S-1

Selective Nucleic Acid Removal via Exclusion (SNARE): Capturing mRNA and DNA from a single sample

Lindsay Strotman[1], Rachel O'Connell[1], Ben Casavant[1], Scott Berry[1], Jamie M. Sperger[2], Joshua M. Lang[2,3] and David J. Beebe[1,3]

[1] Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, 1111 Highland Ave., Madison, WI, USA. E-mail: djbeebe@wisc.edu; Tel: +1-608-262-2260
[2] Department of Medicine, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, 1111 Highland Ave., Madison, WI, USA
[3] Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI, 53705, USA

ABSTRACT

We have developed a technique, termed SNARE (Selective Nucleic Acid Removal via Exclusion), that uses pinned oil interfaces to simultaneous purify mRNA and DNA from a single sample. A unique advantage of SNARE is the elimination of dilutive wash and centrifugation processes that are fundamental to conventional methods where sample is typically discarded. This minimizes loss and maximizes recovery by allowing non-dilutive re-interrogation of the

sample. The information found in this supplementary gives the primers and probes used in this manuscript. We also established a standard curve to show the efficiency of mRNA and DNA extraction using SNARE. Next, we looked at other genes to show SNARE's broad ability to isolate different genes using qPCR. Finally, we used different cell lines to show SNARE's broad utility for different samples.

qPCR Primers

Table 1: Primers and Probes			
Gene Expression Assay	Forward Primer (5'->3')	Reverse Primer (3'->5')	Probe Sequence (Roche Universal Probe Library)
AR mRNA	gccttgctctctagcctcaa	ggtcgtccacgtgtaagttg	ctgggaga (#14)
PSA mRNA	tccgtgacgtggattggt	cagggttgggaatgcttct	ggaggctg (#75)

Table S-1. Primers and probes used for detection in qPCR of mRNA androgen receptor (AR) and mRNA prostate specific antigen (PSA)

Table 2: TaqMan® Gene Expression Assay (Life Technologies)	
GAPDH mRNA	Hs02758991_g1 (Cat. # 4331182)
AR DNA	Hs04272737_s1 (Cat. # 4351372)
PSA DNA	Hs00377590_s1 (Cat. # 4351372)
GAPDH DNA	Hs9999905_m1 (Cat #. 4331182)

Table S-2. Taqman® gene expression assays for detection in qPCR of mRNA glyceraldehyde 3-phosphate dehydrogenase (GAPDH), DNA GAPDH, DNA AR and DNA PSA.

GAPDH mRNA and DNA Standard Curve

Methods & Materials. To estimate the threshold cycle values (C_t) for different LNCaP cell dilutions, we purified mRNA (Dynabeads® mRNA Direct™ Kit, Invitrogen) and DNA (MagaZorb®, Promega) separately from 10^6 LNCaP cells according to manufacturer's directions. Total mRNA and DNA amount was quantified using Qubit® 2.0 Fluorometer (Life

Technologies, USA). A standard curve was established for both mRNA and DNA using the GAPDH gene expression assay. It was assumed that a single cell expressed approximately 3 pg mRNA (Dynabeads® mRNA Direct™ Kit) and 6.6 pg of DNA[1]. These values were then used to confirm appropriate C_t values were obtained using the SNARE technique.

Results & Discussion. In Figure S-1A & B the GAPDH expression for mRNA and DNA is shown for nucleic acids purified from 1000, 100, 10 & 1 LNCaPs using the Qiagen DNA/RNA AllPrep kit and SNARE. Both techniques were graphed along with a standard curve. For both mRNA and DNA the standard curve was established by assuming LNCaPs have 3 pg of mRNA and 6.6 pg of DNA per a cell. Both standard curves show the gene expression values obtained using either Qiagen or SNARE technique are within the correct ranges. Finally, the mRNA standard curve shows lower efficiency but the amount of mRNA can greatly differ between cell types as compared to the DNA.

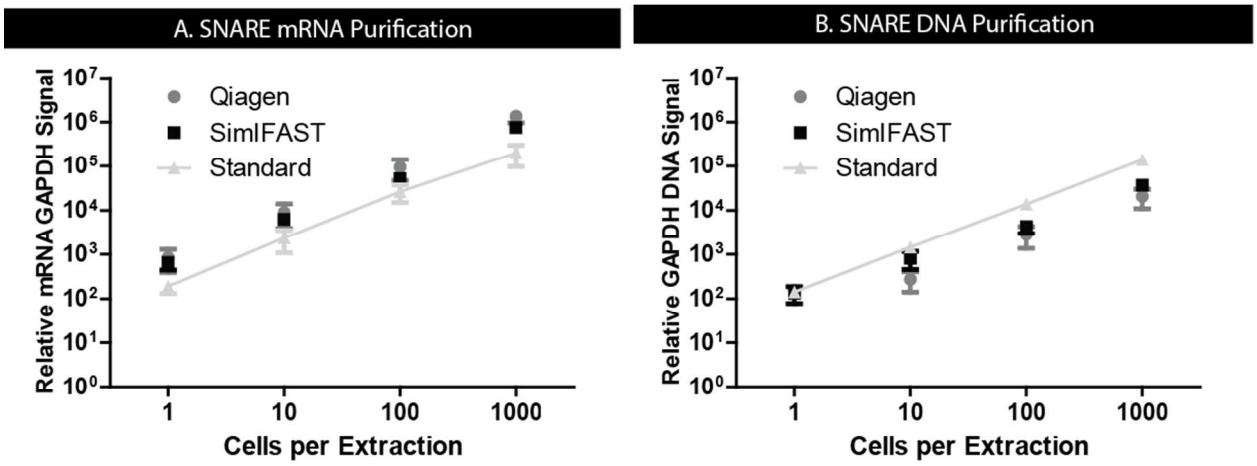

Figure S-1: A) GAPDH mRNA Expression of Qiagen vs. SNARE as compared to standard curve of mRNA extracted using Dynabeads® mRNA Direct™ Kit with 1 cell equal to 3 pg/cell B) GAPDH DNA Expression of Qiagen vs. SNARE as compared to standard curve of DNA extracted using MagaZorb® with 1 cell equal to 6.6 pg of DNA/cell

AR & PSA Relative Signal SNARE vs. Qiagen

Methods & Materials. mRNA elution from LNCaPs containing PMPs was reverse transcribed using a High Capacity cDNA Reverse Transcript kit (ABI, Foster City, CA) according to manufacturer's directions. For PSA and AR mRNA gene expression assays, 4 µL of template was mixed with 10 µL LightCycler 480® probes master mix (Roche, USA), 0.3 µM forward and reverse primers, 0.2 µM probes (Universal Probe Library, Roche, USA) and 5.2 µL NF water (Primers and probes specified in Supplementary Table T-1). For all other gene expression assays, 1 µL TaqMan® Gene Expression Assay (Life Technologies, USA) replaced the primers and probes used previously (TaqMan® Gene Expression Assays specified in Supplementary Table T-2). Each reaction was amplified as previously described and relative gene signal was quantified and normalized using, $(2^{-(45-C_p)})$.

Results & Discussion. Figure S-2A shows both the SNARE and Qiagen method were able to detect AR and PSA using mRNA purified from approximately a single cell by qPCR. However, SNARE was able to detect AR and PSA in all samples at approximately a single cell, whereas the Qiagen kit could only detect 75 % of the samples. Higher variability in mRNA isolation was also observed for the Qiagen kit as sample size decreased. The differences could be due to sample lost through additional fluid transfer steps, centrifugal forces that result in fluid shear stresses and partial elution in wash buffers. In Figure S-2B, using SNARE we show AR signal is reduced to 75 % for 10 cell samples and PSA gene signal to 75 % for 100 cell samples. For the Qiagen kit AR signal was reduced to 50 % for 100 cell samples and PSA gene expression to 50 % for 1000 cell samples. The higher sensitivity seen with that of GAPDH DNA expression could be due to copy number aberrations from aberrant karyotypes in LNCaPs[2], a feature common to cancer cell lines. For AR and PSA, the lower sensitivity could also be due to primer design,

especially for PSA as there are three highly homologous isoforms[3].

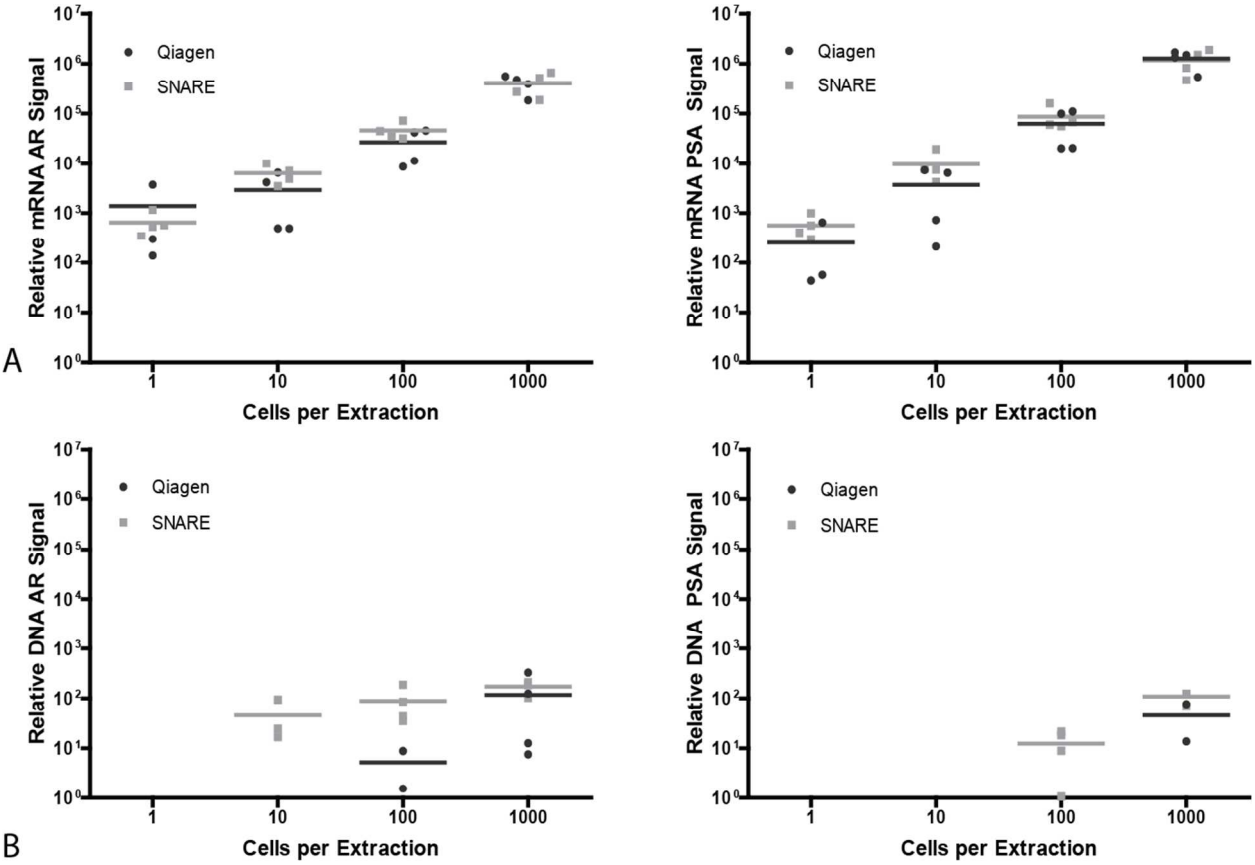

Figure S-2: Comparison of A) relative AR and PSA mRNA, and B) AR and PSA DNA signal purified from 1000, 100, 10 or 1 LNCaPs using SNARE (grey dots) or Qiagen (black dots). Each dot represents a nucleic acid purification procedure with horizontal lines representing the mean of the individual experiments. Sample size per a group n=4.

SNARE Method Using Two Different Cell Lines

Methods & Materials. Two different cell lines were used to verify mRNA and DNA extraction and purification using SNARE. All cells were cultured at 37 °C and maintained under 5 % CO_2 in polystyrene flasks until confluent. Human myocardio fibroblasts (HMFs) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Mediatech, Herndon, VA) containing 10 % fetal

calf serum (FCS) and 1 % Pen-Strep/ Human acute monocytic leukemia cell line (THP-1) were cultured in Cornig Cellgro® RPMI 1640 Medium (VWR, USA) containing 10 % fetal bovine serum (FBS) and 1 % Pen-Strep. Cells were released using a 0.05 % trypsin/EDTA solution and collected via centrifugation. A 1:10 serial dilution of 100,000 to 1000 LNCaPs/mL of 1x PBS was performed. 10 µL of each serial dilution (n=2) was processed using SNARE, which correlated to 1000, 100, 10 and 1 LNCaP per a device. 10 µL of each serial dilution was processed using the SNARE method. GAPDH gene expression assays were performed on both mRNA and DNA and delta C_t values calculated.

Results and Discussion. To determine that SNARE could be used for a variety of cells lines, two cell lines of monocyte and fibroblast origin were processed. Both mRNA and DNA were able to be purified from the low cell number population using the SNARE technique. With both cell lines, single cell sensitivity was achieved (See Figure S-3). However, for DNA the sensitivity was reduced to 10 cells. The decrease in relative DNA expression sensitivity could be due to the hypothesis that LNCaPS, a cancer cell line, might have more copies of GAPDH due to aberrant chromosome numbers.

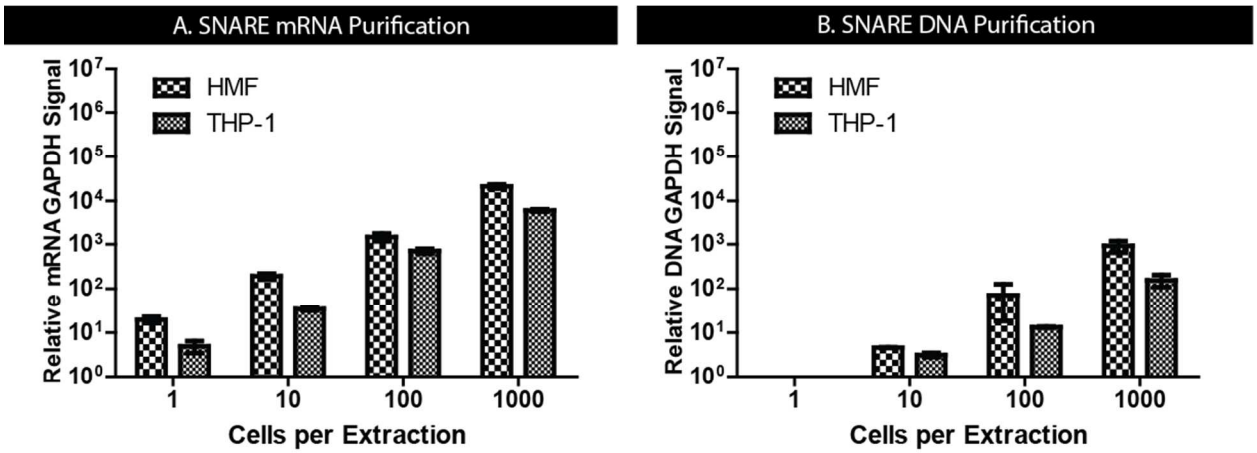

Figure S-3: A) Comparison of GAPDH relative mRNA expression purified from 1000, 100, 10 or 1 using the SNARE method for HMF or THP-1 cell lines. B) Comparison of GAPDH relative DNA expression purified from 1000, 100, 10 or 1 using the SNARE method for HMF or THP-1 cell lines.

REFERENCES

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