# PIPETMAX®: Automation of the Agencourt® AMPure® XP PCR Purification System

## APPLICATION NOTE AN1010

### APPLICATION BENEFITS

Solid phase reversible immobilization (SPRI) beads are commonly used to clean up polymerase chain reaction (PCR) and DNA samples for downstream applications, such as sequencing and cloning.

### SOLUTIONS

This application note demonstrates a flexible and reliable automated procedure for next-generation sequencing (NGS) library cleanup. This script can process up to 96 samples in a single run, increasing throughput and providing consistency for a challenging procedure.

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## ABSTRACT

The Agencourt® AMPure® XP PCR purification system was automated on PIPETMAX®, creating a simple, reliable, and flexible method for a process that is often laborious and technically challenging. Genomic DNA from *Escherichia coli* was prepared for whole genome sequencing using the Illumina® Nextera® XT DNA Library Preparation Kit. As part of this process, the Agencourt® AMPure® XP PCR purification system was used to clean up the library after amplification and before normalization. A custom magnetic bead separator rack (Figure 1) was used to easily and rapidly purify up to 96 samples in a single run. The automated protocol was created with the ability to modify several variables, depending on the needs of the application, including the number of samples to process, sample volume, bead volume, number of wash steps, and incubation times. This application allows users to easily integrate PIPETMAX into multiple workflows.


## INTRODUCTION

The purification of PCR amplicons is needed for several downstream applications, including Sanger and next-generation sequencing (NGS), genotyping, single nucleotide polymorphism (SNP) detection, cloning, fragment analysis, and primer walking. This clean up procedure removes primers, unbound nucleotides, salts, and enzymes, which are essential for DNA amplification, but are considered contaminants for many downstream reactions.[1]

Several approaches exist for purifying PCR amplicons, including the use of agarose gels, spin columns, functional tips, enzymatic digestions, and magnetic beads. Each approach may serve a specific need (cost, speed, quality); however, magnetic beads are commonly used for NGS applications as they are more amenable to automation and are readily scalable.[2,3] Illumina specifically recommends the use of the Agencourt® AMPure® XP PCR purification system for the clean up step within the Nextera® XT DNA Library Preparation workflow (Figure 2 on page 2). Many NGS library preparation workflows utilize the AMPure XP bead clean-up, including Ion Torrent, Roche, KAPA, New England BioLabs, and TATAA.


**Figure 1**
The custom magnetic bead separator rack (part number SPL2294) contains 24 magnets which can be raised or lowered to be in close contact with a 96-well plate. Each magnet is positioned between four wells, pulling the magnetic beads to the corner of each well.

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**Figure 2**
Illumina Nextera® XT DNA Library preparation workflow. Samples were processed through the standard route; tagmentation, amplification, clean up using the AMPure® XP system, normalization, and pooling, before moving on to sequencing.

The AMPure XP PCR purification system utilizes solid-phase reversible immobilization (SPRI) paramagnetic beads in an optimized buffer to selectively bind fragments larger than 100 bp. Subsequent wash steps remove unwanted PCR components before a final elution step yields the purified PCR amplicons.

The automated method described here was used to clean up *E. coli* gDNA libraries prior to whole genome sequencing. The PIPETMAX® protocol used a custom magnetic bead separator rack (part number SPL-2294), which allows the user to process up to 96 samples per run.

## MATERIALS AND METHODS

### Library Construction

NGS libraries were created from *E. coli* K12 gDNA, using the Nextera® XT DNA Library Preparation Kit (Illumina® part number FC-131-1005) on PIPETMAX®, according to the manufacturer's specifications.[4] Twelve unique primer sets were used to prepare gDNA samples (1 ng at 0.2 ng/µL) following tagmentation.

### AMPure XP Automated Protocol

Default values were used for all protocol variables, except for Library Wells to be Processed (Figure 3).

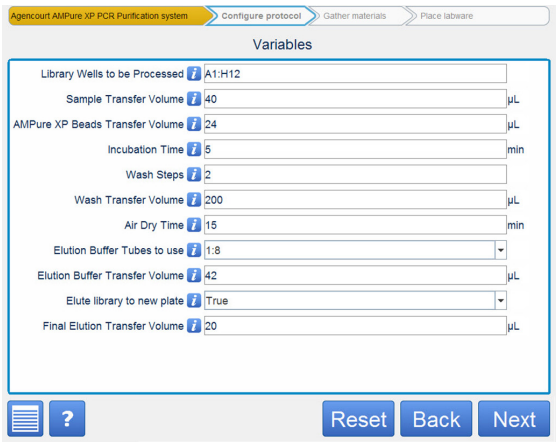


**Figure 3**
User interface for modifying variables such as Library Wells to be Processed, Sample Transfer Volume, AMPure® XP Beads Transfer Volume, Wash Steps, etc.

All labware and reagents were placed on the PIPETMAX bed at the start of the run, including tips (DSF200ST, part number F172513), PCR plate (Eppendorf part number 0030 128.575) each well containing 50 µL amplified libraries in a support base (Applied Biosystems part number N8010531), liquid reservoir (Seahorse part number 201256-100) containing 2 mL AMPure® XP beads (Beckman Coulter part number A63881) and 10 mL 80% ethanol, 0.2 mL MicroAmp 8-tube strip (Applied Biosystems part number N8010580) each tube containing 95 µL RSB resuspension buffer, an empty Midi plate (Thermo part number AB-0859) on the bed, and another on the magnetic bead separator (part numbers SPL-2294F and SPL-2294E) (Figure 4).

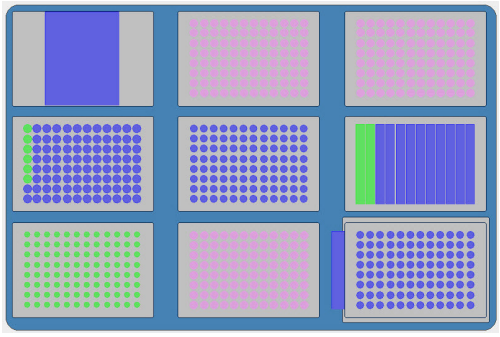

**Figure 4**
User interface for bed layout on PIPETMAX®. Labware and reagents from left to right, back to front: Tip Waste Chute, DSF200ST Tip Rack, DSF200ST Tip Rack, RSB Resuspension buffer in 0.2 mL 8-Tube Strips, empty Midi plate for final eluted libraries, liquid reservoir containing AMPure® XP beads and 80% Ethanol, starting material (amplified Libraries, DSF200ST Tip Rack, and another empty Midi plate on the magnetic bead separator for the clean up procedure.

AMPure XP beads (24 µL) were premixed and transferred to twelve wells of the Midi plate on the magnetic bead separator rack. The twelve amplified libraries (40 µL) were transferred by PIPETMAX to the Midi plate containing the AMPure XP beads and mixed. The magnetic bead separator was engaged, bringing the magnets in contact with the Midi plate and pulling down the beads. After a short wait, the supernatant was removed from each well and discarded. Two washes were then performed on the beads where wash solution (200 µL 80% ethanol) was added and removed to waste. The beads were

allowed to air dry for 15 minutes to remove any residual ethanol. The magnetic bead separator rack was disengaged, removing the magnetic field from the Midi plate before RSB (42 µL) was added to each library and mixed. Finally, the magnetic bead separator rack was engaged, bringing the magnets in contact with the Midi plate and pulling down the beads, and the cleaned-up libraries were transferred to the fresh Midi plate.

### Fragment Size Analysis

Following PCR cleanup, DNA fragment size was assessed for sample quality and consistency. A small amount (1 µL) of each sample was loaded on to an electrophoretic chip and analyzed by an Agilent 2100 Bioanalyzer, producing an electropherogram trace for each sample.

### Results and Discussion

The Agencourt AMPure XP PCR purification system was used to clean up *E. coli* K12 gDNA libraries for whole genome sequencing as part of the Illumina Nextera XT DNA Library Preparation process. The protocol was carried out on a PIPETMAX equipped with an on-bed magnetic bead separator. An electropherogram trace shows high quality samples were produced. The majority of fragments were between 700 and 2000 base pairs with no observable contamination (Figure 5).

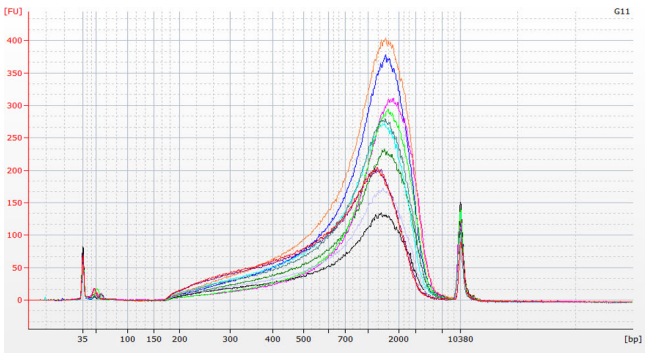


**Figure 5**
Electropherogram of *E. coli* K12 gDNA fragments, processed with the Nextera® XT DNA Library Preparation Kit and cleaned up with the AMPure® XP PCR purification system on PIPETMAX®.

The automated PIPETMAX script can be quickly and easily modified to fit into many different workflows and application needs, by adjusting run time variables (Table 1). Variables for samples to be processed, volumes for each reagent, number of washes, and elution volume are included to provide a highly flexible solution.

**Table 1**
Variables that can be adjusted at run time for the automated PIPETMAX® script for the Agencourt® AMPure® XP PCR Purification System

| VARIABLE | UNITS | DEFAULT | INFORMATION |
|---|---|---|---|
| Library Wells to be Processed | N/A | A1:H12 | The wells in the Midi plate to use for cleaning up PCR reactions. |
| Sample Transfer Volume | µL | 40 | The volume of PCR reaction to transfer to Midi plate for clean up procedure. |
| AMPure XP Beads Transfer Volume | µL | 24 | The volume of beads to transfer to the Midi plate for clean up procedure. |
| Incubation Time | Minutes | 5 | The amount of time to allow the samples and beads to incubate at room temperature for initial binding. |
| Wash Steps | N/A | 2 | The number of times to wash the PCR reactions. |
| Wash Transfer Volume | µL | 200 | The volume of wash solution to transfer to the Midi plate for each wash step. |
| Air Dry Time | Minutes | 15 | The amount of time to allow the samples and beads to incubate at room temperature to allow the remaining ethanol to evaporate. |
| Elution Buffer Tubes to Use | N/A | 1:8 | The tubes which contain elution buffer. |
| Elution Buffer Transfer Volume | µL | 42 | The volume of elution buffer to transfer to the Midi plate for final elution step. |
| Elute Library to New Plate | N/A | True | After adding elution buffer, optionally transfer Final Elution Volume to a new plate. |
| Final Elution Transfer Volume | µL | 20 | The volume of eluted sample to move to a new plate at the end of the script. |

## REFERENCES


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2. Head, S., Komori, H. K., LaMere, S.A., Whisenant, T., Van Nieuwerburgh, F., Salomon, D.R., and Ordoukhanian, P. Library construction for next-generation sequencing: Overviews and challenges. BioTechniques., 56: 61-77 (2014).
3. Bronner, I.F., Quail, M.A., Turner, D.J., Swerdlow, H. Improved Protocols for Illumina Sequencing. Curr Protoc Hum Genet., 18.2, (2009).
4. Nextera® XT DNA Library Prep Reference Guide. 15031942 v01. San Diego, CA, USA. 2016. Print.





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