The effects of phenol on nucleic acid preparation and downstream applications

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Phenol contamination of nucleic acid preparations (prepared using either a commercially available kit or a conventional method), can adversely affect the yield and quality of the nucleic acid obtained. Carryover of phenol also inhibits or reduces the efficiency of downstream applications such as PCR, sequencing, and screening.

Here we demonstrate how phenol contamination can affect both nucleic acid preparation and downstream reactions. We further show that QIAGEN® purification technologies allow the isolation of pure nucleic acids suitable for use in any downstream reaction, and save time when compared to purification methods using phenol.

Materials and methods

Nucleic acids were purified using conventional phenol–chloroform extraction (1), a commercial acid-phenol method, or the relevant QIAGEN kit (RNeasy® Kits for isolation of total RNA, QIAprep® Kits for purification of plasmid DNA, QIAamp® Kits for purification of genomic DNA, and MinElute™ and QIAquick® Kits for cleanup of DNA from enzymatic reactions). In some cases phenol was added to QIAGEN kit-purified nucleic acids, in order to assess the effects of phenol on downstream applications.

Results

Variable and reduced nucleic acid yields using phenol

UV scans of total RNA purified using an acid-phenol method show widely varying yields. The UV peaks are slightly shifted due to absorbance of phenol at 270 and 275 nm* (Figure 1A). There is also increased absorbance at 220–230 nm. In addition, variable and reduced yields are apparent on a formaldehyde agarose gel (Figure 1B). ▶

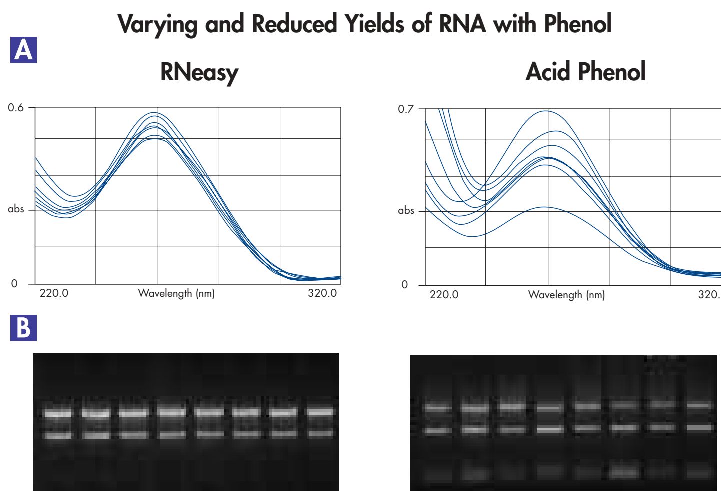


Figure 1 Total RNA was isolated from 1 x 10^6 HeLa cells using the RNeasy Mini Kit (QIAGEN) or a commercial acid-phenol–extraction method (Supplier L). The RNA isolation was performed 8 times with each method. RNA was eluted (RNeasy) or resuspended after ethanol precipitation (phenol method) in 100 µl RNase-free water. **A** Aliquots were diluted in 10 mM Tris·Cl, pH 7.5, and analyzed by UV spectrophotometry. **B** 10 µg of each sample, based on the A_{260} readings in (A), was analyzed on a formaldehyde agarose gel.

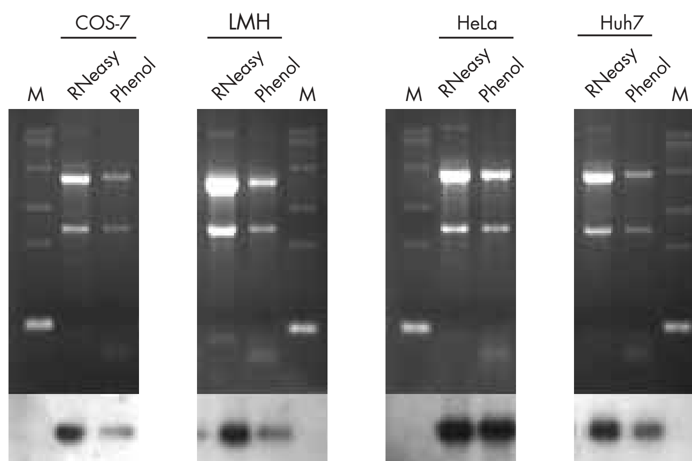


Figure 2 Various cell lines grown in single wells of a 24-well plate (approximately 2.5 x 10^5 cells). RNA was isolated using the RNeasy Mini Kit (QIAGEN; **RNeasy**) or a commercial acid phenol method (Supplier L; **Phenol**). RNA was eluted (RNeasy) or resuspended (Phenol) in 100 µl RNase-free water, and 20 µl was loaded per lane. Blots were hybridized with a ^{32}P-labeled GAPDH probe. **M**: 0.24–9.5 kb ladder.

* In addition this can affect the A_{260}/A_{280} ratio (see reference 2).

References

1. Sambrook, J., Fritsch, E.G., and Maniatis, T., eds. (1989) Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

2. Stulnig, T.M. and Amberger, A. (1994) Exposing contaminating phenol in nucleic acid preparations. BioTechniques **16**, 403.

Formaldehyde agarose gels and northern blots show that consistently higher yields of RNA were obtained with RNeasy Kits than with acid-phenol methods (Figure 2). Higher yields were obtained with a variety of different cell lines using the same number of cells per prep (approximately 2.5 x 10^5). In the acid-phenol preparations, tRNAs and small rRNAs are seen (Figures 1 and 2). RNeasy purification provides an enrichment for mRNA, and small RNAs (i.e., 5S rRNA and tRNA) are selectively excluded. The presence of these small RNAs in the acid-phenol preps can contribute to mispriming during RT-PCR.

Phenol Shortens Read Lengths in Sequencing

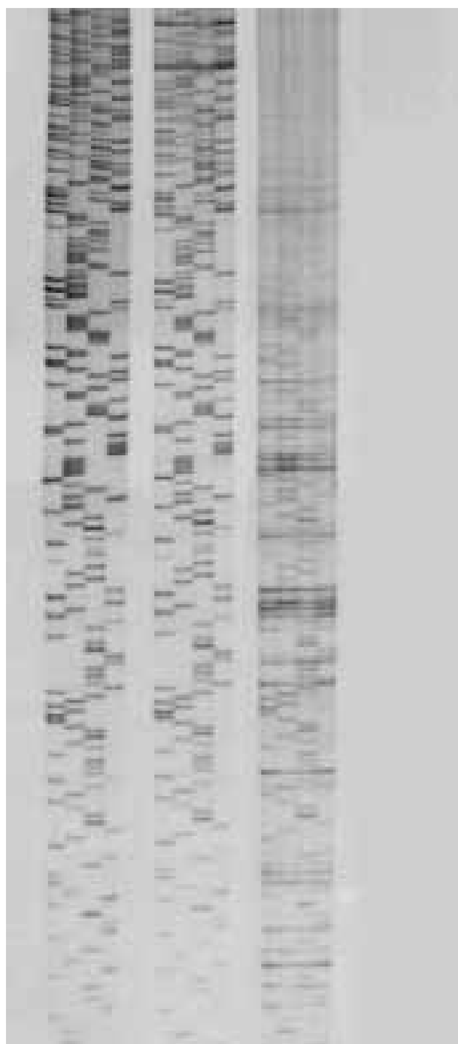


Figure 3 *Sequencing profile of plasmid DNA purified using the QIAprep procedure and sequenced in the absence (**C**: Control) or presence of phenol (concentrations indicated) prior to the sequencing reactions.*

Phenol contamination adversely affects sequencing and screening

The effect of phenol contamination on the quality of sequencing data was examined. Phenol was added to plasmid DNA prior to sequencing, so that the final phenol concentration in the sequencing reactions was between 0.1 and 2.25% (w/v). This corresponds to 0.01–0.25 µl saturated phenol solution in a 10 µl sequencing reaction. Above 1% (w/v) phenol, more compression bands were found in the sequence profiles, making reading of the sequence difficult or impossible (Figure 3). At phenol concentrations greater than 1.5% no sequence profiles could be obtained. Although phenol can be used efficiently to remove nuclease contamination from plasmid preparations, subsequent sequencing reactions are highly sensitive to traces of phenol contamination.

Phenol Decreases Accuracy in Screening

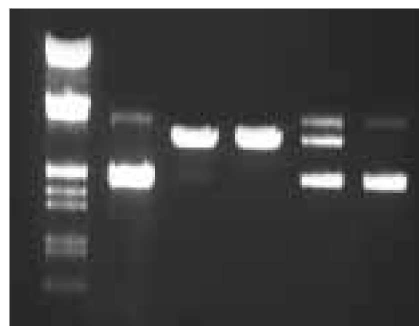


Figure 4 *Agarose gel analysis of DNA samples from Figure 3 digested with EcoRI. **U**: Undigested DNA; **M**: lambda HindIII-EcoRI markers.*

Analysis of plasmid DNA quality by agarose gel electrophoresis can be misleading since the phenol-contaminated plasmid DNA appears to be as pure as the uncontaminated DNA. Differences in plasmid quality due to phenol contamination can only be seen on agarose gels when the DNA is analyzed enzymatically, for example with a restriction enzyme (Figure 4). The phenol contamination leads to incomplete restriction digest and therefore to ambiguous screening results. The effects of phenol arise from its ability to denature proteins, thus inhibiting enzymatic reactions.

Phenol contamination affects PCR performance

Home-made PCR template preparation methods usually include a phenol extraction step. We examined the effects of phenol on PCR template preparation by purifying genomic DNA using the QIAamp DNA Blood Mini Kit and spiking preparations with final concentrations of 0.2% and 0.5% phenol.

Phenol, used for the removal of proteins such as proteases and nucleases, decreased the yield of PCR product when present at a final concentration of 0.2%. At 0.5% phenol, no PCR product was detectable (Figure 5).

Faster DNA cleanup with QIAquick and MinElute Systems

DNA cleanup of an enzymatic reaction or PCR sample was compared using QIAquick and MinElute DNA Cleanup Systems from QIAGEN with a conventional procedure using phenol–chloroform extraction and ethanol precipitation (1). Phenol extractions typically took 65 minutes from starting sample to ready-to-use DNA. In comparison, the MinElute procedure yielded ready-to-use DNA in only 6 minutes, and the QIAquick procedure in just 5 minutes — more than 10 times faster than phenol extractions. The QIAquick and MinElute DNA Cleanup Systems can be used for purifying DNA from all enzymatic reactions, including dephosphorylation, ligation, restriction digestion, end-labeling, primed synthesis, and nick-translation.

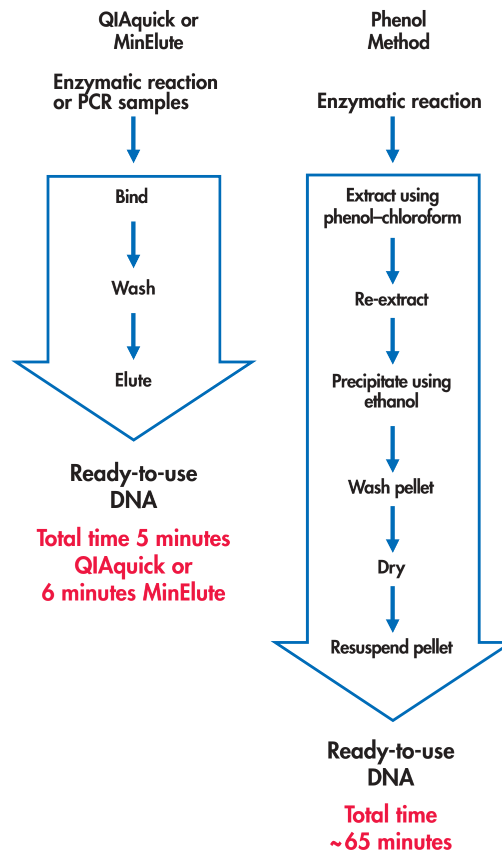
Conclusions

- Phenol can adversely affect the yield and quality of both RNA and DNA.
- Phenol carryover inhibits downstream enzymatic reactions. Nucleic acids purified using QIAGEN kits are suitable for even the most sensitive downstream applications
- QIAGEN kits are up to 12 times faster than phenol methods and yield reproducibly high quality nucleic acids
- QIAGEN offers fast, reliable, and convenient alternatives to phenol preps for all applications and throughput and purity requirements. ■

Effect of Phenol on Templates for PCR

Figure 5 *PCR was carried out according to the "Standard PCR Protocol" in the Taq PCR Handbook provided with QIAGEN Taq DNA Polymerase and Taq PCR Core Kits. 10 µl of each sample was analyzed after PCR on a 1% agarose gel. PCR templates were amplified in the absence (**Control**) and presence of phenol (concentrations indicated). **M**: markers.*

Faster Cleanup with QIAquick and MinElute Kits



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Ordering Information

Product	Contents	Cat. No.
QIAprep Spin Miniprep Kit — for isolation of plasmid DNA from bacterial cultures		
QIAprep Spin Miniprep Kit (50)*	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
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MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
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QIAamp Viral RNA Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	52904
QIAamp RNA Blood Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Mini Spin Columns, 50 QIAshredder™ Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
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RNeasy Plant Mini Kit (20)*	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Protect Mini Kit (50)*	RNA*later*™ RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124
RNeasy Protect Bacteria Mini Kit (50)	RNeasy Mini Kit (50) and RNAprotect™ Bacteria Reagent (2 x 100 ml)	74524

** Larger kit sizes available; please inquire.*