Automation of PCR Reaction Setup and AMPure XP Purification Using the Biomek 4000 Workstation

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Abstract

Both polymerase chain reaction (PCR) reaction setup and AMPure XP PCR reagent-mediated purification were automated on the Biomek 4000 Laboratory Automation Workstation.

The graphic user interface for the Biomek 4000 PCR Reaction Setup method provides users the flexibility to setup PCR reactions through a visual-well-selection process with a throughput of 1 to192 samples in a 96-well plate format. The method allows the use of two master mixes, two primers, and two sample sources, as well as creating master mix from single components. The AMPure XP reagent system provides highly efficient PCR purification that delivers superior quality DNA with no salt carryover. The AMPure XP system uses solid phase reversible immobilization (SPRI) magnetic bead-based technology; no centrifugation or filtration is required. With a simple three-step protocol (Bind-Wash-Elute) the reaction contaminants, including primers, dNTPs, primer dimers and salts, are effectively removed. The Biomek 4000 AMPure XP method allows users to select the number of samples to be purified, as well as user ID, sample and reagent lot number tracking via LIMS data collection.

To demonstrate system functionality, 285 base pair human β-actin gene amplifications (Promega, Madison, WI) were performed using default techniques and labware definitions that come with PCR application. The results from the block PCR were free of well-to-well cross-contamination and the real-time PCR showed 1.74% CV in Ct values. The automated AMPure XP application was demonstrated by purifying 100 base pair pGEM DNA fragments (Promega, Madison, WI). The system yielded concentrations (260nm) and purity (260/230 ratio) equivalent to or better than manually- purified samples, with no well-to-well cross-contamination as measured by real-time PCR quantification.

Our data illustrate that the Biomek 4000 platform can automate PCR Reaction Setup and AMPure XP reagent-mediated PCR purification with excellent precision and without risk of cross-contamination. The increased throughput and reproducibility that comes with automating these processes can greatly accelerate medical and biological research in today's laboratory.

Disclaimer: The Biomek 4000 Workstation, PCR and AMPure XP reagent-mediated purification applications are not intend or validated for use in the diagnosis of disease or other conditions.

Introduction

PCR has widespread application in the field of medical, forensic and biological research, including genetic testing, tissue typing, infectious disease identification, genetic fingerprinting, DNA sequencing and cloning, and gene expression. In addition, the variation or types of PCR, such as PCR, RT_PCR, qPCR, Multiplex PCR, Inverse PCR, Miniprimer PCR, have made PCR reaction setup even more intricate and time consuming. With the increase in the number of samples in studies, a simple, easy to use, and high throughput automation solution for PCR reaction setup is necessary.

The AMPure XP reagent system uses solid-phase paramagnetic bead technology for high-throughput purification of PCR amplicons. It utilizes an optimized buffer to selectively bind PCR amplicons 100bp and larger to the paramagnetic beads, so the excess primers, nucleotides, salts and enzymes from the reaction can be removed using a simple **Bind-Wash-Elute** procedure. As the simple procedure requires no centrifugation or vacuum filtration, the AMPure XP reagent system is highly amenable to automation.

In this poster, we will describe the PCR and AMPure XP system automation solutions on the Biomek 4000 Laboratory Automation Workstation - the newest addition to Beckman Coulter's research automation line. With new pipetting tools, updated software and Window 7 compatibility, the Biomek 4000 Workstation applications improve the ease and speed of PCR and AMPure XP reagent setups. 1 to192 PCR sample reactions can be setup in two 96-well PCR plates from up to two plates of master mixes, two primers and two sample sources, including master mix made from individual components. 192 PCR samples (10-70µL) can be purified via the AMPure XP reagent system in under 90 minutes with features like sample selection by column, and choices of 1-2 PCR plates, LIMS sample data collection, user ID/reagents lot # tracking, and automation procedure recovery.

Due to its smaller footprint and improvements including AccuFrame framing, automatic tool selection, orbital shaker integration, wash tool, MP1000 tool, runtime patterns, and single channel serial dilution, the Biomek 4000 Workstation and its new applications can provide automation solutions to meet the diverse needs of genomic laboratories.

Figure 1: The Biomek 4000 Laboratory Automation Workstation is shown with an optional enclosure (In development) (Beckman, PN: A99749)

Materials and Methods

Reagents

- KAPA SYBR Fast Universal qPCR Kit (KAPA Biosystems, Woburn, MA)
- Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, part number A63882)
- Nuclease-free Water (Life Technologies, Carlsbad, CA)
- Denatured 70% Ethanol (American Bioanalytical, Natick, MA)
- AccuPrime Supermix I (Life Technologies)
- pGEM DNA vector (Promega, Madison, WI)
- M13 Forward Primer: 5'-TAA TAC GAC TCA CTA TAG GG-3' (IDT, Coralville, IA)
- 1000bp_pGEM Reverse Primer: 5'-TCT AGT GTA GCC GTA GTT AGG-3' (IDT)
- Human Genomic DNA (Promega)
- β-actin Primer Pairs (Promega)
- PCR Master Mix (Promega)
- 2% agarose gel (Life Technologies)

PCR Reaction Setup

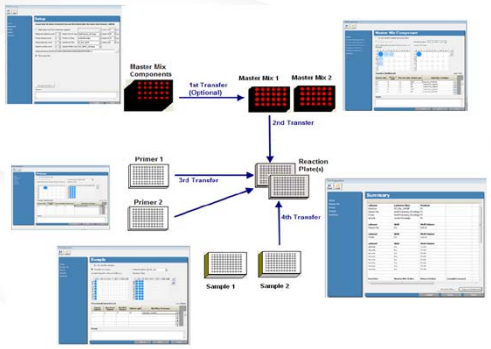

Figure 2. PCR Reaction Setup Process: following the pop-up User Interface, two 96-well PCR plates can be setup from different reagent resources, including plate or tube formats for master mixes, primers and samples. This application also comes with default automation transfer techniques and a number of ready-to-use PCR plate and other labware definitions.

AMPure XP Purification

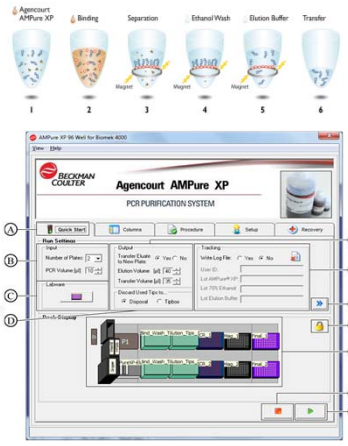

Figure 3. Standard workflow of AMPure XP reagent-mediated PCR purification (right) and Biomek 4000 AMPure XP Graphic User Interface (below) :

Quick Start Tab (A)
Input Values (B)
Labware (C)
Discard Used Tips (D)
Output (E)
Tracking (F)
Forward Button (G)
Lock Indicator (H)
Deck Display (I)
Abort Button (J)
Run Button (K)

Results

Both Biomek 4000 PCR Reaction Setup and AMPure XP Purification showed excellent results from these two robust automation processes.

- **Block PCR Amplification and Cross-Contamination Test for Biomek 4000 PCR Reaction Setup:** The 285bp Human β-actin gene was amplified using a ready-to-use PCR master mix and default automation transfer techniques. Results showed no well-to-well PCR cross-contamination on a 2% agarose gel (Figure 4).
- **Real-time PCR Amplification Test for Biomek 4000 PCR Reaction Setup:** The 285bp Human β-actin gene was also amplified using a ready-to-use KAPA SYBR qPCR master mix and default automation transfer techniques. The data showed highly consistent amplification with a CV of 1.74 % across 32 amplified samples (Figure 5).
- **Automation Efficiency Test for Biomek 4000 AMPure XP Purification:** 70 µL of 100bp pGEM plasmid DNA PCR fragments were purified via automation and produced equivalent yield (µg/mL) and purity (260nm/230nm Ratio) when compared to samples from the manual process (Table 1).
- **Cross-Contamination Test for Biomek 4000 AMPure XP Purification:** qPCR re-amplification of automation-purified 452bp PCR fragments showed no well-to-well cross-contamination in AMPure XP reagent-purified samples (Figure 6-7).

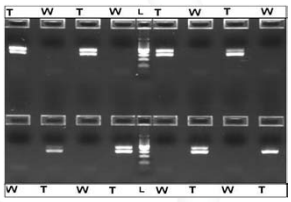

Figure 4. Cross-Contamination Results (25 µL/reaction): Human genomic DNA (24 ng/reaction), β-actin primer pairs and ready-to-use PCR master mix (Promega) were used for automated PCR setup. The results show no presence of the β-actin amplicons (285 bp) in any negative wells where human gDNA template (T) was replaced by water (W). The middle lane is 100bp DNA Ladder (L) (Promega).

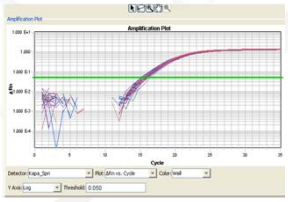

Figure 5. Real-Time PCR amplification (20 µL/reaction): The data show the β-actin real-time PCR reaction results from 32 samples amplified on the ABI 7900HT Fast Real-time PCR System (Life Technologies). Each β-actin qPCR reaction amplified 8 ng human genomic DNA (Promega), using β-actin primer pairs (Promega), and KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems). The samples gave an average Ct value of15.72 with 1.74% CV, illustrating consistent liquid transfers.

	Automation (Column #1-3)	Manual (Column #4)	Automation/Manual (%)
Yield (µg/mL)	5.5823	5.8491	95.4
Purity (260 nm/ 230 nm Ratio)	2.02	1.97	102.5

Table 1: AMPure XP PCR automation purification efficiency data. 100bp pGEM plasmid DNA PCR fragments were purified using Biomek 4000 AMPure XP and manual processes. Yield (95.4%) and purity (102.5%) were compared between samples purified by automation to those purified manually.

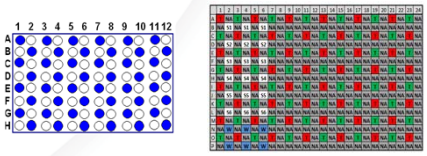

Figure 6: Cross-contamination sample plate patterns: 70 µL of 452bp PCR fragments (white wells) and water (blue wells) were purified using Biomek 4000 AMPure XP method (left). The purified samples were re-amplified by qPCR in a 384-well plate format (right). T= AMPure reagent eluate samples, S = Standards 1-6, W = Water controls, NA = empty wells. Red Wells are "Negative" samples; Green Wells are "Positive" samples.

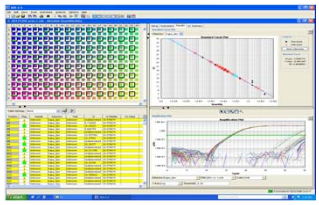

Figure 7: Cross-Contamination Test by qPCR Quantification: The AMPure XP reagent-purified 452bp PCR fragment and water were re-amplified using the ABI 7900HT Fast Real-time PCR System and KAPA SYBR FAST qPCR Master Mix. Amplification was observed only from the wells containing the 452bp PCR fragments.

Conclusion

Automation solutions for both PCR reaction Setup and AMPure XP reagent-mediated purification on Biomek 4000 Workstation can help users from small, medium and high throughput laboratories improve efficiency and results. The templates are simple, fast, and easy to use, and can improve efficiency and results in many areas in medical, forensic and biological research.

Biomek 4000 Workstation PCR Reaction Setup: the key feature for this application is *flexibility*. With the visual-well-selection user interface, a user can easily setup two 96-well PCR plates for various combinations of master mixes, primers, and samples, as well as creating master mixes from single components. This flexibility allows automation to be used for numerous PCR reaction setup conditions.

Biomek 4000 Workstation AMPure XP Reagent-Mediated Purification: The key feature for this application is *speed*. Two 96-well PCR plates of samples can be purified under 90 minutes for volumes between 10 to 70 µL. The PCR amplicons purified using the Biomek 4000 Workstation matched the yield and purity of samples purified manually. Agencourt AMPure XP chemistry has also been used in many other downstream applications such as sequencing (Sanger and Next Gen), genotyping and SNP detection, fragment analysis, primer walking, and cloning, which all can be enhanced with automated high-throughput AMPure XP purification.