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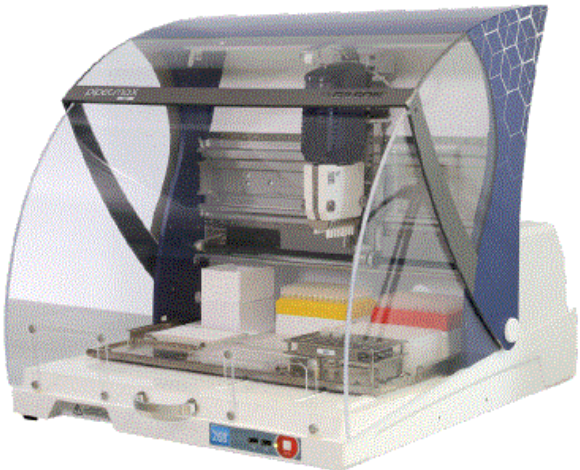
GILSON PIPETMAX 268 Lab Automated Liquid Handling Platform



BENEFITS

- Automation on PIPETMAX® 268 simplifies the process, making PCR purification more reliable and reproducible.
- The custom magnetic bead separator enables rapid purification of up to 96 samples in a single run, increasing efficiency.
- The automated protocol is flexible and customizable, allowing users to adjust variables to fit different workflows and applications

ADDRESSED ISSUES

- PCR purification is often laborious and technically challenging, requiring manual effort and precision.
- Manual handling limits sample throughput, making it time-consuming to process multiple samples.
- Protocols can lack flexibility, with limited ability to adjust parameters such as volume, washes, or incubation times.

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 cleanup procedure removes primers, unbound nucleotides, salts, and enzymes, which are essential for DNA amplification, but are considered contaminants for many downstream reactions1.
- 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). Many NGS library preparation workflows utilize the AMPureXP bead clean-up, including Ion Torrent,

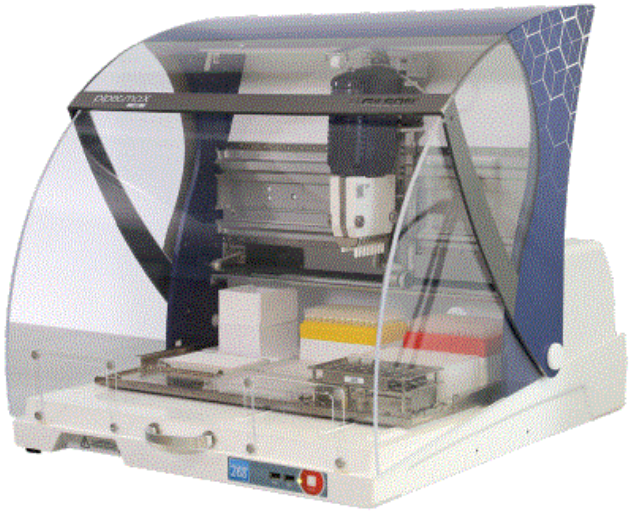


Figure 1
PIPETMAX® 268

- Roche, KAPA, New England BioLabs and TATAA.
- 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.

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 automated method described here was used to clean up E. coli gDNA libraries prior to whole genome sequencing. The PIPETMAX 268 protocol used a custom magnetic bead separator (part number SPL-2294), which allows the user to process up to 96 samples per run (Figure 3).

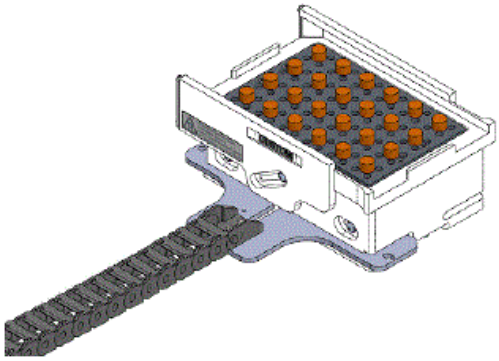


Figure 3

- The custom magnetic bead separator (part number SPL-2294) 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.

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 268 according to the manufacturer's specifications4. Twelve unique primer sets were used to prepare gDNA samples (1 ng at 0.2 ng/uL) following tagmentation.

AMPure XP Automated Protocol

Default values were used for all protocol variables, except for Library Wells to be Processed (Figure 4).

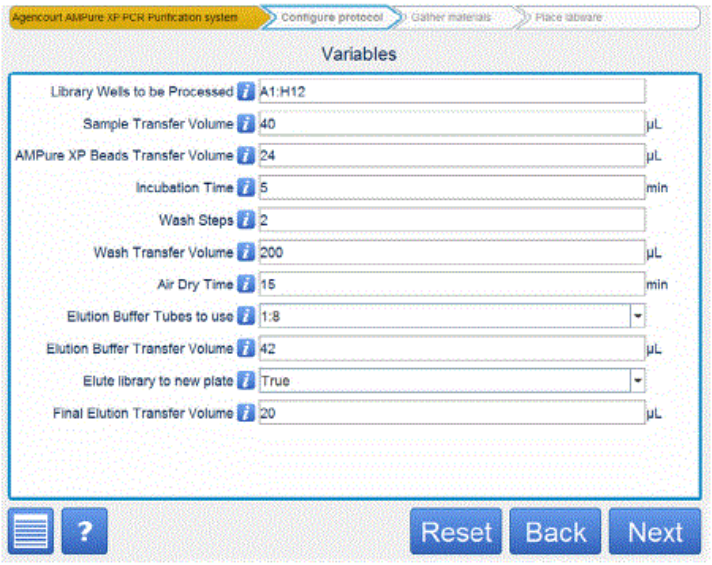


Figure 4

- User interface for modifying variables such as Library Wells to be Processed, Sample Tranfer Volume, AMPure® XP Beads Transfer Volume, Wash Steps, etc.
- All labware and reagents were placed on the PIPETMAX 268 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, another on the magnetic bead separator (part numbers SPL-2294J and SPL-2294E) (Figure 5).

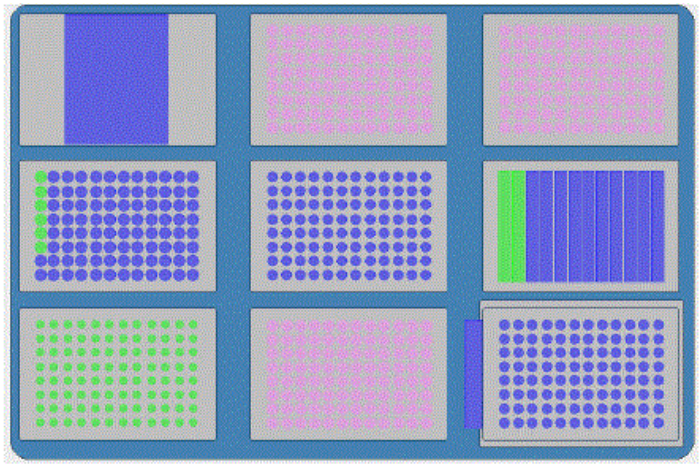


Figure 5

- User interface for bed layout on PIPETMAX 268. Labware and reagents from left to right, back to front: Tip Waste Chute, DSF200ST Tip Rack, DSF200ST Tip Rack, RSB Resuspension buffer in 0.2mL 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. The twelve amplified libraries (40 µL) were transferred by PIPETMAX 268 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 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 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 electro-pherogram 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 performed on PIPETMAX 268 with the use of the 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 6).

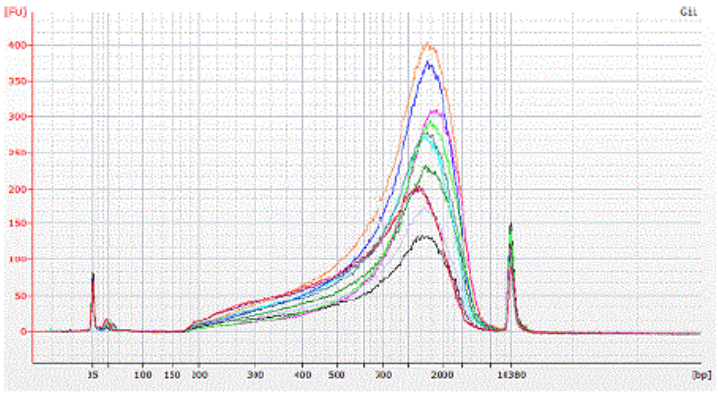


Figure 6

- 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 268.
- The automated PIPETMAX 268 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 268 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).	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	min.	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 reacti ons.
Wash Transfer Volum e	µL	200	The volume of wash solution to transfer to th e MIDI plate for each wash step.
Air Dry Time	min	15	The amount of time to allow the samples an d beads to incubate at room temperature to allow the remaining ethanol to evaporate.
Elution Buffer Tubes t o Use	N/A	1:8	The tubes which contain elution buffer.
Elution Buffer Transfe r Volume	µL	42	The volume of elution buffer to transfer to th e 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 n ew plate at the end of the protocol.

ORDERING INFORMATION

PART NUMBER	DESCRIPTION
32100001	PIPETMAX 268 with Cover Cutouts
FC10021	MAX8x200 Pipette Head
FC10022	MAX8x20 Pipette Head
32000091	PIPETMAX 268 Tray 384 Well.
32000175	PIPETMAX 268 Tip Adapter Blocks (x 3)
32000321	TRILUTION® micro Installed on a

32000177	Tip Storage Riser for PIPETMAX 268
SPL-2294J-HDW	Magnetic Bead Separator Rack
SPL-2294E-HDW	Magnetic Bead Separator Magnet
40000026	Thumb Screw (x 6)
32000292	Reload Block Clip (x 4)
4214540393	Washer (x 4)
32000303	Microamp Short 96 PCR Tube Rack
F172513	DSF200ST Diamond Tips in Blister Packaging

CONCLUSION

- E. coli K12 gDNA libraries were prepared for whole genome sequencing as part of the Illumina Nextera XT DNA Library Preparation kit.
- The Agencourt AMPure XP PCR purification system is commonly used for the clean-up step in Next Generation Sequencing workflows.
- This application note demonstrates the auto-mated PIPETMAX 268 protocol, which uses the magnetic bead separator.
- Variables allow the user to modify the protocol at run time, creating a dynamic solution for a tedious and technically challenging procedure.

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-genertion 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.

ACKNOWLEDGMENTS

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- WI, USA.
- www.gilson.com/contactus

FAQ

What downstream applications can be performed using purified PCR amplicons?

Purified PCR amplicons are essential for Sanger and next-generation sequencing, genotyping, cloning, fragment analysis, and primer walking.

How many samples can be processed per run with the custom magnetic bead separator?

The custom magnetic bead separator allows processing of up to 96 samples in a single run.

What is the technology used in the AMPure XP PCR purification system?

The system utilizes solid-phase reversible immobilization (SPRI) paramagnetic beads to selectively bind PCR fragments larger than 100 bp.

Documents / Resources

	GILSON PIPETMAX 268 Lab Automated Liquid Handling Platform [pdf] Instructions PIPETMAX 268 Lab Automated Liquid Handling Platform, PIPETMAX 268 , Lab Automated Liquid Handling Platform, Automated Liquid Handling Platform, Liquid Handling Platform, Handling Platform

References

- User Manual

Gilson

Automated Liquid Handling Platform, Gilson, Handling Platform, Lab Automated Liquid Handling Platform, Liquid Handling Platform, PIPETMAX 268, PIPETMAX 268 Lab Automated Liquid Handling Platform

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