

Ultra-Sensitive Purification of Microbial Nucleic Acids Using the MasterPure™ Complete DNA and RNA Purification Kit

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Nucleic acid amplification has become the method of choice for identification and typing of microorganisms. In many cases, the limiting factor for sensitive detection is the method used for extraction of the nucleic acid. The MasterPure Complete DNA and RNA Purification Kit is specifically designed to provide research laboratories with a rapid, sensitive, non-hazardous, and extremely simple method for isolating microbial DNA or RNA. The method uses a modified salt precipitation protocol that incorporates a co-precipitant* that substantially improves sensitivity and permits the extraction of RNA and/or DNA from virtually any microbial sample.

The gold standard for nucleic acid extraction involves the removal of proteins from nucleic acids using organic solvents such as phenol and/or chloroform.¹ The hazardous nature of these methods has led to the development of column-based methods that eliminate the organic solvents. Unfortunately, column technologies introduce other problems including: contamination due to repeated contact with the columns; complicated manipulation of buffers while loading and washing the columns; frequent labeling and handling of microcentrifuge tubes; sensitivity problems due to non specific binding; and finally, "drop-out" issues where no nucleic acid is detected. What is needed is a method that combines the consistency of organic methods, with the safety of column methods.

In this article, we compare the MasterPure Complete DNA and RNA Purification Kit with a major manufacturer's spin column method for DNA extraction. We found that the spin column method resulted in reduced yields at low cell numbers relative to the MasterPure Complete Kit. At very low cells numbers, the column method produced no detectable DNA. These data suggest columns have non-elutable binding sites that reduce the sensitivity of the method.

Methods

MasterPure Complete Protocol

The MasterPure Complete technology is based on the salt precipitation method described by Miller *et al.*;² specific protocols are available on our web site at www.epicentre.com/lit/mpclit.htm. Twenty µl samples from serial 10-fold dilutions of a liquid *E. coli* strain MC1061-lambda (containing 2×10^7 to 200 organisms per 20 µl sample) were processed as follows. Bacteria were added to 300 µl of Tissue and Cell Lysis Buffer (RNase A was added for DNA-only samples) and treated with

proteinase K. After chilling on ice, 160 µl of Protein Precipitation Reagent were added and the debris pelleted. The supernatant was poured into a fresh microcentrifuge tube and the nucleic acid precipitated with isopropanol. After washing with 70% ethanol, the pellet was resuspended in 10 µl of sterile water. DNA concentrations were assayed using a Hoefer DyNA Quant™ fluorimeter and Hoechst 33258 fluorescent dye. Purity of nucleic acids was assayed by spectrophotometry.

Spin Column Method

The spin column method was performed according to the manufacturer's instructions. The same dilution series described above was processed as follows. Twenty µl of diluted cells were pelleted and resuspended in the manufacturer's cell lysis solution and treated with proteinase K. After loading and washing the spin column, the DNA was eluted in 50 µl of sterile water as suggested by the manufacturer for samples containing small amounts of DNA.

PCR Amplification

A 188 bp region of an integrated lambda genome was amplified using the following conditions; PCR reactions contained 0.5 µM of each primer (TACACAACCGCC-CAACTGC, forward primer; CGGGAACGGATAACCTCAC, reverse primer), 25 µl of MasterAmp 2X PCR Buffer E (web address [epicentre.com/lit/maoptlit.htm](http://www.epicentre.com/lit/maoptlit.htm)), 0.25 µl MasterAmp™ Taq Thermostable DNA Polymerase (<http://www.epicentre.com/lit/taqlt.htm>); and water to 50 µl total volume. Cycling conditions included a denaturation step at 94°C for 1 min., followed by 30 cycles of: 94°C for 1 min.; 60°C for 1 min., 72°C for 1 min. Amplification products were resolved on 2% agarose gels and stained with ethidium bromide.

Results and Discussion

Yield Comparisons

The advent of spin columns provided an alternative to organic solvents for the extraction of nucleic acids. While spin columns have substantial problems, such as limited binding capacity and cumbersome protocols, these disadvantages have not prevented columns from becoming a popular method for nucleic acid purification because they eliminate hazardous solvents. Other potential problems with spin columns are non-elutable binding and dead volumes that may limit sensitivity. Most nucleic acid purification protocols were developed under situations where the amount of starting material was not limiting.

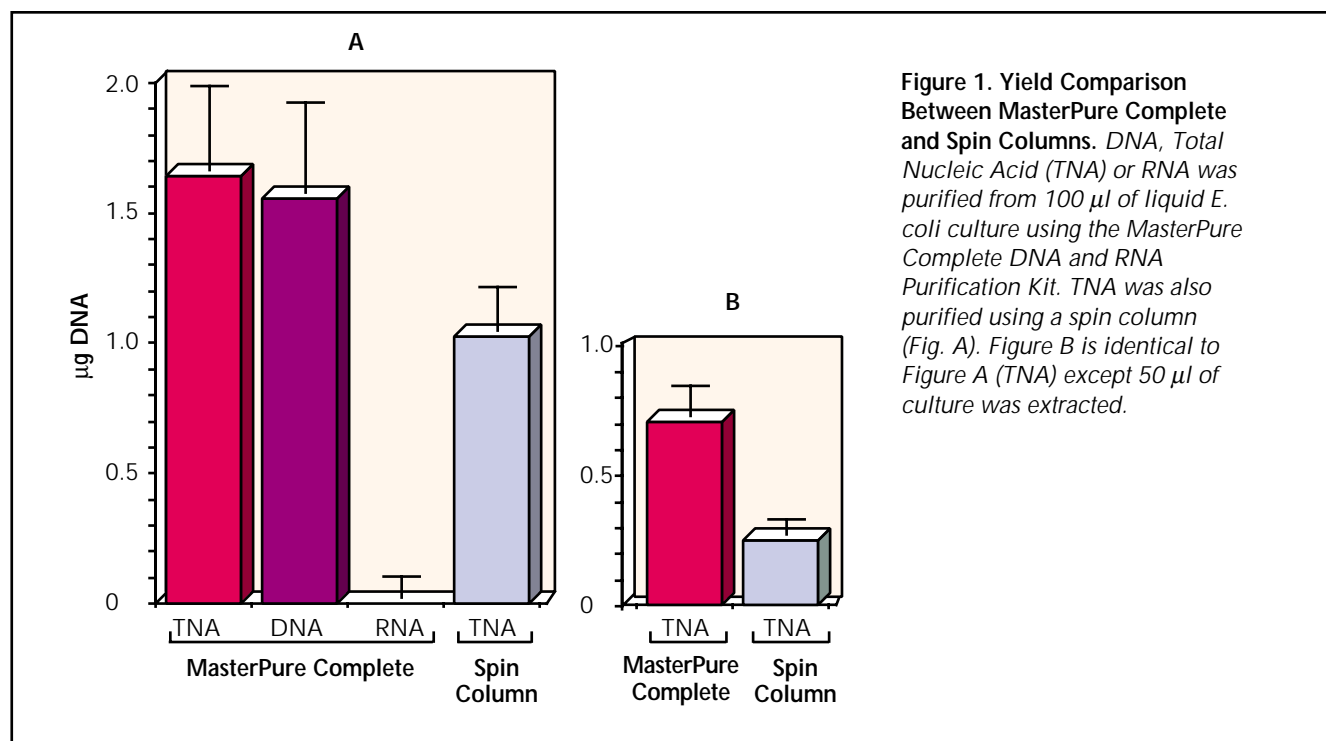


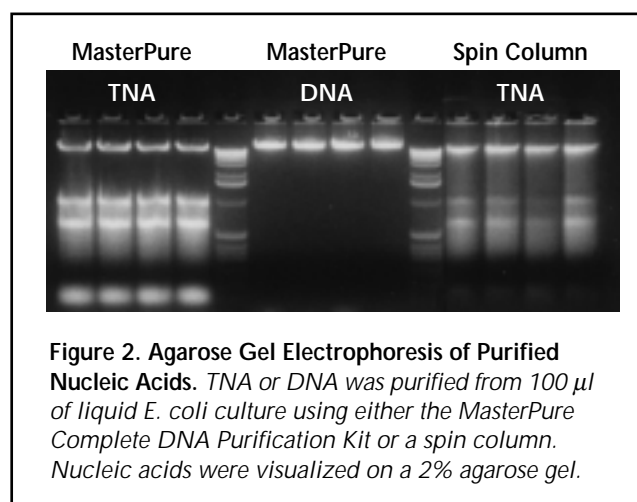
Figure 1. Yield Comparison Between MasterPure Complete and Spin Columns. DNA, Total Nucleic Acid (TNA) or RNA was purified from 100 µl of liquid *E. coli* culture using the MasterPure Complete DNA and RNA Purification Kit. TNA was also purified using a spin column (Fig. A). Figure B is identical to Figure A (TNA) except 50 µl of culture was extracted.

Many research laboratories and essentially all clinical microbiology laboratories, however, need extraction protocols that give the highest sensitivity possible.

We have recently developed a technology for nucleic acid isolation that combines the simplicity and sensitivity of non-column based methods with the safety of non-organic methods. The MasterPure Complete DNA and RNA Purification Kit uses a strong ionic detergent (and proteinase K, if necessary) to lyse cells and high salt to precipitate contaminating proteins. Nucleic acids are then concentrated by isopropanol precipitation, which allows resuspension in any volume necessary to assure sensitive detection.

This study was designed to determine the yield of DNA from small samples and at the low concentrations of microorganisms. Figure 1 compares the DNA yields obtained with the MasterPure Complete DNA and RNA Purification Kit versus the spin column method. The MasterPure Complete protocols for the isolation of total nucleic acid (TNA) and DNA are identical except the DNA samples are treated with RNase A to eliminate intact RNA. Figure 1A shows that the yield of DNA (as assayed by fluorimetry, which is insensitive to RNA) from 3.5×10^8 cells is similar using either the DNA or TNA protocol. The spin column method yields substantially less DNA relative to either of the MasterPure Complete protocols. Specifically, the yield of DNA using the TNA protocol was 63% lower (0.53 µg less) using the spin column method than with the MasterPure Complete protocol. To help clarify the reason for lower yields with the spin column method the experiment was repeated

with half the number of cells. As shown in Figure 1B the column method yielded 0.46 µg less DNA. This suggests that the difference is due to non-elutable binding of approximately 0.5 µg of DNA to the column. Nucleic acids purified by the two methods were analyzed by agarose gel electrophoresis and stained with ethidium bromide (Figure 2). The TNA and spin column methods purify both RNA and DNA. Treatment with RNase A in the

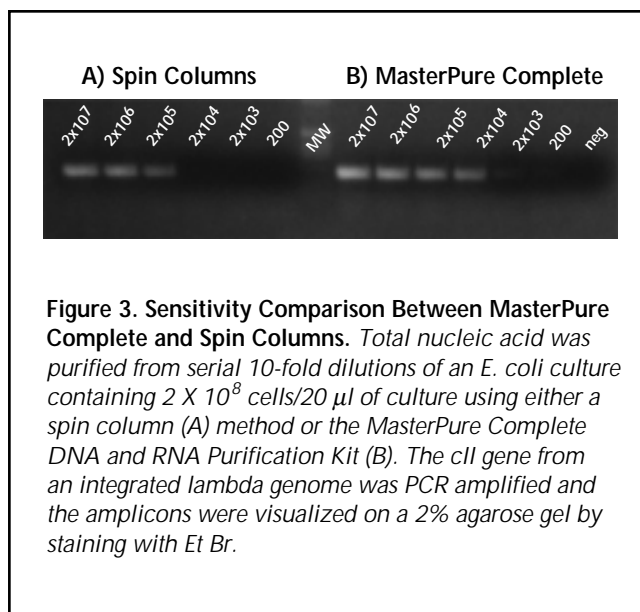


MasterPure DNA protocol eliminates the intact RNA.

The reduced DNA yield using the spin column method suggested that the MasterPure method may be more sensitive for extraction from small numbers of organisms. To investigate whether this is the case, DNA was extracted

continued

from 10-fold serial dilutions of an overnight culture of *E. coli* cells. The purified nucleic acid was used to amplify a region of the lambda *cII* gene. Consistent with the yield data, the spin column method was substantially less



sensitive in extracting DNA from small numbers of cells (Figure 3).

Conclusion

As molecular technology evolves away from first generation extraction protocols, new methods must be developed that permit rapid learning and optimization. Older technologies for extracting DNA, like phenol-chloroform and spin column methods, were not designed to be rapid or particularly simple. In contrast, the MasterPure Complete DNA and RNA Purification Kits were specifically conceived and developed for easy use. The method is useful for the full range of samples extracted by research or clinical laboratories, including samples containing extremely low numbers of organisms.

References

1. Sambrook, J. *et al.* (1989) in: *Molecular Cloning: A Laboratory Manual* (2nd ed.), Cold Spring Harbor

MasterPure Complete DNA and RNA Purification Kit

MC89010	10 Purifications (trial size)
MC85200	200 Purifications

MasterPure DNA Purification Kit (for isolating TNA or DNA)

MCD85201	200 Purifications
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MasterPure RNA Purification Kit (for isolating RNA only)

MCR85102	100 Purifications
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Related Products:

MasterPure™ Yeast DNA Purification Kit
 Masterpure™ Plant Leaf DNA Purification Kit
 Masterpure™ DNA Purification Kit for Blood
 Masterpure™ Buccal Swab DNA Extraction Kits

For more information about the MasterPure™ Complete DNA and RNA Purification Kit, please visit our website at www.epicentre.com/catalog/mpc.htm or circle reader service number N635 on the reply card found in the center insert.

MasterPure Complete DNA and RNA Purification Kit

MC89010	10 Purifications (trial size)
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