Clean Up 384 Wells at a Time

High-Throughput DNA Fragment Purification Using the MagneSil® Automated 384-Well Clean-Up Systems

By Brad Larson, B.A., and Tom Strader, B.S., Promega Corporation

Abstract

Automated fluorescent sequencing using energy transfer dyes is the current method of choice for DNA sequence determination. If not removed prior to analysis, certain reaction components such as buffer salts and unincorporated dye-labeled terminators can interfere with data collection in a capillary-based automated sequencer. Likewise, the presence of contaminants such as salts, unincorporated dNTPs and primers can interfere with analysis of PCR fragments by capillary electrophoresis or microarray spotting. Promega has developed high-throughput, validated, walkaway systems to remove these contaminants using the Beckman Coulter Biomek® FX liquid handler and the flexible format of the MagneSil® Paramagnetic Particles.

The MagneSil® 384-well clean-up methods provide an automated, walkaway solution for high-throughput purification of PCR products and fluorescent dye terminator sequencing reactions.

Introduction

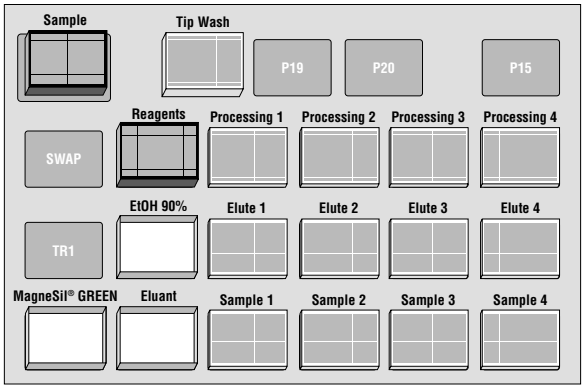
Automated fluorescent sequencing and PCR[a] continue to be industry standards in the field of genomics research. These applications, while continuing to evolve and improve, still require the removal of excess nucleotides, salts, primers and nontargeted amplification products for analysis and downstream applications. This fact, coupled with the need to test samples in a rapid yet efficient manner, has created the need for high-throughput, 384-well purification systems. Common purification methods based on gel filtration and/or precipitation by ethanol are problematic for these high-throughput applications, as they require (multiple) centrifugation and/or vacuum drying steps.

We have developed fully automated 384-well methods to meet the demands of a high-throughput purification system. These methods use MagneSil® Paramagnetic Particles[b] (PMPs) for PCR fragment and fluorescent dye terminator DNA sequencing reaction purification. The methods described in this article were developed on Beckman Coulter's Biomek® FX liquid handling workstation. MagneSil® technology in a 384-well format significantly increases throughput and reduces cost compared to a 96-well format. With the integration of multiple plate methods, this combination provides rapid, high density, hands-off solutions to purification needs. It requires few active ALPs (automated labware positioners), fits onto most deck configurations and can be scaled to any format.

Automated Methods

These methods were written for a Beckman Coulter Biomek® FX Liquid Handler using a minimum number of labware positions and only one active ALP, the 384-well Tipwash Station (Figure 1). This ALP is used for all tip rinsing and supernatant removal steps, reducing the number of labware positions required in the deck configuration when compared to using individual plates for these steps.

A. 384-Well Sequencing Reaction Clean-Up Method Deck



B. 384-Well PCR Clean-Up Method Deck

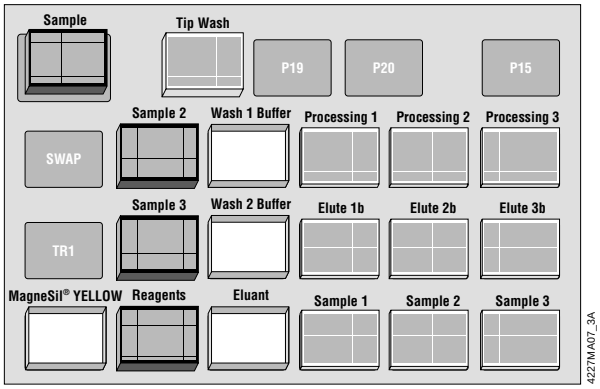


Figure 1. Simplified deck layout for processing multiple plates on the Beckman Coulter Biomek® FX robotic platform. With a fully utilized deck four plates can be processed simultaneously with the 384-Well MagneSil® Sequencing Reaction Clean-Up method (**Panel A**), and three plates can be processed simultaneously with the 384-Well MagneSil® PCR Clean-Up method (**Panel B**) on a single-POD Biomek® FX.

The purification processes, using MagneSil® PMPs, follow a simple 3- or 4-step procedure for the Wizard MagneSil® Sequencing Reaction Clean-Up method[b] and Wizard MagneSil® PCR Clean-Up method[b], respectively. PCR or sequencing extension products are bound to the MagneSil® PMPs, primers and other nonspecifically bound material are removed with a specialized wash buffer and/or an ethanol wash, and the purified products are eluted in water (Figure 2). This makes it possible to create multiple plate methods that can process over 1,000 templates in under an hour without the need for stacking. With the addition of a stacker carousel, or other plate-stacking capabilities, it would be possible to process over 10,000 templates in an 8-hour shift (Table 1).

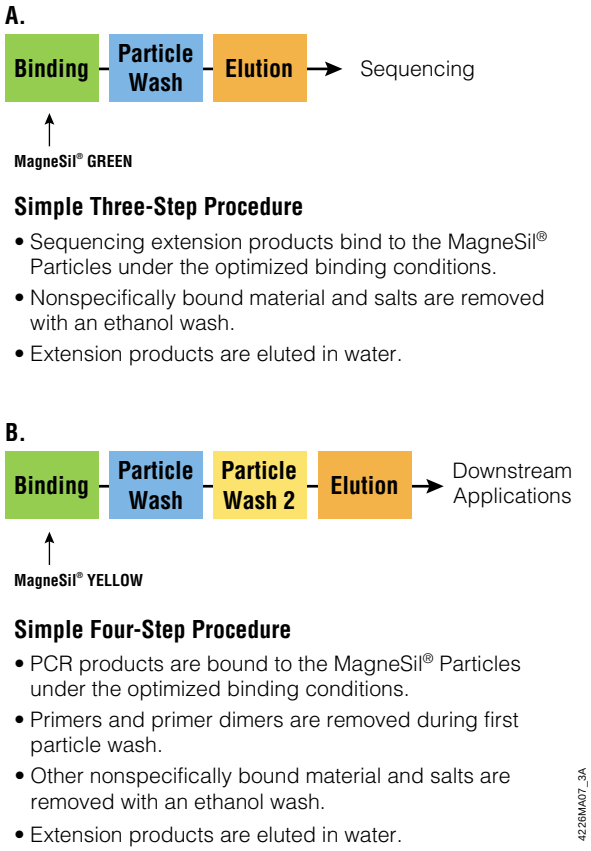


Figure 2. Highlights of the MagneSil® Sequencing Reaction Clean-Up (Panel A) and MagneSil® PCR Clean-Up (Panel B) methods.

Table 1. Throughput of the 384-Well MagneSil® PCR Clean-Up and Sequencing Reaction Clean-Up Multiplate Methods on the Biomek® FX.

MagneSil® Method	Method Length	Templates per Run	Templates per 8-Hour Shift*
PCR Clean-Up	50 minutes	1,152	10,368
Sequencing Reaction Clean-Up	45 minutes	1,536	12,288

*Using plate stacking.

MagnaBot® 384 Magnetic Separation Device

The MagnaBot® 384 Device is a high-density magnet array designed for processing 384-well plates. The magnet has a standard footprint and will fit onto any ALP position of the Biomek® FX workstation. The 425 individual magnets are arrayed across the bottom of the separation device. The magnets draw the MagneSil® particles to the sides or corners of 384-well PCR, flat-bottom, and pyramid-bottom plates, allowing for complete removal of liquids during the methods (Figure 3).

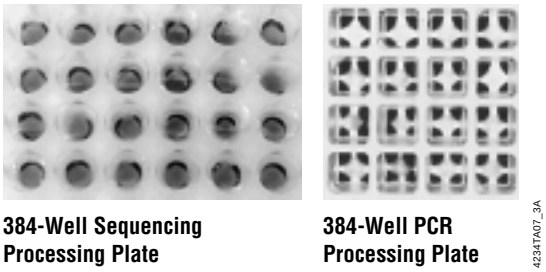


Figure 3. Magnetic separation using the MagnaBot® 384 Device. The MagnaBot® 384 Device is constructed such that the magnetic field draws the particles to the outer edges of the conical-well processing plate or to the corners of the square-well processing plate. This allows access to the bottom of the well for complete removal of liquids during the method.

Results

384-Well MagneSil® PCR Clean-Up Method (MagneSil™ YELLOW)

We purified PCR amplification products using the 384-well MagneSil® PCR Clean-Up method as described in Automated Protocol #EP009. A portion of each purified PCR fragment was rearrayed into a fresh 384-well PCR plate. These samples were then used for downstream sequencing. The data from the last column of Table 2 shows that the samples purified with the MagneSil® PCR Clean-Up method provide excellent sequencing data with Phred >20 scores above 650, start sites between 15–20 bases past the primer, and accuracy greater than 99% at 600 bases past the start site.

Table 2. Sequencing Results from Reactions Purified Using the 384-Well Purification Methods.

	Sequencing Reaction Clean-Up					PCR Clean-Up
	ABI 3700		ABI 3730			
Property	V.3	V.3.1	V.3	V.3.1	Amersham	ABI 3700
Phred >20	675	677	714	731	662	668
Average total signal	3176	1721	2002	1858	685	338
Start site	21.85	29	27.34	40.04	43.32	16.42
% Accuracy at 600 bases	99.58	99.54	99.64	99.72	99.53	99.04

Sequencing reactions were performed using ABI BigDye® V.3 or V.3.1 chemistries or Amersham DYEnamic™ ET chemistry. These reactions were purified using the 384-well MagneSil® Sequencing Clean-Up method on a Biomek® FX. Purified ABI sequencing reactions were run on the ABI PRISM® 3700 and 3730xl DNA Analyzers. Purified Amersham samples were run on the MegaBACE™ 1000. PCR products purified with the PCR Clean-Up method were sequenced by SeqWright DNA Technology Services (Houston, TX).

The remaining volume from each purified sample was used to show fragment recovery and the removal of nonspecific amplification products. Figure 4A shows >80% recovery of a 1,000bp PCR fragment with quantitative removal of the ~100bp primer/primer-dimer. Figure 4B further demonstrates the removal of 100bp fragments by the MagneSil® PCR Clean-Up method.

PCR products purified with this system were tested in microarray analysis using Cy®3- and Cy®5-target hybridizations. Figure 5 demonstrates the consistency and reproducibility of microarray analysis using PCR products purified by MagneSil® PCR Clean-Up.

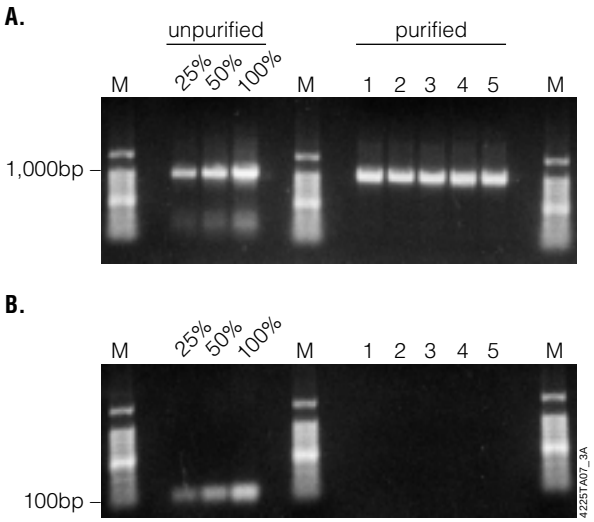


Figure 4. Purification of 1,000bp (Panel A) and removal of 100bp (Panel B) PCR products with the 384-well MagneSil® PCR Clean-Up method. Five separate purified PCR amplifications are compared to 25%, 50%, and 100% of an unpurified reaction. Lane M, 100bp DNA Ladder (Cat.# G2101).

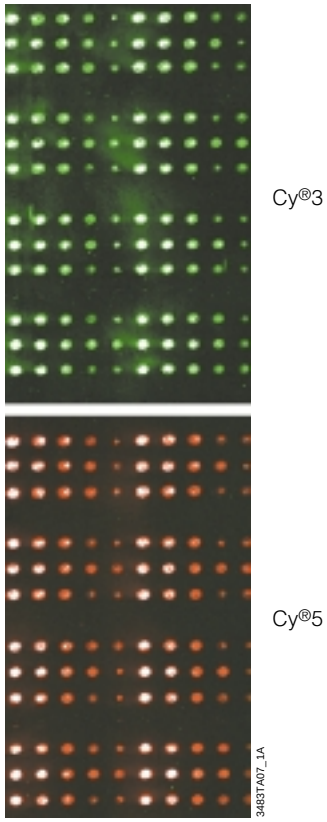


Figure 5. Microarray analysis of MagneSil®-purified PCR fragments. A sample quadrant of kanamycin-control reactions for both Cy®3- and Cy®5-target hybridizations. Each quadrant consists of four identical groups of three rows each. The three rows correspond to three 96-well plates containing replicates of the purified probes. Each row is a duplicate group of five spots corresponding to undiluted, 1:5, 1:25, 1:100 diluted RT-PCR products and a blank 3X SSC spot.

384-Well MagneSil® Sequencing Reaction Clean-Up Method (MagneSil® GREEN)

The 384-well MagneSil® Sequencing Reaction Clean-Up method was tested using the ABI BigDye® Terminator V.3 and V.3.1 chemistry, as well as the Amersham ET Chemistry (Table 2). The clean-up method was performed as described in Automated Protocol #EP010. This method provided high-quality data from purified samples using multiple sequencing chemistries and various DNA analyzers. All samples yielded Phred >20 scores above 650 with many exceeding 700. Average accuracies from the same test sequences were above 99% at 600 bases past the start site, while nearly all remained above 98% at 700 bases.

Conclusions

The 384-well MagneSil® Clean-Up methods provide an automated, walkaway solution for high-throughput purification of PCR products and fluorescent dye terminator sequencing reactions. These methods require no user interventions and are easily adaptable to single- or dual-arm Beckman Coulter Biomek® FX automated workstations with minimal deck requirements. The 384-well MagneSil® PCR Clean-Up method gives efficient recovery of fragments larger than 150bp, with >99% removal of primer and primer-dimer. The 384-well Sequencing Clean-Up method consistently delivers Phred >20 scores that are equal to or greater than other manual, semi-automated, or automated methods such as ethanol precipitation, gel filtration and other particle methods. The flexibility, scalability, and cost efficiency of the methods make them an excellent solution for the needs of the high-throughput genomics researcher.

Protocols

- *Automated Wizard® MagneSil® 384-Well PCR Clean-Up System Automated Protocol* #EP009, Promega Corporation. (www.promega.com/tbs/ep009/ep009.html)
- *Automated Wizard® MagneSil® 384-Well Sequencing Reaction Clean-Up System Automated Protocol* #EP010, Promega Corporation. (www.promega.com/tbs/ep010/ep010.html)

Brad Larson, B.A.
Process Development Scientist

Tom Strader, B.S.
Product Manager

Ordering Information

PCR Clean-Up Method

Product	Size	Cat.#
MagneSil® YELLOW*[b]	100ml	A9231
Wash Solution*	500ml	A8241
MagnaBot® 384 Magnetic Separation Device*	1 each	V8241
384-Well Plate, Flat (Polystyrene)	10/pack	V5291

*For Laboratory Use.

Sequencing Reaction Clean-Up Method

Product	Size	Cat.#
MagneSil® GREEN*[b]	100ml	A8231
MagnaBot® 384 Magnetic Separation Device*	1 each	V8241
384-Well Plate, Conical (Polypropylene)	10/pack	V5311

*For Laboratory Use.

[a] 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.

[b] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.