Complete Automation of the Stratagene StrataPrep 96 PCR Purification Kit with the Agilent Bravo Automated Liquid Handling Platform and Agilent Automated Centrifuge

Application Note

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Abstract

A new method was developed to automate PCR purification in a 96-well format using the Bravo Automated Liquid Handling Platform, Automated Centrifuge and StrataPrep 96 PCR Purification Kit from Agilent Technologies (Automation Solutions and Stratagene divisions, respectively). Current PCR purification protocols require extensive manual processing including repetitive pipetting and vacuum filtration steps. Using Agilent's VWorks Automation Control software, a protocol was developed for the Bravo Platform to aspirate, dispense, mix, and transfer sample from a source 96-well PCR plate to the binding and collection plates of the StrataPrep 96 kit as well as to automate sample wash steps using an accessory vacuum filtration unit on the Bravo Platform. Sample recovery was accomplished using the Agilent Automated Centrifuge. DNA recovery quality was assessed on the Agilent BioAnalyzer 2100.

Introduction

The StrataPrep 96 PCR purification kit provides a rapid method to simultaneously purify 96 PCR products from oligonucleotide primers, unincorporated nucleotides, buffer components, and enzymes. This simple method of DNA purification eliminates the need for tedious manipulation of resins, toxic phenol–chloroform extraction, and the time-consuming ethanol precipitation used in other DNA purification methods. The StrataPrep 96 kit does rely on repeated washes, filtration, and centrifugation steps that are amenable to automation. The Bravo Platform has nine plate deck positions, which along with an Agilent BenchCel Microplate Workstation and an Agilent Automated Centrifuge and Loader, can fully accommodate the StrataPrep 96 protocol without manual intervention. Described below is a protocol, written for a workstation containing a Bravo, a BenchCel Microplate Workstation and an Agilent Microplate Centrifuge to automatically handle binding, washing and elution of isolated PCR products.

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Workstation Configuration

An automated workstation was assembled using Agilent Automation Solutions instrumentation and components. An Agilent Bravo Automated Liquid Handling Platform (G5409A) with gripper attachment for moving plates on the deck and a 96-channel pipetting head (04730-202) to handle Agilent 200 µL Disposable Pipette Tips (06880-102) was configured with the 9 deck positions as follows (see **Figure 1**): 1) Vacuum Filtration Station (G5432A), 2) 200 µL pipette tips for washing, 3) reservoir for binding buffer, 4) reservoir for wash buffer, 5) 200 µL pipette tips for binding or elution, 6) filter plate disassembly, 7) PCR product plate, 8) elution buffer, 9) hand-off position to handle plates to and from Agilent BenchCel 6R Microplate Workstation containing six plate handler racks configured with 2 racks of pipette tips (capacity of 30), 1 rack with 12 filter plates, 1 rack with 24 96-well collection / waste plates, 1 rack of PCR product plates, and 1 empty rack for receiving the purified product plates. The BenchCel was also used to hand plates to an Agilent Automated Centrifuge with optional microplate loader. A trash chute for expended labware and tips was installed above the centrifuge. The workstation was controlled by Agilent VWorks Automation Control software.

Generation of PCR Products

PCR products were prepared using the Stratagene PfuUltra II Hotstart PCR Master Mix (#600850) to amplify a 582 bp fragment of Phage Lambda DNA following the recommended protocol. Reactions (50 µL each) were assembled into single 96-well plates (Eppendorf 951020401) with columns 6 and 12 used as no-template controls (NTC). Plates were sealed using the Agilent PlateLoc Thermal Microplate Sealer prior to thermal cycling. To compare the efficacy of the automated method against the hand method, each 1/2 plate (columns 1-6 or 7-12) was purified using the process below either manually or with the automated workstation.

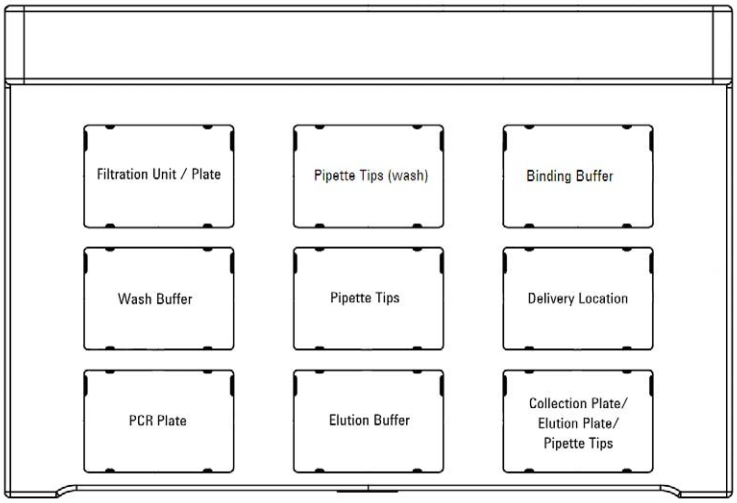

Figure 1. Agilent Bravo Configuration

Purification Protocol Workflow

PCR products were purified using the Stratagene StrataPrep 96 PCR Purification Kit (400774, 400775, 400776 for 50, 2, and 10 PCR plates, respectively) 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. Accessories such as the vacuum filtration unit and tip boxes are predefined in the software. Plates and tips are automatically stacked and unstacked as needed and transferred between the Bravo, BenchCel and centrifuge when required without user intervention.

Briefly, the automated workflow was as follows: the 2 reservoirs on the Bravo deck were charged with binding and elution buffer, and the plates were placed into the BenchCel stacker locations as described above. The protocol automatically transferred all required plates and tips to the Bravo deck. Two racks of tips, and a PCR product plate were placed onto their positions, and a filter plate was placed on the vacuum station. Binding buffer was added to the PCR products plate, mixed, and then the contents of each well were added to the filter plate. A vacuum (400 mbars) was applied to the filter plate for 5 minutes and then 1X PCR wash buffer was added to the plate before removing by vacuum for about 5 minutes. The filter plate was placed onto one of the collection plates included in the kit. The assembled plates were transferred to the Automated Centrifuge, centrifuged at 1000 X g for 10 minutes, then returned to the Bravo where the filter plate was placed onto a fresh collection plate. Using fresh tips, the Bravo added 50 µL of elution buffer to each well of the filter plate. After 5 minutes, the filter/collection plate was centrifuged for 5 minutes at 1000 X g. The assembly was separated on the Bravo and the collection plate containing the purified products was transferred to the BenchCel. As a control, PCR products from the second half-plate (columns 7 – 12) were purified using the same hardware, but with no robotic handling, or pipetting.

Purification Analysis

Results from 4 wells from different portions of the plate assessed quality using an Agilent BioAnalyzer 2100 on a DNA 1000 Nanochip, and were assessed for sample quality using BioAnalyzer 2100 Expert software version B.02.06. The figure shows overlaid samples from both manually-purified and robot-purified samples. The single peak is correctly sized within 1% of the predicted PCR product size and there are no contaminating peaks or primers.

Results and Conclusions

Effectiveness of using the automated platform was compared with the manual purification process by processing half-plates from using each method. **Figure 2** shows representative electropherograms produced using the Bioanalyzer 2100. Results from both hand and robot-purified plates are overlaid and show identical sizing and purity. Thus the Agilent Bravo Automated Liquid Handling Platform and Agilent Automated Plate Centrifuge can be used to effectively and fully automate the StrataPrep 96 PCR purification kit. The hardware and protocol can be adjusted to allow for different plate capacities by modifying the workstation components. For example, using an Agilent BenchCel Microplate Workstation will allow up to 12 full-skirted PCR plates to be processed in a single batch. It is also possible to stack PCR plates on the Bravo deck to allow batches of 4 plates to be processed using the BenchCel 2R Microplate Workstation.

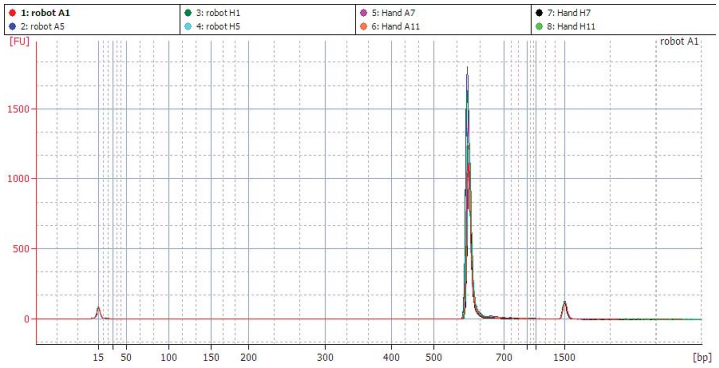

Figure 2. Co-migration of manual and robot-purified PCR products.
The large peak at just over 600 bp shows the major product formed during PCR of Lambda DNA with the selected primers. Note the absence of contaminating peaks and the comparable relative abundance of the product. The smaller peaks at 15 and 1500 bp are markers introduced by the DNA ladder used with the DNA 1000 Nanochip.

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