



Purify RNA from 10 to 10,000 Eukaryotic Cells Using the ArrayPure™ Nano-scale RNA Purification Kit

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The ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from a few hundred eukaryotic cells, a quantity typically obtained with Laser Capture Microdissection procedures.^{1,2} The kit contains only aqueous solutions and requires no toxic organic solvents.³ This nano-scale protocol has been specifically designed for and tested on 10 to 10,000 eukaryotic cells. To purify RNA from a greater number of cells, the original MasterPure™ RNA Purification Kit is recommended.

Purify RNA from 10 cells

The ArrayPure Kit was tested and compared to two other commercially available kits. Those two kits gave results comparable to each other, so the data from only one kit is shown here. Ten-fold serial dilutions of intact HeLa cells were dispensed into tubes at 10⁴, 10³, 10², 10 or 0 (medium control) cells per tube. These were dilutions of living cells, not dilutions

of a cell lysate, which other vendors have used to claim low cell numbers. The RNA at each cell dilution was purified using the ArrayPure Nano-scale RNA Purification Kit or the other supplier's kit, in duplicate.

The ArrayPure method initially uses RNase-free DNase I. In subsequent steps the DNase is removed from the purified RNA by precipitation. Tests showed no detectable double-stranded DNA and no residual DNase activity in the final purified RNA.

For 10⁴ and 10³ cells, RNA was quantitated using RiboGreen® fluorescence. The average total RNA obtained from 10⁴ and 10³ cells with the ArrayPure Kit was 213 and 21 ng, respectively, and with the other supplier's kit was 197 and 15 ng, respectively. The amount of RNA purified from 100 cells and 10 cells was below the RiboGreen® assay detection limit; so RNA from all samples was compared by quantitative RT-PCR.

Prepare cDNA for real-time PCR

To compare the amount of RNA purified from each cell dilution, cDNA was prepared from the RNA and used in real-time PCR reactions. Figure 1 shows the amplification plots resulting from the ArrayPure samples (1A) and from the other supplier's samples (1B). The ArrayPure samples have lower threshold cycles (C_T) at lower cell concentrations, indicating that the reactions contain more template. Data for the standard curves derived from the amplification plots are shown in Table 1, on page 6. An acceptable range for PCR efficiency is 90 to 110%. The low PCR efficiency (83.5%) for the samples prepared with the other supplier's kit indicates that the standard curve is too inaccurate to use for template quantification.

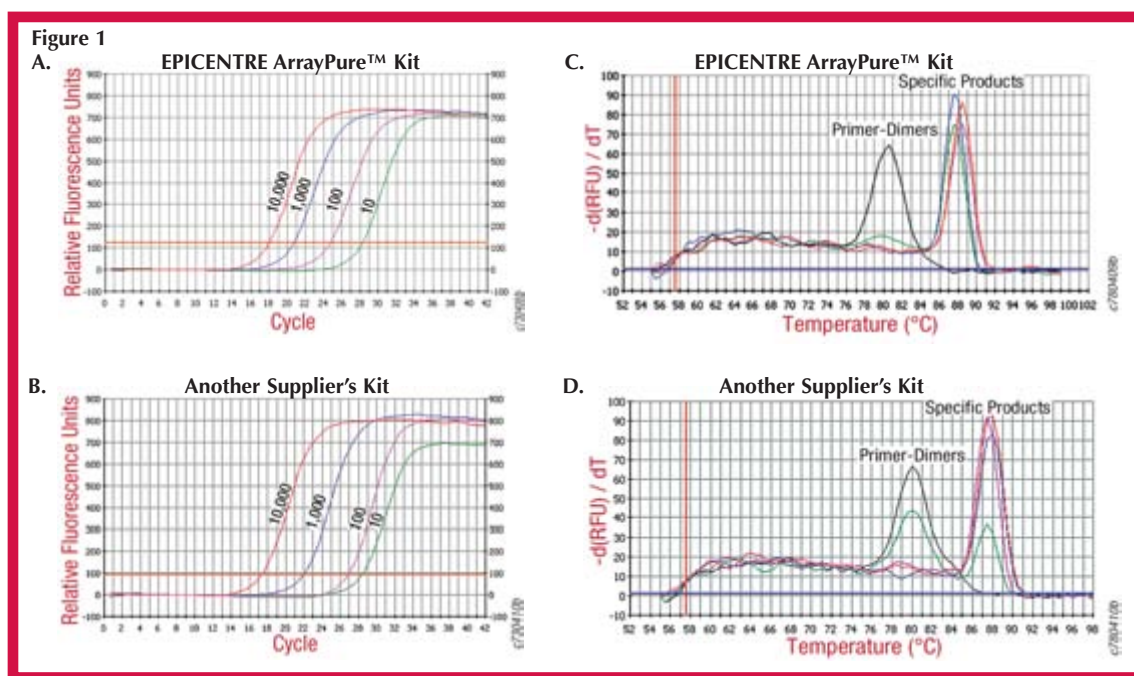
Melt curve analyses (1C and 1D) indicate that the medium control (0 cells) purified by ArrayPure (1C) had no RNA and

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Figure 1. Quantitative RT-PCR amplification plots were generated using cDNA prepared from RNA purified with the ArrayPure™ Nano-scale RNA Purification Kit or with another supplier's kit. A. RNA purified with the ArrayPure Kit. **B.** RNA purified with another supplier's kit. Intact HeLa cells were serially diluted ten-fold in growth medium, washed with PBS, and dispensed at 10⁴, 10³, 10², 10 or 0 (medium control) cells per tube. RNA was purified using either the ArrayPure Kit or the other supplier's kit, in duplicate, for each cell concentration. Purified HeLa RNA was converted to cDNA using EPICENTRE's MMLV reverse transcriptase. The corresponding cDNAs were amplified using the FailSafe™ Real-Time PCR System with SYBR® Green I dye on a Bio-Rad iCycler

iQ™ Real-Time PCR Detection System. Cycling conditions were 95°C (2 minutes) and 45 cycles of 95°C (20 seconds), 53°C (30 seconds), and 72°C (30 seconds). Primers were for human cyclophilin A (peptidylprolyl isomerase A) 5'- CAT ACG GGT CCT GGC ATC TTG and 5'- GCC ATT CCT GGA CCC AAA GC.

Melt Curve Analysis of the Quantitative RT-PCR. C. RNA purified with ArrayPure. PCR amplification of cDNA corresponding to RNA purified from 10⁴, 10³, 10² and 10 HeLa cells all yielded specific PCR products (peaks at 88°C). The 0-cell (medium control) sample yielded only primer-dimers (peak at 80°C to 81°C) indicating the absence of detectable RNA, as expected. **D.** RNA purified with another supplier's kit. The cDNA corresponding to RNA purified from 10 HeLa cells produced primarily primer-dimers and the 0-cell (medium control) sample produced only primer-dimers.



Purify Microbial DNA from Water Samples. . . (continued from page 4)

source, PCR may yield additional, slower migrating amplicons. These heteroduplex amplicons are created when rDNA from different species anneal together, and can be prevented by a method known as "PCR reconditioning."⁴ In this method the amplification reaction is diluted 1:10 in fresh reaction mix before the last 3 PCR cycles.

Conclusion

The EPICENTRE WaterMaster DNA Purification Kit effectively purifies microbial DNA from environmental water sources using a simple procedure, with no toxic solvents or bead beating. DNA is recovered in a low, 60-µl

volume and is ready for PCR or other techniques, as described in the poster presented at the 12th International Meeting on Microbial Genomes (2004) <http://www.epicentre.com/posters/enviroDNAposterweb.pdf>.

References

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www.epicentre.com/watermaster.asp

WaterMaster™ DNA Purification Kit

WM04005	5 Purifications
WM04020	20 Purifications

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PURIFICATION KIT

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produced only primer-dimers, as expected. ArrayPure RNA from as little as 10 HeLa cells produced cDNA that gave a specific real-time PCR product. RNA purified from 10 HeLa cells with the other supplier's kit did not produce a significant amount of cDNA as indicated by the substantial primer-dimer peak in the 10-cell PCR reaction (1D).

RNA amplification

The ArrayPure Nano-scale RNA Kit is designed to prepare RNA that can be amplified, which is often necessary for

microarray work. From 2.5×10^4 cells, 1 round of RNA amplification was sufficient to amplify 220 ng of purified RNA to 40 µg of RNA. With 20 HeLa cells (400 pg RNA), 2 rounds of amplification produced 32 µg of amplified RNA. After both 1 and 2 rounds of amplification, the RNA could be used for real-time PCR.

Acknowledgments

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References

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www.epicentre.com/arraypure.asp

ArrayPure™ Nano-scale RNA Purification Kit

MPS04050	50 Purifications
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RNA PURIFICATION KIT

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Table 1. Standard curve data generated from quantitative RT-PCR amplification plots.

RNA Purification Kit	Correlation Coefficient	Slope	PCR Efficiency (%)
ArrayPure™ Kit	0.998	-3.412	96.4
Other supplier's kit	0.980	-3.793	83.5