

Automation of PCR¹ Purification Using Agencourt[®] AMPure[®] Reagents on the Biomek[®] 3000 Laboratory Automation Workstation from Beckman Coulter



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ABSTRACT

The reliable and high-quality purification of PCR products is essential for a successful sequencing process, but manual PCR purification is both labor intensive and time consuming. Therefore, we developed an automated method to provide a complete walk-away system for purification of PCR products. The information included in this poster describes the utilization of the Biomek 3000 Laboratory Automation Workstation for the removal of unincorporated dNTPs, primers, salts and other contaminants from PCR products using Agencourt AMPure[®] reagents. The user interface allows the user to customize an individual run by selecting a full or partial 96-well plate and inputting certain reaction parameters.

The information presented here includes the full description of the automated methods and subsequent results. Representative data obtained from the purification of PCR products using this system are shown. Data from capillary sequencing on the CEQ[™] 8800 Genetic Analysis System from Beckman Coulter demonstrate the suitability of the purified PCR products for stringent assays such as capillary sequencing.

¹The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.

Process Overview of AMPure PCR Purification

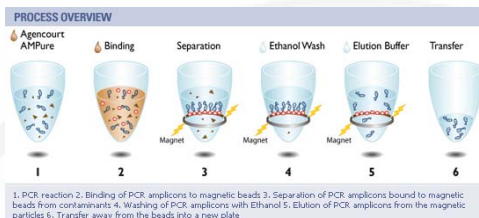


Figure 3. Schematic representation of the Agencourt AMPure PCR purification protocol.

AMPure Reservoir Volume Calculator

Column 1	Column 2	Column 3	Column 4
2400	3987	8400	Empty
μL of Elution Buffer	μL of AMPure	μL of 70% ETOH	Empty

*Always Use Freshly Prepared 70% Ethanol

Figure 6. Each reagent volume will be automatically computed according to the number of columns and reaction volume input via the user interface.

Capillary Sequencing Chromatogram



Figure 9. A representative sequencing chromatogram of purified human β -Globin gene (5' end of 1,453-bp fragment) PCR product sequenced on the ABI PRISM[®] 3100 Genetic Analyzer. The first 400 base pairs of sequence are shown.

INTRODUCTION

The automated Agencourt AMPure PCR purification method, developed for the Biomek 3000 Laboratory Automation Workstation, provides high-quality purified amplicons for downstream applications. This method utilizes the positions in the base module section of the Genomelab deck configuration, so that it will run on any configured Biomek 3000 workstation deck with the appropriate tools, labware and tip box holders shown in Figure 1.



Figure 1. The Genomelab deck configuration is used for an automated method of purification of PCR products using the Biomek 3000 workstation. This configuration contains five positions for labware (P1-P5), four manual latch positions for tip boxes (ML1-ML4), a tool rack with gripper (Rack1), two dedicated positions for the Peltier Thermoshaker and Static devices and a disposal position for waste management. A customer provided thermocycler can be placed in position P5.

MATERIALS & METHODS

Agencourt AMPure PCR Purification Instrument Setup

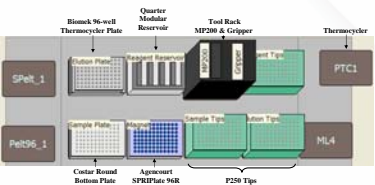


Figure 2. Instrument setup for the Agencourt AMPure PCR purification method. A 96-well microplate in P1 position works as elution plate. P2 position (sample position) can be either a 96-well microplate or a Costar round bottom plate. A Quarter Modular Reservoir in P3 position is used for reagents and liquid waste. The Agencourt SPRIPure[®] 96R magnet plate is situated in position P4. MP200 tool and gripper are designated for transferring liquid and moving labware.

Instructions for the Automated PCR Purification Method

Figure 4. Instructions for the automated Agencourt AMPure PCR purification method.

User Interface for the Automated PCR Purification Method

Figure 5. By inputting the start and stop columns and reaction volume for processing via the user interface, the required parameters for each reaction will be automatically calculated in the corresponding cells. Users can use the default values or change those parameters based on their specific needs.

RESULTS

Gel Electrophoresis Results for Cross Contamination Test

A. Cross-contamination test of automated purification method.



B. Re-amplification of purified PCR products after cross-contamination test.

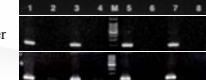
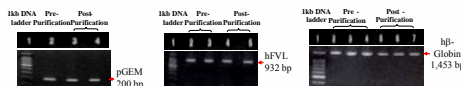


Figure 7. Cross contamination study of pGEM[®] PCR products (200 bp) by 2% agarose gel electrophoresis after automated AMPure PCR purification method. A. PCR products from amplifications with DNA (+) or water only (-) were loaded every other well of the 96-well microplate prior to purification. B. The purified samples from above were reamplified and the results shown above indicate that no cross contamination was detected.

PCR Products Post-Purification Recovery Rate



A. 2% Agarose gel analysis of pGEM (200 bp, left), hFVL (932 bp, middle) and h β -Globin (1,453 bp, right) PCR products pre- and post- purification.

PCR Products	Plate name	Pre-Purify Concentration (ng/ μ l)	Post-Purify Concentration (ng/ μ l)	Recovery Rate (%)	Sample number
pGEM (200 bp)	Plate 1	2.48 \pm 0.09	2.45 \pm 0.16	98.7%	48
	Plate 2	1.10 \pm 0.02	1.08 \pm 0.11	98.6%	24
hFVL (932 bp)	Plate 1	13.68 \pm 0.31	12.15 \pm 0.46	88.8%	24
h β -Globin (1,453 bp)	Plate 1	8.97 \pm 0.64	7.68 \pm 0.96	85.6%	12

B. Quant-iT[™] PicoGreen[®] dsDNA assay of PCR products recovery rates after purification.

Figure 8. Purification results of different size PCR products. A. 2% Agarose gel analysis. B. Quant-iT PicoGreen assay of three different size PCR products recovery rates after purification method. Results indicate that the recovery rates of the PCR products are more than 85% after automated purification.

Read Length and Phred20 Pass Rate

Human β -Globin (1,453 bp)	Read Length in base pair	Percent Accuracy (%) at 600 bp	Phred 20 Read Length Pass Rate [®] (%)	Sample Number
Data obtained from CEQ 8800	859	98.8	100	24
Data obtained from ABI 3100	871	99.0	100	24

[®] Average Phred value greater than 20 for bases greater than 600bp.

Figure 10. The summarized sequencing results of purified human β -Globin gene amplicons obtained from both the CEQ 8800 Genetic Analysis System and ABI PRISM 3100 Genetic Analyzer. The average percent accuracy of the sequence obtained from both systems is approximately 99%. The average read lengths are greater than 800 base pairs and the read length pass rates for Phred value greater than 20 are 100%.

SUMMARY & CONCLUSIONS

- The automated Agencourt AMPure PCR purification method is developed for the Biomek 3000 workstation utilizing the base module section of the Genomelab deck configuration, so it will run on any appropriately configured Biomek 3000 workstation deck.
- The graphical user interface allows for the processing of a full or partial 96-well plate; the functions automatically compute reagent volumes according to or based upon user input. Users can select default parameter values or change according to their specific needs.
- The recovery rates of purified PCR products, both small size (200 bp) and large size (932 bp and 1,453 bp), are > 85%; the result indicates no cross contamination was detected.
- Purified PCR products are suitable for cycle sequencing, generating an average Phred 20 of \geq 600 bp.
- Data presented here demonstrate that the completely walk-away automated method results in high-quality purification of PCR amplicons for sequencing.