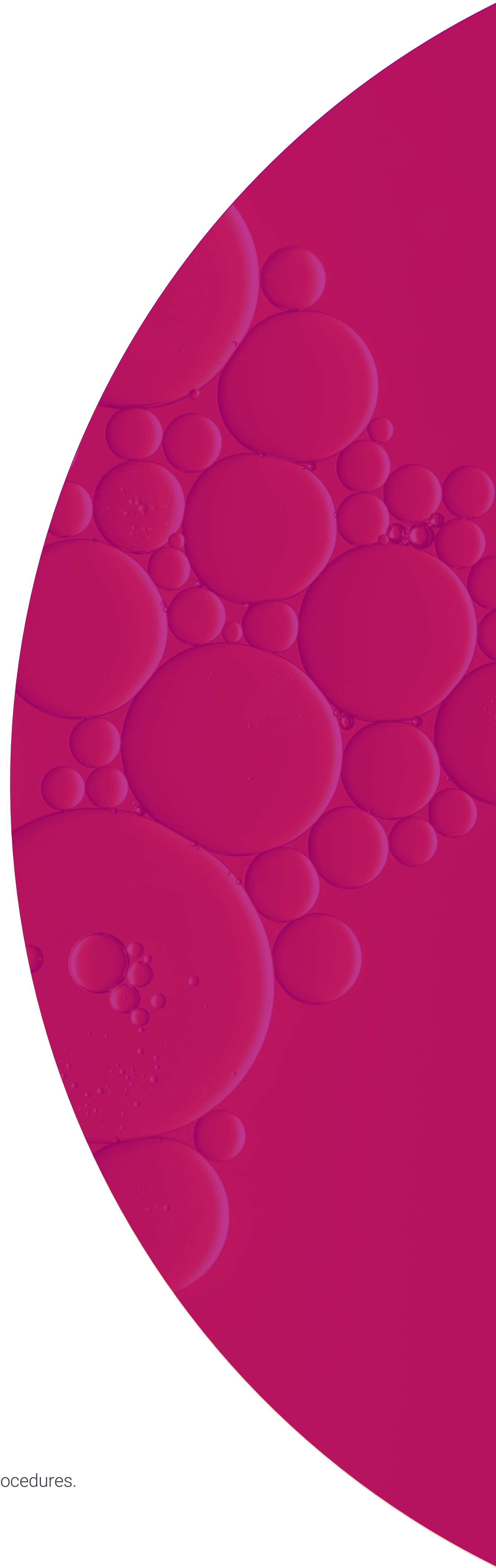
PacBio



Automated 3.1x diluted AMPure PB for saliva gDNA on the Hamilton NGS STAR MOA system

Guide & overview

103-801-500 REV01 FEB2026

Introduction

This procedure describes the automated workflow for performing 3.1x dilute AMPure® PB bead cleanup on saliva gDNA using the Hamilton NGS STAR MOA system. This step is necessary to improve coverage of saliva samples for PureTarget™.

Overview

Overview	
Samples	96
Workflow step	**Automation run time**
gDNA cleanup with diluted AMPure PB	1.0 hour
DNA input	
DNA input Quantity	2.0–6.0 µg in 45 µL of Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water

Required materials and equipment

Consumables	Catalog Number
Hard Shell 96 well PCR plate	Bio-Rad, HSP9601
50 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235948
300 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235903
1000 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235905
20 mL Reagent Reservoir	Hamilton, 10161052
300 mL Reservoir	Agilent, 201244-100
MicroAmp Clear Adhesive Film	ThermoFisher Scientific, 00146104
Equipment	**Catalog Number**
Hamilton NGS STAR MOA	Contact Hamilton representative
Vortex Mixer	Any major lab supplier (MLS)
Microcentrifuge	Any MLS
Qubit 4 or Qubit Flex Fluorometer	ThermoFisher Scientific, Q33238 (Qubit 4), Q33327 (Qubit Flex)
Recommended DNA sizing	**Catalog Number**
Femto Pulse System	Agilent Technologies, Inc. M5330AA
Femto Pulse gDNA 165kb analysis kit	Agilent Technologies, Inc. FP-1002-0275
AMPure® PB size selection	**Catalog Number**
AMPure® PB	PacBio® PN: 100-265-900
Elution buffer (note: Elution buffer included in PureTarget™ bundle is sufficient)	PacBio® PN: 101-633-500

General Best Practices

DNA input

Saliva is not a supported sample type for PureTarget 96 due to performance variability. However, performance of this sample type can be improved with an additional upfront AMPure PB bead cleanup. The AMPure PB bead size selection step will deplete DNA shorter than 3 kb and may remove contaminants that inhibit library preparation. This step is recommended for saliva genomic DNA to achieve better performance.

> **Important**: To ensure that there is sufficient mass for the gDNA repair step, 2 µg or more of Nanobind saliva gDNA is the recommended input mass for 3.1x dilute (35%) AMPure PB. Recoveries range from 50–80%. gDNA with less than 50% recovery from 3.1x dilute AMPure PB may indicate low sample quality and low coverage may be observed. It is recommended to start at the gDNA repair step with 1.3–1.5 µg per sample. Note that samples with a higher bacterial content will have lower target coverage. Bacterial contamination will vary between samples.

For information on the performance of **unsupported samples** (saliva and other extraction methods), see Application note - Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing.

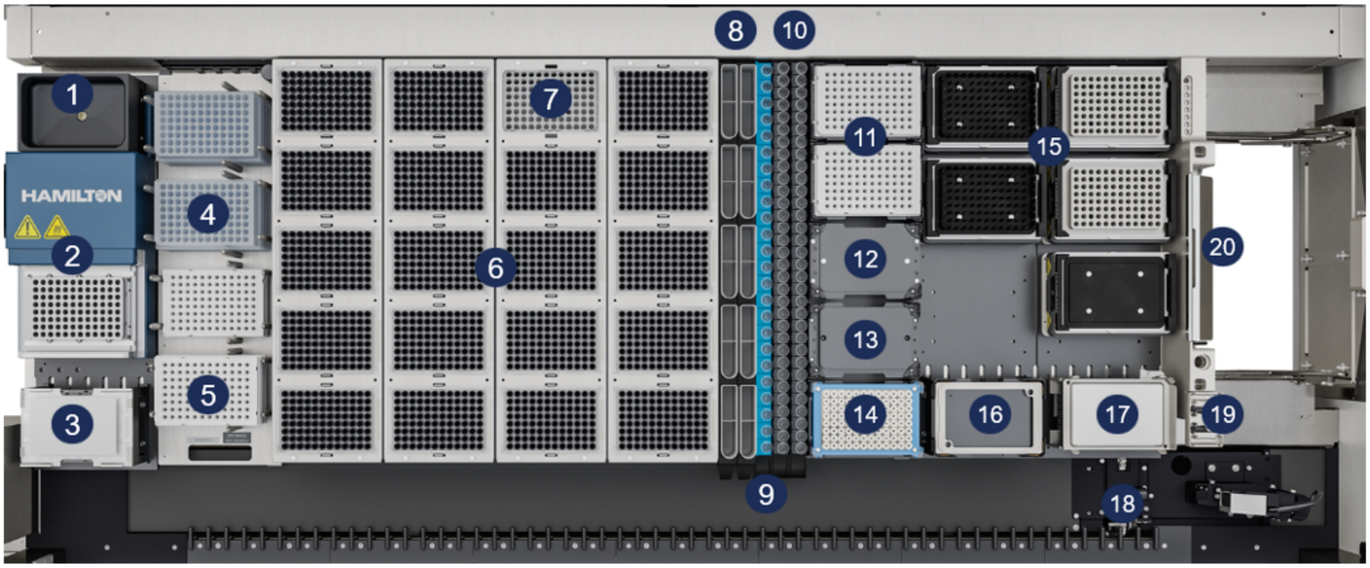
Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Reagent and sample handling

- Room temperature is defined as any temperature in the range of 18–23°C for this protocol.
- Bring AMPure PB beads to room temperature. Always vortex immediately prior to use.
- Bring Qubit reagents to room temperature prior to use.

Hamilton NGS STAR MOA Deck



1. Gravity liquid waste for Multi Probe Head (MPH)
2. On-Deck Thermal Cycler (ODTC) - iSWAP included
3. Comfort Lid parking position
4. Deep well plate stacking positions on plate stacker carrier (N/A)
5. 96 well PCR plates stacking position on plate stacker carrier
6. 4 Tip Carriers
7. Core II Tip support adapter for MPH
8. Reagent reservoir carriers (Only 1 out of 2 carriers used for 20 mL reservoirs)
9. 15–17mm Tube carrier (N/A)
10. Microtube carriers
11. 96 well PCR plate positions on the plate carrier
12. Deep well plate position on the plate carrier
13. 300mL reservoir position on the plate carrier
14. Alpaqua Magnum FLX magnetic plate position on the plate carrier (without springs)
15. Hamilton Heater Shakers (HHS) on risers (2 96-well PCR plate adapters and 1 flat bottom adapter, 2 Deep well plate HHS are N/A)
16. Inheco CPAC with 96-well PCR plate adapter (CPAC 1)
17. Inheco CPAC with 2mL tube adapter (N/A)
18. Autoloader
19. CO-RE gripper paddles
20. Solid/Liquid waste for the 1000 µL channels

Note: Contact your Hamilton representative for installation and deck details.

Quality checks

Refer to the below recovery tracking table if performing quality checks (QC). Measure DNA concentration with a Qubit fluorometer using the **1X dsDNA HS kit.**

Protocol Step	gDNA recovery
Starting Input	100%
Post 3.1x 35% AMPure PB	50 – 80%

Workflow steps

Preparation of the Hamilton NGS STAR MOA System:

- The automation run is designed for and requires 96 samples.
- Instrument prompts will provide reagent volumes and will indicate where and when to load based on start and stop process selected at the beginning of the run.

3.1X Diluted Ampure PB cleanup

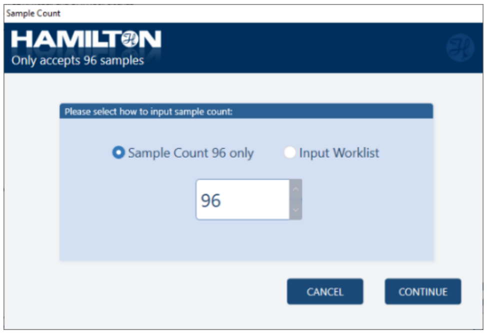
1. Start the PacBio method “**PacBio PureTarget 3.1x AMPure cleanup for saliva**”.

2. **Enter a “USER ID” for run.** To initiate the run, enter the desired run name. This name will be recorded in the run file.

Please type User ID

Type	Value	Description
USER ID		Please type USER ID

3. **Sample count selection.** There are two ways to enter the sample count:
    1. In “Sample count input” enter 96. Only 96 samples are compatible with this workflow.
    2. In “Worklist input,” a worklist input file can be uploaded for sample tracking. Reference the Appendix for instructions.


Sample Count

HAMILTON

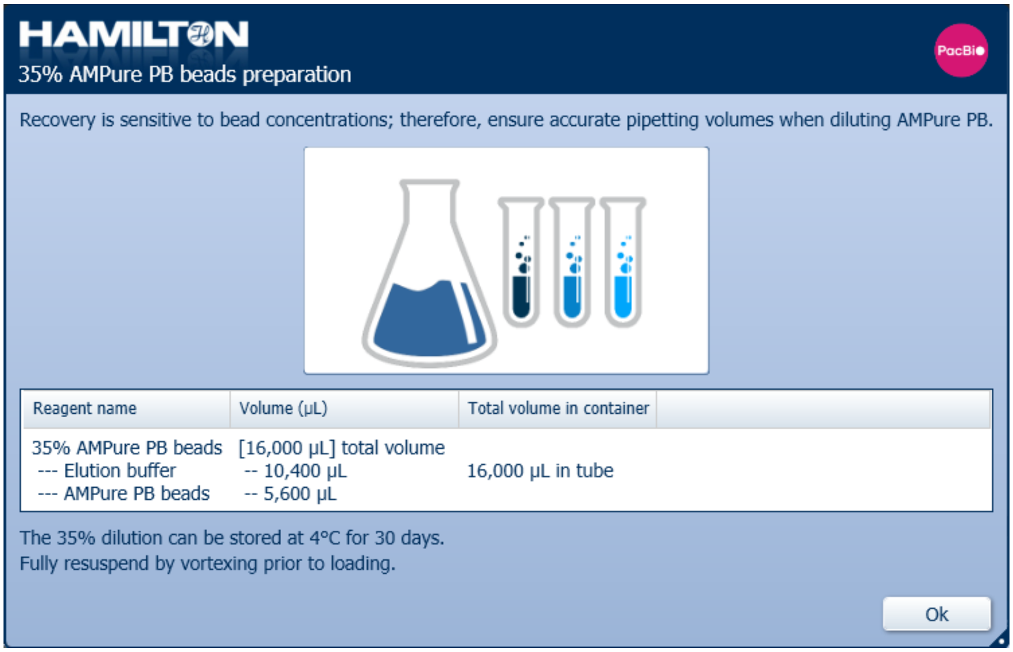
Only accepts 96 samples

Please select how to input sample count:

● Sample Count 96 only ○ Input Worklist

96

4. **Prepare reagents.** Prepare the 35% AMPure PB dilution according to the provided instructions in a 50 mL conical tube. Vortex AMPure PB beads immediately prior to dilution.


HAMILTON

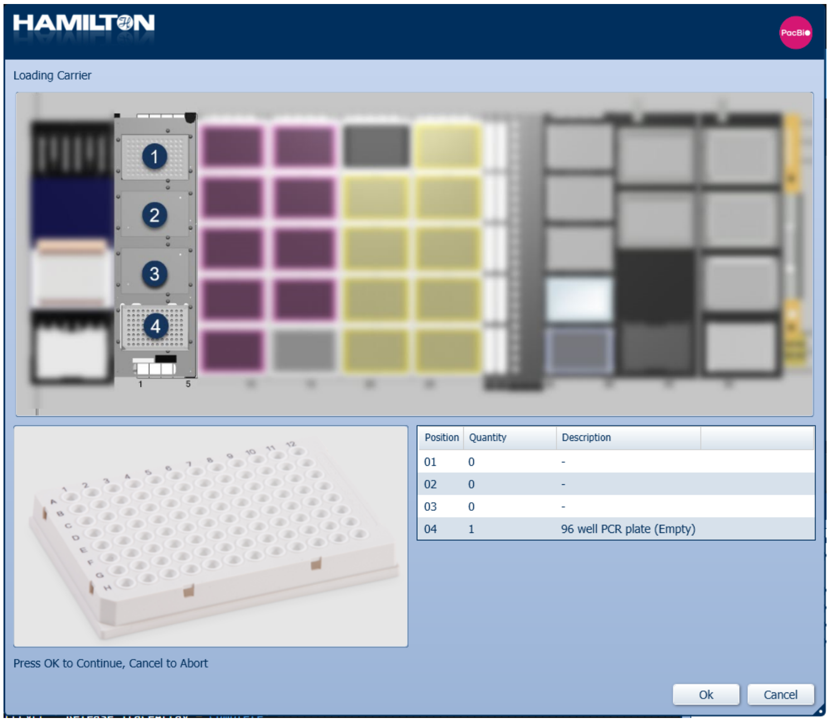
35% AMPure PB beads preparation

Recovery is sensitive to bead concentrations; therefore, ensure accurate pipetting volumes when diluting AMPure PB.

Reagent name	Volume (µL)	Total volume in container
35% AMPure PB beads --- Elution buffer --- AMPure PB beads	[16,000 µL] total volume -- 10,400 µL -- 5,600 µL	16,000 µL in tube

The 35% dilution can be stored at 4°C for 30 days.
Fully resuspend by vortexing prior to loading.

5. **Load plate.** Place new 96 well PCR plate onto the plate stacker in position 4. Make sure position A1 is in the upper left-hand corner.



Loading Carrier

Position	Quantity	Description
01	0	-
02	0	-
03	0	-
04	1	96 well PCR plate (Empty)

Press OK to Continue, Cancel to Abort

6. **Ensure Tip Support is empty.** Verify that the Tip Support is empty. The Tip Support is required for the 96 Multiprobe head (MPH) and is located in tip carrier 3, position 1.

Loading Carrier

Position	Quantity	Description
01	1	Tip Support Adapter (Empty)
02	0	-
03	0	-
04	0	-
05	0	-

Press OK to Continue, Cancel to Abort

7. **Tip deck layout.** A prompt will display the tip positions, including the Tip Support position. There are four tip carriers. Refill with new tips according to the specified positions for each tip size: 50 µL filtered conductive tips, 300 µL filtered conductive tips, and 1000 µL filtered conductive tips.

Insert new tips to the tip carriers and press 'ok'.

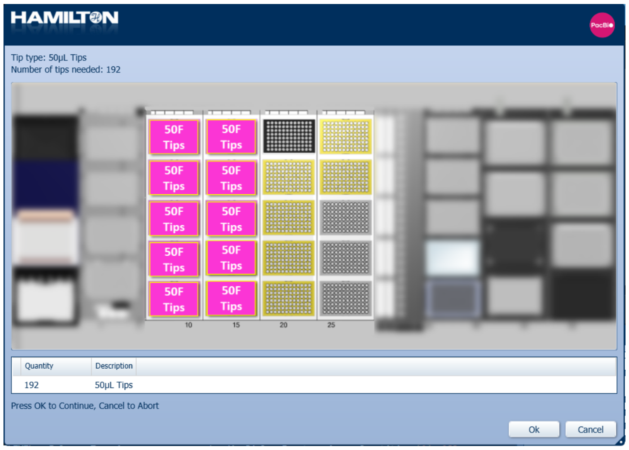
8. **Load the tips and ensure the tip count matches what is on the deck.** Load the 50 µL, 300 µL, and 1000 µL tips into their designated positions on the instrument.

   Click ‘Ok” to continue to tip deck matching display. To match tips to the deck, click on the corresponding tip positions so they are highlighted. Once the selections match the deck layout, click “OK” to continue.

   Note: If tips run out during the run, the system will pause and prompt for a refill.

Note: It is critical that these selections match exactly what is loaded on the deck. Inaccurate tip selections may cause collisions or sample failure.

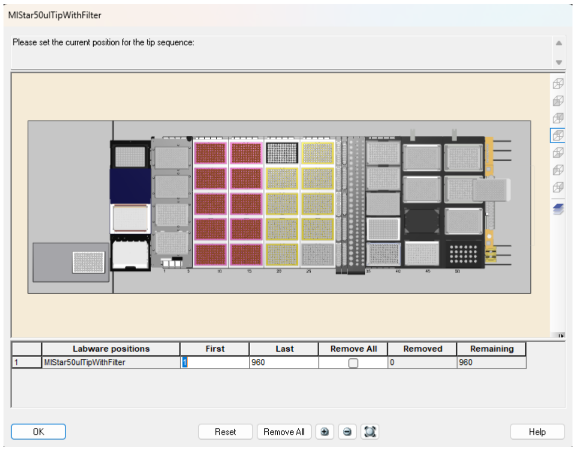
Labware	Minimum required quantity
50 µL filtered conductive tips	192
300 µL filtered conductive tips	480
1000 µL filtered conductive tips	16



Tip type: 50µL Tips
Number of tips needed: 192

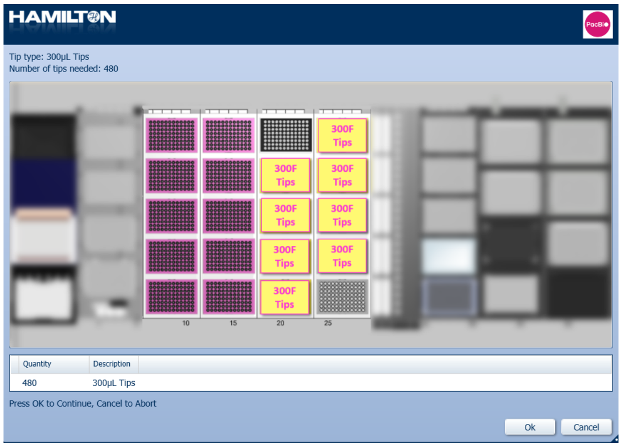
Quantity	Description
192	50µL Tips

Press OK to Continue, Cancel to Abort



Please set the current position for the tip sequence:

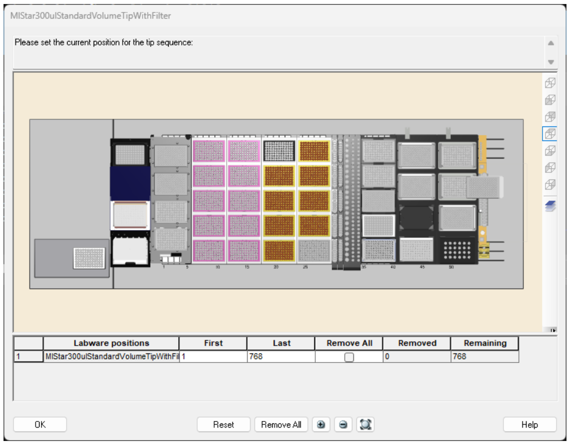
	Labware positions	First	Last	Remove All	Removed	Remaining
1	MlStar50ulTipWithFilter	1	960	☐	0	960



Tip type: 300µL Tips
Number of tips needed: 480

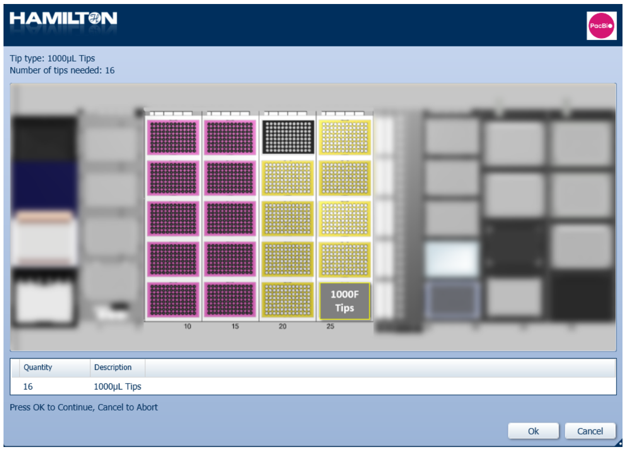
Quantity	Description
480	300µL Tips

Press OK to Continue, Cancel to Abort



Please set the current position for the tip sequence:

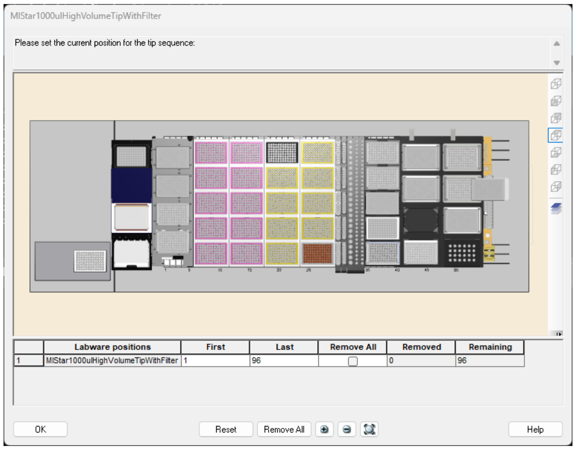
	Labware positions	First	Last	Remove All	Removed	Remaining
1	MlStar300ulStandardVolumeTipWithFil	1	768	☐	0	768



Tip type: 1000µL Tips
Number of tips needed: 16

Quantity	Description
16	1000µL Tips

Press OK to Continue, Cancel to Abort



Please set the current position for the tip sequence:

	Labware positions	First	Last	Remove All	Removed	Remaining
1	MlStar1000ulHighVolumeTipWithFilter	1	96	☐	0	96

9. **Load 20 mL reservoirs in the reagent trough carrier.** Place the 20 mL reservoirs containing their respective reagents into the reagent trough carrier located in track 31. Refer to the table below for the 35% dilute AMPure and Elution buffer volumes to load onto the instrument. Vortex the diluted Ampure PB immediately prior to transfer to a 20 mL reagent reservoir.

Reagent	Reagent amount (µL)
35% v/v dilution of AMPure PB beads	16,000
Elution buffer	6,500

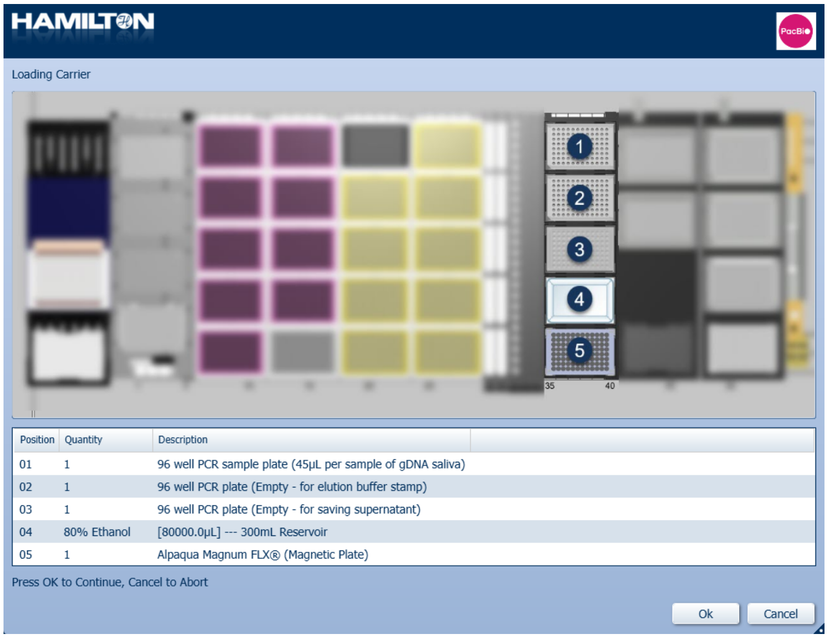
Position	Quantity	Description
01	0	-
02	Elution buffer	[6500.0µL] --- 20 mL Reservoir
03	35% v/v dilution of AMPure PB beads	[16000.0µL] --- 20 mL Reservoir
04	0	-
05	0	-

Press OK to Continue, Cancel to Abort

10. **Load the plate processing carrier.** Load the sample input plate, 2 new 96-well PCR plates for Elution buffer and supernatant, and an 80% ethanol reservoir onto the carrier in the positions shown in the loading prompt. Fill the 300 mL reservoir with the volume of 80% ethanol specified in the table. Ensure the sample input plate is spun down prior to loading.

    Note: The Alpaqua Magnum FLX magnetic plate is located in position 5 and remains stationary.

Reagent	Reagent amount (µL)
80% ethanol	80,000

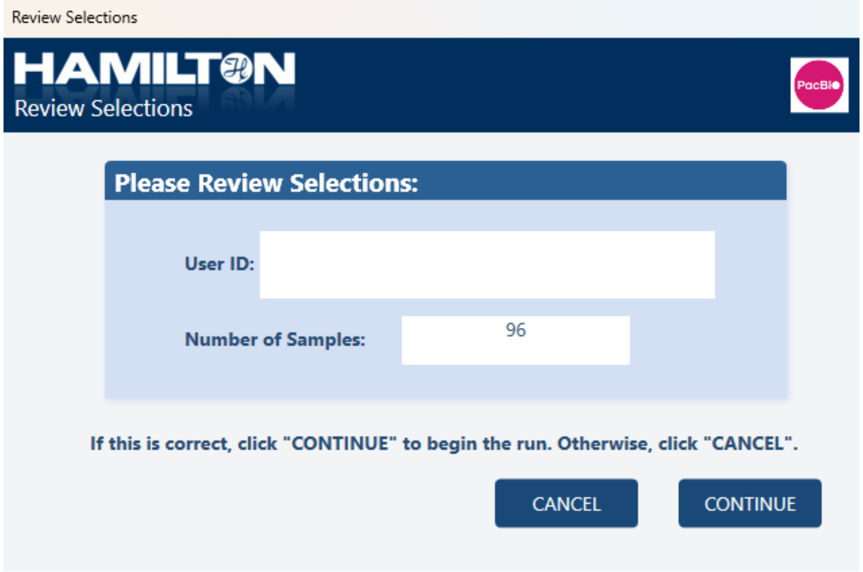


Loading Carrier

Position	Quantity	Description
01	1	96 well PCR sample plate (45µL per sample of gDNA saliva)
02	1	96 well PCR plate (Empty - for elution buffer stamp)
03	1	96 well PCR plate (Empty - for saving supernatant)
04	80% Ethanol	[80000.0µL] --- 300mL Reservoir
05	1	Alpaqua Magnum FLX® (Magnetic Plate)

Press OK to Continue, Cancel to Abort

11. **Review selections.** Click **CONTINUE** to begin the run .



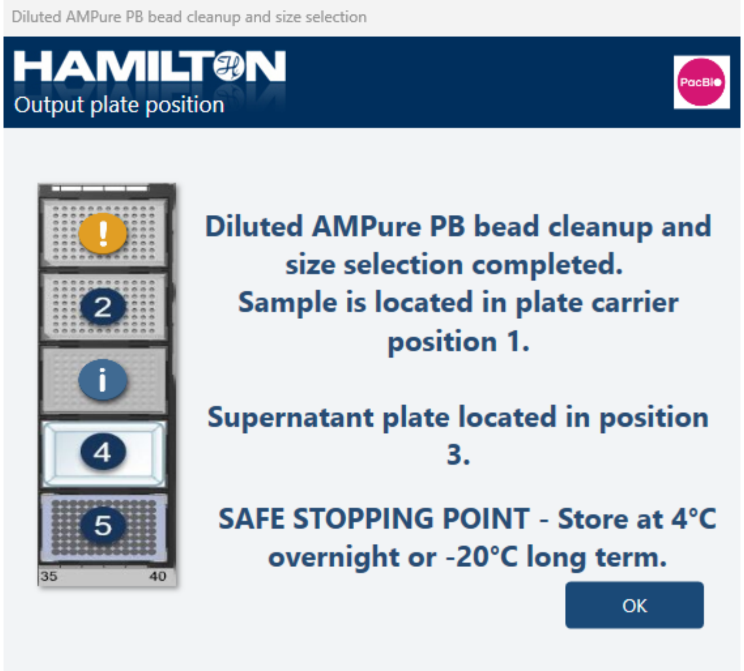
Please Review Selections:

User ID:

Number of Samples: 96

If this is correct, click "CONTINUE" to begin the run. Otherwise, click "CANCEL".

12. **3.1X diluted Ampure PB cleanup complete.** After the 3.1X diluted Ampure PB cleanup completes, the sample output plate is in position 1 on the plate carrier. The supernatant plate is located at position 3 and can be disposed of once it is confirmed that the expected recovery is present in the sample output plate.



Diluted AMPure PB bead cleanup and size selection completed.
Sample is located in plate carrier position 1.

Supernatant plate located in position 3.

SAFE STOPPING POINT - Store at 4°C overnight or -20°C long term.

13. **Method complete.** Empty the tip waste, check the liquid waste container, and remove and store reagents and processing plates. Select “No” to close the run software. Select “Yes” to have the instrument re-rack tips.

Method Completed. Please perform the following actions:

1. Check/empty the tip waste
2. Check/empty the liquid waste container
3. Remove and store reagents and processing plates

Do you want to defragment the tip racks?

Appendix

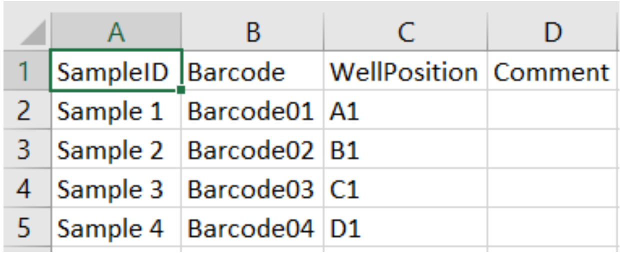
Input file:

Users may enter a `.csv` file containing sample tracking information. An example file can be found in the installation path:

`C:\Program Files (x86)\HAMILTON\NGSStar\PacBio\PureTarget\Files\Example Worklists`

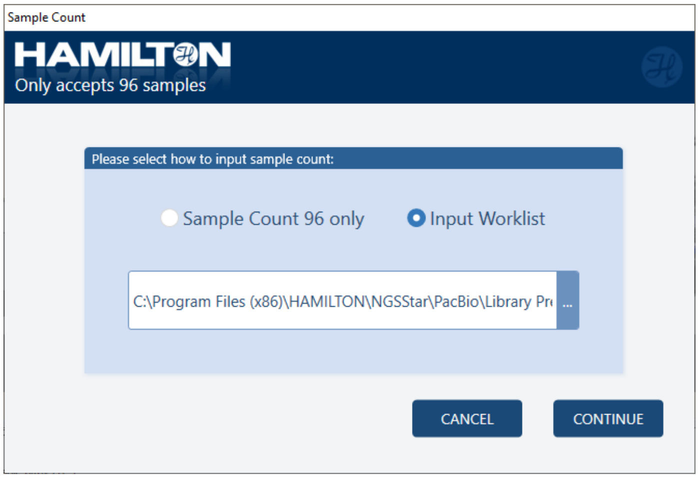
Download the example file, edit as needed, and save the updated `.csv` to a known location.

Note: When editing the file, ensure the sample count is **96**.



	A	B	C	D
1	SampleID	Barcode	WellPosition	Comment
2	Sample 1	Barcode01	A1	
3	Sample 2	Barcode02	B1	
4	Sample 3	Barcode03	C1	
5	Sample 4	Barcode04	D1	

When sample count is prompted at start-up, navigate to edited file with updated sample information and select.



Sample Count

HAMILTON

Only accepts 96 samples

Please select how to input sample count:

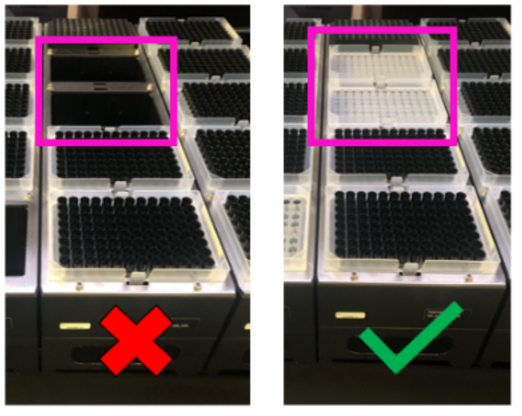
○ Sample Count 96 only ● Input Worklist

C:\Program Files (x86)\HAMILTON\NGSStar\PacBio\Library Pr

Empty tip racks: If leaving a 96-tip position empty, place only the tip wafer in the tip carrier to prevent possible instrument crashes or incomplete liquid transfers with the Multiprobe Head (MPH).

The examples below illustrate correct and incorrect empty tip rack setups:

- ❌ *What not to do*
- ✅ *What to do*



Sample location:

DNA location for each start and stop module selection is shown in the chart below.

Sample location	**Safe Start**	**Safe stop**	**Input plate location & input volume**	**Output plate location & output volume**
3.1X diluted Ampure PB cleanup	x	x	Plate carrier position 1 (A1–H12), 45 µL	Plate carrier position 1 (A1–H12), 30 µL

Revision history (description)	**Version**	**Date**
Initial release	01	February 2026