PacBio

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

Guide & overview

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Introduction

This Guide & overview describes the sample, reagent, and consumable preparation for Short Read Eliminator (SRE), DNA shearing, and post-shearing 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-shearing 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-shearing cleanup	20 minutes	40 minutes	1 hour	

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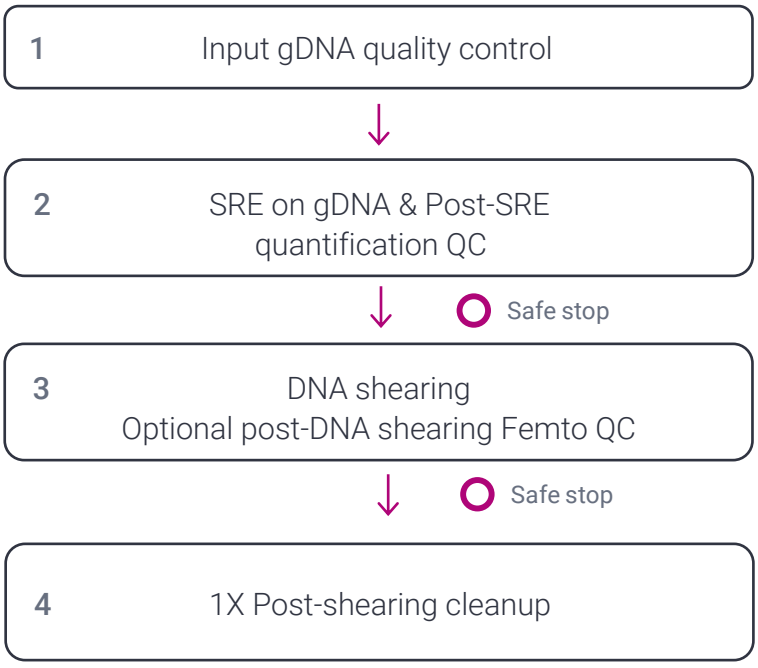
Workflow overview



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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 kit	PacBio® 102-208-300 (24 rxns) PacBio® 103-124-500 (96 rxns)
SMRTbell® cleanup beads	PacBio® 103-306-300, included in library prep kit
Buffer LTE HT	PacBio® 103-306-100, included in SRE HT kit
Elution buffer	PacBio® 101-633-500, included in library prep kit
Other supplies	
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

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1000 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235905
60mL Reagent Reservoir (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

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Before you begin

Hamilton Microlab Prep configuration

The Hamilton Microlab Prep scripts provided by PacBio are compatible with instruments configured with a multi-channel 8-Probe Head (8 MPH). The 8-Probe Head is used for pipetting steps while the two independent channels for the CO-RE paddles move labware.

Genomic DNA (gDNA) QC and input amount recommendations

PacBio Nanobind® DNA extractions kits are recommended to ensure sufficient amounts and quality of high molecular weight DNA for this protocol.

gDNA quality QC

The Agilent Femto Pulse system is recommended for the accurate sizing of gDNA. Please see the PacBio Technical note for more details.

Recommended guidelines for evaluating gDNA quality for this protocol:

- Use the Femto Pulse gDNA 165 kb analysis kit (Agilent FP-1002-0275)
- Dilute samples to 250 pg/µL
- 70% or more of the DNA should be ≥10 kb for this protocol. This corresponds to a genome quality number (GQN) of 7.0 or higher at 10 kb. If the GQN at 10 kb is less than 7.0, higher gDNA inputs may be required.
- If the majority of DNA is less than 10 kb, Short read eliminator is not recommended.
- Shearing may be bypassed if the sample is already in the appropriate size-range.

gDNA input mass

It is highly recommended to use a quantification assay specific for double stranded DNA (dsDNA) such as the Qubit 1X dsDNA high sensitivity assay kit. When a high number of samples will be prepared, we recommend using the Quant-iT 1X dsDNA high sensitivity assay kit with the Varioskan LUX multimode microplate reader. Please follow manufacturer's instructions for the assay being used.

We *do not* recommend quantification with UV-Vis Spectrophotometers (e.g., NanoDrop).

Low mass vs High mass

Using the Revio® system with SPRQ™ chemistry reduces the gDNA mass required for library preparation by reducing the SMRT® Cell loading volume. **While the "High mass" option is still available, there is now a "Low mass" option with updated parameters for SRE and DNA shearing when using 0.5–1.25 µg of gDNA.** Low mass SRE has been optimized to provide comparable recoveries to High mass SRE with the same size selection performance. Low mass shearing has been optimized to maintain the size distribution between 15–20 kb. It is recommended to stay consistent for both SRE and shearing steps (i.e. **use Low or High mass settings for *both* SRE and shearing**).

Note that High mass and Low mass samples cannot be included in a single SRE/shearing automation run. **If 0.5–1.25 µg gDNA is available and Revio SPRQ chemistry is being used, the Low mass workflow will provide enough library for a Revio SMRT Cell (+SPRQ)**; however, High mass parameters can also be used for Revio SPRQ chemistry if excess library is desired. **For Revio non-SPRQ chemistry and Vega™, 2 µg gDNA input is still recommended for loading of 1 SMRT Cell.**

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Table 1. Recommended DNA input amounts by starting gDNA quality

gDNA quality	Low mass Compatible with SPRQ	High mass	Expected SRE recovery (dependent on DNA quality)
70% >10 kb	0.5 – 1.25 µg	2–4 µg	60–95%
<70% >10 kb	1–1.75 µg	4–5 µg	40–60%
<10 kb (no SRE)	0.25–1 µg	1–3 µg	Not recommended

Starting with 500 ng and 2 µg of genomic DNA will typically provide enough library to load at least 1 Revio SMRT Cell (+SPRQ) and 1 Revio/Vega™ SMRT Cell (non-SPRQ), respectively (Table 2, Table 3).

If gDNA mass available is between 1.25 µg and 2 µg, use the "High mass" workflow; however, note that if sequencing on Vega or Revio without SPRQ, there may not be enough final library for loading at an optimal on-plate loading concentration for a single SMRT Cell.

Table 2. Polymerase-bound library mass necessary for loading on a Revio SMRT Cell.

Mean insert size	Revio +SPRQ (250 pM)	Revio/Vega (non-SPRQ) (250 pM)
15,000 bp	61 ng	243 ng
18,000 bp	73 ng	293 ng
21,000 bp	85 ng	341 ng

SRE and Post-shear 1X SMRTbell bead cleanup stepwise recoveries

The overall recovery is dependent on gDNA quality and size. See Table 3 for step recoveries from SRE and 1X SMRTbell bead post-shear cleanup.

Table 3. Expected stepwise recoveries of DNA from SRE and post-shear cleanup. Post-SRE recovery will vary with the quality of the DNA input. The better the quality of DNA, the higer the recovery post-SRE.

Protocol Step	DNA or SMRTbell step recovery	Expected size (Femto Pulse)
Starting Input	100%	$GQN_{10\ kb}$ >7.0
Post-SRE	65-95%	$GQN_{10\ kb}$ >9.3
Post-shear SMRTbell bead cleanup	80-95%*	15–20 kb

*This can vary based on extraction methods. As low as 60% step recovery has been observed.

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Reagent handling

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

SRE kit

Buffer SRE and Buffer LTE are room temperature reagents. Quick spin Buffer SRE tube in microcentrifuge to collect liquid at bottom prior to use.

SMRTbell cleanup beads

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 reservoir.

Pipette-mix all bead binding and elution steps until beads are distributed evenly in solution.

Bring Elution buffer to room temperature for 30 minutes prior to use (if stored at 4°C).

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.

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Workflow steps

1. Short Read Eliminator (SRE)

Short read elminator (SRE) will progressively deplete fragments up to 25 kb from genomic DNA samples. **SRE should not be done on gDNA samples that are <10 kb.**

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. Determine whether to use "Low mass" or "High mass" parameters**. Pipette gDNA ($GQN_{10\ kb}$ >7.0), diluted with Buffer LTE according to the table below into a 96-well PCR plate (Bio-Rad, HSP9601) starting with position A1. Proceed to fill the plate by column in a downward position as shown in Figure 1 with the exact volume specified. 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.

	Low mass	High mass
Sample volume	25 µL	50 µL
DNA concentration	20–50 ng/µL	40–80 ng/µL
Recommended max gDNA mass	1.25 µg	4 µg
Elution volume (Buffer LTE)	200 µL	300 µL
Shearing mass limit	≤1 µg	≤3 µg

Note: If working with low quality gDNA with a $GQN_{10\ kb}$ <7.0, input mass and concentration can be increased if the expected recovery (40–60%, Table 1) matches the pipette-tip shearing mass limit for each respective workflow. For example, 2 µg of gDNA can be used with the Low mass workflow if expected recovery is 40% (800 ng).

Note: The script must be run in mutliples of 8. If your sample count is not in multiples of 8, fill the remaining wells in the column with 25 µL or 50 µL of blanks (Buffer LTE or water).

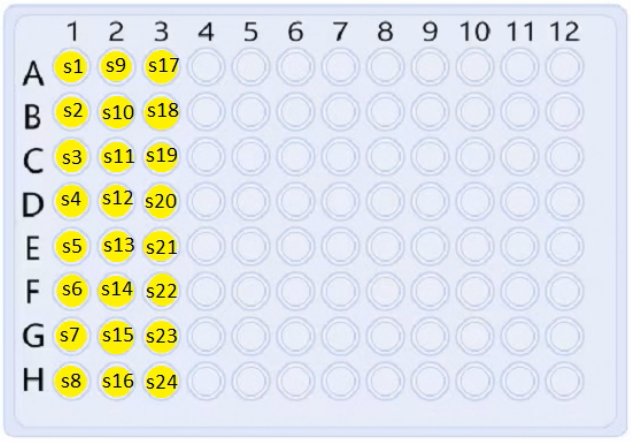

Figure 1. Sample input plate example for SRE, 24 samples (s1-s24). Prepare in 96-well PCR plate (Bio-rad, HSP9601).

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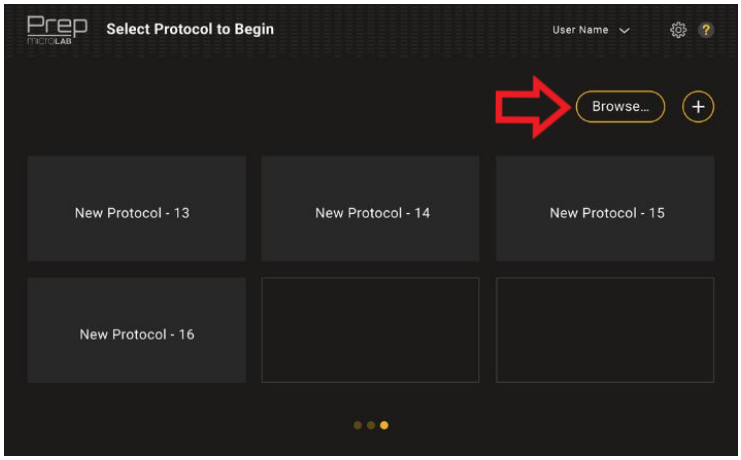
2. **Prepare the reagent plates for SRE.** Manually pipette Buffer SRE and Buffer LTE in designated plate according to the table below, starting with position A1. Proceed to fill the plate by column in a downward position as shown in Figure 1 with the exact volume specified. Seal with an adhesive seal and spin down the plates to collect liquid at the bottom of the well and remove bubbles.

Reagent	Low mass	High mass	Labware
Buffer SRE	30 µL	55 µL	96 well PCR plate (Bio-Rad, HSP9601)
Buffer LTE	210 µL	310 µL	96 deep well plate (ThermoFisher Scientific, AB0859)

3. **Select SRE protocol.** On the Hamilton Microlab Prep home touch screen, select the appropriate SRE protocol:

Parameter	Protocol Name
Low mass	PacBio SRE Low mass (25uL Input)
High mass	PacBio SRE High mass (50uL Input)



Note: If it is not a favorite on the home screen, tap “Browse” and search for SRE method.

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

Note: The prompt example is for the SRE Low mass protocol.

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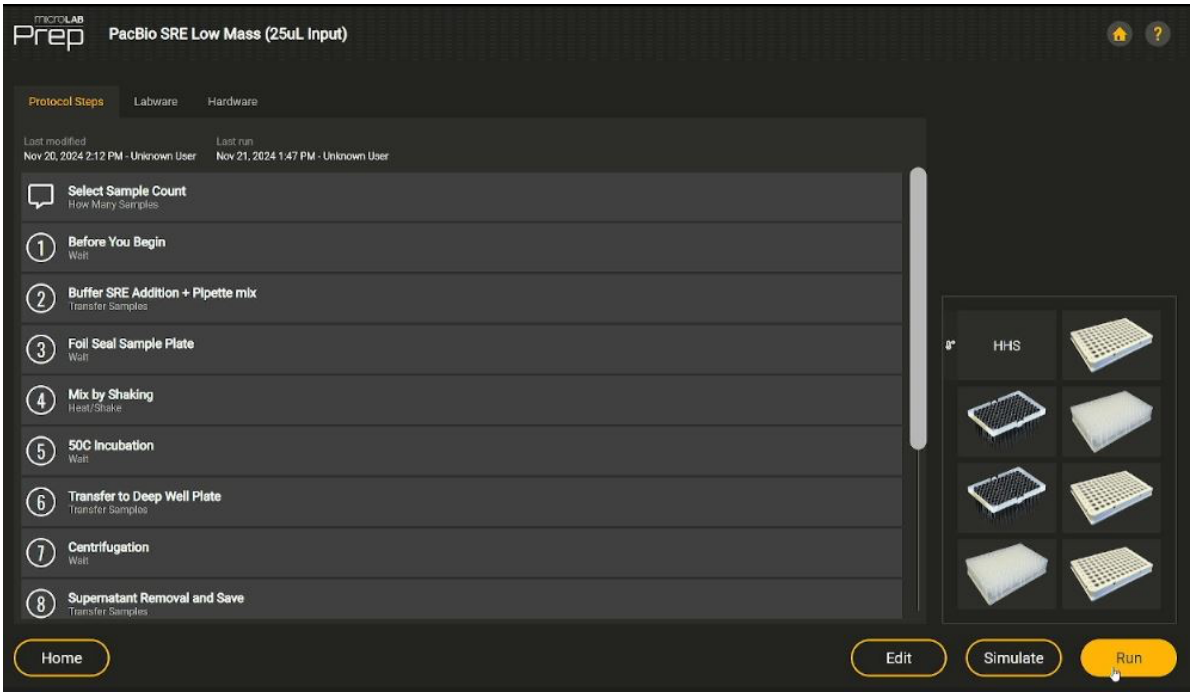
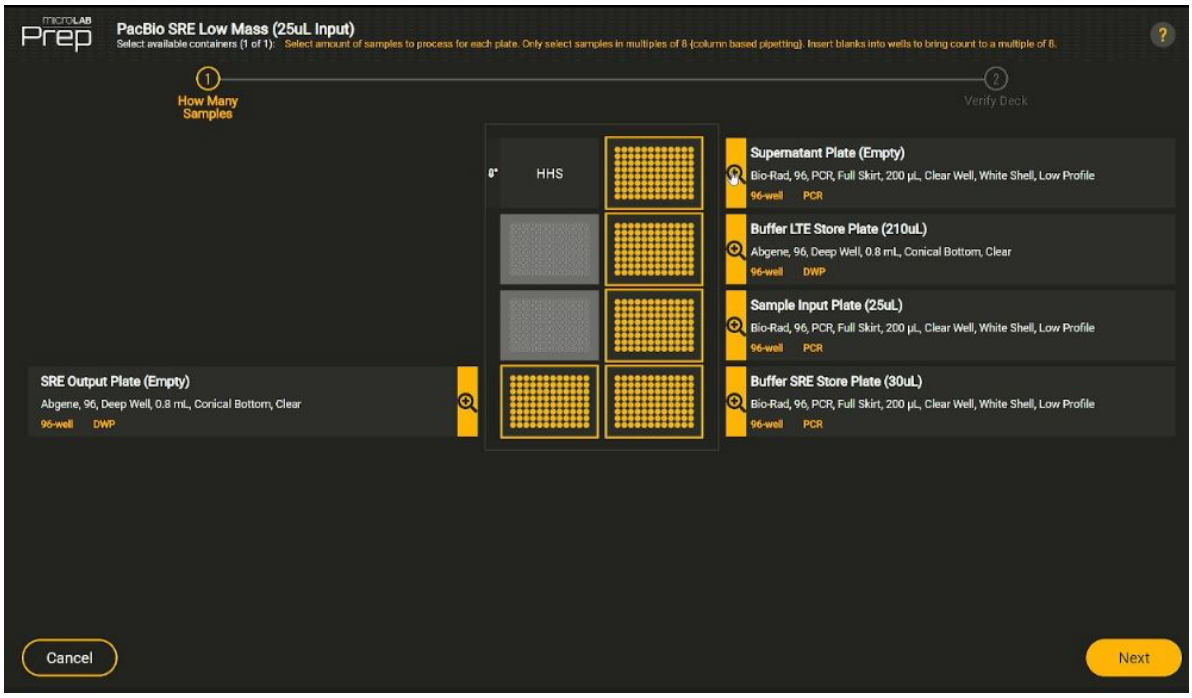


4. **Select the number of samples to process for each plate.** This must be done for every plate on the deck. To select the sample positions on each plate, tap on the magnifying glass next to the plate to open the selection menu.

Note: The prompt example is for the SRE Low mass protocol.



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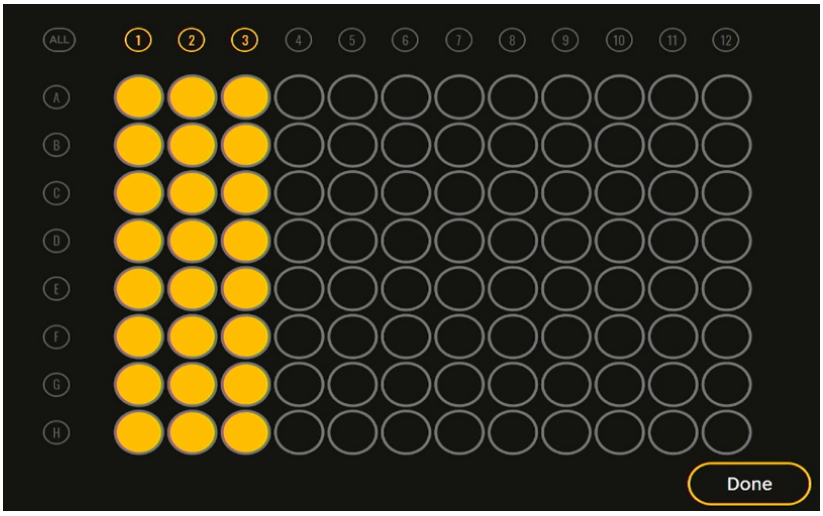
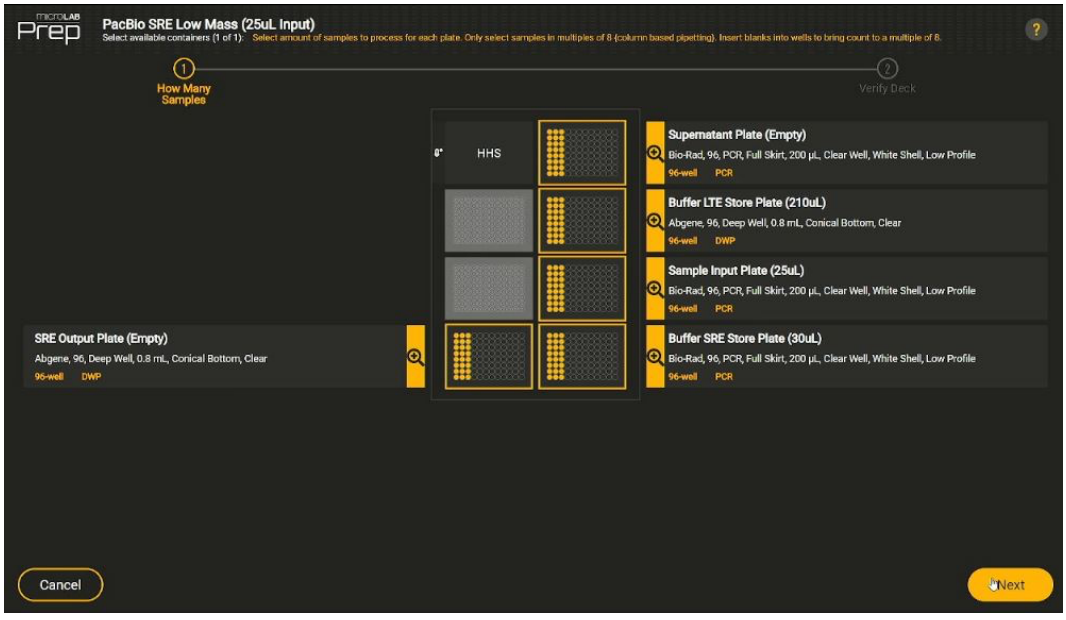
Select the appropriate sample wells by tapping, or touch and drag in multiples of 8. Tap “Done” after sample count is selected for each plate.



Select “Next” to continue.

Note: The prompt example is for 24 samples using the SRE Low mass protocol.



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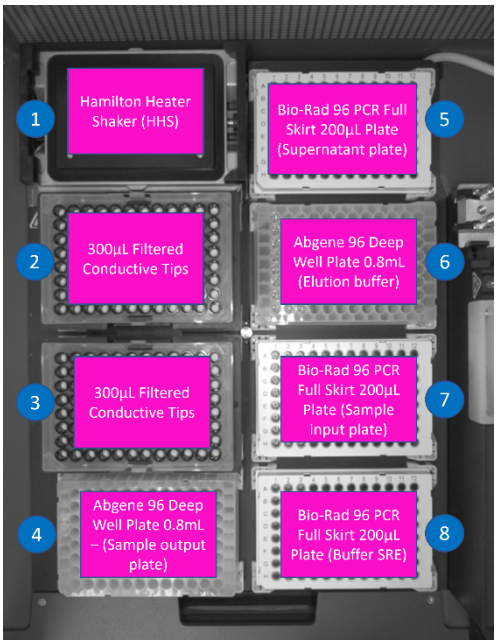
5. **Load consumambles.** Place the consumambles on the deck as indicated in the figure below. Quick spin all plates and remove seals prior to loading deck.
    - Position 2: Tip stand & 300 µL tips
    - Position 3: Tip stand & 300 µL tips
    - Position 4: Abgene 96 deep well plate 0.8 mL (Empty)
    - Position 5: 96 well PCR full skirt 200 µL plate (Empty)
    - Position 6: Abgene 96 deep well plate 0.8 mL (210 µL or 310 µL of Buffer LTE)
    - Position 7: 96 well PCR full skirt 200 µL plate (25 µL or 50 µL of sample)
    - Position 8: 96 well PCR full skirt 200 µL plate (30 µL or 55 µL of Buffer SRE)



6. **Verify the deck.** The required labware to load the deck will display. Tap “Detect” for the Microlab prep to capture a photo of the current deck.

Note: The prompt example is for the SRE Low mass protocol.

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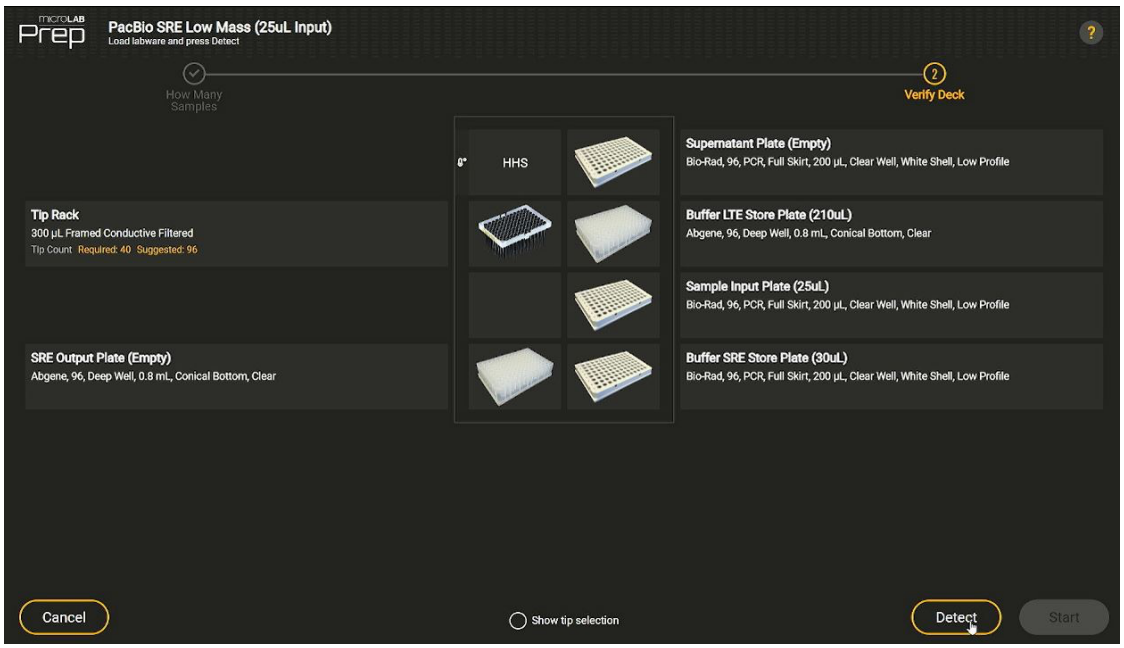
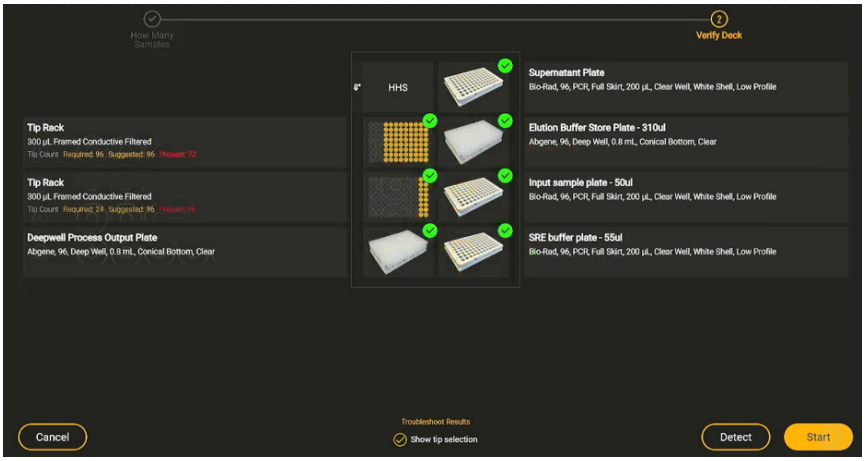


7. **Confirm labware.** Resolve all deck verification errors to proceed. Tap “Start” to continue.

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

Note: Note: The prompt example is for the SRE Low mass protocol.



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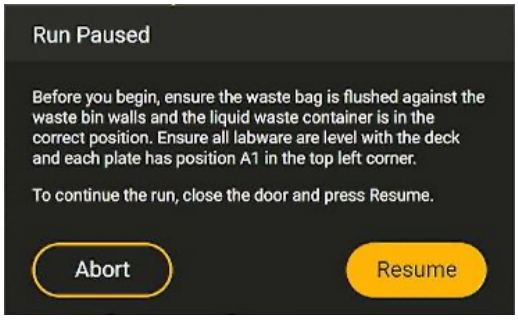
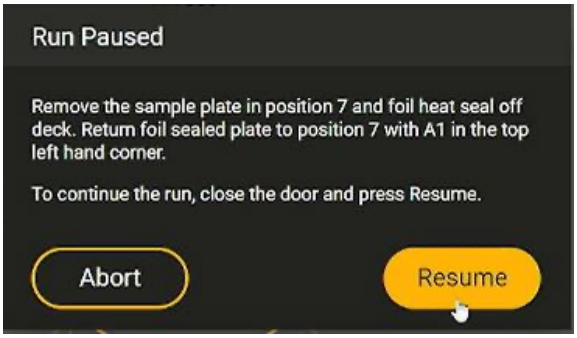
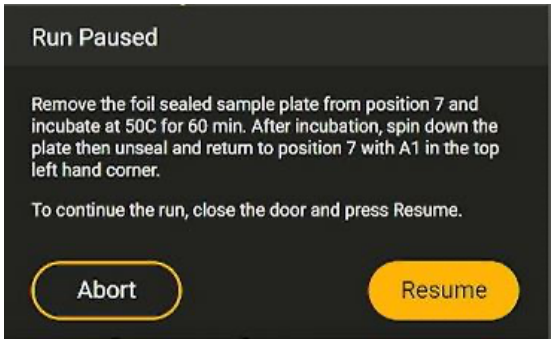
8. **Deck check.** Ensure that the deck is properly loaded. Tap “Resume” to continue. Automated SRE will start. Buffer SRE will be added to the sample plate.



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



10. **Incubation.** When prompted, remove the foil sealed sample plate from position 7 to incubate off deck for 1 hour at 50°C. After incubation, spin down, carefully unseal and return the sample plate to position 7. Tap “Resume” to continue. The sample will be transferred to a deep well plate for centrifugation.



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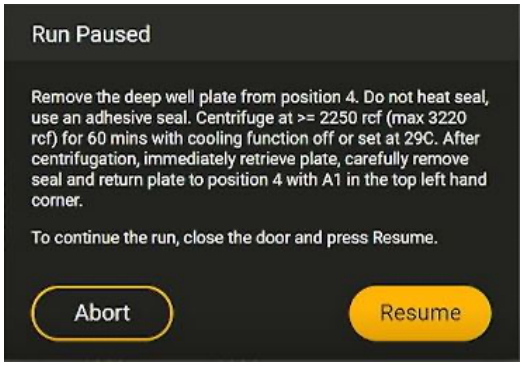
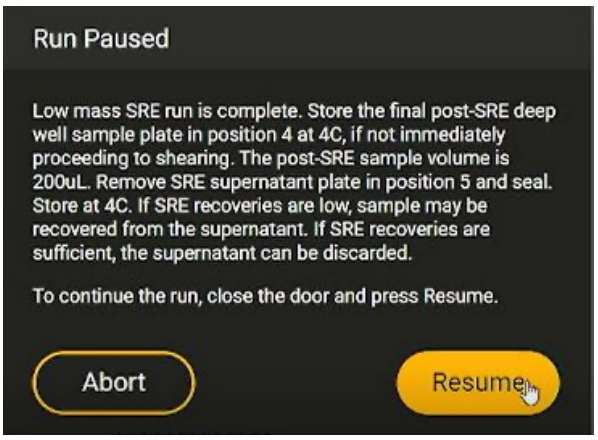
11. **Centrifugation.** When prompted, remove the deep well plate containing the samples in position 4. Seal the plate with an adhesive seal. Centrifuge at ≥ 2250 rcf (max 3220 rcf) for 1 hour at room temperature. If using a centrifuge with temperature control specify a target temperature set point higher than ambient room temperature (e.g., 29°C). Once the spin cycle is complete, unseal and immediately place the deep well plate back to position 4. Tap "Resume" to continue.



12. **Method completion and QC.** Once the SRE protocol is complete, retrieve the final post-SRE deep well sample plate in position 4 to quantify the samples using the Qubit 1x dsDNA HS assay. Seal and store the sample plate at 4ºC for up to 2 weeks if not proceeding to DNA shearing. Next, seal the SRE supernatant plate in position 5 and store at 4ºC. If SRE recoveries are sufficient, the supernatant can be discarded. If SRE recoveries are lower than 50%, vortex sample and requant. If SRE recoveries are low, sample may be recovered from the supernatant, refer to the troubleshooting section in the Appendix.

Note: The prompt example is for the SRE Low mass protocol.



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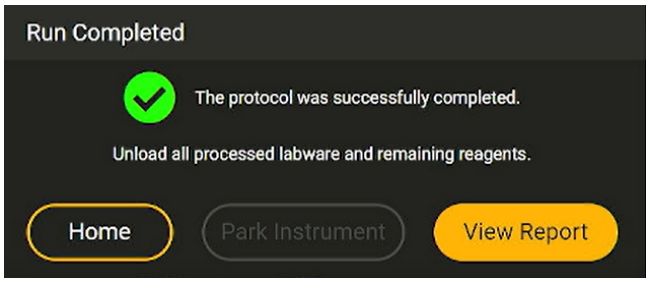
13. **Final instrument prompt.** Clean up deck and discard all used consumables and tip waste.



2. DNA Shearing

Important: A mean fragment size between 15 to 20 kb with a narrow distribution (typically ~10 – 35 kb) is recommended for this protocol. **If gDNA is within these ranges or lower, the DNA shearing step can be bypassed**. 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 DNA concentration to ≤5 ng/µL in 200 µL or ≤10 ng/µL in 300 µL, if necessary (e.g., if more than 1 µg or 3 µg of gDNA was recovered from SRE). Use Buffer LTE to dilute samples **in a 96 deep well plate (Thermofisher Scientific, AB0859). Deviating from the DNA concentration or volume specified will result in inefficient shearing. Parameters for shearing on the Microlab Prep, or Hamilton assay-ready workstations are listed in the table below.**

Parameter	Low mass setting	High mass setting
DNA concentration	≤5 ng/µL	≤10 ng/µL
Volume of Buffer LTE	200 µL	300 µL
Number of mixes	300 cycles	300 cycles
Pipette mixing speed	400 µL/sec	500 µL/sec
Liquid following	83% volume	83% volume
Pipette tip	300 µL CO-RE II tips (filtered, black, non-sterile)	300 µL CO-RE II tips (filtered, black, non-sterile)

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 200 µL (Low mass workflow) or 300 µL (High mass workflow) of sample in the 96 deep well plate (Thermofisher Scientific, AB0859) starting with position A1. Proceed to fill the plate with samples by column (as shown in Figure 1). 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.

Note: The script must be run in multiples of 8. If your sample count is not in multiples of 8, fill the remaining wells in the column with 200 µL or 300 µL of blanks (Buffer LTE or water).

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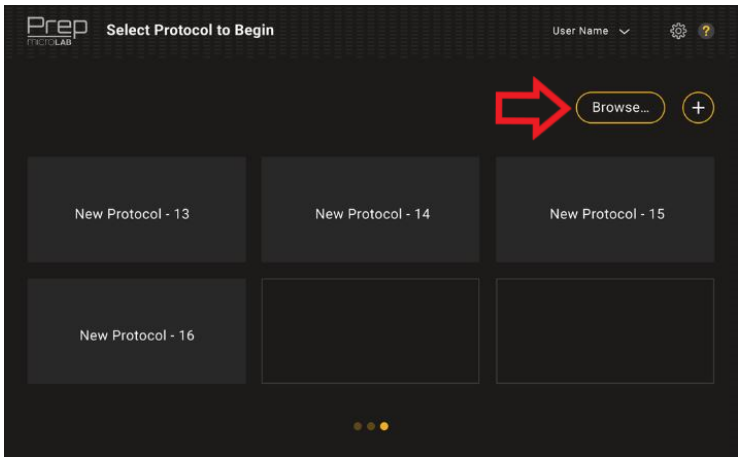
3. **Select shearing protocol**. On the Hamilton Microlab Prep home touch screen, select the appropriate shearing protocol:

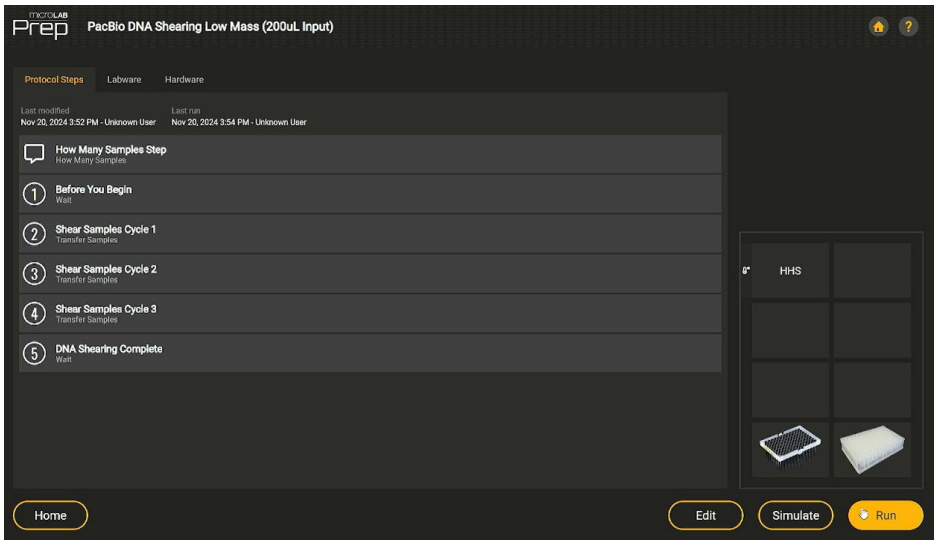
Parameter	Protocol Name
Low mass	PacBio DNA Shearing Low mass (200µL Input)
High mass	PacBio DNA Shearing High mass (300µL Input)

Note: If it is not a favorite on the home screen, tap “Browse” then search for the shearing protocol.



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

Note: The prompt example is for the Low mass shearing protocol.



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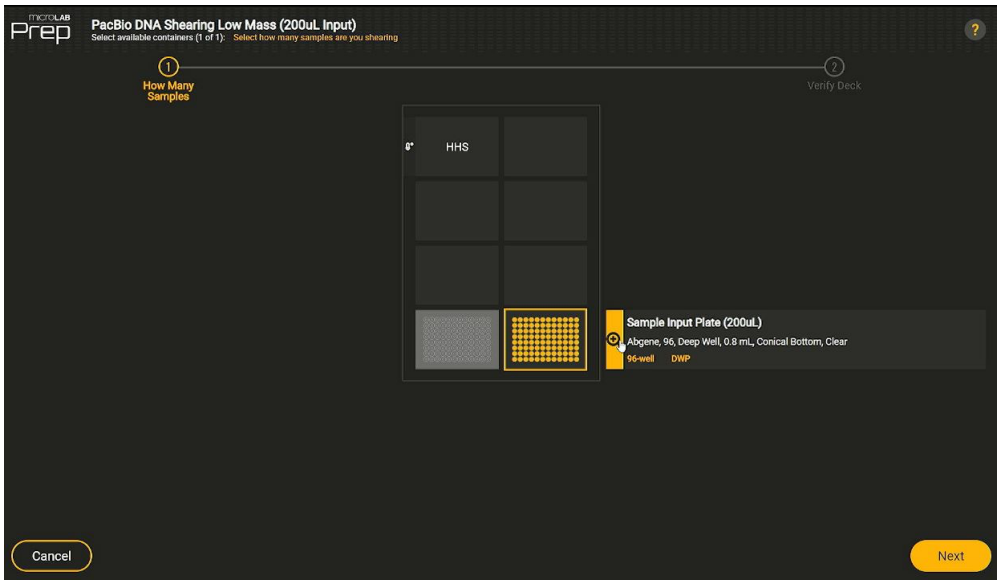
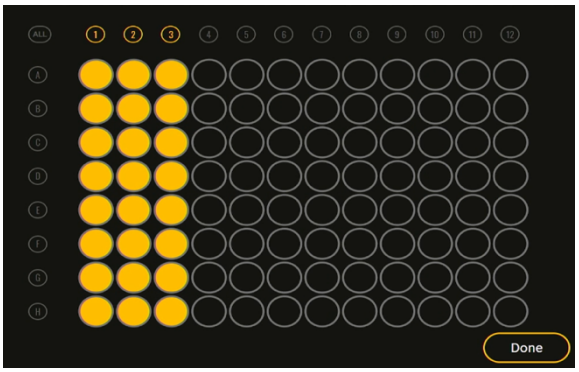
4. **Select the number of samples to process for each plate**. This must be done for every plate on the deck. To select the sample positions on each plate, tap on the magnifying glass next to the plate to open the selection menu.

Note: The prompt example is for the SRE Low mass protocol.



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.

Note: The prompt example is for the Low mass shearing protocol with 24 samples selected.

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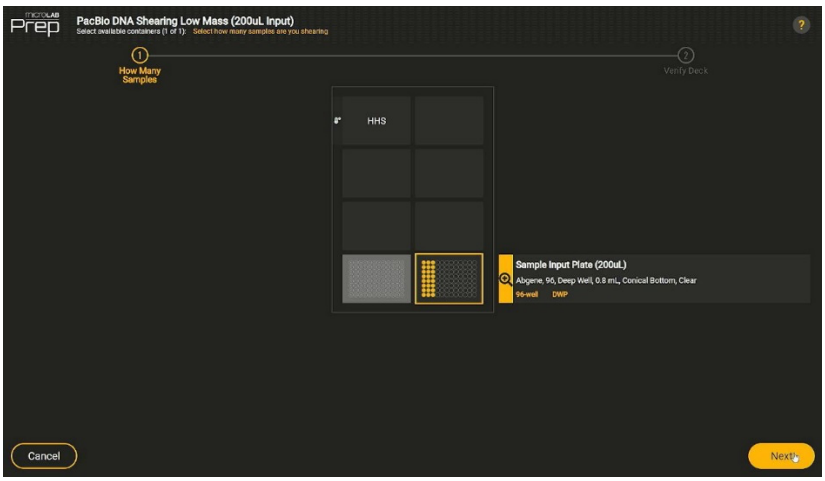
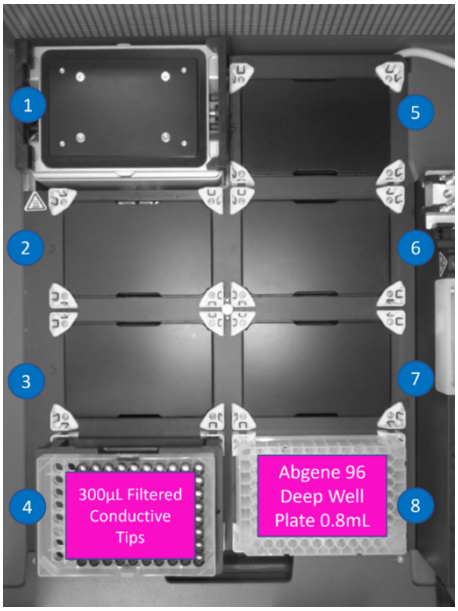


5. **Load the deck and begin the DNA shearing protocol.** Place the consumables on deck as indicated in the figure below. Position 4 should contain a tip stand and 300 µL tips. Position 8 should contain a 96 deep well plate (Thermofisher Scientific, AB0859) with DNA samples (either 200 µL at ≤ 5 ng/µL or 300 µL at ≤ 10ng/µL).



6. **Verify the deck**. The required labware to load the deck will display. When "Detect" is selected, the Microlab Prep captures a photo of the current deck.

Note: 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.

Note: The prompt example is for the Low mass shearing protocol.

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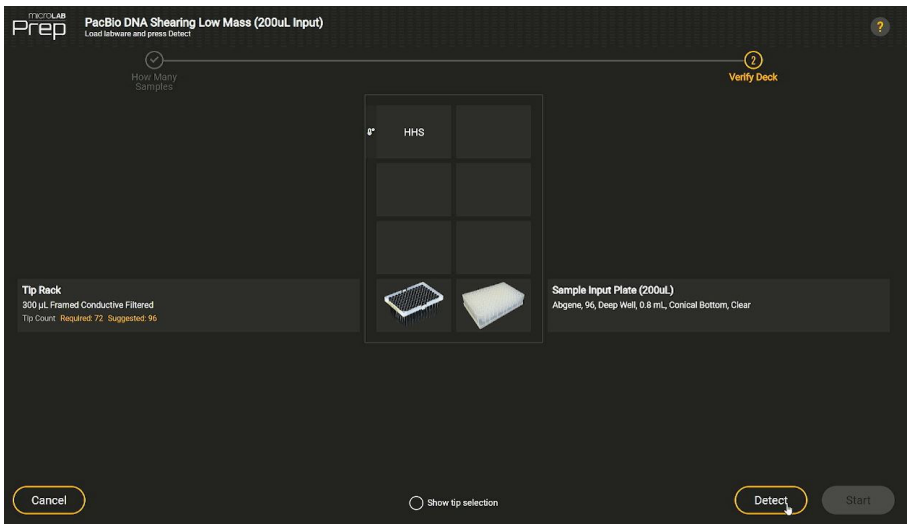
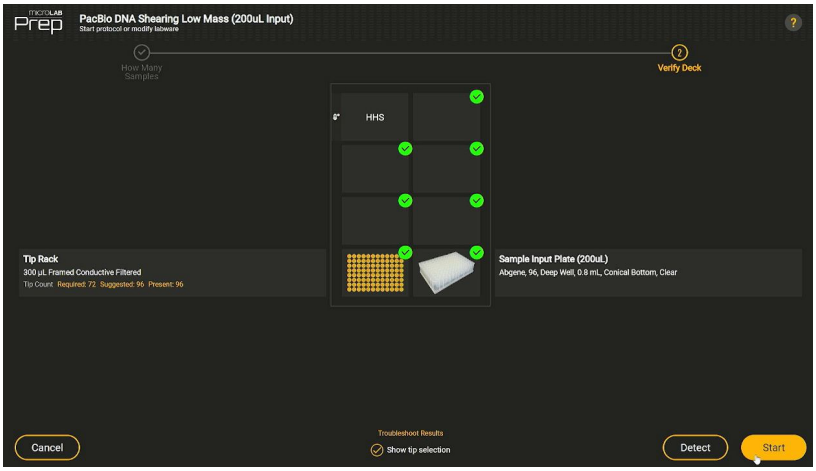
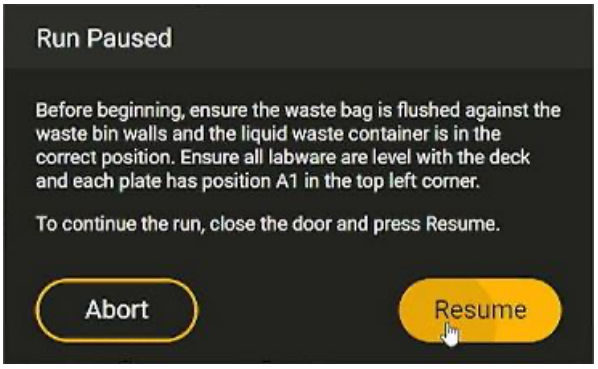


7. **Confirm labware**. Resolve all deck verification errors to proceed. Tap “Start” to continue.



8. **Deck check**. Ensure that the deck is properly loaded. Tap “Resume” to continue. DNA shearing will start.



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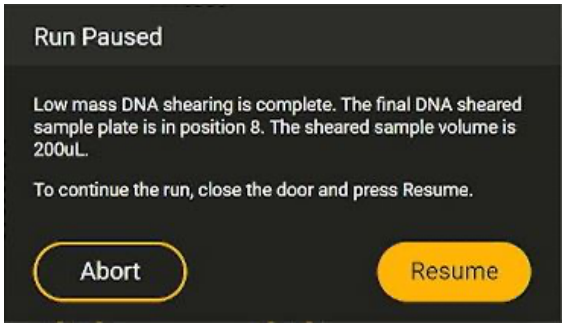
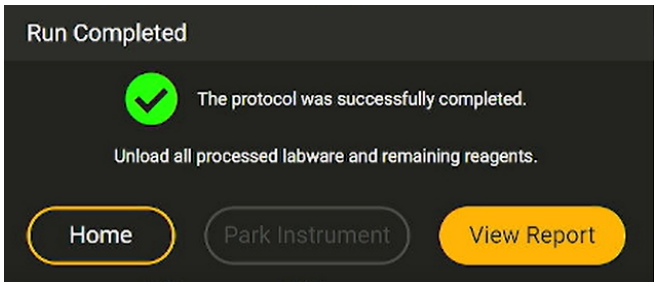
9. **Shearing complete**. At the end of DNA Shearing, remove the 96 deep well (PN: AB0859) output plate in position 8. Perform optional QC using the Femto Pulse to check size distribution (range of 15–20 kb). If your samples did not shear to the expected range, see Appendix. Continue to 1X SMRTbell post-shear cleanup protocol.

Note: The prompt example is for the Low mass shearing protocol.



10. **Final instrument prompt**. Clean up deck and discard all used consumables and tip waste.



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3. 1x SMRTbell cleanup

1. **Prepare sample plate for post-shearing cleanup.** If pipette DNA shearing has been run (Section 2), the samples are already in a deep well plate with a 200 µL (Low mass) or 300 µL (High mass) sample volume and this step can be skipped. If beginning here, manually pipette 200 µL or 300 µL of sample diluted with Buffer LTE into a deep well plate (Thermofisher Scientific, AB0859). Start at position A1 then proceed to fill the plate with samples in a column-based down direction (as shown in Figure 1). 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 load the deck.

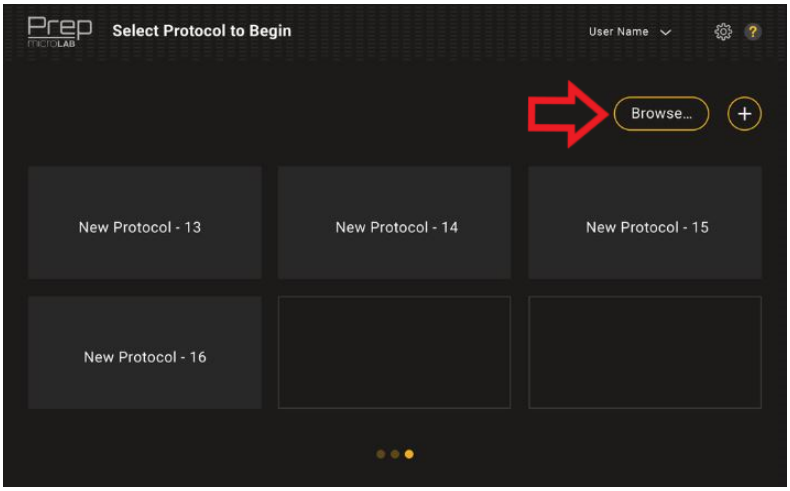
Note: The script must be run in multiples of 8. If your sample count is not in multiples of 8, fill the remaining wells in the column with 200 µL or 300 µL of blanks (Buffer LTE or water).

2. **Select 1x post-shear cleanup protocol.** Determine which 1x post-shear cleanup protocol to run based on the table below. The difference between the post-shear cleanup methods are the input volume and elution volume.

Note: To determine the post-shear cleanup method to use, please see companion documentation.

Library preparation workflow	Protocol name	Elution volume (µL)
HiFi prep Low mass (WGS)	PacBio Post-Shear 1xCleanup Low Mass (200 µL Input, 50 µL Output)	50
HiFi prep High mass (WGS)	PacBio Post-Shear 1xCleanup High Mass (300 µL Input, 50 µL Output)	50
HiFi plex prep (mulitplex)	PB Post Pipette Shearing Cleanup (Multiplex)	25.5

Note: If it is not a favorite on the home screen, tap “Browse” then search for the post-shear cleanup protocol.



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

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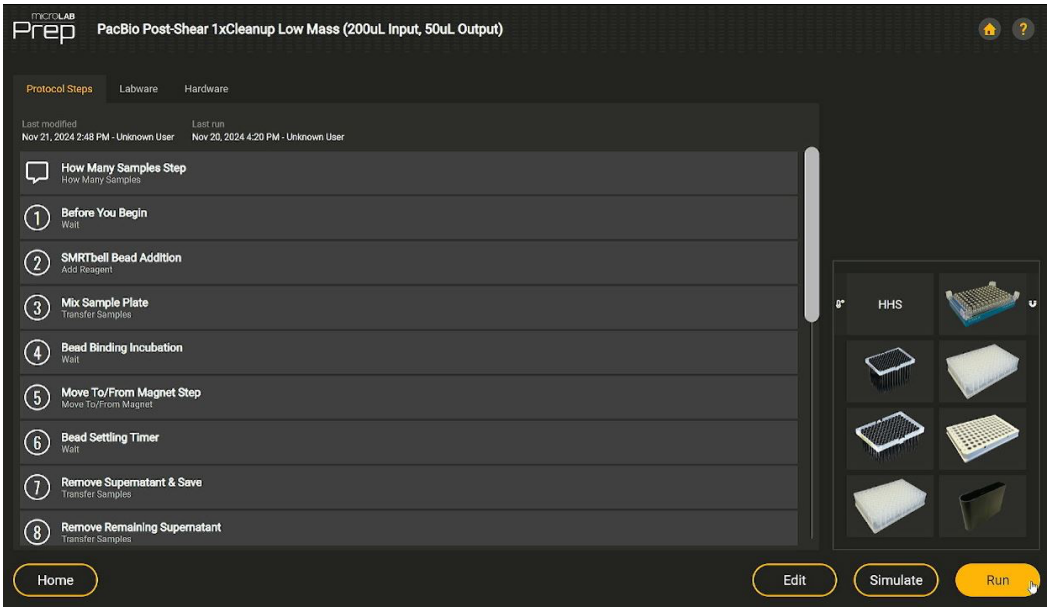
Note: The prompt example is for the post-shear cleanup Low mass protocol.



3. **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.

Note: The prompt example is for the post-shear cleanup Low mass protocol.

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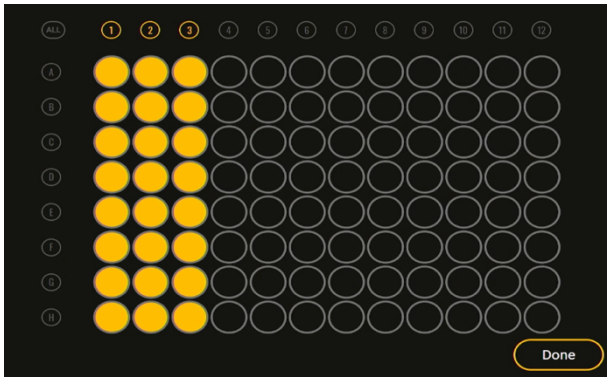
Select the appropriate sample wells by holding down and dragging in multiples of 8. Tap “Done” after the appropriate sample count is selected.



Tap “Next” to continue.

Note: The prompt example is for the post-shear cleanup Low mass protocol with 24 samples selected.

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

Note: Before loading the reagent reservoir into the reservoir rack, remove the middle insert from the black 60 mL reservoir as shown in the image below.

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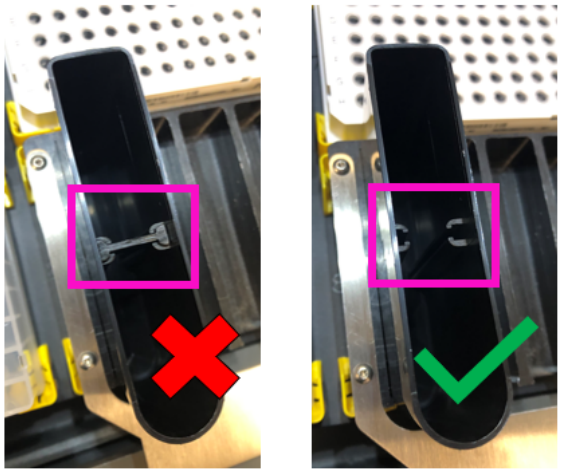
Important: User must enter reagent volumes manually for every run. The volumes will automatically display the reagent volumes from the previous run, so these must be modified if the sample count is different.



Post-shear cleanup workflow	HiFi prep Low mass (WGS)			HiFi prep High mass (WGS)/HiFi plex prep (mulitplex)		
Sample count	8	16	24	8	16	24
SMRTbell cleanup bead (µL)	3,600	5,200	6,800	4,400	6,800	9,200
Elution buffer (µL)	2,400	2,800	3,200	2,400	2,800	3,200
80% ethanol (µL)	3,600	5,200	6,800	3,600	5,200	6,800

Note: The prompt example is for the post-shear cleanup Low mass protocol.

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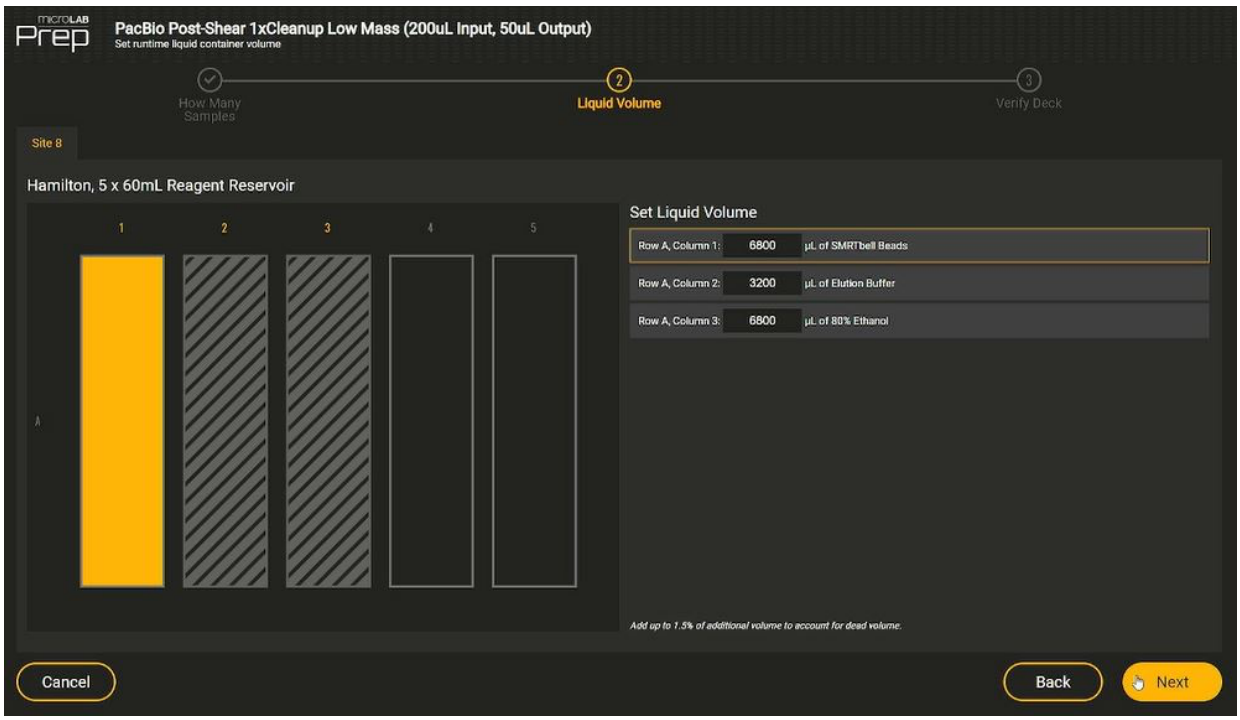

5. **Load the deck and begin post-shear cleanup protocol**. Place the consumables on deck as indicated in the figure below. To place the 1000 µL tips on deck, remove the base plate in position 2. Then place the tip stand in position 2. The 1000 µL tips will dip below the deck when secured in the tip stand. Quick spin sample plate and remove any seals prior to loading deck.

    - Position 2: Tip stand & 1000 µL tips (base plate removed)
    - Position 3: Tip stand & 300 µL tips
    - Position 4: Abgene 96 deep well plate 0.8 mL (Empty)
    - Position 5: Alpaqua Magnum FLX magnet plate
    - Position 6: Abgene 96 deep well plate 0.8 mL with samples (200 µL or 300 µL)
    - Position 7: 96 well PCR full skirt 200 µL plate (Empty)
    - Position 8: 60 mL Reagent reservoir (three 60 mL reservoirs)

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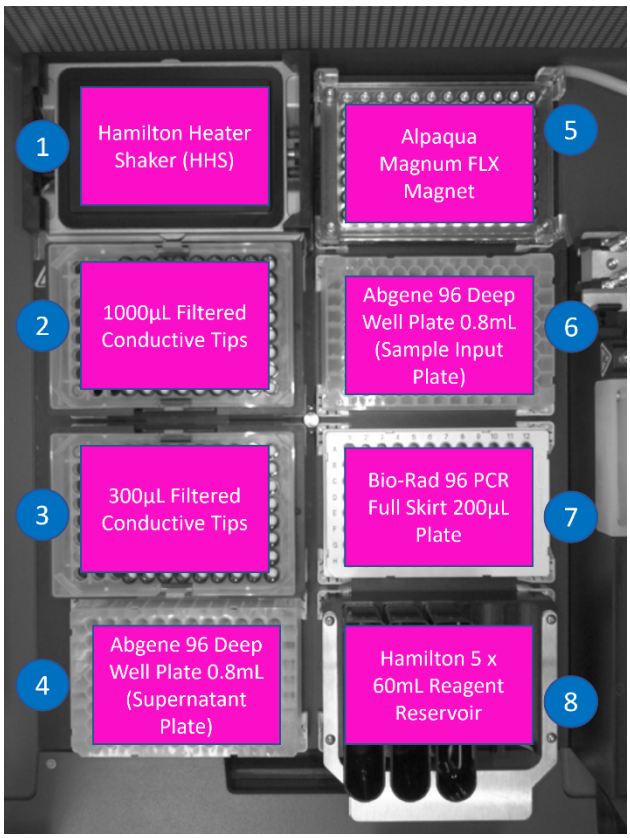
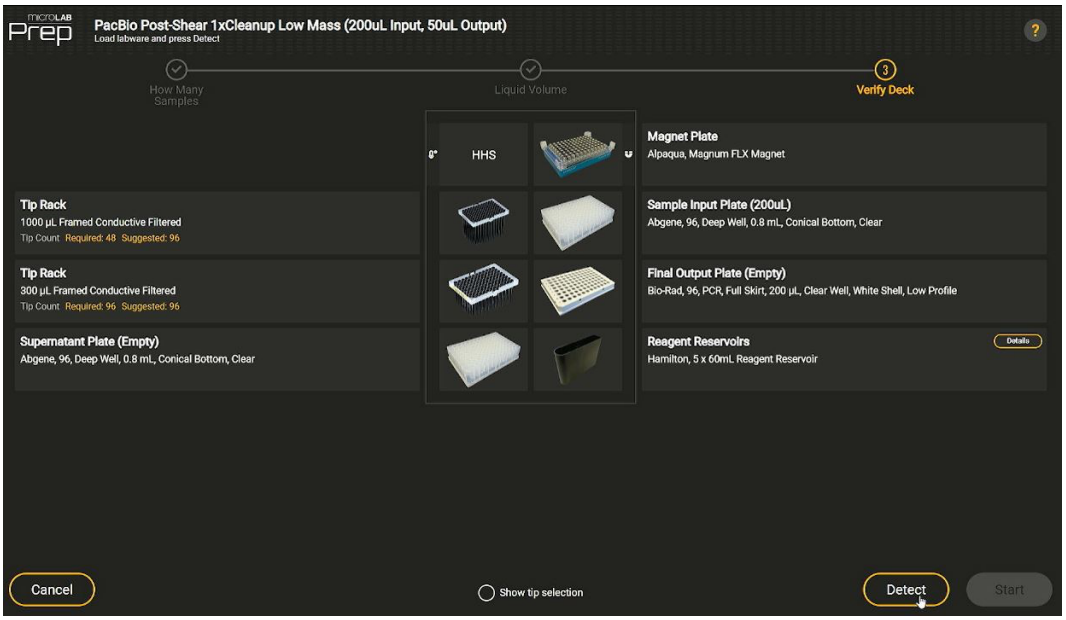


6. **Verify the deck**. The required labware to load the deck will display. Tap “Detect”. When “Detect” is selected, the Microlab Prep captures a photo of the current deck.

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



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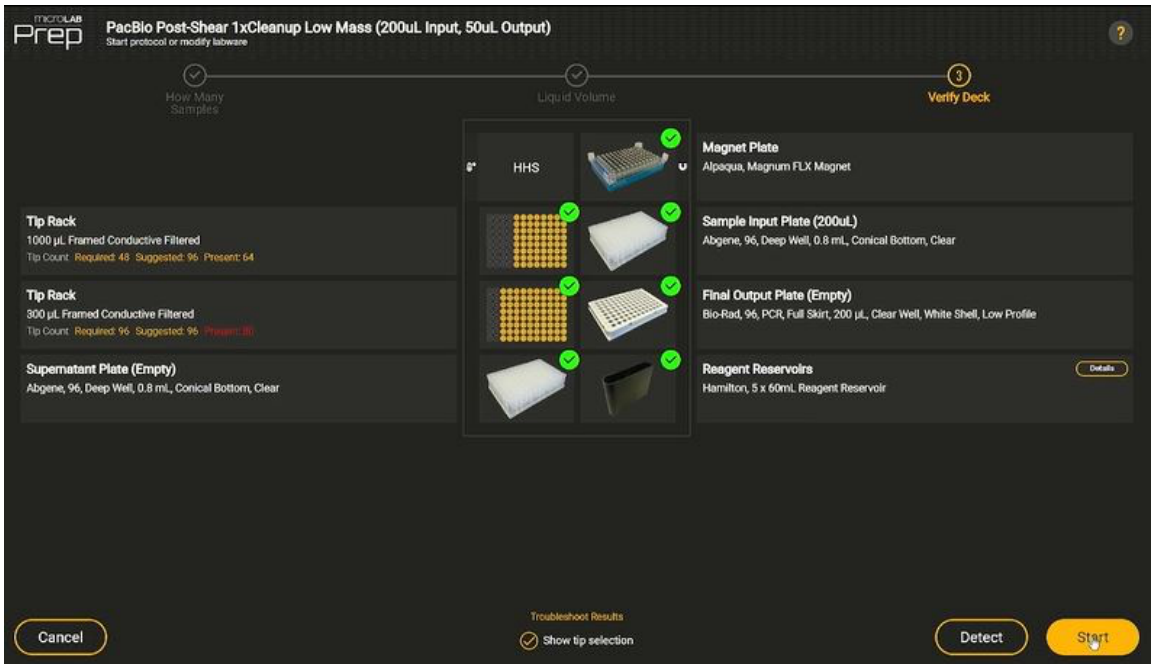
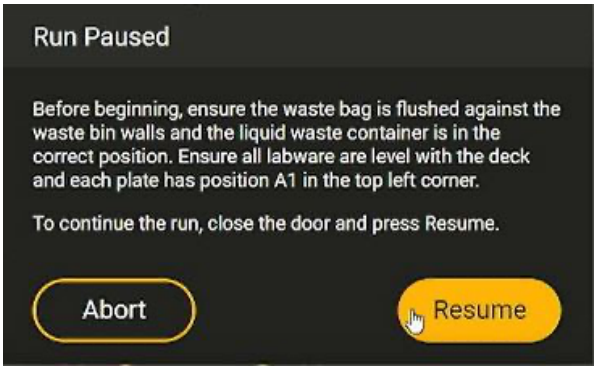
7. **Confirm labware.** Resolve all deck verification errors to proceed. Tap “Start” to continue.



8. **Before you begin.** A last check to ensure the deck is properly loaded. Tap “Resume” to continue. 1X post-shear bead cleanup will start.



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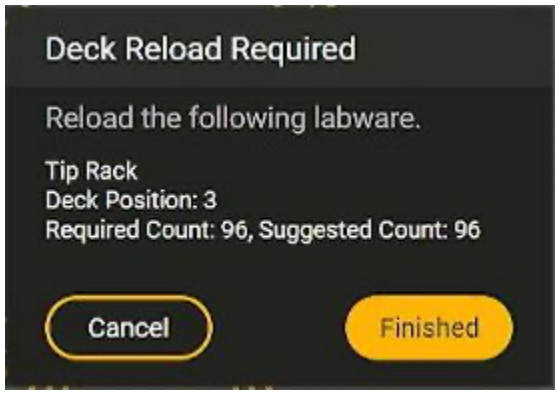
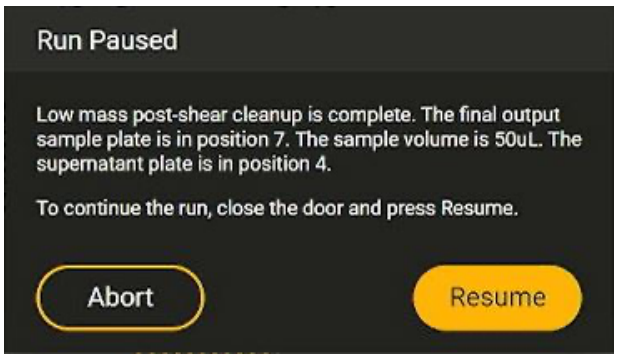
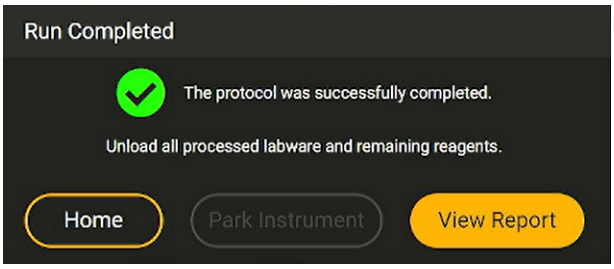
9. **Reload tips.** If running 24 samples, a prompt will require a full rack of 300µL tips.



10. **Post-shear cleanup method complete.** Once complete, the samples are ready to continue with library preparation step, End Repair and A-Tailing. Seal and store DNA at 4°C for up to 2 weeks.



11. **Final instrument prompt.** Clean up deck and discard all used consumambles and tip waster.



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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: ".mlplq"

Note: ".mlppe" is a protocol file ".mlplq" 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.

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	2. Input volume or SRE Buffer volume not accurate	2. Sample input volume in Buffer LTE buffer must be: High mass: 50 µL per well. Buffer SRE must be 55 µL per well. Low mass: 25 µL per well. Buffer SRE must be 30 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 for High mass, 5uL for Low mass. 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 positioning 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.

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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%.
	3. Custom liquid class not imported	3. Make sure the shearing liquid class has been imported correctly. Import the custom liquid class file for your Microlab system. If a 2-channel only system, import "PB_Shearing2ChannelMixing.mlplq". If a MPH system, import "PB_Shearing.mlplq".

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
Updated for SPRQ chemistry and the Vega system	02	December 2024
Clarified compatibility with multi-channel 8-Prob Head instruments	03	February 2025

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