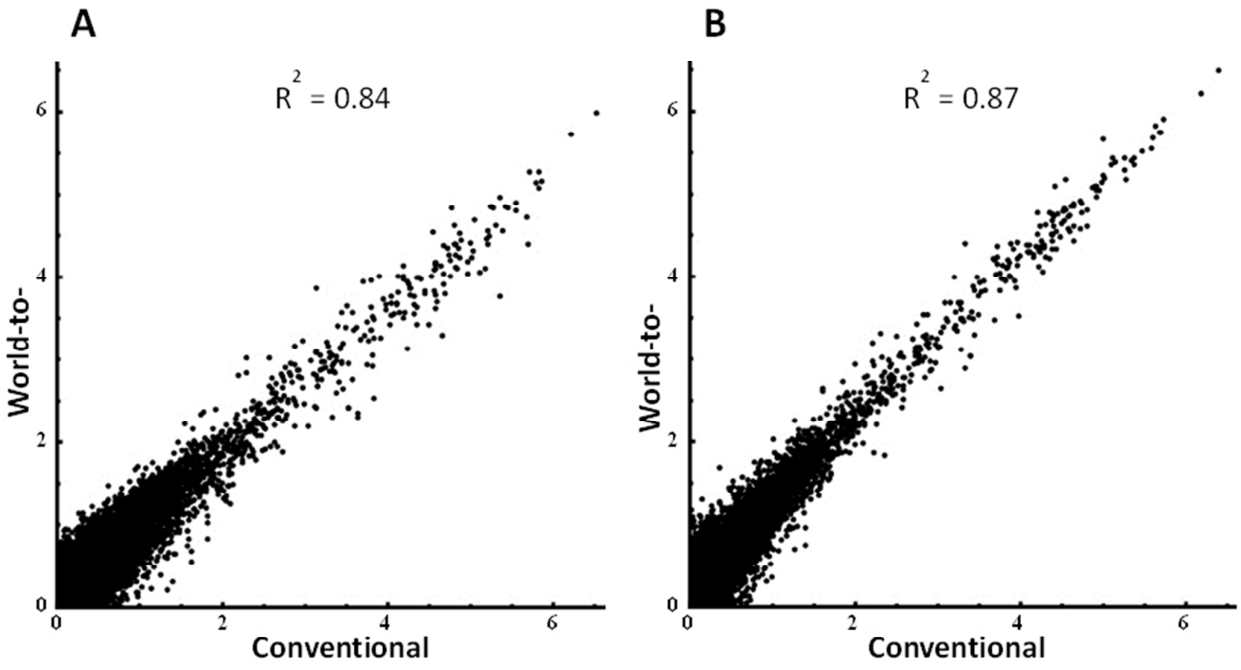

Figure S-4. RNA-Seq analysis of total RNA extracted and purified from human whole blood using the world-to-DMF system. Total RNA was prepared from 50 µL aliquots of whole blood from three different human donors, using the world-to-DMF system *versus* the conventional approach. Each RNA sample served as template in generating a cDNA library for Second Generation Sequencing (SGS). After depleting highly-abundant sequences (primarily rRNA) through molecular normalization, the six individually-barcoded libraries were mixed together in equal ratios, and the multiplexed library loaded into a MiSeq sequencer for a 50 bp single-end run. High-quality reads were mapped to the reference human genome, and transcript abundance [*i.e.*, fragments *per* kilobase of exon model *per* million mapped reads (FPKM)] was calculated for each of the genes "hit". The scatter plot shows the Log_{10} FPKM value for each gene, as measured using RNA prepared from a single blood specimen by the world-to-DMF system *versus* the conventional approach. The overall similarity of the FPKM profiles is indicated by the coefficient of determination (R^2) value. Results from blood specimens 2 (panel A) and 3 (panel B) are shown.

	World-to-DMF			Conventional		
Volume (µL)	25	50	100	25	50	100
A_{260}/A_{280}	1.65 ± 0.04	1.94 ± 0.12	1.99 ± 0.21	1.98 ± 0.17	1.89 ± 0.03	1.70 ± 0.14
RIN	6.8 ± 0.5	6.9 ± 0.7	6.2 ± 0.5	7.1 ± 0.4	6.5 ± 0.9	6.7 ± 0.3

Table S-1. Purity and integrity of total RNA extracted and purified from human whole blood using the world-to-DMF system. RNA samples were prepared from aliquots of different volumes (25, 50, or 100 µL) from the same blood specimen, using the world-to-DMF system *versus* the conventional approach. Three independent preparations of RNA *per* condition were analyzed for RNA purity (A_{260}/A_{280}) and integrity [RNA integrity number (RIN)]. The mean ± standard deviation for the three RNA samples is indicated.

Blood Specimen	RNA Preparation	cDNA Library Yield (ng)	Raw Reads (Millions)	Passed Qfilter (% of Raw)	Mapped to Human (% of Qfilter)	R^2 Value
1	World-to-DMF	83.2	2.8 2.8	95.9 95.9	99.0 99.0	0.89
	Conventional	89.6	3.1 3.0	95.5 95.5	99.0 99.0	0.89
2	World-to-DMF	110.0	2.4 2.3	96.0 96.0	99.4 99.4	0.84
	Conventional	81.6	3.4 3.4	95.1 95.2	98.9 98.9	0.83
3	World-to-DMF	72.0	2.8 2.8	95.7 95.7	98.8 98.8	0.87
	Conventional	81.6	2.5 2.4	95.6 95.6	99.2 99.2	0.87

Table S-2. RNA-Seq library yields and sequencing results. The world-to-DMF system *versus* the conventional approach was used to prepare total RNA from 50 μL aliquots of whole blood from three different human donors. Each RNA sample served as template in generating a cDNA library for Second Generation Sequencing (SGS); library yields are indicated. After depleting highly-abundant sequences (primarily rRNA) through molecular normalization, the six individually-barcoded libraries were mixed together in equal ratios, and the multiplexed library loaded into a MiSeq sequencer for a 50 bp single-end run. A duplicate MiSeq run was carried out to assess run-to-run variability. The total number of reads, percentage of reads passing qfilter, and percentage of high-quality reads mapping to the reference human genome are indicated for each library and MiSeq run. Transcript abundance [*i.e.*, fragments *per* kilobase of exon model *per* million mapped reads (FPKM)] was calculated for each of the human genes "hit", and the overall similarity of transcriptomes (FPKM profiles) generated from world-to-DMF *versus* conventionally prepared RNA is indicated by each coefficient of determination (R^2) value.