Automation of Agencourt AMPure Purification Kit for the Purification of Next-Generation Sequencing Sample Preparation Reactions on the Agilent Bravo Automated Liquid Handling Platform

Application Note

Figure 1: Bravo Automated Liquid Handling Platform (G5523A/G)

Authors

Brian Minie, Justin Abreu, Christopher Friedrich, and Andrew Barry
Broad Institute of MIT and Harvard
Cambridge, MA USA

Randy Dyer
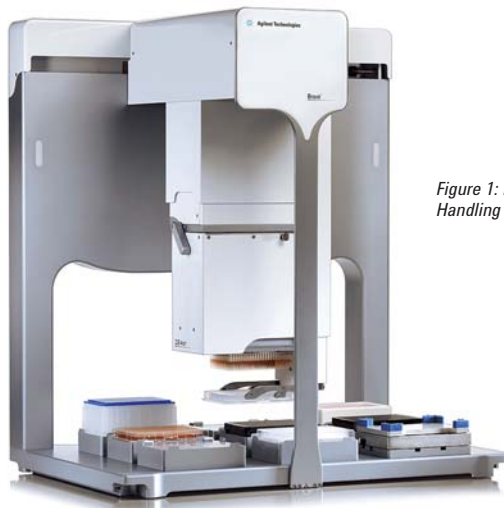Agilent Technologies, Inc.
Santa Clara, CA USA

Abstract

A new method was developed to automate library construction for the Illumina sequencing platform using the Agilent Bravo Automated Liquid Handling Platform and the Agencourt Ampure PCR purification kit. Current Next-Generation sequencing workflows require extensive manual processing steps including repetitive pipetting and the use of individual centrifugation columns for cleanups of enzymatic steps. Using the Agilent VWorks Automation Control software, a protocol was developed for the Bravo platform to aspirate and dispense enzyme master-mixes, and perform cleanups of these reactions through the transfer of the Agencourt AMPure reagent to the reaction, transfer of the microtiter plate to a magnet using the integrated gripper, and subsequent sample wash and elution steps. Sample quality and quantity were assessed using the Agilent Bioanalyzer 2100 and Illumina Genome Analyzer IIX.

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Introduction

Next-Generation sequencing platforms offer unparalleled access to DNA sequence data both in terms of cost per basepair and yield per run. These developments have enabled a wide range of applications for which sequence data can be used.

Automation of sample preparation for these applications is critical not only to achieve throughput, but also to reduce sample-to-sample variability. Magnetic bead purification of enzymatic reactions relies solely on liquid handling to separate desired reaction products from reaction buffers and unincorporated oligonucleotide and dNTP molecules. Described below is a protocol for the Agilent Bravo Automated Liquid Handling Platform capable of automatically handling the binding, washing, and elution of isolated next generation sample preparation products. We demonstrate the capability of this protocol through the reaction clean-up following the final step in the preparation of samples for sequencing genomic DNA, where we enrich the Adapter-Modified DNA fragments by PCR. It is important to note that this clean-up method can be used for all of the enzymatic reaction clean-ups throughout the Illumina "Preparing Samples for Sequencing Genomic DNA protocol," as well as other Next- Generation sequencing sample preparation protocols. More information on the Bravo Automated Liquid Handling Platform is available at www.agilent.com/life-sciences/automation and in the Data Sheet (5990-3480EN).

Workstation Configuration

An automated workstation was assembled using Agilent Automation Solutions instrumentation and components. A Bravo Automated Liquid Handling Platform (G5523A/G) with gripper attachment for moving plates on the deck and a 96-channel LT pipetting head (Option 178) to handle Agilent 200 µL Disposable Pipette Tips (06880-102) was configured with the 9 deck positions as follows (see **Figure 2**): 1) 200 µL pipette tips for binding, 2) 200 µL pipette tips for 70% ethanol washing, 3) 200 µL pipette tips for elution, 4) 96-well Eppendorf PCR plate containing Agencourt Ampure beads, 5) 96-well Eppendorf PCR plate containing 70% ethanol, 6) 96-well Eppendorf PCR plate containing elution buffer, 7) 96-well Eppendorf PCR plate for elate transfer, 8) PCR product, 9) 96-well side magnet plate (Invitrogen). The workstation was controlled by Agilent VWorks Automation Control software.
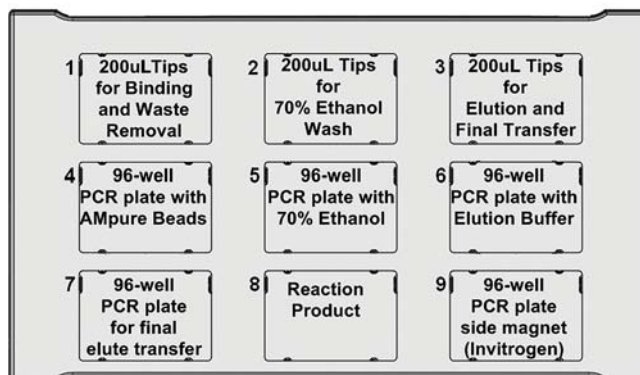

Figure 2: Agilent Bravo configuration.

Generation of Reaction Products

Reaction products were prepared using E. coli genomic DNA (Sigma) according to the method described by Illumina "Preparing Samples for Sequencing Genomic DNA" through the purification of Ligation Products. Enrichment for Adapter-Modified DNA Fragments by PCR was performed using the Stratagene PfuUltra II Hotstart PCR Master Mix (#600850) to amplify the 220 bp adapter-ligated fragments of E. coli DNA following the recommended protocol. To compare the efficacy of the automated method against the manual Qiaquick column based method, as well as the manual Agencourt Ampure purification method, samples were purified using the process below either manually or with the automated workstation.

Purification Protocol Workflow

PCR products were purified using the Agencourt Ampure beads following the recommended protocol. Using VWorks Automation Control software, one protocol was written to complete the entire purification process. Unique labware was defined in the software to allow the Bravo Platform to properly position the tip head relative to the deck plate pad. Labware such as the tip boxes are predefined in the software. Plates are automatically stacked and unstacked as needed and transferred between the Bravo positions when required without user intervention. Briefly, the automated workflow was as follows: all plates and tips were placed on the Bravo deck positions as described above. The VWorks software protocol automatically transferred all liquids and attached and removed tips when required. Agencourt Ampure beads were

added to the PCR products plate and mixed before the PCR plate was moved onto the magnet at position 9 using the Bravo gripper for an incubation of 8 minutes. After the incubation, the supernatant was removed and dispensed into the Agencourt Ampure 96-well plate. A fresh set of tips was used for the 70% ethanol wash, after which the PCR plate was removed from the magnet using the Agilent Bravo gripper and placed at position 8 for 6 minutes. Using fresh tips, the Bravo added 40 µL of elution buffer to each well of the PCR plate, mixed 12 times, followed with a 2 minute incubation. After 2 minutes, the PCR plate was placed onto the magnet using the Bravo gripper for an additional 3 minute incubation. After 3 minutes, the eluate was transferred to a clean 96-well PCR plate in position 7. For comparison, Qiaquick purifications were performed using manufacturer's suggested protocol.

Purification Analysis

Assays were conducted to both quantify the amount of sample which was retained as well as to analyze the purity of the sample. Sample quantification was conducted using the Invitrogen Quant-iT PicoGreen dsDNA assay (P7581), in which the PicoGreen dye was diluted 1:200 and run on a Perkin Elmer Victor microplate reader. Sample quality was assessed by running purified product on an Agilent BioAnalyzer 2100 (G2938C) using a DNA 1000 Nanochip (5067-1504), and BioAnalyzer 2100 Expert software version B.02.06.
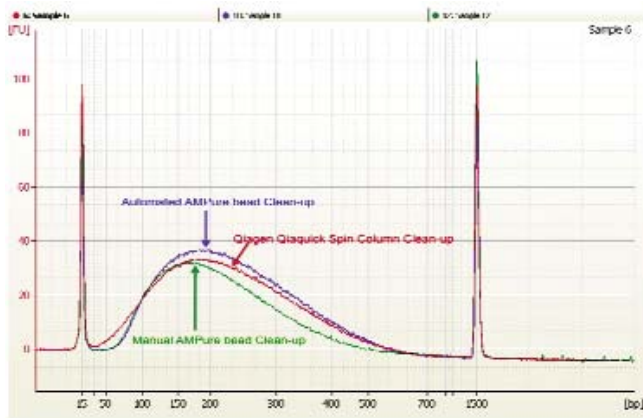
Figure 3: Agilent Bioanalyzer traces showing representative sample size distributions using various purification methodologies.
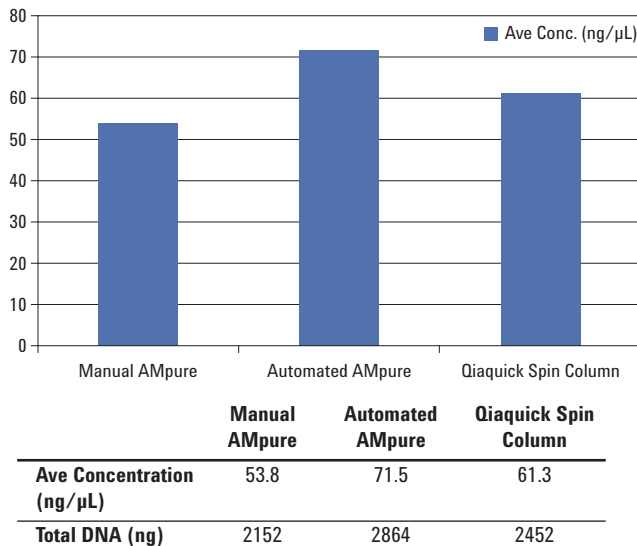
Post-Fragmentation Clean-up Methods

	Manual AMpure	Automated AMpure	Qiaquick Spin Column
Ave Concentration (ng/µL)	53.8	71.5	61.3
Total DNA (ng)	2152	2864	2452

Figure 4: Reaction yields using varied input into automated purification as compared with standard centrifugation column purification.

Results and Conclusions

The automated Agencourt Ampure bead-based purification method was compared to both the manual Ampure purification method and the traditional Qiaquick Spin Column method to determine the effectiveness of the automated procedure. When comparing all three purification methods in terms of yield, the automated method performed similarly, if not better than the manual version, and the Qiagen Qiaquick Spin column method. The sample electropherograms generated using the Bioanalyzer 2100 show almost identical sample sizing and sample purity (**Figure 3**). Comparison of samples post fragmentation shows more DNA was retained using the automated method (**Figure 4**). The Bravo Automated Liquid Handling Platform in combination with Agencourt Ampure Beads can be used to effectively and fully automate the cleanup of enzymatic steps associated with sample library construction. The automated Ampure purification protocol may be altered to allow for a wide variety of bead concentrations as well as volumes. This automated process will allow for 96 samples to be processed simultaneously while maintaining the success observed for a single sample using the manual protocol defined by Agencourt.

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