

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

SETUP GUIDE FOR APPLICATION NOTE AN1010

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OVERVIEW

Use this setup guide to configure the PIPETMAX®, prepare bed elements, and run scripts on TRILUTION® micro for *Application Note AN1010: PIPETMAX®: Automation of the Agencourt® AMPure® XP PCR Purification System*.

Description

The AMPure XP PCR purification system is used to clean up polymerase chain reaction (PCR) samples for various downstream applications, including next-generation sequencing (NGS), Sanger sequencing, genotyping, single-nucleotide polymorphism (SNP) detection, cloning, fragment analysis, and primer walking. The automated script has been created and tested for use on the PIPETMAX, and it can be quickly and easily modified to fit into many different workflows and application needs by adjusting run time variables.

Prerequisites

Read the *PIPETMAX® 268 User's Guide* (available at www.gilson.com) and technical resource documents for the AMPure XP PCR purification system (available at www.beckmancoulter.com) before setting up the application. Read all hardware, software, and safety instructions for equipment, reagents, and materials before performing the application. This setup guide is provided as a general resource and without warranty. Email Gilson technical support (techsupport@gilson.com) for additional assistance.



Figure 1

The custom magnetic bead separator rack (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.

SETUP

To complete the script, you will need a PIPETMAX® configured with the following: a tip storage riser, MAX8x20 and MAX8x200 pipette heads, tip chute, waste bin, and the items listed in the following tables.

Required Gilson Parts and Accessories

DESCRIPTION	PART NUMBER	QTY.	DETAILS
PIPETMAX with Cover Cutouts	32100001	1	
TRILUTION® micro on Tablet	32000321		Software used to run PIPETMAX. Only one copy of the control software (for tablet or PC) is required.
TRILUTION® micro for PC	32000320	1	
MAX8x200 Pipette Head	FC10021	1	8-channel head (20–200 µL) for PIPETMAX.
MAX8x20 Pipette Head	FC10022	1	8-channel head (1–20 µL) for PIPETMAX.
PIPETMAX Tray, 384 Well	32000091	1	Removable tray with nine bed positions that includes clips for securing microplates.
PIPETMAX Tip Adapter Blocks	32000175	3	Reusable support racks for holding pipette tips.
Tip Storage Riser for PIPETMAX	32000177	1	Riser for off-bed tip disposal. Includes on-bed waste chute and under-instrument waste bin.
Magnetic Bead Separator Rack	SPL-2294F-HDW	1	Base for PIPETMAX magnetic bead rack.
Magnetic Bead Separator Magnetic Plate	SPL-2294E-HDW	1	Magnetic insert for PIPETMAX magnetic bead rack.
PIPETMAX Tip Block Clip Set	32000311	3	The tip block clips are used to secure the magnetic bead rack and orbital shaker to the tray.
MicroAmp® Short 96-Well PCR Tube Rack	32000303	2	Solid base that holds the semi skirted PCR plate
DSF200ST Diamond Tips in Blister Packaging	F172513	1	Sterile filter tips with 200 µL capacity.
Agencourt AMPure XP PCR Purification System.sqlite	Custom	N/A	PIPETMAX run file. Available from techsupport@gilson.com

Required User Supplied Materials and Equipment

DESCRIPTION	PART NUMBER	QTY.	DETAILS
PCR Plate	Eppendorf 0030 128.575	1	96-well semi-skirted microplate suitable for use in a thermal cycler.
4-Column Reservoir	Agilent Seahorse 201308-100	1	12-column reservoir.
Midi Plate	Thermo AB-0859	2	96-well deep well plate used for library normalization script.
Centrifuge	N/A	1	Compatible with microplates.
Thermal Cycler	N/A	1	Compatible with the PCR plate.
AMPure XP PCR 5 mL	A63880 (5 mL)		Refer to the manufacturer's specifications.
AMPure XP PCR 60 mL	A63881 (60 mL)		
AMPure XP PCR 450 mL	A63882 (450 mL)	1	

SCRIPT: AGENCOURT® AMPURE® XP PCR PURIFICATION

Overview

This script makes use of a custom magnetic bead separator for PIPETMAX®. The script allows PIPETMAX to automatically engage or disengage the magnets in order to capture or release the magnetic beads during incubation, washes, and elution. Variables can be modified at run time to customize the script according to the user's needs.

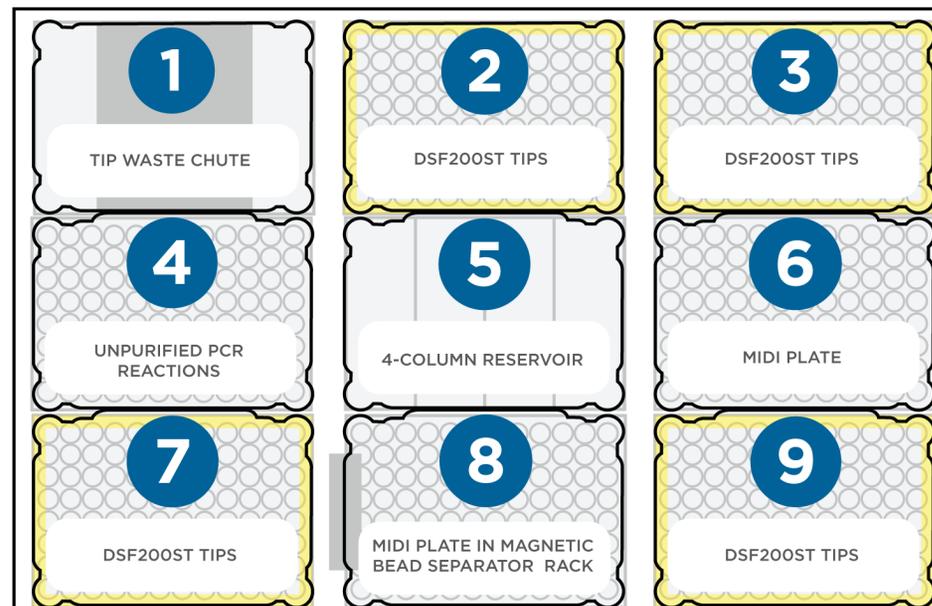
Automation Steps

- Disengage magnets (move magnetic bead separator to home position).
- Transfer 24 µL AMPure® beads to target wells in Midi plate on magnetic bead separator.
- Transfer 40 µL unpurified PCR reactions from PCR plate to Midi plate on magnetic bead separator.
- Incubate beads and samples at room temperature.
- Engage magnets on magnetic bead separator. Wait. Remove supernatant.
- Add 200 µL 80% ethanol wash. Wait. Remove supernatant.
- Add 200 µL 80% ethanol wash. Wait. Remove supernatant.
- Disengage magnets on magnetic bead separator.
- Add 42 µL elution buffer.
- Mix beads and elution buffer to elute.
- Engage magnets on magnetic bead separator. Wait.
- Transfer eluent to new Midi plate.

Optional: After completion of the Bead Cleanup script, you may wish to carry out BioAnalyzer analysis of 1 µL from one or more purified PCR reactions.

Script Variables

DESCRIPTION	DEFAULT VALUE
Library Wells to be Processed	A1:H12
Sample Transfer Volume	40 µL
AMPure XP Beads Transfer Volume	24 µL
Incubation Time	5 Minutes
Wash Steps	2
Wash Transfer Volume	200 µL
Air Dry Time	15 Minutes
Elution Buffer Tubes to Use	1:8
Elution Buffer Transfer Volume	42 µL
Elute Library to New Plate	True
Final Elution Transfer Volume	20 µL



Bed Layout

POSITION	BED ELEMENT	INITIAL VOLUME	DETAILS
1	Tip Waste Chute	N/A	Tip waste chute, underbed bin, and bed riser.
2, 3, 7, 9	DSF200ST Tips	N/A	Sterile filter tips, Gilson part number F172513.
4	Unpurified PCR Reactions	50 µL	96V PCR twin.tec Semi Skirted Eppendorf 128.575 in Thermo MicroAmp Base part number N8010531.
5	AMPure XP in Column 1 80% Ethanol in Column 2 Elution Buffer in Column 3	4 mL AMPure XP Beads 45 mL 80% Ethanol 5.5 mL Elution Buffer	Reservoir, 4 column, Seahorse part number 201256-100.
6	Purified and Eluted PCR Reactions	0 µL	Thermo part number AB-0859 (Midi plate).
8	Purification Plate	0 µL	Thermo part number AB-0859 ("Midi plate") in magnetic bead rack SPL-2294F-HDW.

FREQUENTLY ASKED QUESTIONS (FAQ)

Q: The instructions specify particular labware for the PIPETMAX script. Can I use different labware?

A: No, this script has been validated with specific labware. Any deviation from the specified plates and rack may result in incorrect pipetting or crashes. Contact technical support for further assistance if alternate labware is required (techsupport@gilson.com).

Q: Can PIPETMAX accurately dispense small volumes?

A: Yes, PIPETMAX exceeds the Maximum Permissible Errors for piston operated multichannel pipettes (ISO 8655) at 1µL on the MAX8x20 pipette head (refer to the table below):

VOLUME	ERROR COMPONENT	PIPETMAX MAX8X20	ISO 8655
1 µL	Systematic Error	±0.08 µL (8%)	±0.10 µL (10%)
1 µL	Random Error	±0.05 µL (8%)	±0.10 µL (10%)

Q: What is the procedure for placing the custom magnetic bead separator (SPL-2294F-HDW) onto the PIPETMAX tray?

A: Place thumbscrews, reload block clips, and washers at the corners of position nine (front right) on the removable tray. Place the magnetic bead separator rack on the removable tray in position nine and secure it in place with the thumbscrew and clips tightened against the small ridge built into the white plastic of the rack.

Q: How many tips are needed? Can I use the partial tip box from one run in a second run?

A: The number of tips required will depend on the number of samples and the number of washes that are being processed. Partial tip boxes may be used in a subsequent run; to do this enter the number of missing tips when prompted during tip setup in the step-by-step wizard.

Trademarks

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MicroAmp® (ThermoFisher Scientific)

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Gilson, Inc.

3000 Parmenter Street · PO Box 620027 · Middleton, WI 53562, USA
T 608-836-1551 or 800-445-7661 · F 608-831-4451

Gilson S.A.S.

19 avenue des Entrepreneurs · 95400 Villiers-le-Bel, France
T +33 (0) 1 34 29 50 00 · F +33 (0) 1 34 29 50 20

www.gilson.com/ContactUs