Both PCR reaction setup and AMPure XP reagent-mediated purification were automated on the Biomek 4000 Laboratory Automation Workstation. The graphical user interface for the Biomek 4000 PCR Reaction Setup Method setup provides the flexibility to setup PCR reactions through a visual-well selection process with a throughput of 1.150 reactions per hour. The Biomek 4000 Workstation can perform two sample sources, as well as creating master mix from single components. The Biomek 4000 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, 265 base pair human β-actin gene amplifications (Promega, Madison, WI) were performed using default techniques and labware decontaminants that come with PCR application. The results from the bioc laid-free of well-to-well cross-contamination and the real-time PCR showed 1.5% CV or less variation. The automated Biomek-AMPure XP application was demonstrated by purifying 103 base pair pGEM DNA fragments (Promega, Madison, WI). The system yielded consistent amplifications and purity (260nm/230nm ratio equivalent to 1.8) from two purified master mix samples, with no well-to-well cross-contamination as measured by KAPA SYBR Green qPCR quantification.

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

Introduction

AMPure XP 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, minipipette PCR, leave PCR reaction setup even more interesting and time consuming. With the increase in the number of samples to be studied, a simple, easy to use, and high through-put automation solution for PCR reaction setup is necessary.

The Biomek 4000 Workstation AMPure XP reagent-mediated purification system uses a solid-phase paramagnetic bead technology for high-throughput purification of PCR fragments. The system optimizes buffer to selectively bind PCR fragments 100bp and larger for the paramagnetic beads, so the excess primers, nucleotides, salts and enzymes from the reaction can be removed using a simple bind/wash/elute protocol. As the simple procedure requires no centrifugation or vacuum filtration, the AMPure XP system is highly tolerant 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 printing tools, updated software and Windows 7 compatibility, the Biomek 4000 Workstation applications improve the speed and flexibility of PCR reactions. The system is capable of purifying two sample sources, as well as creating master mix from single components. 192 PCR samples (70 µl) can be purified on the AMPure XP system in under 90 minutes with no carryover or risk of cross contamination.

Due to its smaller footprint and improvements such as AcuPipette™ barcode detection, automated tube selection, optical analyzer integration, wash tool, MP1000 tool, run-time patterns, and single channel liquid delivery, the Biomek 4000 Workstation and its new applications can provide automation solutions to meet the diverse needs of genomic laboratories.

Materials and Methods

Reagents

• KAPA SYBR Fast Universal PCR Kit (KAPA Biosystems, Wilmington, MA)
• Agencourt Ampure XP Kit (Beckman Coulter, Brea, CA; part number AS0401)
• Agencourt RNaseInhibitor Kit (Beckman Coulter, Brea, CA; part number AS0342)
• Agencourt DNA Clean & Concentrator-5 (Beckman Coulter, Brea, CA; part number AG0525)
• Agencourt spin Sepharose (Beckman Coulter, Brea, CA; part number AS0341)
• Agencourt 18S rRNA (Sigma-Aldrich, St Louis, MO; part number A8376)
• G3PDH Shaker vector (Promega, Madison, WI)
• PCR forward primer: 5'-TCT AGT GTA GCC GTA GTT AGG-3' (IDT)
• 100ng qPCR Master Mix (KAPA, 2x) with KAPA SYBR Green qPCR Kit (KAPA, 2x)
• Human β-actin Primers (Promega)
• β-actin Amplification Primers: 5'-TCC AGT GAC TCA TAG GGG G-3' (KAPA, 2x)
• 2% agarose gel (Life Technologies)

Results

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

• Black PCR Amplification and Cross-Contamination Test for Biomek 4000 PCR Reaction Setup: The 285bp Human β-actin gene was amplified using two-ready-to-use PCR master mix and custom 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 Green qPCR master mix and custom 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: 75 µl of 100bp pGEM plasmid DNA fragments were purified via automation and produced equivalent yield (95.4%) and purity (102.2%) compared between samples purified by automation to those purified manually (Table 1).

Conclusion

Automation solutions for both PCR reaction setup and AMPure XP reagent-mediated purification on the 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 the ability to select source/destination trays and manage time settings for each PCR reaction. The reaction setup is shown in Figure 4 and a DIY plate setup is shown in Figure 5. The system can also be used for various combinations of master mixes, primers, and samples, as well as creating master mixes from two single components. This flexibility allows automation to be used for numerous PCR reactions on the Biomek 4000 Workstation.

Biomek 4000 Workstation AMPure XP Purification: the key feature for this application is speed. Two 96-well plates of samples can be purified under 90 minutes for volumes between 100-1.2µl. The PCR reagents used for purification were purified using the KAPA SYBR Green qPCR kit and the system 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, genotyping and SNP detection, fragment analysis, primer walking, and cloning, which can all be enhanced with automated high-throughput Agencourt XP purification.

Figure 1. The Biomek 4000 Laboratory Automation Workstation is shown with an optional enclosure (top) (Kapa Biosystems), (middle) (Beckman Coulter, Inc.), (bottom) (IDT)

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 plates of tube formats for master mixes, primers, and samples. The application also comes with default automation transfer techniques and a number of ready-to-use PCR plates and other reagent containers.

Figure 3. Standard workflow of AMPure XP reagent-mediated purification; (right) and Biomek 4000 AMPure XP Graphic User Interface (below): (a) Quick Start Test (A): output locations (B); (b) Discrete Unit Operations (C): sample Transfer (D), (right) and Biomek 4000 AMPure XP Graphic User Interface (below): (c) Quick Start Test (A): output locations (B); (d) Discrete Unit Operations (C): sample Transfer (D)

Table 1. AMPure XP PCR Purification efficiency data. 100bp pGEM plasmid DNA PCR fragments were purified using Biomek 4000 AMPure XP and manual processes. Yield (93.4%) and purity (102.2%) were compared between samples purified by automation to those purified manually.

Table 2. PCR amplification efficiency data. 285bp Human β-actin gene was amplified using a ready-to-use PCR master mix and custom automation transfer techniques. Results showed no well-to-well PCR cross-contamination on a 2% agarose gel.

Table 3. Real-time PCR analysis data. The data show the qPCR results from both manual and automated assays were amplified using two ready-to-use primers. Each 285bp-PCR reaction showed 1.5% CV or less variation. The automated Biomek-AMPure XP application was demonstrated by purifying 103 base pair pGEM DNA fragments (Promega, Madison, WI). The system yielded consistent amplifications and purity (260nm/230nm ratio equivalent to 1.8) from two purified master mix samples, with no well-to-well cross-contamination as measured by KAPA SYBR Green qPCR quantification.

Table 4. Automation Efficiency Test for Biomek 4000 AMPure XP Purification: 75 µl of 100bp pGEM plasmid DNA fragments were purified via automation and produced equivalent yield (95.4%) and purity (102.2%) compared between samples purified by automation to those purified manually.