PacBio

Automated HiFi plex prep 96 for the Hamilton NGS Microlab STAR system

Guide & overview

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Introduction

This Guide and overview describes the workflow for PacBio high-throughput semi-automated long read sample prep utilizing the HiFi Prep Plex 96 kit that is designed for a minimum of 24 and maximum of 96 samples per automated run.

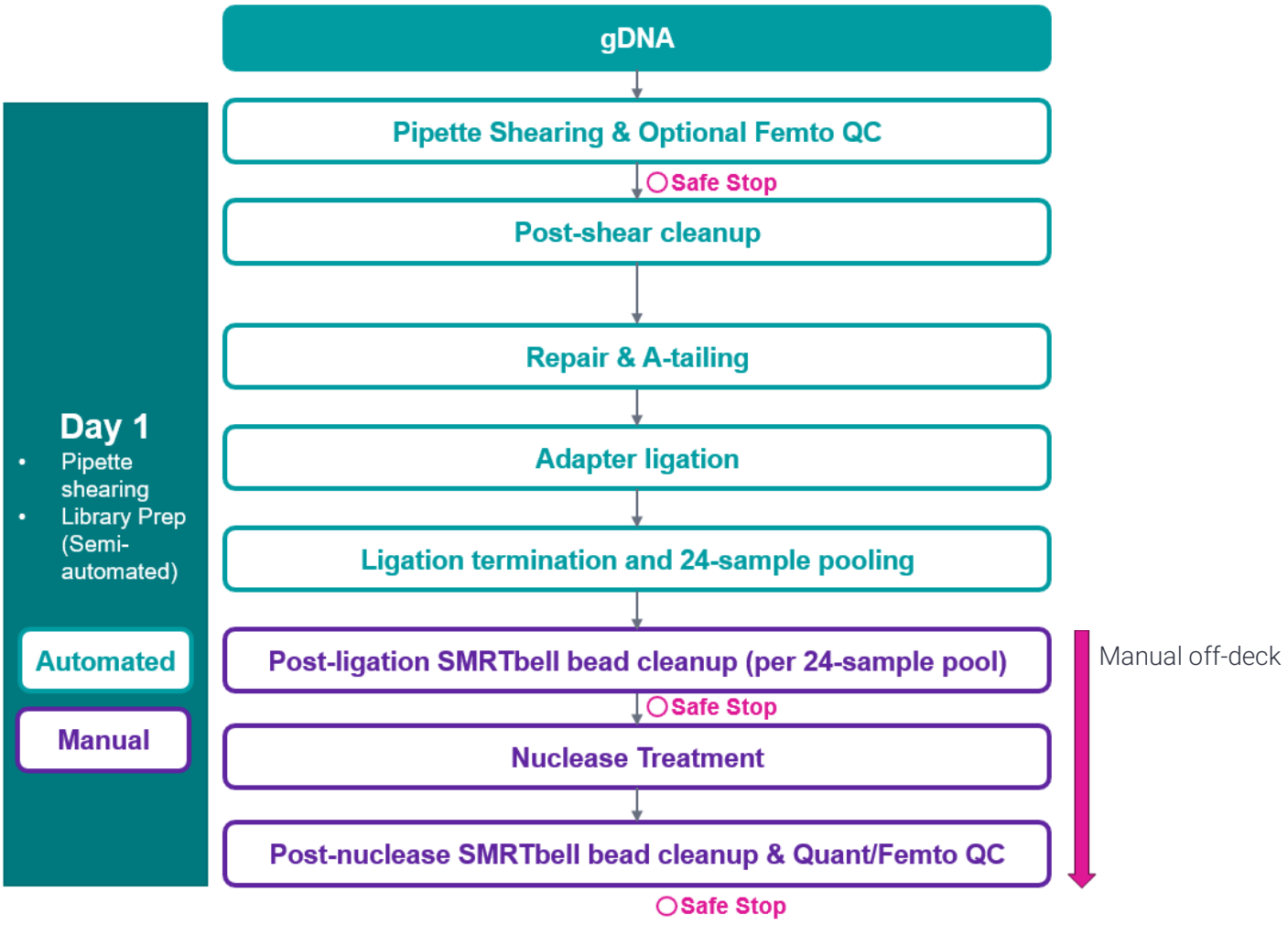
Overview

Overview	
Applications	• Microbial WGS • Metagenome shotgun sequencing • Amplicon sequencing • Long read low pass WGS
Samples	24 – 96 per kit
Minimum batch size supported	4 x 24
Maximum batch size supported	96
	Hamilton NGS STAR
Shearing	10 min for 24–96 samples
Post-shearing cleanup	1 hour for 96 samples
Automated steps (ER/DDR to pooling)	4 hours for 96 samples
Manual steps (Post-ligation cleanup to post-nuclease cleanup)	1.5 hours for 96 samples (4 pools of 24 samples)
Average total time	7 hours

DNA input	gDNA	Amplicons
Per sample input	50 - 300 ng	20 - 200 ng
DNA shearing	Automated pipette-tip shearing	N/A
Target fragment lengths	13–20 kb*	Any size >1 kb

*Smaller fragment lengths can be used when working with lower quality DNA samples (see gDNA quality recommendations)

Workflow overview



Required materials and equipment

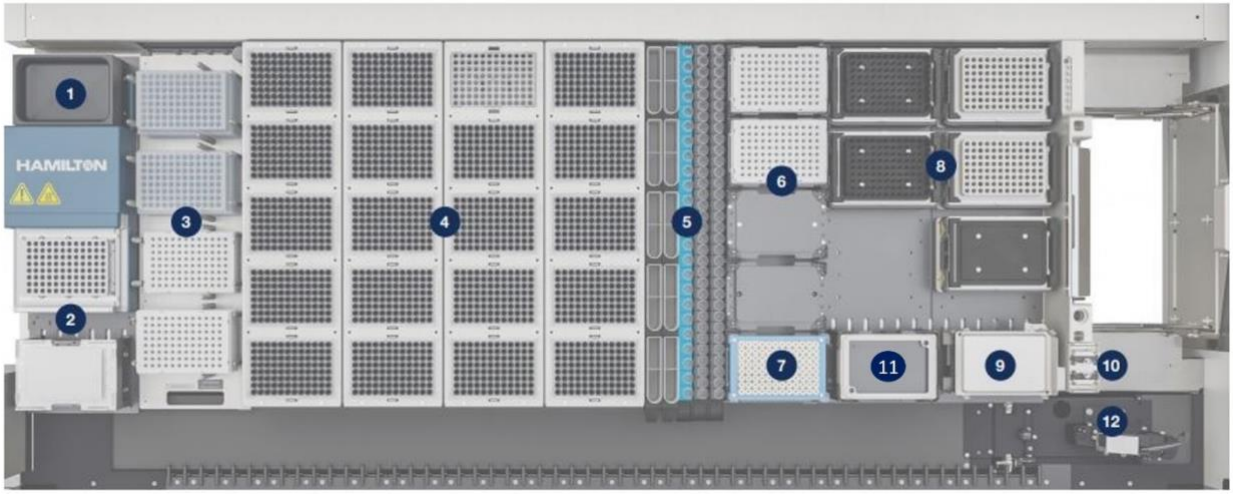
Consumables	Catalog Number
Automation	
Hard Shell 96 PCR Plate	Bio-Rad, HSP9601
Abgene 96 Well 0.8 mL Polypropylene Deepwell Storage Plate	ThermoFisher Scientific, AB0859
50 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235948
300 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235903
1000 µL CO-RE II Tips (Filtered, Conductive)	Hamilton, 235905
60 mL Reagent Trough Self-Standing	Hamilton, 194051
Heat Sealing Foil	Thermo Scientific, AB-0757
MicroAmp Clear Adhesive Film	ThermoFisher Scientific, 00146104
2 mL Sarstedt Tubes	Sarstedt Inc, 72.694.306
300 mL Reservoir	Hamilton, 56669-01
Manual	
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708
Magnetic bead rack for PCR tubes or plates	Any major lab supplier (MLS)
Magnetic bead rack for tubes	ThermoFisher Scientific 12321D
Equipment	
Hamilton NGS MOA star	Contact Hamilton representative
Vortex Mixer	Any MLS
Microcentrifuge	Any MLS
ALPS 50 V-Manual Heat Sealer	ThermoScientific , AB-1443A
Plate Centrifuge with 2250 g force capability	Any MLS

Femto Pulse System	Agilent, M5330AA
Qubit 4 or Qubit Flex Fluorometer	ThermoFisher Scientific, Q33238 (Qubit 4), Q33327 (Qubit Flex)
Varioskan LUX multimode microplate reader	ThermoFisher Scientific, VL0L00D0
Thermocycler	Any MLS

Reagents	Catalog Number
HiFi plex prep kit 96, includes: HiFi plex prep kit 96 SMRTbell® cleanup beads—52 mL Buffer LTE HT Elution buffer	PacBio®, 103-381-300
SMRTbell® adapter index plate 96 (A, B, C, and D)	Plate A – PacBio® 102-009-200 Plate B – PacBio® 102-547-800 Plate C – PacBio® 102-547-900 Plate D – PacBio® 102-548-000
200 Proof ethanol, molecular biology or ACS grade	Any MLS
Nuclease-free water, molecular biology grade	Any MLS
Femto Pulse gDNA 165kb Analysis Kit	Agilent, FP-1002-0275
Qubit 1x dsDNA HS (High Sensitivity) Assay Kit	ThermoFisher Scientific, Q33231
Quant-iT 1X dsDNA HS assay kit (for Varioskan)	ThermoFisher Scientific, Q33232

Hamilton NGS Star MOA System

Note: Contact your Hamilton representative for installation and deck details



Deck Layout

1. Gravity liquid waste for Multi Probe Head (MPH)
2. On-Deck Thermal Cycler (ODTC) with lid parking position
3. Plate stacker
4. Tip carriers with MPH tip support adapter
5. Reagent carriers
6. Plate carrier
7. Alpaqua Magnum FLX magnetic plate
8. Hamilton Heater Shakers with 96-well PCR PCR plate adapters and flat bottom
9. Inheco CPAC with 2mL tube cold block adapter (CPAC 2)
10. CO-RE gripper paddles
11. Inheco CPAC with 96-well PCR plate adapter (CPAC 1)
12. Autoloader with barcode reader

Before you begin

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 highly 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 (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.

Because HiFi reads are single molecules of DNA, the total base yield and mean read length of a sequencing run is directly proportional to the quality of the genomic DNA input and the fragment lengths generated after shearing.

gDNA and amplicon input amount

It is highly recommended to use a quantification assay specific for double stranded DNA (dsDNA) such as the Qubit dsDNA high sensitivity assays. Please follow manufacturer's instructions for the assay being used.

We *do not* recommend quantification with UV-Vis Spectrophotometers (e.g. NanoDrop) that measure all nucleic acids in a sample. For example, measuring all nucleic acid will inflate the true concentration of gDNA in samples.

Remove all RNA from genomic DNA samples prior to beginning. Residue RNA can inhibit sequencing primer annealing and therefore prevent polymerase binding and sequencing.

gDNA	Amplicons
50 to 300 ng per sample	20 to 200 ng per sample

Important:

- Do not exceed >300 ng of gDNA per sample going into the Repair and A-tailing step. Too much DNA may overwhelm enzymatic reactions and lead to poor library recovery.
- At least 24 samples need to be used when using lower input amounts (e.g., 50 ng).
- Samples must be pooled after ligation.

Multiplexing

Important: This procedure requires one of the four available SMRTbell adapter index plates:

- SMRTbell adapter index plate 96A
- SMRTbell adapter index plate 96B
- SMRTbell adapter index plate 96C
- SMRTbell adapter index plate 96D

To balance the number of reads per sample, please consider the following:

- Shear all gDNA samples to similar mean fragment sizes and distributions.
- Normalize DNA input across all samples.

To pool more than 96 samples per SMRT® Cell combine multiple HiFi plex preps after the final cleanup. Each prep needs to use a different SMRTbell adapter index plate. A total of 384 samples can be pooled for sequencing on a single SMRT Cell. A total of 1,536 (4 cells x 384 samples) can be sequenced on a single Revio run.

DNA shearing

This protocol recommends shearing gDNA using automated liquid handler systems to a size between 13 – 20 kb.

For more details, please see the high-throughput DNA shearing Technical note.

Microbial and metagenomic samples often have degraded DNA where the majority is already < 13 kb in length. To better balance the number or reads between samples it may be necessary to shear all samples to a mean size of ~10 kb. For shearing below <13 kb, we recommend the following equipment:

SPEX SamplePrep *1600 MiniG* homogenizer:

- Speed = 1500 RPM
- Time = 3 minutes
- DNA input = 300 ng – 3 µg
- Volume = Up to 300 µL

For more details, please see the PacBio Technical note describing experimental conditions for shearing using the 1600 MiniG.

FastPrep96 (contact MP Bio for latest protocol on shearing for microbial samples). PacBio recommendations below. Conditions may need to be adjusted based on DNA input and volume used.

- Speed = 1800 RPM
- Time = 60 seconds
- DNA input = 300 ng
- Volume = 50 µL

Reagent and sample handling

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

Thaw the Repair buffer M96, Nuclease buffer M96, Index plate, and Stop solution M96 at room temperature. Once thawed, place on ice prior to loading the deck.

Quick-spin all reagents in a microcentrifuge to collect liquid at bottom prior to use.

Keep all temperature-sensitive reagents on ice.

Temperature-sensitive reagents		
Step used	**Tube color**	**Reagent**
Repair and A-tailing	Blue	End repair mix M96
	Green	DNA repair mix M96
Adapter ligation	Yellow	Ligation mix M96
	Red	Ligation enhancer M96
Nuclease treatment	Light green	Nuclease mix M96

Bring 1X dsDNA HS reagents to room temperature for 30 minutes prior to use.

Bring SMRTbell cleanup beads to room temperature for at least 1.5 hours prior to use. Alternatively, beads can be left out overnight if being used the next morning.

Samples can be stored at 4°C at all safe stopping points listed in the protocol.

80% ethanol should be made fresh for each run.

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

Preparation of the Hamilton NGS Star MOA System:

- Set CPACs (Cold Plate Air Cooled device) to 4°C before thawing and preparing reagents and consumables.
- Ensure that tip support adapter is empty before starting a run.

1. Hamilton NGS STAR HiFi plex prep DNA shearing module to pooling module loading procedure

1. **Prepare sample plate.**

   Manually pipette 300 µL of gDNA into a 96-well deepwell plate (ThermoFisher Scientific, AB0859) starting with position A1. Proceed to fill the plate by column as shown in Figure 1 below.

Note: This script requires multiples of 8. If sample count is not in multiples of 8, fill the remaining wells in the column with 50µL of Buffer LTE. The reagent kit supports 4 sets of 24 sample runs.

Figure 1: 24 sample input plate example (s1 – s24). Prepare in the 96-well PCR 200µL plate (Bio-rad, HSP9601).

2. Gather the following reagents and consumables as shown in the table below. Instrument prompts will guide when to load and prepare each reagent.

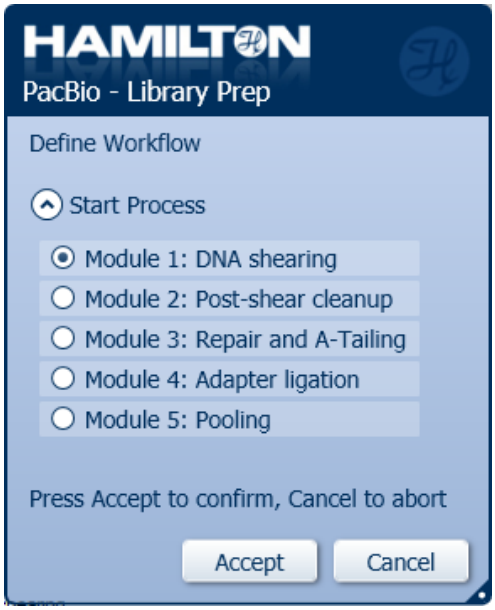
Note: Master mix preparation will be described at step 9.

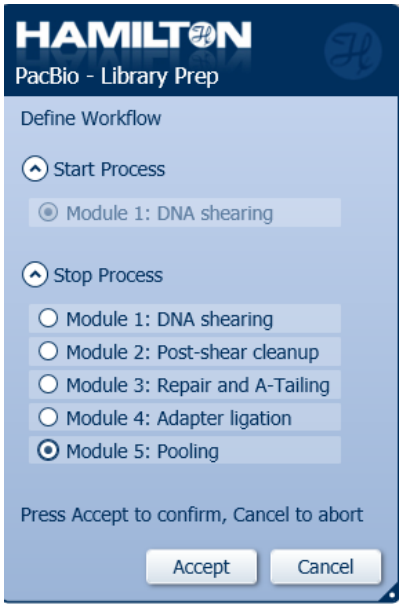
Reagent	Consumables
SMRTbell cleanup beads	60 mL trough
Elution Buffer	60 mL trough
Stop Solution M96	60 mL trough
80% Ethanol	300 mL reservoir
SMRTbell Adapter Plate	96 Well PCR Plate part of kit
End Repair Master Mix • Repair Buffer M96, • End Repair M96, and	Designated master mix 96 well hard shell plate (Bio-rad, HSP9601) in column 1

- DNA Repair M96

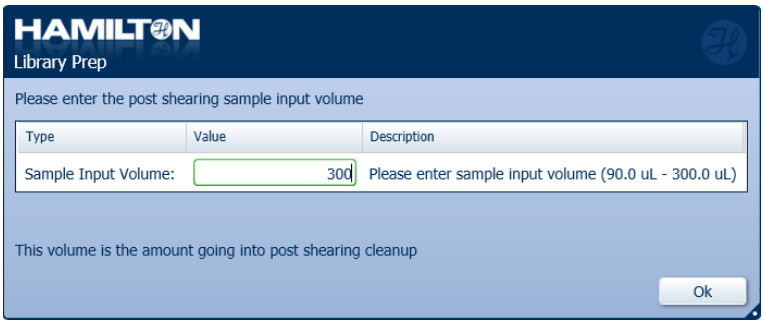
Ligation Master Mix	
• Ligation mix M96 • Ligation Enhancer M96	Same as above plate (Bio-rad, HSP9601) but in column 2

3. Start the Hamilton library prep script: Select “PacBio HiFi Plex Prep v1.0.0”.
4. **Define workflow.** Select “DNA shearing” for the start process and “Pooling” for the stop process. Select “Accept” to continue.





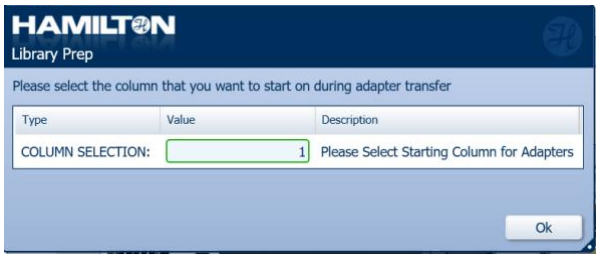
5. **Sample Input Volume.** If pipette tip shearing is performed, enter “300” µL as the “Sample Input Volume” for post-shear cleanup. If an alternative shearing method is performed or amplicons are the input, the “Sample Input Volume” range is compatible with 90–300 µL. Click “Ok” to continue.



6. Enter a “USER ID” for run. Click “Ok” to continue.

7. **Sample Count Input.** There are two ways to enter the sample count:
(1) In “Sample Count Input”, type in a number that is in a multiple of 8.
(2) In “Worklist Input”, a worklist input file can be uploaded for sample tracking. Reference Appendix for instruction.
Click “CONTINUE”.

8. **Adapter Column Selection.** Enter the start column number (range 1–12) for the indexed adapter plate transfer. This prompt will only display if the sample count is ≤88 samples.

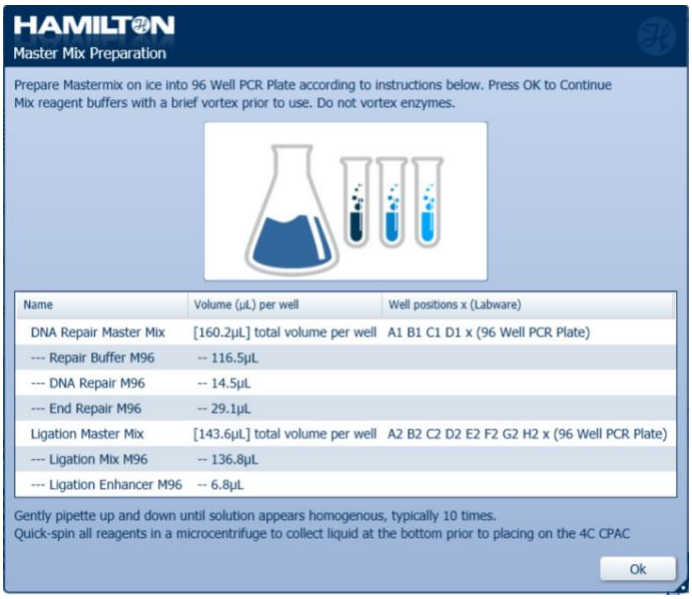


9. **Master mix preparation for DNA repair and ligation steps.**
   The table below describes how to prepare the DNA repair master mix and the ligation master mix for 24, 48, 72, 96 samples in a 96-well PCR plate (Bio-Rad, HSP9601). The displayed prompt is an example for a 96-sample run. Gently pipette mix master mixes and spin down the master mix plate to remove any bubbles. Place master mix plate on ice until prompted to load on the deck.

Note: Do not vortex Master Mix plate.

Note: Prepare all master mixes in 24-sample batches (as shown in table below). For example, if running 96 samples, prepare master mix for a 24-sample batch four times. We do not recommend preparing a bulk master mix due to pipetting volume loss.

	Master Mix for Library Preparation (Automation) DNA Repair Master Mix				
Sample Number	24-samples	24-samples	48-samples	72-samples	96-samples
# of wells in plate		1	2	3	4
Well Locations		A1	A1, B1	A1, B1, C1	A1, B1, C1, D1
Reagent Name	Reagent Volumes (µL)				
Repair Buffer 96	116.5 µL	Prepare 24-sample master mix 1-time for each well	Prepare 24-sample master mix 2-times for each well	Prepare 24-sample master mix 3-times for each well	Prepare 24-sample master mix 4-times for each well
DNA Repair 96	14.5 µL				
End Repair 96	29.1 µL				
Total Volume per well	160.2 µL				
	Ligation Master Mix				
Sample Configuration	24-samples	24-samples	48-samples	72-samples	96-samples
# of wells in plate		2	4	6	8
Well Locations		A2, B2	A2, B2, C2, D2	A2, B2, C2, D2, E2, F2	A2, B2, C2, D2, E2, F2, G2, H2
Reagent Name	Reagent Volumes (µL)				
Ligation Mix 96	136.8 µL	Prepare 24-sample master mix 2 times for each well	Prepare 24-sample master mix 4 times for each well	Prepare 24-sample master mix 6 times for each well	Prepare 24-sample master mix 8 times for each well
Ligation Enhancer 96	6.8 µL				
Total Volume per well	143.6 µL				



10. Load the stacker carrier. Load the stacker plate carrier with two 96-well PCR plates stacked (Bio-Rad, HSP9601) in position 4. Select “Ok” to continue.

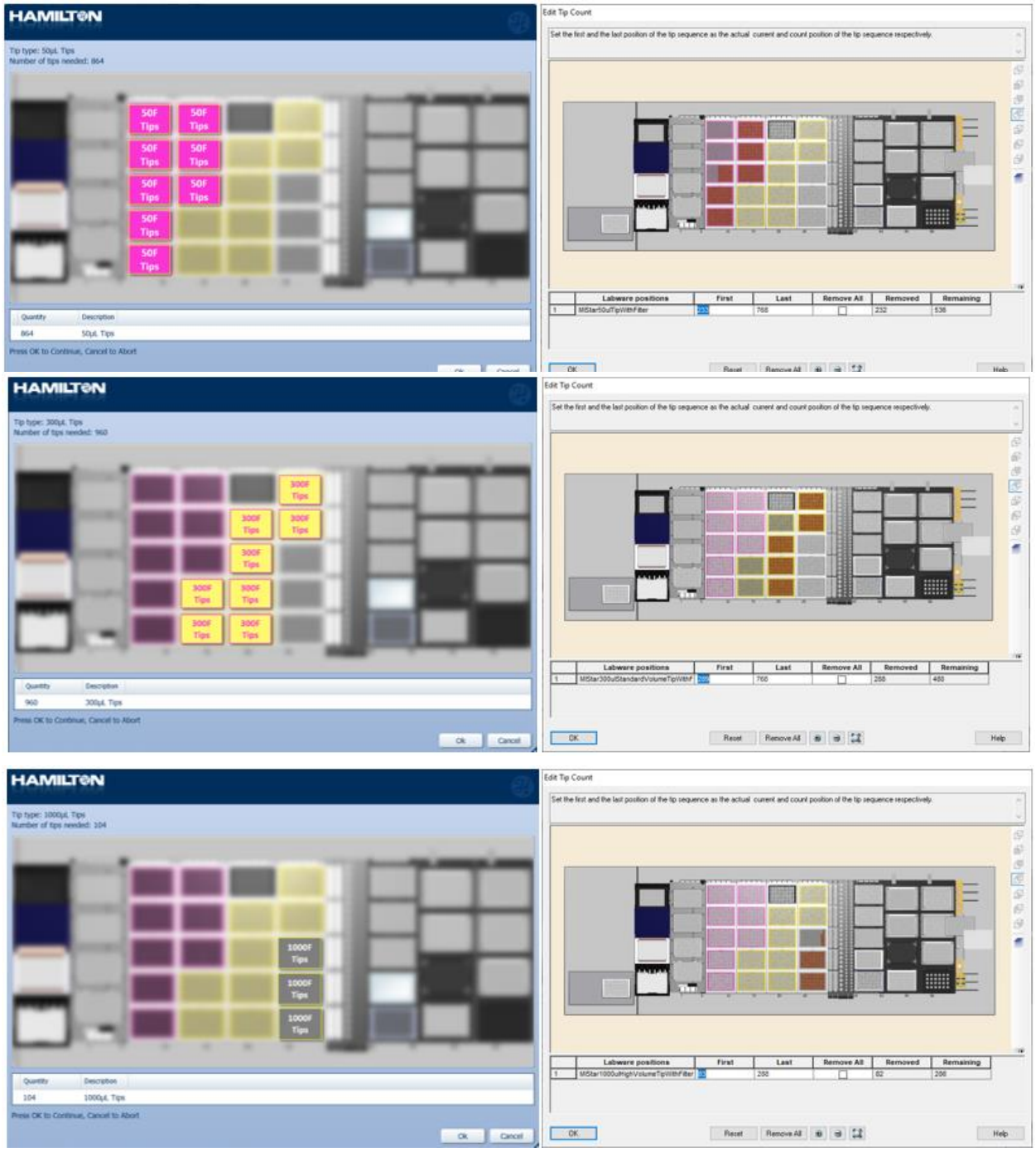
11. **Ensure the tip support for the MPH is empty.** CO-RE I instruments require the CO-RE I tip support. CO-RE II instruments require the CO-RE II tip support. Contact your Hamilton Representative to ensure the configuration is set up correctly with your instrument’s CO-RE technology.

12. **Tip deck layout.** A prompt displaying the tip positions will appear, including the tip adapter position. There are 4 tip carriers. Refill the tips on deck in the positions for each tip size: 50 µL filtered conductive tips, 300 µL filtered conductive tips and 100 µL filtered conductive tips.

13. Select the 50 µL, 300 µL, and 1000 µL tips on the instrument.

The type of tip will be under “Description” and the number of tips needed will be under “Quantity”. Select ‘OK” to continue to tip deck matching display. To select your tips to match the deck, click and drag so the positions are bolded. Click again to de select. Select “OK” to continue once you have matched tip positions to the deck.

Note: It is critical that these tip selections are accurate and to leave an empty tip rack even if no tips are present (See Appendix for example).



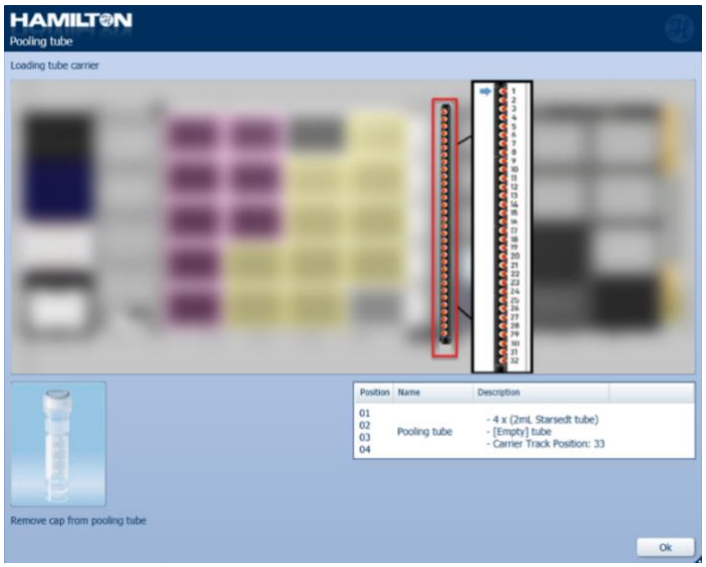
14. **Load 60 mL trough reagent carrier.** Load the SMRTbell cleanup beads, Elution Buffer, and Stop Solution M96 into the 60 mL reagent troughs. Place troughs into the 60 mL reagent carrier in track position 30. Reagent carrier position 1 for SMRTbell cleanup beads, position 2 for elution buffer, and position 3 for Stop Solution M96. The table below shows SMRTbell cleanup beads, elution buffer, and Stop Solution M96 volume amounts based off sample count. The example prompt is for 96-samples. Click "Ok".

Reagent	Consumables	24 Samples	48 Samples	72 Samples	96-Samples
SMRTbell cleanup beads	60 mL trough	10,200 µL	17,400 µL	24,600 µL	31,800 µL
Elution Buffer	60 mL trough	2,520 µL	3,240 µL	3,960 µL	4,680 µL
Stop Solution M96	60 mL trough	2,480 µL	2,960 µL	3,440 µL	3,920 µL

Volume of SMRTbell cleanup beads, elution buffer and Stop Solution M96 required when starting at DNA Shearing to Pooling.

15. **Load the pooling tube(s).** Load the appropriate number of pooling tubes (Sarstedt Inc, 72.694.306) to the tube carrier in track 33, starting at position 1. The table below shows the number of pooling tubes required according to sample count. Un-cap prior to loading. Select "Ok" to continue. The example prompt below is the number of pooling tubes for 96 samples.

Labware	24 Samples	48 Samples	72 Samples	96 Samples
2mL Sarstedt tube	1 tube	2 tubes	3 tubes	4 tubes



16. Load plate carrier.

Load the following on the plate carrier: Position 1 – Empty 96-well PCR plate (Bio-Rad, HSP9601), Position 2 - SMRTbell barcoded adapter plate, Position 3 - 96-well Deepwell plate with samples (Thermofisher Scientific, AB0859), Position 4 – 300 mL Reservoir trough (Hamilton, 56669-01) with 80% ethanol and Position 5 – Alpaqua magnum FLX magnet plate.

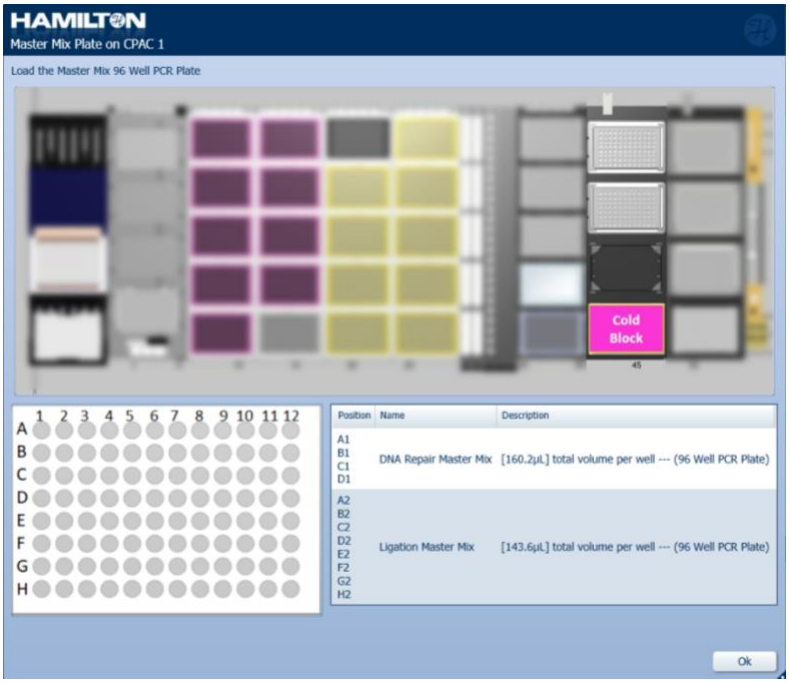
Reagent	Labware	24 Sample	48 Sample	72 Sample	96-Sample
80% Ethanol	300 mL reservoir	44,800 µL	49,600 µL	54,400 µL	59,200 µL

Amount of 80% ethanol needed based on sample count.

Note: Vortex, spin down then remove the seal for the amount of columns needed for the SMRTbell barcoded adapter plate before placing on the deck.

17. **Load Master Mix Plate on Cold Block.** Follow the prompt to load the master mix plate (containing DNA repair and ligation master mixes) on the cold block 96-well PCR plate adapter (CPAC 1) at 4ºC. The example prompt shown below is for 96 samples. Click “Ok”.

Note: If bubbles are present in master mix plate, spin down to remove prior to loading on cold block.



18. **Review selections.** The example prompt below is set for 96 samples starting at the DNA Shearing module and stopping at pooling using adapter column 1. The run begins DNA shearing after clicking “Continue”.

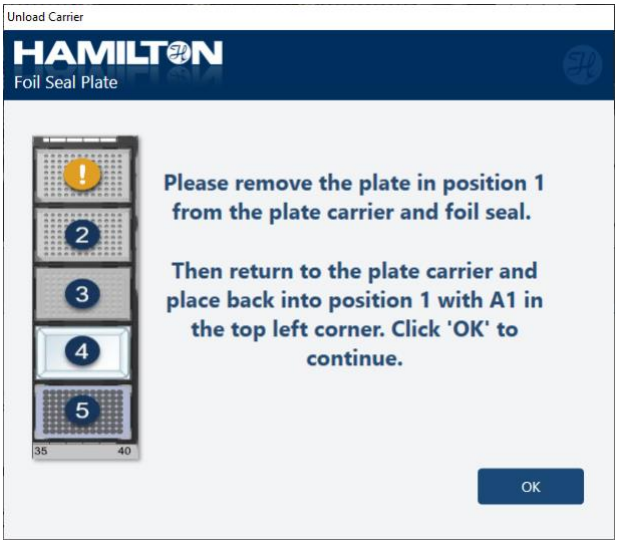
Note: For a 96 sample run, the next prompt will display after ~1 hour.

19. **Post DNA Shearing Optional QC.** Measure the concentration and size distribution of the sheared DNA.

20. **Seal plate for incubation during end repair step.**
    The instrument will prompt the user to unload the sample plate from the carrier position 1. Manually heat foil seal the 96-well PCR sample plate for incubation. Return plate to position 1. Click “Ok”. After incubation, the instrument will prompt the user to spin down the plate and remove the seal prior to loading back onto the carrier.

Note: Carefully remove the seal to prevent contamination. The instrument will pull the carrier back in and continue the run when “OK” is selected.

Note: For a 96 sample run, the next prompt will display after ~1 hour.

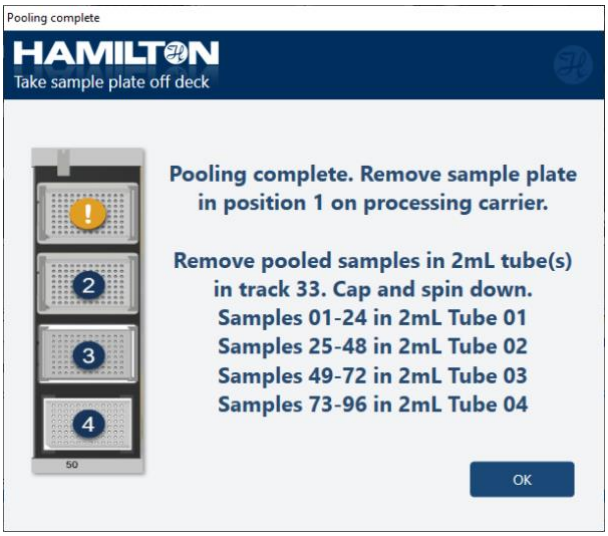


21. **Unseal plate after incubation.** The instrument will prompt the user to retrieve sample plate in position 1 to spin down and remove the seal off deck prior to loading back onto the same position carrier. Click “Ok”. The instrument proceeds to adapter ligation and post-ligation cleanup.

Note: Carefully remove the seal to prevent contamination.

Note: For a 96 sample run, refill the 300 tips after ~1.5 hours.

22. Run complete. Once the automated run has completed, a prompt displays. Gather the 2 mL pooling tubes in the tube carrier and continue the library prep off-deck. Cap and spin down to remove bubbles. Proceed to 1x SMRTbell bead cleanup manually off deck. The plate containing leftover volume of sample in plate carrier position 1 should be sealed and stored.



2. Manual off-deck post-ligation cleanup, nuclease treatment, and final SMRTbell cleanup procedure

Post-ligation SMRTbell cleanup bead purification of pooled libraries

Bring SMRTbell cleanup beads to room temperature prior to the purification step.

1. Add **960 µL** (1.0X) of resuspended, room-temperature SMRTbell cleanup beads to each pool.

Note: If using less than 960 µL, add 1.0X (v/v) concentration of SMRTbell cleanup beads.

2. Pipette mix or invert the sample until the beads are evenly distributed. Quick spin the samples to collect liquid.
3. Leave at room temperature for 10 minutes to allow DNA to bind beads.
4. Place samples on a magnet and allow beads to separate fully from the solution. The solution should be clear and beads pelleted to the magnet.
5. Slowly pipette off the supernatant without disturbing the beads. Discard the supernatant.
6. Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol to each sample. After 30 seconds, pipette off the 80% ethanol and discard.
7. Repeat the previous step.
8. Remove residual 80% ethanol:

    - Remove the samples from the magnet and quick spin to collect liquid.
    - Place samples back on the magnetic and wait until beads separate fully from the solution.
    - Carefully pipette off the residual 80% ethanol without disturbing the bead pellet and discard.

9. Remove samples from the magnet and immediately **add 40 µL of elution buffer**.

    Resuspend the by pipetting mixing until beads are evenly distributed in solution. Quick spin samples if necessary to collect liquid.

10. Leave samples at room temperature for **5 minutes** to elute DNA off beads.
11. Place samples back on the magnet and allow beads to separate fully from the solution. The solution should be clear, and beads pelleted to the magnet before proceeding.
12. Slowly pipette off the cleared supernatant without disturbing the beads. Transfer supernatant to a new LoBind tube. Discard old tube with beads.
13. Proceed to next step (nuclease treatment), or store samples at 4°C.

SAFE STOPPING POINT - Store at 4°C

Nuclease treatment

1. Add the following components in the order and volume listed below to each of the pools from the previous step.

Nuclease mix		
Tube	Component	Volume
Light purple	Nuclease buffer M96	5 µL
Light green	Nuclease mix M96	5 µL
	Total volume	10 µL

2. Pipette mix and spin down to collect liquid. Total volume should equal 50 µL.
3. Run the Nuclease treatment thermocycler program. Set lid temperature to 75°C if programmable.

Step	Time	Temperature
1	15 min	37°C
2	Hold	4°C

SMRTbell cleanup bead purification

Bring SMRTbell cleanup beads to room temperature prior to the purification step.

1. For **DNA >3kb, add 50 µL (1.0X)** of resuspended, room-temperature SMRTbell cleanup beads to each sample. For **DNA <3kb, add 65 µl (1.3x)** of resuspended, room-temperature SMRTbell cleanup beads to the sample.
2. Pipette-mix the sample until the beads are evenly distributed. Quick spin the samples to collect liquid.
3. Leave at room temperature for 10 minutes to allow DNA to bind beads.
4. Place samples on a magnet and allow beads to separate fully from the solution. The solution should be clear and beads pelleted to the magnet.
5. Slowly pipette off the supernatant without disturbing the beads. Discard the supernatant.
6. Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol to each sample. After 30 seconds, pipette off the 80% ethanol and discard.
7. Repeat the previous step.
8. Remove residual 80% ethanol:

    - Remove the samples from the magnet and quick spin to collect liquid.
    - Place samples back on the magnetic and wait until beads separate fully from the solution.
    - Carefully pipette off the residual 80% ethanol without disturbing the bead pellet and discard.

9. Remove samples from the magnet and immediately add 25 µL of elution buffer.

    Resuspend by pipetting mixing until beads are evenly distributed in solution. Quick-spin samples if necessary to collect liquid.

10. Leave samples at room temperature for **5 minutes** to elute DNA off beads.
11. Place samples back on the magnet and allow beads to separate fully from the solution. The solution should be clear, and beads pelleted to the magnet before proceeding.
12. Slowly pipette off the cleared supernatant without disturbing the beads. Transfer supernatant to a new LoBind tube. Discard tube with beads.
13. **Recommended:** quality control checkpoint. Use 1 µL from each pool to measure the concentration and size distribution of the completed library.
14. Please note: elutions from the bead cleanup can be pooled together at this stage prior to proceeding to ABC in SMRT Link Sample Setup. The input library concentration into ABC must be <60 ng/µL for >10kb libraries, <20 ng/µL for 3–10 kb libraries, and <10 ng/µL for <3 kb libraries. Failure to dilute library prior to ABC may result in load loading.

PROTOCOL COMPLETE

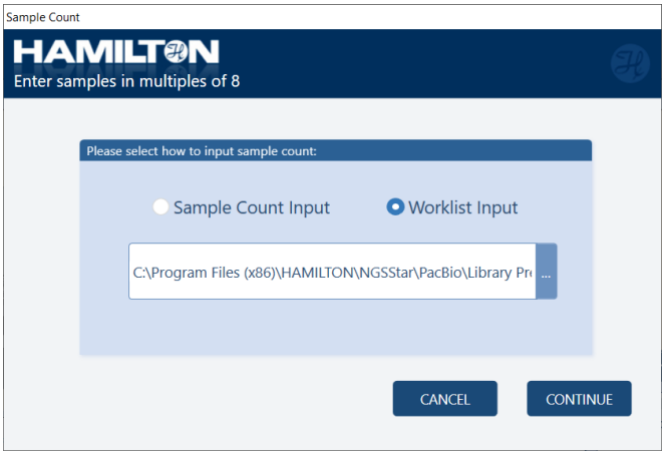
Appendix

Input file: Users can enter a .csv file containing sample tracking information. This file can be found in the path installed with instrument C:\Program Files (x86)\HAMILTON\NGSStar\PacBio\Library Prep\Files\Example Worklists. Download an example and edit the .csv file. Save to a known location.

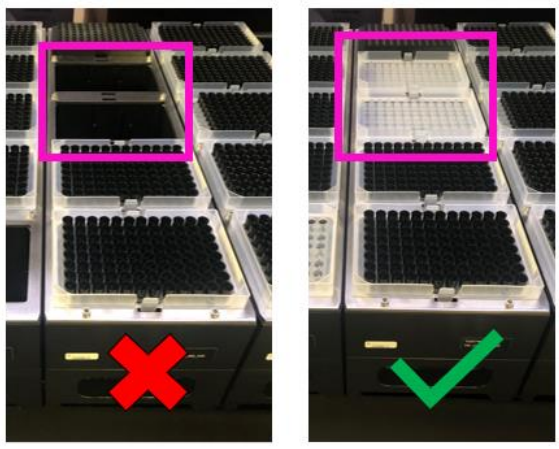
Note: When editing the file ensure sample count is a multiple of 8.

	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.



Empty tip racks: It is possible to have empty tip racks during tip selection. If leaving a tip rack empty, to prevent possible instrument crashes or incomplete liquid transfers for the MPH, place only the tip wafer in the tip carriers. An example of what not to-do (red x) and what to-do (green check mark) for empty tip rack selection is shown below.



Troubleshooting

Note: Beads transferred into sample plate eluate does not adversely affect the procedure, although can interfere with quanting accuracy. When measuring concentrations, place sample plate on a magnetic plate to avoid beads.

DNA Shearing

Issue	Possible cause	Corrective action
Shearing distribution not as expected	1. Concentration per well is more than 10 ng/µL	1 & 2. For shearing to be in the desired size distribution the concentration must be 10 ng/µL or less and the volume must be 300 µL per sample.
	2. Volume per well is not 300 µL	
	3. Quantification method	3. Be sure to use fluorescence for an accurate quant.

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