PacBio

Short Read Eliminator (SRE), DNA shearing, and cleanup for the Hamilton Microlab Prep system

Guide & overview

103-424-100 REV01 MAR2024

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Introduction

This Guide & overview describes the sample, reagent, and consumable preparation for Short Read Eliminator (SRE), DNA shearing, and post-shear cleanup on the Hamilton Microlab Prep (MLP) Liquid Handling system. This Guide & overview is meant to be used with the HiFi prep kit 96, HiFi plex prep kit 96, or SMRTbell® prep kit 3.0 library protocols.

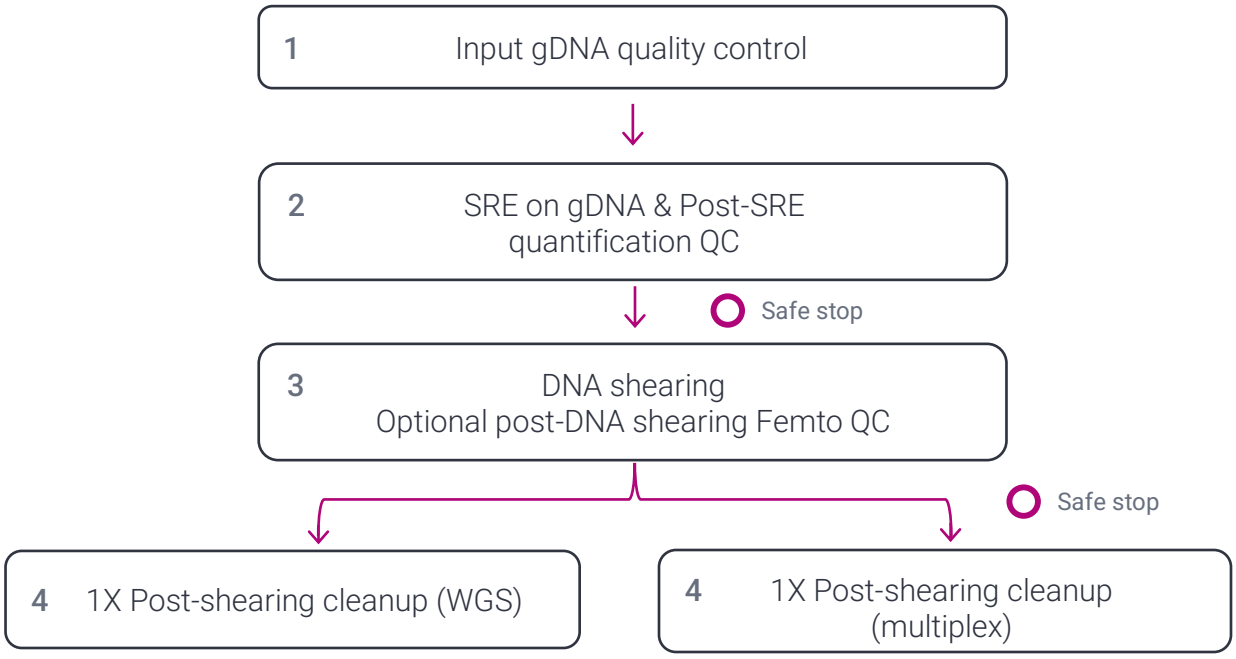
Overview

Overview	
Samples per run recommended	8, 16, 24

24-sample workflow	Prep time off-deck	Instrument run time	Total time per workflow	Total Time
SRE	10 minutes	3 hours	3 hours 10 minutes	
DNA Shearing	0 minutes	22 minutes	22 minutes	5 hours
Post-shear Cleanup	20 minutes	1 hour 10 minutes	1 hour 30 minutes	

8-sample workflow	Prep time off-deck	Instrument run time	Total time per workflow	Total Time
SRE	10 minutes	2 hours 40 minutes	2 hours 50 minutes	
DNA Shearing	0 minutes	7 minutes	7 minutes	4 hours
Post-shear Cleanup	20 minutes	40 minutes	1 hour	

Workflow overview



Required materials and equipment

DNA sizing QC	
Femto Pulse system	Agilent Technologies, Inc. M5330AA
Femto Pulse gDNA 165kb analysis kit	Agilent Technologies, Inc. FP-1002-0275
DNA quantitation QC (one or more of the following may be used)	
Qubit 4 fluorometer	ThermoFisher Scientific, Q33238
Qubit Flex Fluorometer	ThermoFisher Scientific, Q33327
Qubit 1X dsDNA HS assay kit	ThermoFisher Scientific, Q33230
Centrifugation	
Plate Centrifuge with 2250 *g* force capability	Any major lab supplier (MLS)
MicroAmp Clear Adhesive Film	ThermoFisher Scientific 00146104
Incubation	
ALPS 50 V-Manual Heat Sealer	Thermo Scientific, AB-1443A
Easy Pierce Heat Sealing Foil	Thermo Scientifc, AB-0757
C1000 Touch Thermal Cycler with 96-Well Reaction Module[1]	Bio-Rad, 184-1100
Reagents	
SRE	PacBio®, 102-208-300 (24 rxns) PacBio®, 103-124-500 (96 rxns)
SMRTbell® cleanup beads	PacBio®, 103-306-300, or included in library prep kit
Buffer LTE (low TE buffer)	Included in all library prep and bead kits
200 Proof ethanol, molecular biology or ACS grade	Any major lab supplier (MLS)
Nuclease-free water, molecular biology grade	Any MLS
Hard Shell 96 PCR full skirt 200 µL plate	Bio-Rad HSP9601
Abgene 96 Well 0.8 mL Polypropylene Deepwell Plate	ThermoFisher Scientific, AB0859
300 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235903
1000 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235905
60mL Reagent Trough (Black)	Hamilton, 56694-03
Alpaqua Magnum FLX magnet	Alpaqua, ALPQ-0008

Instrument	
Hamilton Microlab Prep - PacBio® configuration 8 channels + 2 independent channels 7 position deck Hamilton 5 × 60 mL reagent reservoir rack HHS 2 tip stands	PacBio®, 103-283-600

[1]Any off-deck heater with 96-well plate adapter block

Before you begin

Genomic DNA (gDNA) QC and input amount recommendations

Please see the respective HiFi prep kit 96, HiFi plex prep kit 96, or SMRTbell prep kit 3.0 protocols for genomic DNA quality and input recommendations.

Note: The maximum mass supported per well for shearing is 3 µg (10 ng/µL in 300 µL of low TE buffer/Buffer LTE).

Reagent handling

Room temperature is defined as any temperature in the range of 18–25°C for this protocol.

Bring SMRTbell cleanup beads to room temperature for 30 minutes (10 mL) – 1.5 hours/overnight (HT kits) prior to use. Vortex SMRTbell cleanup beads immediately before adding to the reagent trough.

Bring Elution Buffer to room temperature for 30 minutes prior to use.

Buffer SRE and buffer LTE should be at room temperature. Quick spin buffer SRE tube in microcentrifuge to collect liquid at bottom prior to use.

Instrument

Microlab Prep software version 3.0.4 or higher is required. See Appendix for how to check software version.

See Appendix for method and custom liquid class installation.

Safety precautions

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

Workflow steps

1. Short Read Eliminator (SRE)

Short read eliminator (SRE) will progressively deplete fragments up to 25 kb from genomic DNA samples. This means that depletion will decrease as fragment sizes approach 25 kb. The depletion occurs in an unbiased manner and results in improved sample quality for HiFi sequencing with most fragments under 10 kb removed.

Important: Use SRE on genomic DNA only. Attempting to use SRE on HiFi libraries (post library construction) will result in poor recoveries and potential loss of the entire library.

1. **Prepare the sample plate for SRE.**
   Bring DNA samples to a concentration of 40 to 100 ng/µL in a total volume of 50 µL using Buffer LTE. The DNA input requirement into shearing is ≤3 µg so gDNA input into SRE will depend on expected recovery. Manually pipette exactly 50 µL of gDNA sample into a 96-well full-skirted PCR 200 µL plate (PN: HSP9601) starting with position A1. Proceed to fill the plate by column (see Figure 1). Seal with an adhesive film and spin down the plate to collect liquid at the bottom of the wells and to remove any bubbles present.

Note: The MLP runs in mutliples of 8. If your sample count is not in multiples of 8, fill the remaining wells in the column with 50 µL of blanks (Buffer LTE or water).

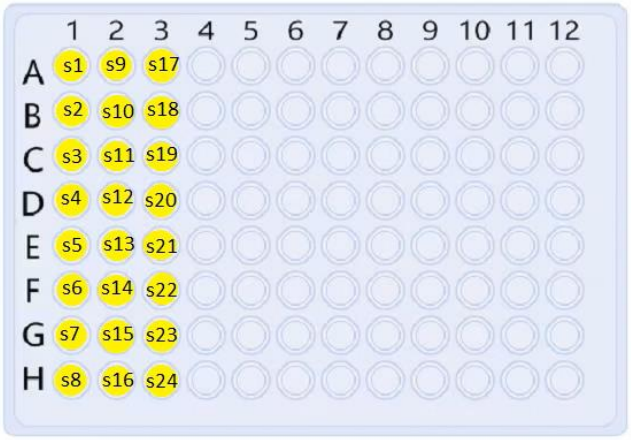
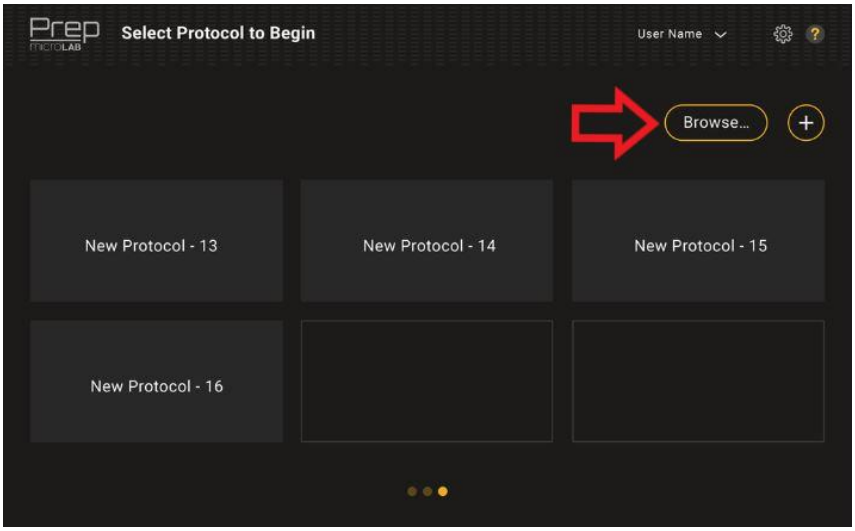


Figure 1. Sample input plate example for SRE for 24 samples (s1–s24). Prepare in a 96-well PCR 200 µL plate (PN: HSP9601).

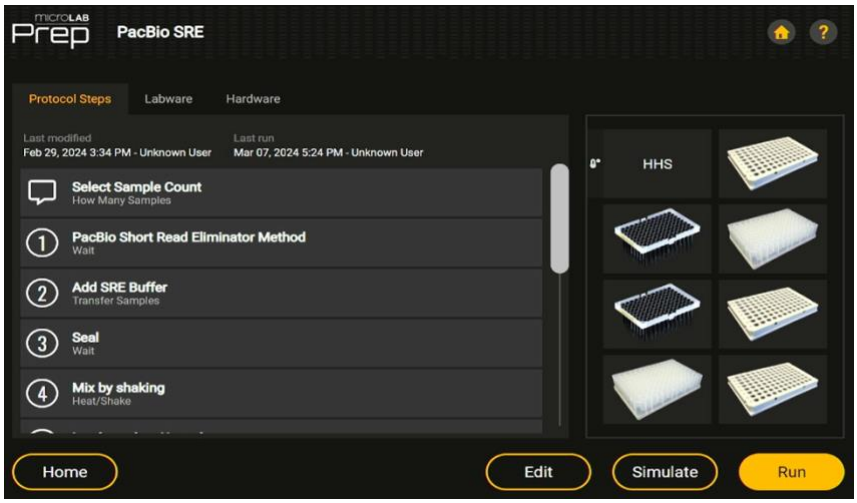
2. **Prepare the reagent plates for the SRE method.**
   - Manually pipette 55 µL of Buffer SRE into a 96-well full-skirted PCR 200 µL plate (PN: HSP9601) in well positions aligning with your sample plate. Seal with an adhesive seal and spin down the plate in a centrifuge to collect liquid at the bottom of the well and remove any bubbles present.
   - Manually pipette 310 µL of Buffer LTE into a 96-well deepwell plate (PN: AB0859) in well positions aligning with your sample plate. Seal with an adhesive film and spin down the plate in a centrifuge to collect liquid at the bottom and remove any bubbles present.

3. **Load the deck and begin the SRE method.**

   On the Hamilton Microlab Prep home touch screen, select the “PacBio SRE” method. If it is not a favorite on the home screen, tap “Browse” and search for “PacBio SRE.”

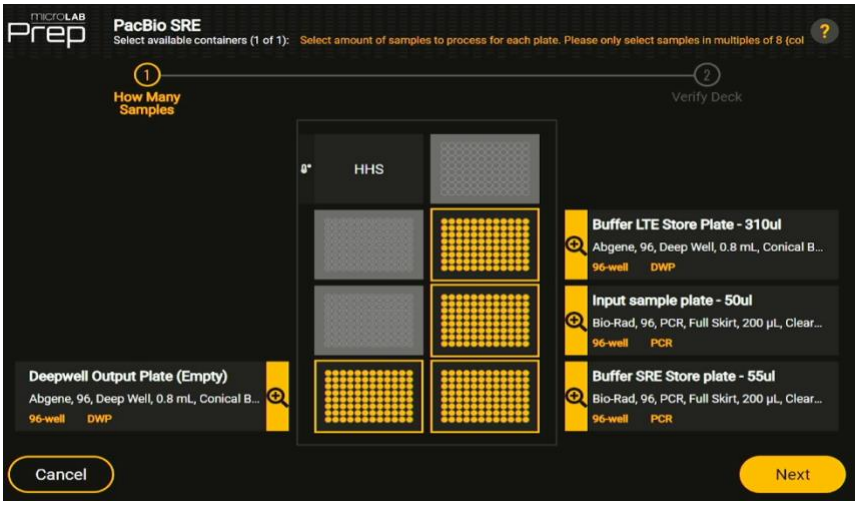


   Select “Run” at the bottom right-hand corner to continue.

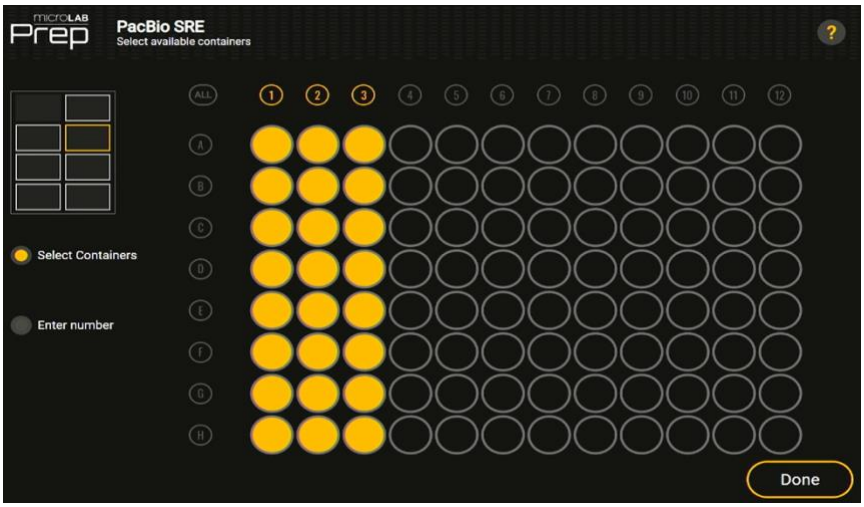


4. **Select the number of samples to process for each plate.**

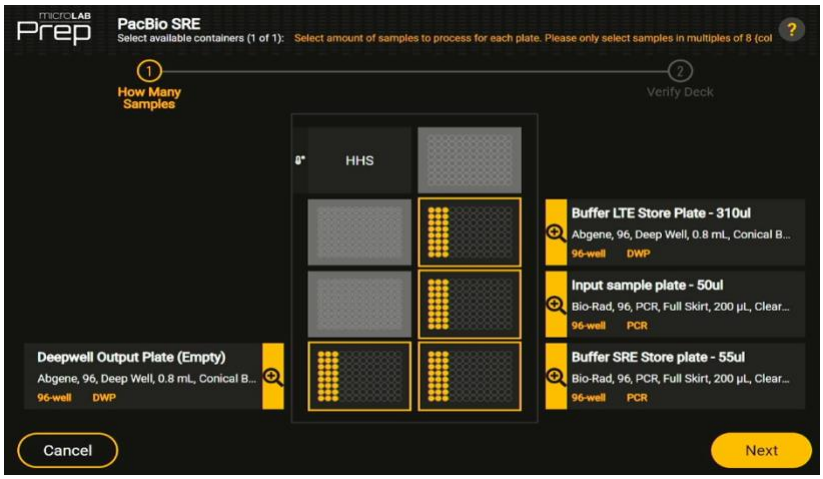
   This must be done for every plate on the deck. To select the sample positions on each plate, tap on the magnifying glass next to the plate to open the selection menu.



Select the appropriate sample wells by tapping, or touch and dragging in multiples of 8. Tap “Done” after sample count is selected for each plate.



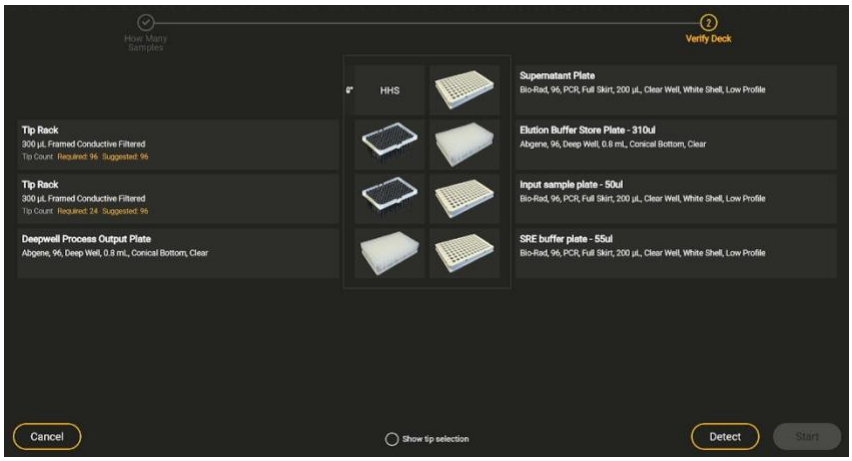
Select “Next” to continue.



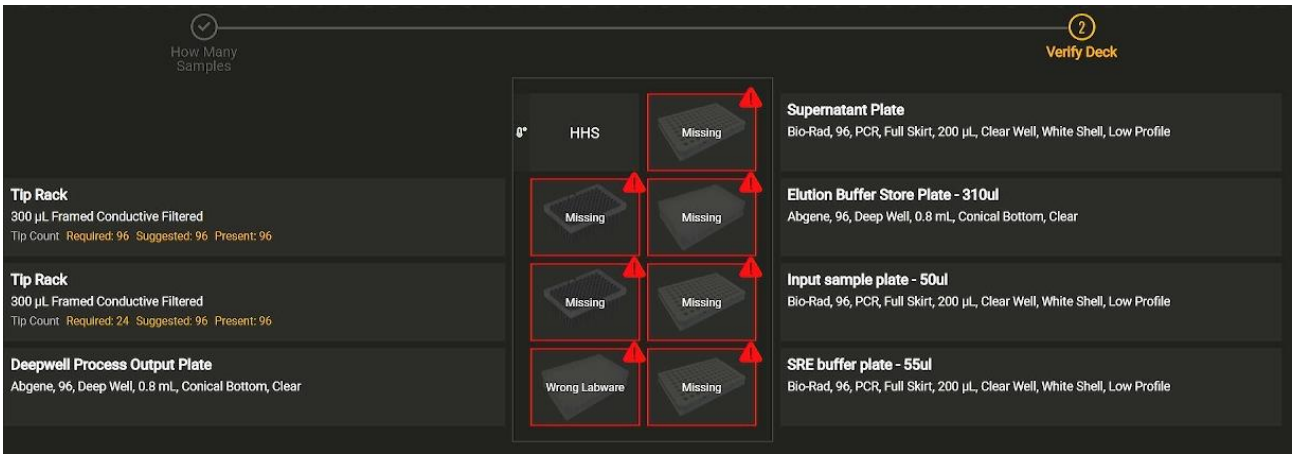
5. Place the consumambles on the deck as indicated in the figure below. Unseal all plates after spinning down.
   - Position 2: Tip stand & 300 µL tips
   - Position 3: Tip stand & 300 µL tips
   - Position 4: Abgene 96 Deepwell plate 0.8 mL (Empty)
   - Position 5: 96 well PCR full skirt 200 µL plate (Empty)
   - Position 6: Abgene 96 Deepwell plate 0.8 mL (310 µL of Buffer LTE)
   - Position 7: 96 well PCR full skirt 200 µL plate (50 µL of Sample)
   - Position 8: 96 well PCR full skirt 200 µL plate (55 µL of Buffer SRE)

6. **Verify the deck.**

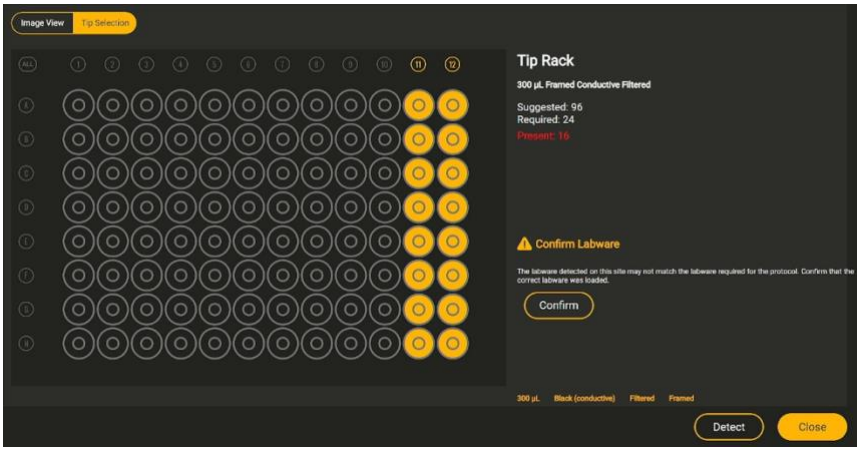
The deck with the required labware will display. Tap “Detect”. When “detect” is selected, the Microlab Prep captures a photo of the current deck.



If a “Missing”, “Wrong Labware”, or “Confirm Labware” warning appears, tap on it to review the required labware for the position.

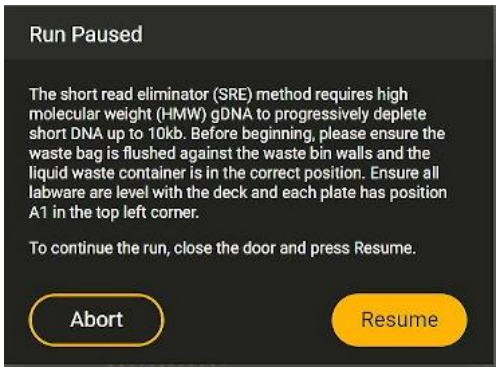


Tap “Confirm” when the highlighted positions match the tips in the rack on-deck or when the plate is present.

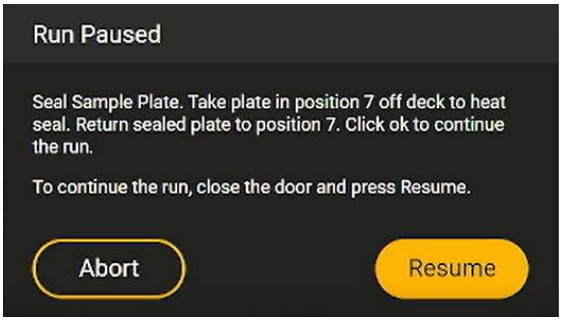


7. Resolve all deck verification errors to proceed. Tap “Start” to begin the SRE run.

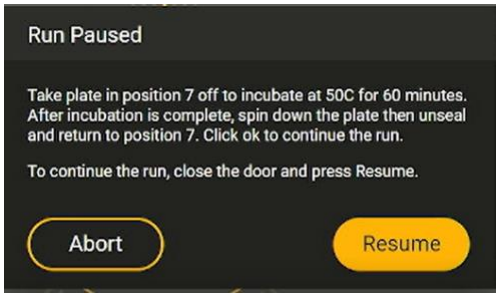
8. A prompt displays a description of the SRE method and guidance on MLP. Tap “Resume” to continue. At this point, the automation will begin. Buffer SRE will be added to the sample plate.



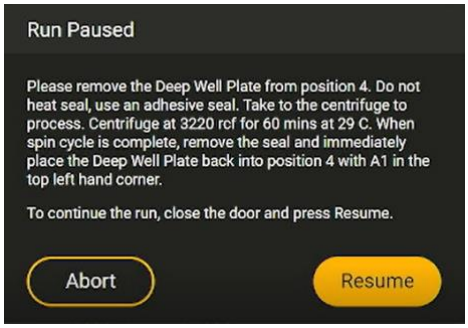
9. **Seal the sample plate.** When prompted, remove the sample plate in position 7 to foil heat seal. Return the sealed plate to position 7 and tap “Resume” to continue. The sample plate will shake to mix on deck.



10. **Incubate at 50°C for 1 hour.** When prompted, take the sealed sample plate off of postion 7 to incubate off-deck. When the incubation is complete, spin down, carefully unseal the foil seal and return the sample plate to position 7. Tap “Resume” to continue. The sample will be transferred to a deep well plate for centrifugation.

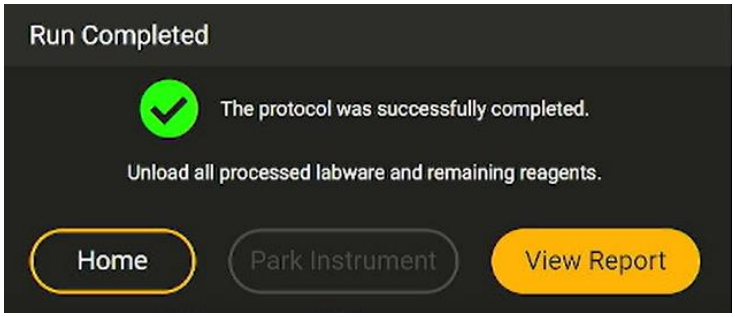


11. **Centrifugation.** When prompted, remove the deepwell plate containing the samples in position 4. Seal the plate with an adhesive seal. Centrifuge at 2250 rcf (max 3220 rcf) for 1 hour at room temperature. If using a centrifuge with temperature control (i.e., cooling function), turn this function off by specifying a target temperature set point higher than ambient room temperature (e.g., 29°C). Once the spin cycle is complete, unseal and immediately place the deepwell plate back into position 4. Tap “Resume” to continue.



12. **Method completion and QC.** Once the SRE protocol is complete, remove the deepwell plate in position 4 to quantify the samples using the Qubit 1x dsDNA HS assay. Seal the supernatant plate in position 5 and discard all used consumables and tip waste. The supernatant plate can be disposed of once it is confirmed that sample loss did not occur. If recovery is lower than 50%, vortex your sample plate and re-quant. If it is still below 50%

recovery, refer to the troubleshooting section in the Appendix. Continue to DNA Shearing or store samples at 4°C for up to 2 weeks.



2. DNA Shearing

Important:

A mean fragment size between 15 to 20 kb is recommended for this protocol. In addition, the distribution of fragment sizes should be narrow and generally between 10 to 30 kb. Fragments that are too short produce less yield per read, and fragments that are too long may result in lower read accuracy and are less likely to produce HiFi reads. Deviating from the concentration and automation settings is not recommended and will result in undersheared DNA.

These shearing parameters are not universal and are specific for only the Hamilton Microlab Prep system, or assay-ready workstations like the NGS STAR, STARlet, and STAR V systems

1. Input DNA quality control

Adjust the DNA concentration to ≤10 ng/µL if necessary (e.g., if more than 3 µg of gDNA was recovered from SRE) or if bypassing SRE. Use Buffer LTE to dilute simples in a 96-well deepwell plate (PN: AB0859). Ensure all samples are 300 µL and ≤10 ng/µL per sample. Using a DNA concentration >10 ng/µL or a volume greater or less than 300 µL will result in inefficient shearing. Parameters for shearing on the Microlab Prep, or Hamilton assay-ready workstations are listed in the table below.

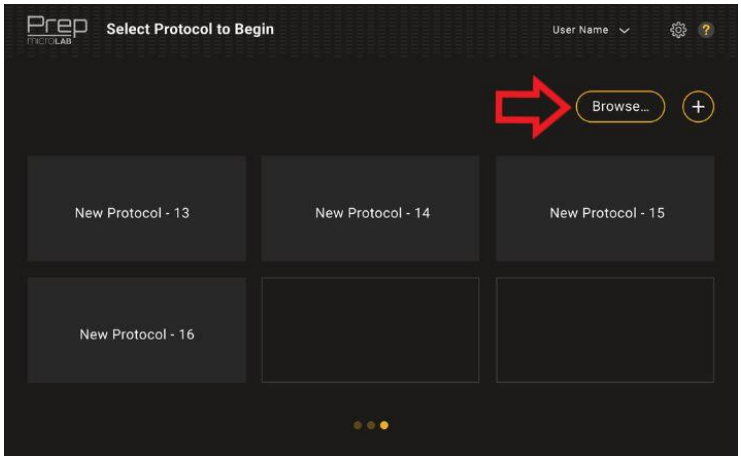
Table 1: The pipette tip shearing parameters are part of the custom liquid class that is imported with the DNA shearing method.

Parameter	Setting
DNA concentration	≤ 10 ng/µL
Volume of Buffer LTE	300 µL
Number of mixes	300 cycles
Pipette mixing speed	500 µL/sec
Mix volume	83% volume
Liquid following, cLLD	On
Pipette tip	300 CO-RE II tips (filtered, conductive)

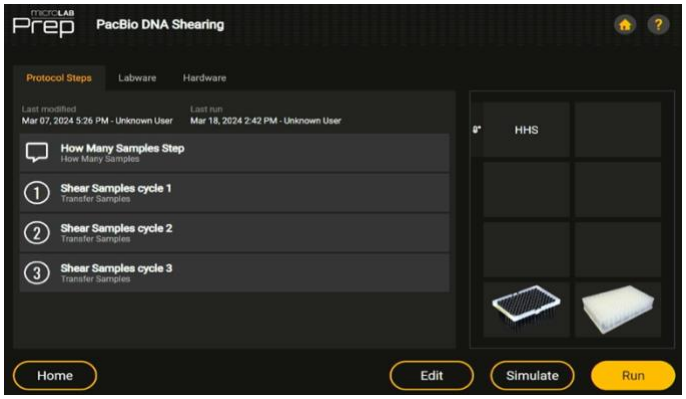
2. Prepare sample plate for shearing

If SRE has been run, the samples are in the output 96-well deepwell plate and this step can be skipped.
If beginning at this method, pipette 300 µL of sample in the 96-well deepwell plate (PN: AB0859) starting with position A1. Proceed to fill the plate with samples by column. Seal with an adhesive seal, vortex and spin down the plate to collect liquid at the bottom and to remove any bubbles present. Remove the seal when ready to place on-deck.

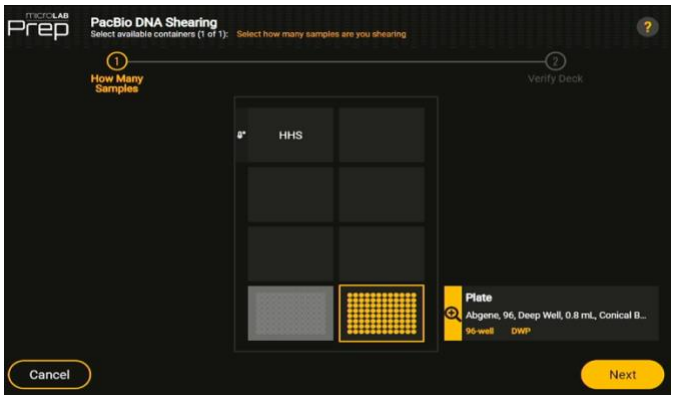
3. **Load the deck and begin shearing method**
When at the Microlab Prep home touch screen, select the “PacBio DNA Shearing” method on the MLP. If it is not a favorite on the home screen, tap “Browse” then search for “PacBio DNA Shearing.



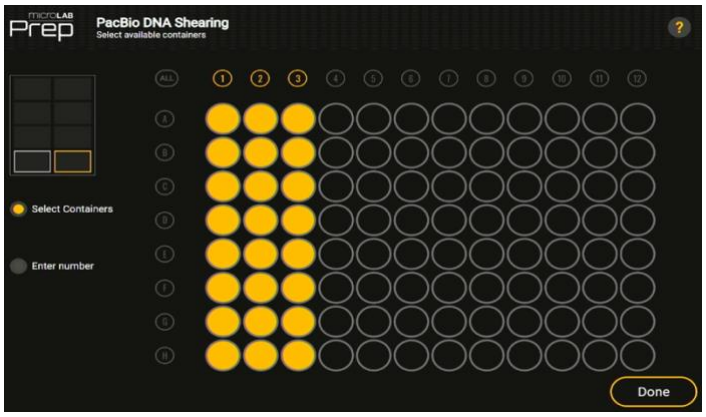
Tap “Run” at the bottom right-hand corner to continue.



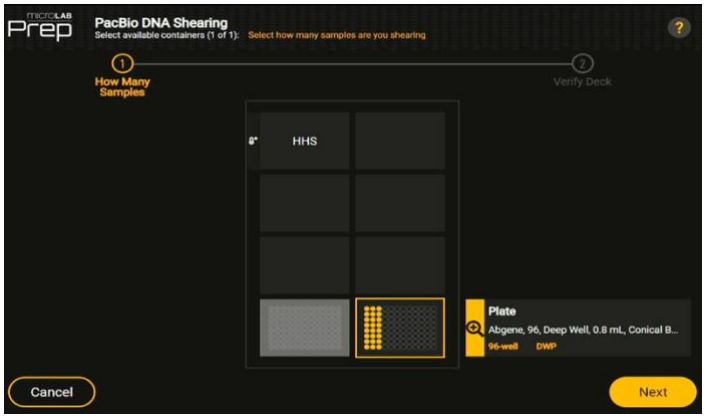
Select your wells by tapping on the magnifying glass icon and highlighting the wells to match the input sample plate.



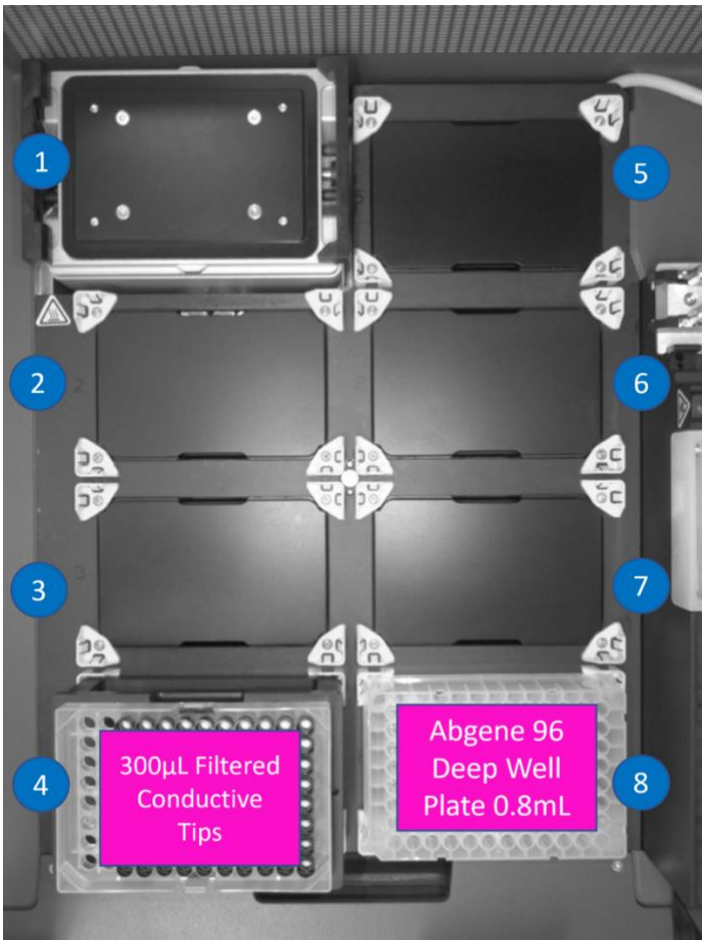
Select the appropriate sample wells by holding down and dragging in multiples of 8. Tap “Done” after the appropriate sample count is selected.



Select “Next” to continue.



4. **Load the deck and begin the DNA shearing method.**

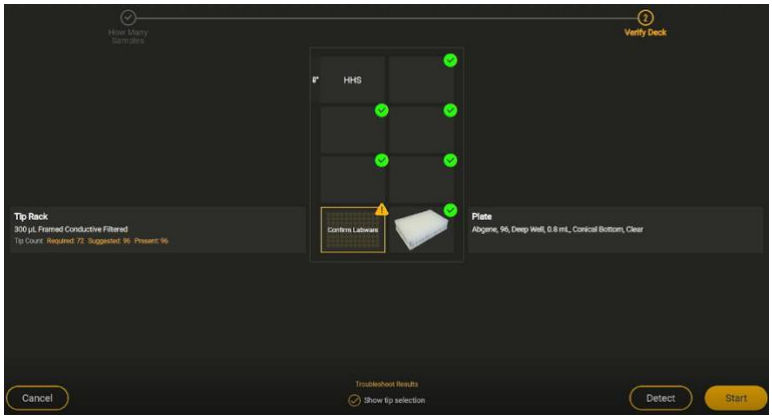


Place the consumables on deck as indicated in the figure below. Position 4 should contain a tip stand and 300 µL tips. Position 8 should contain a 96-well deepwell plate (PN: AB0859) with samples (300 µL at 10ng/µL)

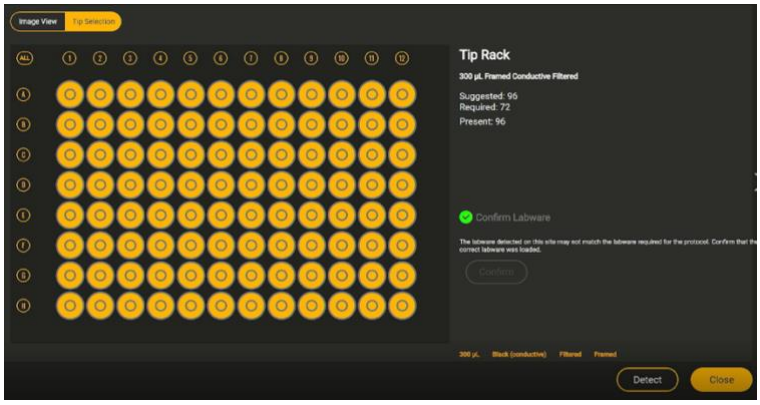
5. **Verify the deck.**

The deck with the required labware will be displayed. When “detect” is selected, the Microlab Prep captures a photo of the current deck.

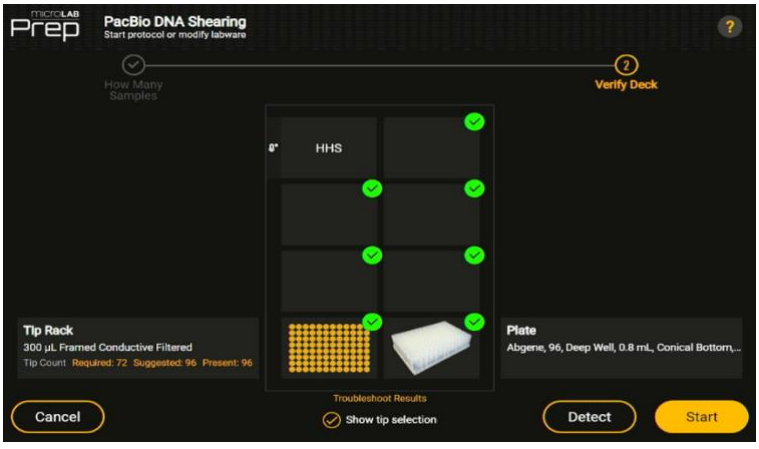
If “Missing”, “wrong labware”, or “Confirm Labware” warning appears, tap on it to review the required labware for the position. Confirm if the labware is correct.



Confirm if the labware is correct.

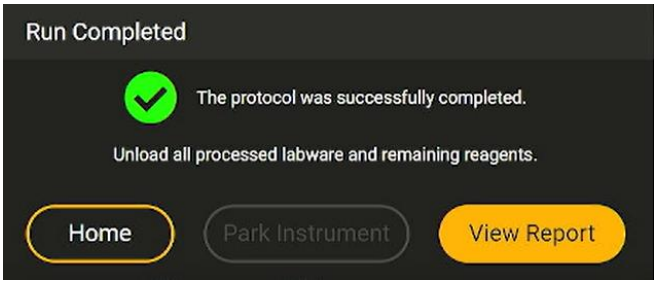


Resolve all deck verification errors to proceed. Tap “Start” to begin DNA Shearing.



6. **Method complete.**

   At the end of DNA Shearing, remove the 96-well deep well (PN: AB0859) output plate in position 8. Perform optional QC using the Femto Pulse to check size distribution (range of 15–20 kb). If your samples did not shear to the expected range, see Appendix. Continue to the PacBio Post-shearing Cleanup process.



3: 1X Post-shear cleanup

1. Prepare sample plate for post-shear cleanup

   If the DNA shearing method has been run, the samples are in the 96-well deepwell output plate and this step can be skipped.
   If beginning here, manually pipette 300 µL of sample diluted with Buffer LTE into a 96-well deepwell plate (PN: AB0859) targeting ≤3 µg input. Start at position A1 then proceed to fill the plate with samples in a column-based down direction.
   Seal, vortex then spin down the plate to collect liquid at the bottom and to remove any bubbles present. Remove the seal when ready to place on-deck.

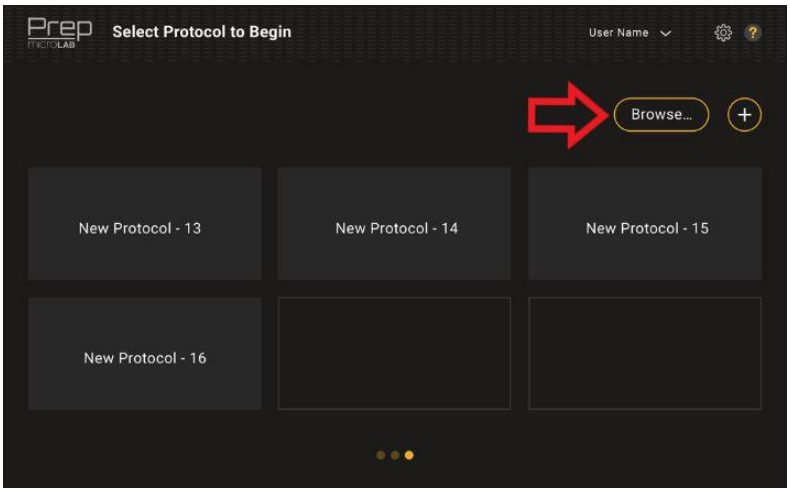
2. Load deck and begin 1X Post-shear cleanup method

   There are two 1X Post-shear cleanup methods to chose depending on the library preparation protocol performing. Reference the table below for guidance on which 1X Post-shear cleanup you will select to run.
   Note: To determine the post-shear cleanup method to use, please see companion documentation.

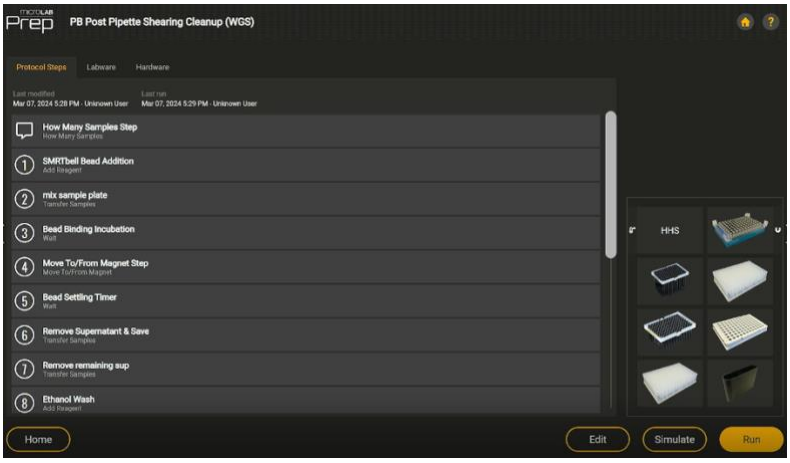
   Table 2: PacBio 1X post-shear cleanup protcols with their associated library prep protocols. The difference between the post-shear methods is the eluate volume.

Library preparation protocol	1X post-shear cleanup protocol	Eluate volume (µL)
HiFi prep (WGS)	1X post-shear cleanup (WGS)	50
HiFi plex prep (mulitplex)	1X post-shear cleanup (multiplex)	25.5

When at the home touch screen, select one of the PacBio 1X post-shear cleanup methods on the MLP. If it is not a favorite on the home screen, tap “Browse” then search for 1X post-shear cleanup method you’re selecting to run.



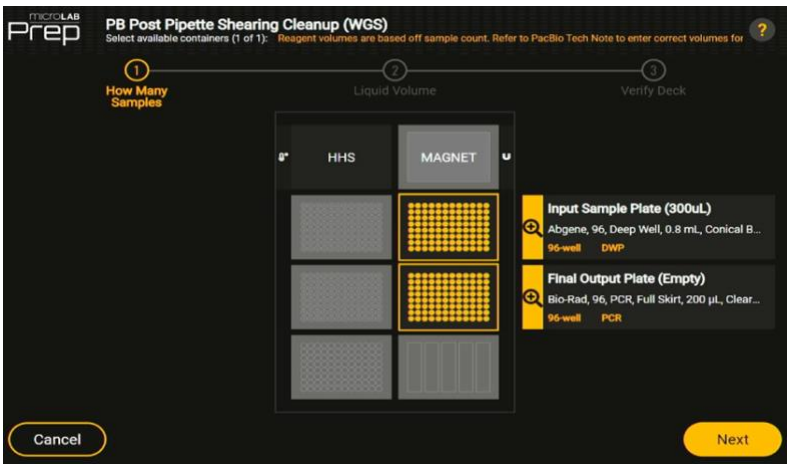
Select “Run” at the bottom right-hand corner to continue.



3. Select sample count for each plate in post-shear cleanup

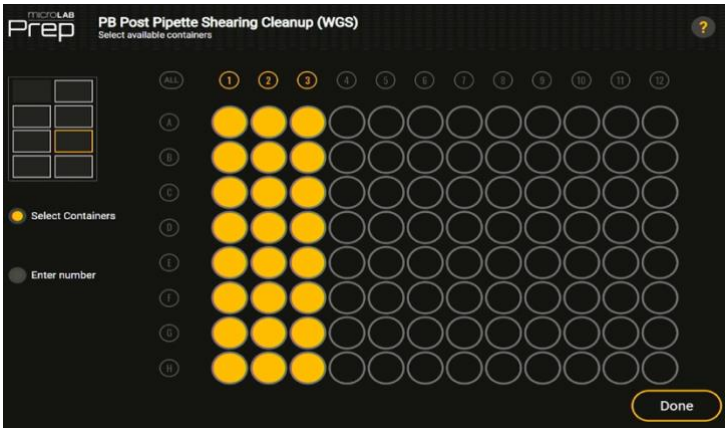
   Select your wells by tapping the magnifiying glass, then highlight the wells to match your sample count.

   - Position 6: 96-well deepwell sample plate (300 µL)
   - Position 7: 96-well PCR plate (Empty)

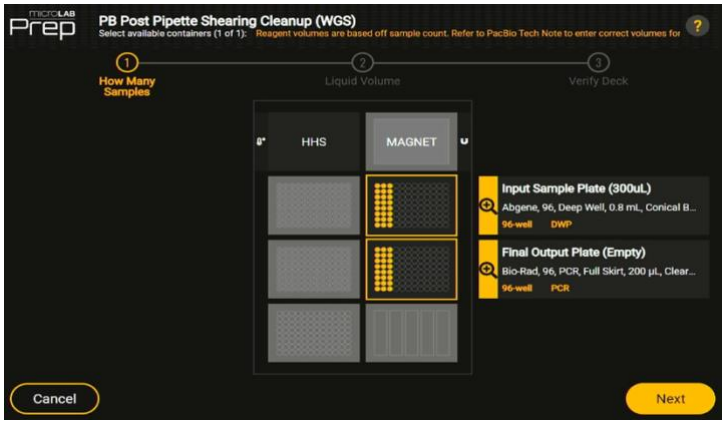


Select your wells by tapping the magnifying glass, then highlight the wells to match the input sample plate.

Select the appropriate sample wells by holding down and dragging in multiples of 8. Tap “Done” after the appropriate sample count is selected.

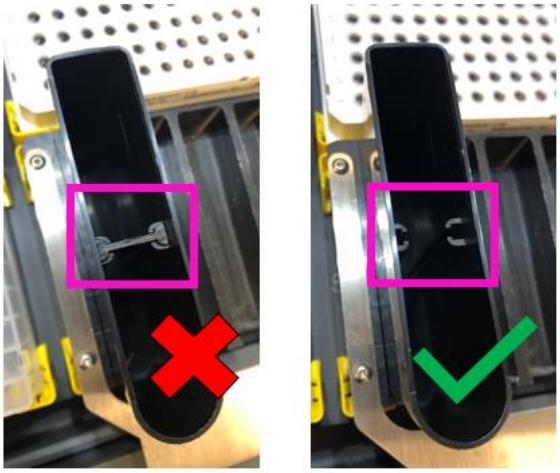


Tap “Next” to continue.



4. Enter reagent volumes for the 60 mL troughs

Note: Before loading the reagent troughs into the reservoir rack, make sure to remove the middle insert from the black 60 mL reservoir.

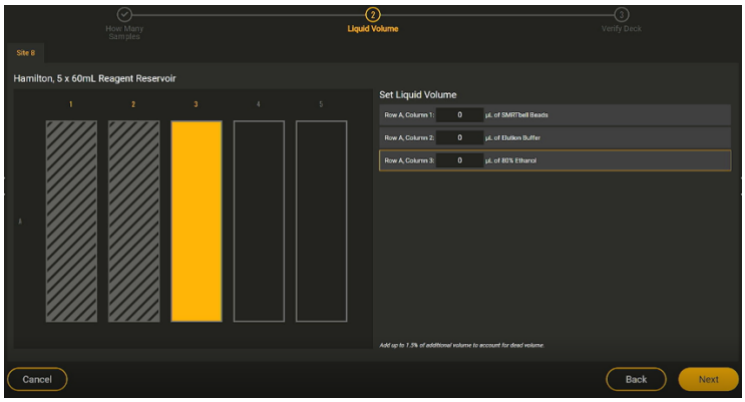


Enter the volume for SMRTbell beads, Elution buffer, and 80% ethanol required. Reference the following table to determine how much reagent volume is needed in each 60mL trough based on sample count.

Note: User must enter reagent volumes manually for every run. The volumes will automatically display the reagent volumes from the previous run, so these must be modified if the sample count is different. Select "Next" to continue.

Table 3: Reagent volumes for 1X post-shear cleanup

Number of Samples	8	16	24
SMRTbell bead volume (µL)	4,400	6,800	9,200
Elution buffer volume (µL)	2,400	2,800	3,200
80% ethanol volume (µL)	3,600	5,200	6,800



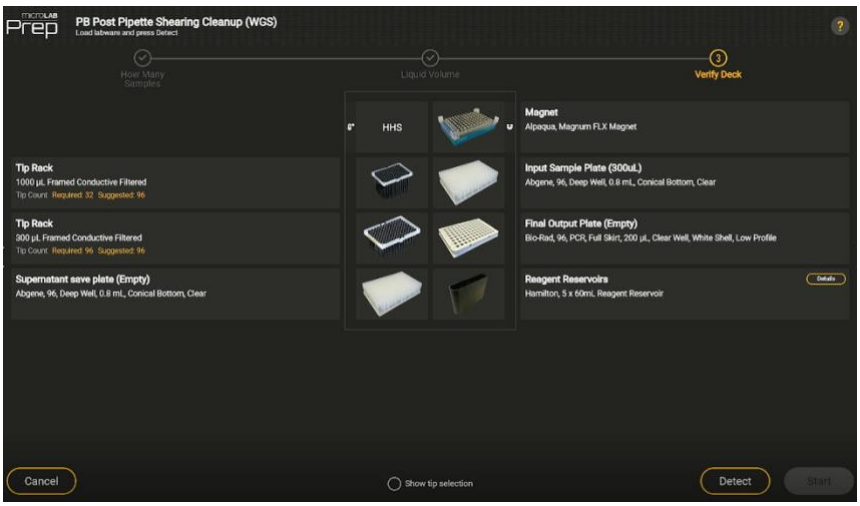
5. Load the deck and begin post-shear cleanup method.

Place the consumables on deck as indicated in the figure below. To place the 1000 µL tips on-deck, remove the base plate in position 2. Then place the tip stand in position 2. The 1000 µL tips will dip below the deck when secured in the tip stand.

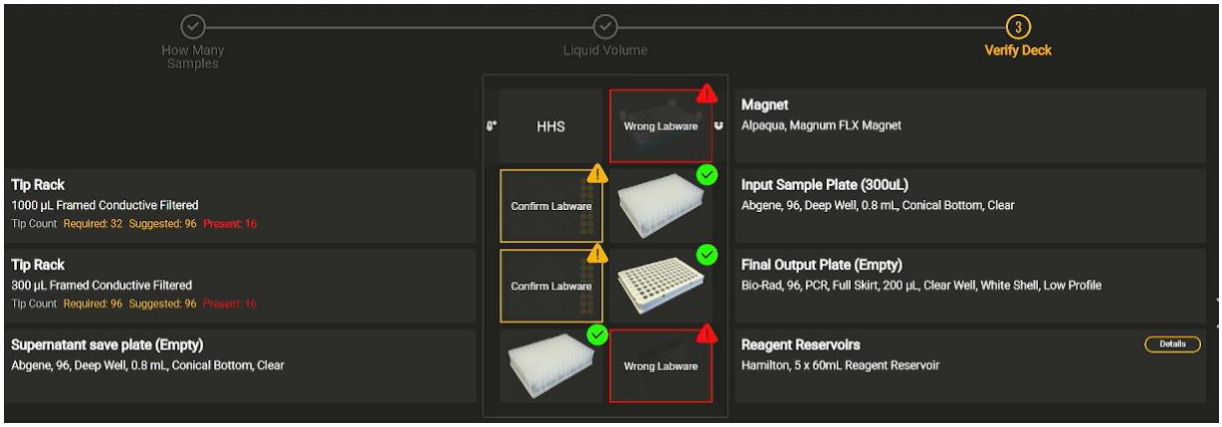
- Position 2: Tip stand & 1000 µL tips (base plate removed)
- Position 3: Tip stand & 300 µL tips
- Position 4: Abgene 96 Deepwell plate 0.8 mL (Empty)
- Position5: Alpaqua Magnum FLX magnet plate
- Position 6: Abgene 96 Deepwell plate 0.8 mL with samples (300 uL)
- Position 7: 96 well PCR full skirt 200 µL plate (Empty)
- Position 8: 60 mL Reagent Reservoir (three 60 mL troughs)

6. **Verify the deck.**

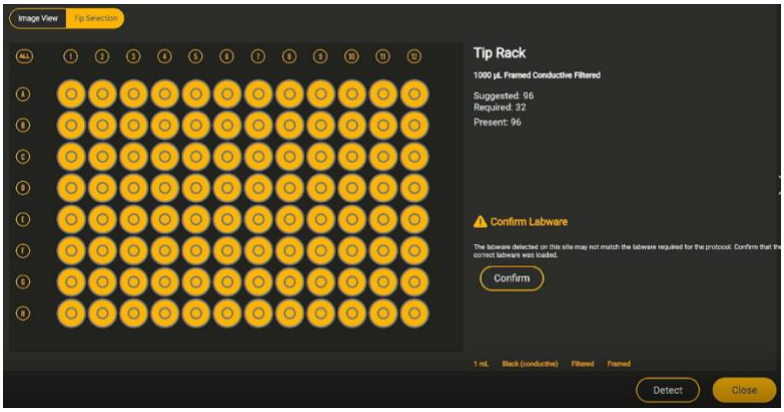
   The deck with the required labware will appear. Tap “Detect”. When “detect” is selected, the Microlab Prep captures a photo of the current deck.



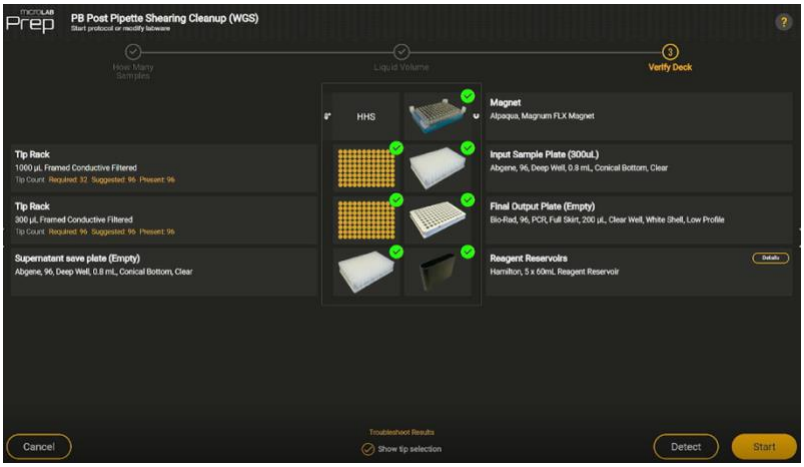
If a “Missing”, “Wrong Labware”, or “Confirm Labware” warning appears, tap on it to review the required labware for the position.



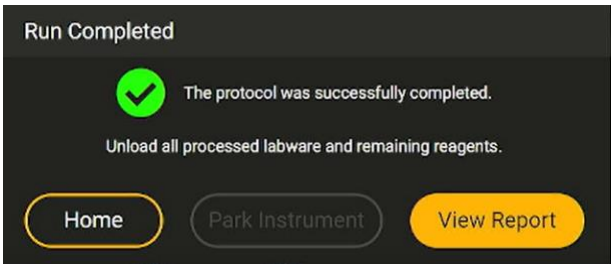
Tap “Confirm” if correct.



Resolve all deck verification errors to proceed. Then tap “Start” to begin the run.



7. **Method Complete.**
   Once complete, the samples are ready to continue with library prep. Seal and store DNA at 4°C for up to 2 weeks until ready to proceed to library preparation.



Appendix

Installation (New User)

Please contact your PacBio FAS to import Microlab Prep methods and liquid class files or visit Hamilton Microlab Prep Support for more information.

1. Plug in the USB drive with the desired files to import
   - Protocol file type: ".mlppe"
   - Custom Liquid Class file: ".mlplc"

Note: ".mlppe" is a protocol file ".mlplc" is a liquid class file

2. Tap "Browse" to open the File Manager
3. In the Protocol tab, select the protocol and tap the three-line menu  (located next to the search bar), then select "Import"
4. In the Liquid tab, scroll to find the liquid class and select the three -line menu, (located next to the search bar), then select "Import"

Troubleshooting

Issue	Possible Cause	Corrective Action
Installation issues	Software not up to date	From the home page, tap the settings button that looks like a gear. In the Software tab, tap "Configuration", then open the "Version" tab. The version will be associated with the Microlab Prep Core Installer.
Instrument error or insufficient volume	1. Not enough volume in the consumable	1. Fill the consumable to the recommended amount specified in the loading dialog
	2. No blanks were inserted into the remaining wells of the last column. Liquid level detection is used.	2. Insert a blank of water or TE buffer into the wells to fill out the column
Low to no recovery at the end of SRE run	1. Not heating effectively during off-deck incubation	1. Ensure plate is heat sealed for the 50°C incubation and the plate is spun down before removing the seal. Ensure the off-deck heating temperature is at 50°C and the appropriate plate adapter is used.

	2. Input volume or SRE Buffer volume not accurate	2. Sample input volume in Buffer LTE buffer must be 50 µL per well. Buffer SRE must be 55 µL per well.
	3. Incorrect centrifuge settings	3. Ensure that plate centrifuge is set at >2250 rcf (max 3220 rcf) at 29°C or room temperature for 60 minutes.
	4. Not enough supernatant left behind	4. (a) Ensure the Prep is leaving behind 8–10 µL of supernatant after removal. It is possible the DNA pellet is aspirated in the tip during supernatant removal. Quant supernant plate to see if the DNA pellet was dispensed into it. If so, the sample can be recovered by transferring into a 1.5mL DNA Lo-Bind tube and recentrifuging as outlined in the HiFi prep kit 96 protocol steps 1.5–1.10. (b) Ensure after centrifugation the plate is not disturbed and immediately placed on deck. (c) The coordinates for the channels could have adjusted: Re-calibrate your instrument. Clean the stop discs and o-rings with DI water and lint free cloth before starting and use a set of new tips when prompted to add them on the deck. • From the Home screen, tap Settings -> Instrument tab -> System Calibration -> Tap Pipetting Tools Calibration -> Run
	5. Sample lost in supernatant	5. DNA pellet aspirated in the tip during supernatant removal. Quant the supernatant plate. If sample is present in supernatant plate, the sample can be recovered by transferring ~100 µL in a 1.5 mL DNA Lo-Bind tube and recentrifuging as outlined in the HiFi prep kit 96 protocol steps 1.5–1.10.
Sample Volume discarded to waste during DNA Shearing	Custom Liquid class for shearing not imported	Ensure the custom liquid class was imported by following the installation procedure in Appendix.
DNA not shearing to expected fragment range	1. Jelly-like HMW gDNA	1. HMW gDNA sometimes contains dense clumps. Vortex sample to fully homogenize and re-QC to ensure quants are ≤10 ng/µL per well in the plate. Then

		re-shear by running the DNA Shearing protocol again.
	2. Initial quant of gDNA not accurate	2. Ensure fluorescence is used for quanting. HMW gDNA may not be homogenized. Vigorously vortex sample right before quanting. If inhomogenous, it may be necessary to take multiple quants to ensure that values are within 20%.

If experiencing mechanical pipetting errors or frequent instrument errors, reach out to Hamilton Support team via Hamilton support ticket submission: https://robotics.hamiltoncompany.com/benchtopticket

Revision history (description)	Version	Date
Initial release	01	March 2024