

Purify RNA from a Single Cell Using the ArrayPure™ Nano-scale RNA Purification Kit

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The recently introduced ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from 1 to 10,000 eukaryotic cells, quantities typically obtained with laser-capture procedures.^{1,2} Previously we showed that ArrayPure RNA from 20 HeLa cells could be successfully used in 1-round and 2-round RNA amplification procedures.³ We also compared the real-time RT-PCR results for RNA purified from 10 to 10,000 HeLa cells by the ArrayPure Kit and another supplier's kit.³ Here we present real-time RT-PCR data for RNA purified from 1, 10, and 100 cells, demonstrating the ability of the ArrayPure Kit to purify RNA from a single cell.

Methods

HeLa cells were grown in tissue culture, aseptically diluted, and trapped inside sterile 5- μ l microcapillary pipets.⁴ The number of cells isolated was verified by observation with an inverted microscope. Cells were eluted by centrifugation from the capillary pipets, washed with phosphate buffered saline, and RNA was purified using the ArrayPure Kit.

To compare the amount of RNA purified from varying numbers of cells, cDNA was prepared from the RNA using EPICENTRE's MMLV Reverse Transcriptase, and the human β -actin gene was amplified by real-time PCR using the FailSafe™ PROBES Real-Time PCR System (See page 18). Figure 1A shows the amplification plot of the cDNA from an average of 1, 10, and 100 cells. The negative control sample contained only tissue culture media that had gone through the purification procedure and that, as expected, did not amplify. Plotting the threshold cycle (C_T) vs log of the average initial cell number gives a standard curve (Figure 1B) that indicates linearity of response over 3 orders of magnitude from 1 to 100 cells with a correlation coefficient of 0.992.

Conclusion

The new ArrayPure RNA Purification Kit successfully prepares RNA from a single eukaryotic cell, with the purity and quality necessary to prepare cDNA or to be amplified in 1-round and 2-round RNA amplification procedures.

Acknowledgments

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References

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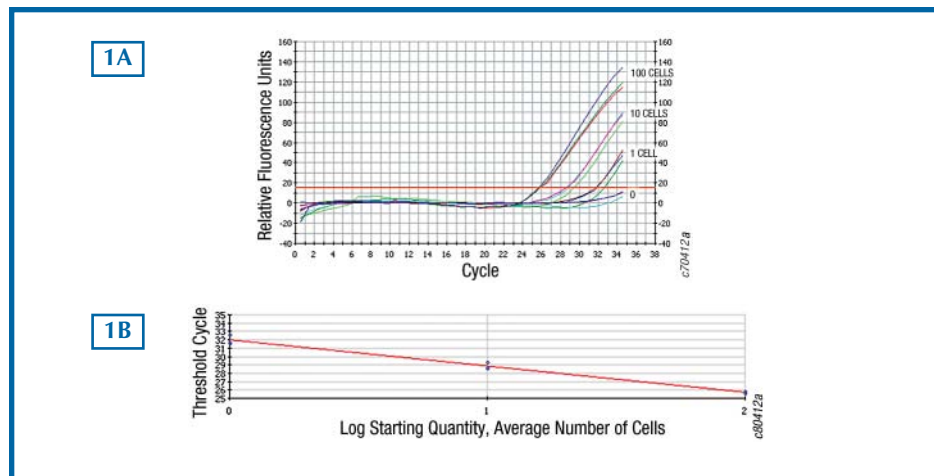


Figure 1. Real-time RT-PCR amplification data shows RNA purification from a single cell.

RNA purified from HeLa cells using the ArrayPure™ Nano-scale RNA Purification Kit was converted to cDNA using EPICENTRE's MMLV Reverse Transcriptase. The corresponding cDNAs were amplified, in triplicate, using the FailSafe™ PROBES Real-Time PCR PreMix-Choice Kit (PreMix 3). Real-Time PCR results were obtained using the Bio-Rad iCycler iQ™ Real-Time PCR Detection System and cycling conditions of 94°C (2 minutes) followed by 35 cycles of 95°C (10 seconds), 60°C (10 seconds), and 72°C (30 seconds). Primers used were for the human β -actin gene: 5'- TGG ACA TCC GCA AAG ACC TG and 5'- CCG ATC CAC ACG GAG TAC TT. The TET/BHQ1 dual-labeled fluorescent probe was 5'- TET-CAC CAC CAT GTA CCC TGG CAT TGC C-3'-BHQ1. **A.** The amplification plot for reactions containing cDNA prepared from an average of 100, 10, 1, and 0 (medium alone) cells. **B.** The standard curve produced by plotting the log of the average initial HeLa cell number against the cycle threshold (C_T) is linear. Slope = -3.14; Correlation Coefficient = 0.992.

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ArrayPure™ Nano-scale RNA Purification Kit

MPS04050 50 Purifications

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