

Clean Up the Competition

Automated Purification of PCR Products Using MagneSil™ Paramagnetic Particles

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Abstract

We have developed a robust system to purify PCR products from reaction contaminants using MagneSil™ Paramagnetic Particles (PMPs). MagneSil™ PMPs act as a “mobile solid phase” to purify PCR products using laboratory robotic systems. Purified PCR products are ready-to-use in downstream applications, demonstrated by microarraying and fluorescent DNA sequencing.

The Wizard® MagneSil™ PCR Clean-Up System can be automated to purify 96 reactions in <1 hour.

Introduction

Researchers routinely purify PCR products to remove contaminating nucleotides, primers and small, nontargeted amplification products (e.g., primer-dimers). The Wizard® MagneSil™ PCR Clean-Up System^(a) removes such impurities while maintaining high yield. Purification of 96 PCR^(b) samples can be performed with complete “walk-away” automation on a robotic workstation. The purified DNA is suitable for spotting microarrays, fluorescent sequencing and many other molecular biology techniques. MagneSil™ systems are scalable, allowing scale-up for higher yield and scale-down for use in a 384 well format.

Fully Automated PCR Product Cleanup

Many post-PCR applications require removal of unincorporated primers, primer-dimers and other reaction components from the PCR product. Traditional purification methods such as ethanol precipitation are difficult to automate. The Wizard® MagneSil™ PCR Clean-Up System can be automated to purify 96 reactions in <1 hour. MagneSil™ PCR Clean-Up is especially amenable to automation because no vacuum or centrifugation steps are required compared to many filter-based methods. The principle behind the Wizard® MagneSil™ PCR Clean-Up System is illustrated in Figure 1.

Size Cutoff and Removal of Primers/Primer-Dimers

Primers and primer-dimers cause problems in microarray spotting by competing with the PCR product for binding sites. Residual primers also interfere with fluorescent DNA sequencing, often seen as elevated background. Some commercially available purification systems and protocols do not provide an adequate size cutoff for removing primers/primer-dimers from the PCR products. However, as evident in Figure 2, the Wizard® MagneSil™ PCR Clean-Up System efficiently removes primers (≤ 50 bp) and primer-dimers (≤ 100 bp).

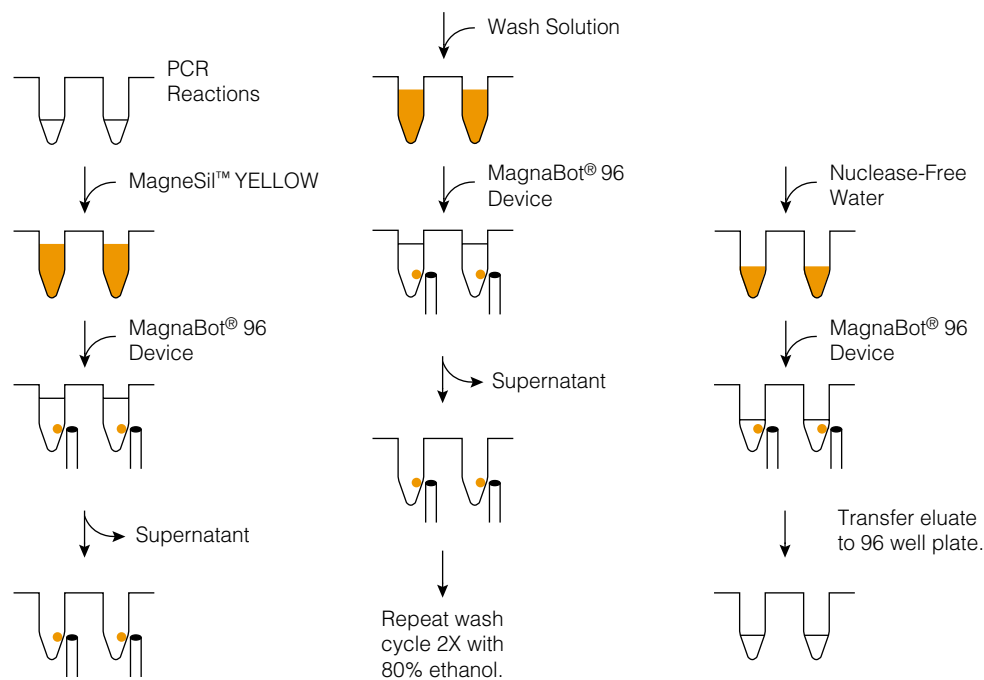
Microarrays Demand High Yields of PCR Products

Researchers preparing microarrays are interested in high yields of PCR products. The Wizard® MagneSil™ PCR Clean-Up System provides yields of $\geq 80\%$ for PCR products ≥ 500 bp. DNA fragments up to 23kb have been recovered with the same high yield (data not shown). The recovery efficiency of PCR products smaller than ~ 500 bp is size-dependent, decreasing with decreasing fragment size (Figure 3).

Many laboratories design and prepare their own arrays, using an assortment of reagents and equipment in a “home-brew” approach (1). cDNA microarrays are typically prepared by spotting purified PCR products on a solid substrate, often treated glass slides. The spotted PCR products serve as coordinately defined probes for interrogating mRNA expression levels. Target cDNAs are typically fluorescently or radioactively labeled during reverse transcription of RNA, prior to interrogation by hybridization (2).

In our hands, unpurified, spotted PCR products do not hybridize well to Cy[®]3- or Cy[®]5-labeled oligonucleotide probes (data not shown). Isopropanol precipitation, the most commonly used method for purifying PCR products is not fully suited to automated processing. Isopropanol precipitation is commonly used as an inexpensive alternative to commercial products. However, the method requires lengthy centrifugation steps and incubations at -20°C . When decanting the supernatant, one risks losing pellets containing the precipitated PCR products.

Reproducibility is an important issue when fabricating microarrays. In order to test the reproducibility of purified PCR products arrayed on poly-L-lysine slides, kanamycin RT-PCR products were purified using the Wizard® MagneSil™ PCR Clean-Up System. The RT-PCR products were purified directly or diluted 1:5, 1:25 and 1:100 prior to purification. The purified DNA was attached to poly-L-lysine-coated glass slides by contact printing in replicate sets (Figure 4). Separate microarrays were hybridized with either Cy[®]3- or Cy[®]5-labeled kanamycin cDNA targets produced using Promega’s ImProm-II™ Reverse Transcription System (Cat.# A3800) as shown in Figure 4. The PCR products were purified independently in three plates using the stated dilutions. Four independent sample series were spotted from each of the three plates. Each sample set was arrayed in two identical sets to address intra-sample spotting reproducibility (Figure 4, columns A and B). High inter- and intra-sample reproducibility were consistently seen, especially with undiluted product (c.v. = 0.31%), indi-



DNA Binding

MagneSil™ YELLOW particles selectively bind PCR products. A magnet is used to capture and hold the particles against the side of the well. Supernatants are removed to waste.

Washing

The plate is removed from the MagnaBot® 96 Device and the particles are resuspended in Wash Solution, mixed and captured. The wash supernatants are removed to waste. The particles are washed twice with 80% ethanol. Following the wash steps, the samples are air-dried.

Elution of DNA

To elute the purified PCR products, the particles are resuspended in Nuclease-Free Water and magnetically captured. The eluate is transferred to a clean plate on a MagnaBot® 96 Device to capture particle fines. The eluate is transferred to a clean plate ready to analyze.

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Figure 1. Schematic of the Wizard® MagneSil™ PCR Clean-Up System protocol.

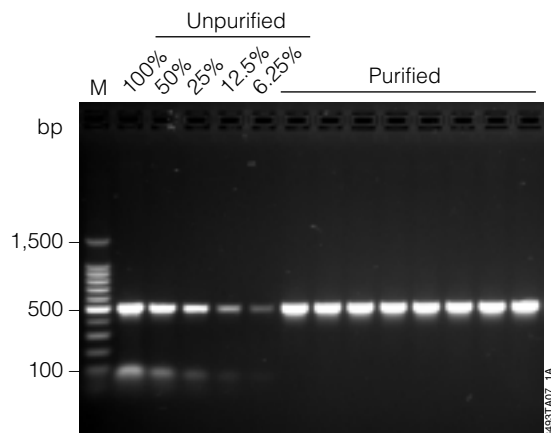


Figure 2. Removal of primer-dimers from PCR products. A titration of unpurified 500bp prothrombin PCR product is shown before purification (100%, 50%, 25%, 12.5% and 6.25% of the PCR sample). The ~100bp primer-dimers are visible in these lanes. Eight replicates purified using the Wizard® MagneSil™ PCR Clean-Up System show the 506bp prothrombin PCR product with no visible primer-dimers. The ethidium bromide-stained 1% agarose gel was imaged using a FluorChem™ fluorescence imaging system (Alpha Innotech). Lane M, 100bp DNA Ladder (Cat.# G2101).

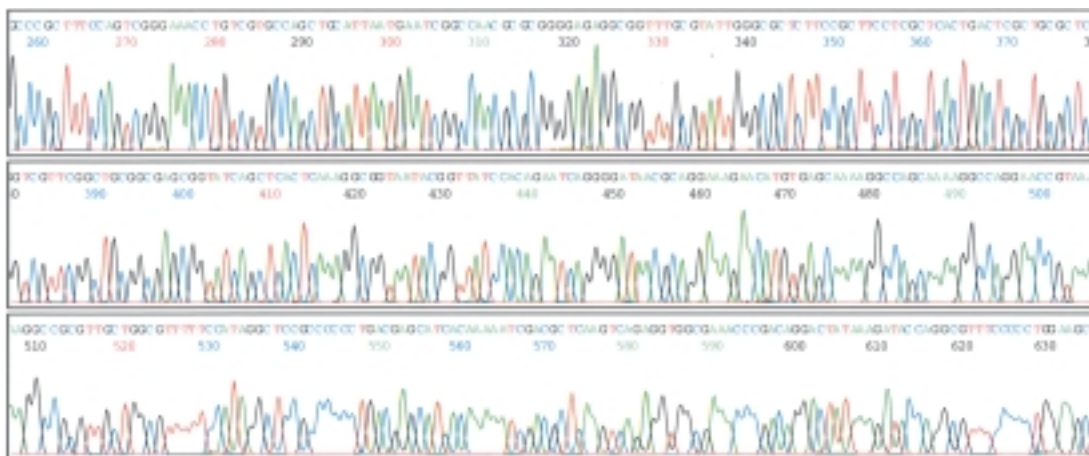


Figure 5. Fluorescent DNA sequencing. Samples from a 1,000bp PCR pool were purified in 96 wells (100µl per well) using the MagneSil™ PCR Clean-Up System. Twenty nanograms of each purified PCR product was sequenced using an ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit. The sequencing reactions were analyzed on an ABI PRISM® 3700 DNA Sequencer, according to the manufacturer's specifications. A representative trace is shown.

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References

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2. Schena, M. (2000) *Microarray Biochip Technology*, BioTechniques Books, Eaton Publishing, Natick, MA.
3. Otto, P. and Bjerke, M. (2001) *Promega Notes* **78**, 2–5.
4. *Wizard® Magnetic 96 DNA Plant System Technical Bulletin* #TB289, Promega Corporation.
5. *Wizard® MagneSil™ Plasmid Purification System Technical Bulletin* #TB286, Promega Corporation.
6. *Wizard® MagneSil™ Sequencing Reaction Clean-Up System Technical Bulletin* #TB289, Promega Corporation.

Protocol

- ◆ *Wizard® MagneSil™ PCR Clean-Up System Technical Bulletin* #TB290, Promega Corporation.
(www.promega.com/tbs/tb290/tb290.html)

Ordering Information

| Product | Size | Cat.# |
|---|----------------|-------|
| Wizard® MagneSil™ PCR Clean-Up System(a)* | 4 x 96 preps | A1930 |
| | 8 x 96 preps | A1931 |
| Wizard® MagneSil™ PCR Clean-Up System, HTP1(a)* | 100 x 96 preps | A1935 |
| MagnaBot® 96 Magnetic Separation Device | 1 unit | V8151 |

*For Laboratory Use.

Related Products

| Product | Size | Cat.# |
|----------------------|----------|-------|
| MagneSil™ YELLOW(a)* | 100ml | A9231 |
| Wash Solution* | 500ml | A8241 |
| Collection Plates | 4 plates | A9161 |
| Plate Clamp 96 | | V8251 |
| Plate Stand | | V8261 |

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(a)U.S. Pat. No. 6,027,945 and other patents pending.

(b)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

Technical Questions?

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