PacBio

Automated PureTarget™ kit 96 for the Hamilton NGS STAR MOA system

Guide & overview

PN 103-740-900 REV05 AUG2026

Introduction

This procedure describes the automated workflow for generating PureTarget libraries using the PureTarget kit 96 on the Hamilton NGS STAR MOA system. The kit is designed for 96 samples per automated run.

Overview

Overview	
Latest Hamilton script version	PacBio_PureTarget96_v3.2.7
Samples	96
Workflow step	**Automation run time**
gDNA Repair & SMRTbell® cleanup (Optional*)	2.5 hours
Dephosphorylation	1.5 hour
Cas9 digestion & SMRTbell cleanup	2 hours
dA-tail	0.5 hours
Adapter ligation, pool & SMRTbell cleanup	2 hours
Nuclease treatment 1, pool & SMRTbell cleanup	1 hour
Nuclease treatment 2 & SMRTbell cleanup	2 hours
PureTarget Library cleanup	1 hour
ABC	1 hour
Average total time	< 16 hours (including off-deck prep)
DNA input	
DNA input Quantity	1.0–1.5 µg in Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >8 on TapeStation system

* gDNA repair is required unless using the Nanobind® extracted blood genomic DNA using RBC lysis method or whole blood extraction.

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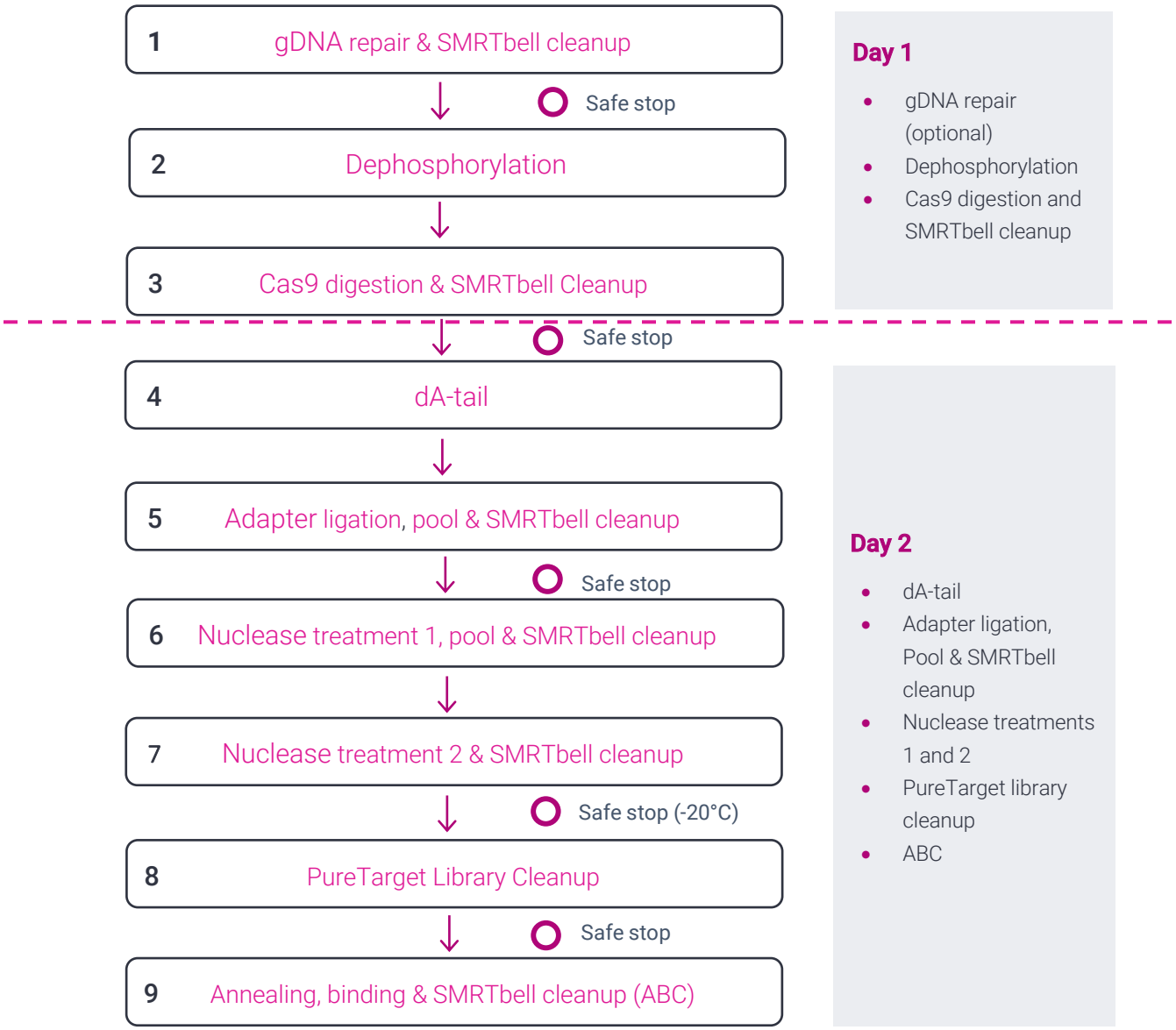
Workflow overview

The recommended automation workflow for PureTarget kit 96 with the Hamilton NGS STAR MOA is shown below.



Day 1

- gDNA repair (optional)
- Dephosphorylation
- Cas9 digestion and SMRTbell cleanup

Day 2

- dA-tail
- Adapter ligation, Pool & SMRTbell cleanup
- Nuclease treatments 1 and 2
- PureTarget library cleanup
- ABC

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Required materials and equipment

Consumables	Catalog Number
Hard Shell 96 well 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
20 mL Reagent Reservoir	Hamilton, 10161052
Comfort Lid	Hamilton, 814300
300 mL Reservoir	Agilent, 201244-100
2 mL Sarstedt Tubes	Sarstedt Inc, 72.694.306
2 mL Amber Tubes	Thermo Scientific, 03-390-27
MicroAmp Clear Adhesive Film	ThermoFisher Scientific, 00146104
1.5 mL DNA LoBind tubes	Eppendorf, 022431021
Equipment	**Catalog Number**
Hamilton NGS STAR MOA	Contact Hamilton representative
Vortex Mixer	Any major lab supplier (MLS)
Microcentrifuge	Any MLS
Qubit 4 or Qubit Flex Fluorometer	ThermoFisher Scientific, Q33238 (Qubit 4), Q33327 (Qubit Flex)
Recommended DNA sizing	**Catalog Number**
Femto Pulse System	Agilent Technologies, Inc. M5330AA

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Femto Pulse gDNA 165kb analysis kit	Agilent Technologies, Inc. FP-1002-0275
TapeStation system	Agilent Technologies, Inc. G2992AA or G2991BA
Genomic DNA ScreenTape Analysis	Agilent Technologies, Inc. 5067-5365/5366
Target enrichment and library preparation	**Catalog Number**
PureTarget™ repeat expansion panel 2.0 or PureTarget™ carrier panel or PureTarget™ control panel	PacBio® 103-633-100 PacBio® 103-633-200 PacBio® 103-633-300
PureTarget™ cleanup beads kit	PacBio® 103-633-000
PureTarget™ cleanup buffer kit	PacBio® 103-682-800
PureTarget™ kit 2.0	PacBio® 103-632-900*
HiFi plex prep kit 96	PacBio® 103-122-800*
SMRTbell® cleanup beads -10 mL (qty 2)	PacBio® 102-158-300*
Elution buffer	PacBio® 101-633-500*
SMRTbell® adapter index plate 96A (for barcoding) or SMRTbell® adapter index plate 96B (for barcoding) or SMRTbell® adapter index plate 96C (for barcoding) or SMRTbell® adapter index plate 96D (for barcoding)	PacBio® 102-009-200 PacBio® 102-547-800 PacBio® 102-547-900 PacBio® 102-548-000
Revio SPRQ™ polymerase kit or Vega™ SPRQ polymerase kit	PacBio® 103-496-900 PacBio® 103-729-600
200 Proof ethanol, molecular biology or ACS grade	Any MLS
Nuclease-free (NF) water	Any MLS
Qubit 1X dsDNA HS assay kit	ThermoFisher Scientific, Q33230

*Sold together as part of the PureTarget™ kit 96 bundle

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General Best Practices

DNA input

For optimal performance, this protocol recommends high-quality, high molecular weight (HMW) human gDNA with at least 50% of the mass of DNA in molecules at ≥30 kb in length (genome quality number (GQN) of ≥5 at 30 kb) based on the Agilent Femto Pulse system, or a DIN value >8 on the Agilent TapeStation. For low-quality human gDNA, it is still feasible to proceed, but lower coverage is expected.

Note: Removal of RNA with RNAse is required for any DNA extraction method used. Failure to remove RNA may result in sequencing inhibition.

The supported sample types are:

- **Human blood genomic DNA**: extracted by Nanobind® RBC lysis PanDNA kit, Nanobind Whole blood manual CBB kit/PanDNA kit, Nanobind HT 200 µL protocol, Nanobind HT 1 mL protocol
- **Human B-lymphocyte cell lines**: extracted by Nanobind CBB kit/ PanDNA kit, Nanobind CBB HT; Coriell cell line gDNA (GQN_{30kb} ≥5).

This protocol supports 1–1.5 µg per sample. 1.3–1.5 µg per sample is highly recommended for samples going through the gDNA repair step to ensure sufficient mass to achieve optimal target coverage. A maximum mass of 150 µg of gDNA input (across 96 samples) is recommended for a single (Revio or Vega system + SPRQ/SPRQ-Nx chemistry) SMRT® Cell.

For information on the performance of unsupported samples (saliva and other extraction methods), see the PureTarget repeat expansion Application note. Note that saliva gDNA requires an additional upfront cleanup for optimal performance. A Hamilton NGS Star MOA script is available (**PacBio PureTarget 3.1x AMPure® cleanup for Saliva**). Reach out to your local Hamilton representative for installation.

Multiplexing samples

Revio or Vega system with SPRQ/SPRQ-Nx chemistry

Each PureTarget kit 96 supports the preparation of 96 samples using the automation protocol.

Safety precautions

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

Reagent and sample handling

- For a quick guide on master mix preparation and plating, see Appendix.
- Room temperature is defined as any temperature in the range of 18–23°C for this protocol.
- Do not vortex enzymes.
- Bring SMRTbell cleanup beads to room temperature. Always vortex immediately prior to use.
- Bring Qubit reagents to room temperature prior to use.

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- Thaw frozen reagents at room temperature. Place on ice after thawing.
- Keep master mixes involving temperature-sensitive reagents on ice until use.
- Quick-spin all reagents in a microcentrifuge to collect liquid at the bottom prior to use.
- Samples can be stored at the specified temperature at the safe stopping points listed in the protocol.

Library preparation reagents

Kits stored at -20°C.

PureTarget kit 2.0		
Step used	**Tube**	**Reagent**
Dephosphorylation	Blue	Phosphatase
Cas9 digestion	Green	Cas9 Nuclease
Dephosphorylation/Cas9 digestion	Red	Cas9 buffer
dA tailing/Nuclease treatment	Orange	dA tail buffer
dA tailing	Violet	*Taq* DNA Polymerase
dA tailing	Yellow	dATP
Nuclease treatment	White	Pure Target Nuclease mix
PureTarget panel		
Cas9 digestion	White	Repeat expansion panel 2.0 *or*
	Purple	Carrier panel *or*
	Orange	Control panel
HiFi plex prep kit 96		
gDNA repair	Purple	Repair buffer M96
gDNA repair	Blue	End repair mix M96
gDNA repair	Green	DNA repair mix M96
Adapter ligation	Yellow	Ligation mix M96
Adapter ligation	Red	Ligation enhancer M96

Nuclease treatment	Light green	Nuclease mix M96
SMRTbell adapter index plate		
Adapter ligation	White plate	SMRTbell adapter index plate 96A, 96B, 96C, or 96D

PureTarget cleanup Bead Kit

Keep at room temperature.

Component	Tube color
PureTarget cleanup wash buffer*	Bottle, white
PureTarget cleanup binding buffer	Bottle, white
PureTarget cleanup beads	Clear

*Important: Prior to the 1st use of the PureTarget cleanup beads kit, add 15 mL of 200 proof ethanol to PureTarget cleanup wash buffer and mix well. Ensure bottle cap is tightly screwed on and store bottles upright to prevent leakage.

PureTarget cleanup buffer Kit

Keep at room temperature.

Component	Tube color
PureTarget cleanup buffer 1	Red
PureTarget cleanup buffer 2	Blue
PureTarget cleanup buffer 3	Green

Revio or Vega SPRQ polymerase kit

Kit stored at -20°C.

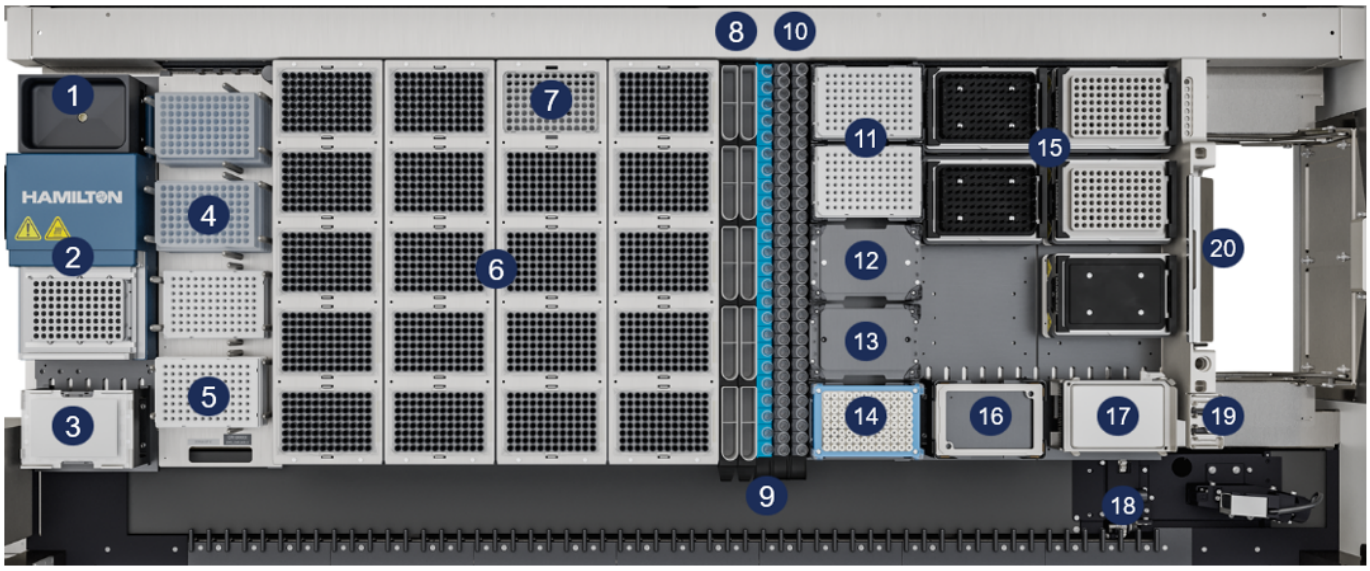
Note: Bring the Loading buffer to room temperature prior to use. The Loading buffer is light sensitive and should be protected from light when not in use.

Component	Tube color
Annealing buffer	Light blue
Standard sequencing primer	Light green
Polymerase buffer	Yellow
Loading buffer	Green
Dilution buffer	Blue
Sequencing polymerase	Purple

Sequencing control	Red

Note: The Revio SPRQ polymerase kit supplies reagent volumes sufficient for 12 SMRT Cells for PureTarget. The Vega SPRQ polymerase kit supplies reagent volumes sufficient for 4 SMRT Cells for PureTarget.

Hamilton NGS STAR MOA Deck



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

20. Solid/Liquid waste for the 1000µL channels

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

Quality checks

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

Protocol Step	DNA or SMRTbell step recovery	DNA or SMRTbell overall recovery
Starting Input	100%	100%
Post-gDNA repair	70-90%	70-90%
Post-Cas9 SMRTbell bead cleanup	50-80%	40-70%
Post-ligation SMRTbell bead cleanup*	60-80%	30-60%
Post-nuclease cleanup 1	0.1-4%	0.1-2%
Post-nuclease cleanup 2	1-20%	0.01-0.1%
Post- PureTarget library cleanup	40-60%	0.005-0.05%
Post-ABC cleanup	70-90%	0.005-0.05%

* In post-ligation SMRTbell bead cleanup, make a 1:10 dilution by taking 1 µL aliquot from each sample and dilute with 9 µL of Elution buffer.

Workflow steps

Preparation of the Hamilton NGS STAR MOA System:

- Manually set CPAC (Cold Plate Air Cooled device) to 4°C before prior to the run to prevent delays when the run is initiated.
- The automation run is designed for and requires 96 samples.
- Instrument prompts will provide reagent volumes and will indicate where and when to load based on start and stop process selected at the beginning of the run.

Day 1: gDNA Repair to Cas9 digestion and SMRTbell cleanup

1. **Prepare gDNA sample plate for gDNA Repair.** Pipette gDNA diluted with Elution buffer, according to the table below, into a 96-well **PCR** plate (Bio-Rad, HSP9601). For Nanobind extracted blood (whole blood or RBC lysis method) gDNA, gDNA Repair (module 1) **can** be skipped.

Description	Quantity
Sample volume per well	30 µL
DNA concentration	33 - 50 ng/µL

2. **Prepare reagents and consumables.** Gather the following reagents and consumables.

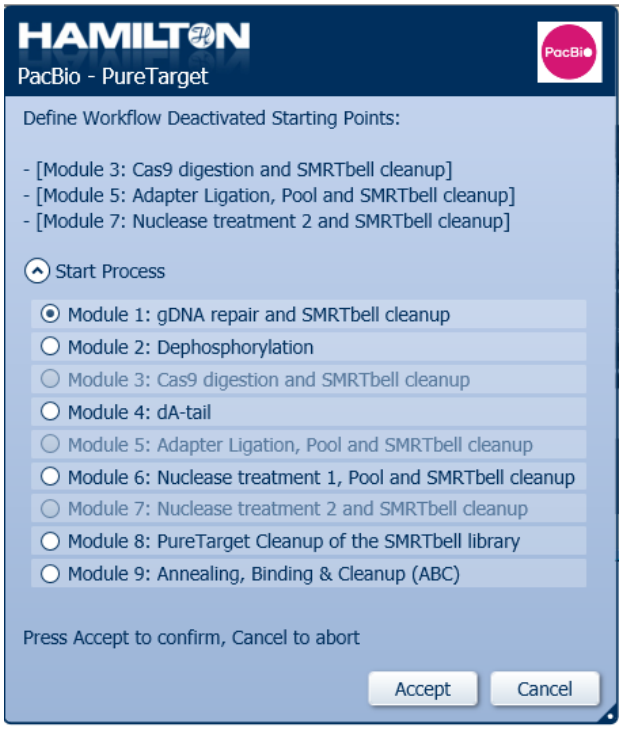
Component	Labware
SMRTbell beads	20 mL reservoir
Elution buffer	20 mL reservoir
Reagent plate	96 well PCR plate
Sample input plate	96 well PCR plate
3 empty plates stacked	96 well PCR plate
80% ethanol	300 mL reservoir
gDNA repair master mix	2 mL tube – for master mix prep only
Dephosphorylation master mix	2 mL tube – for master mix prep only
Cas9 master mix	2 mL tube – for master mix prep only
3 ODTC lids stacked	Comfort Lid

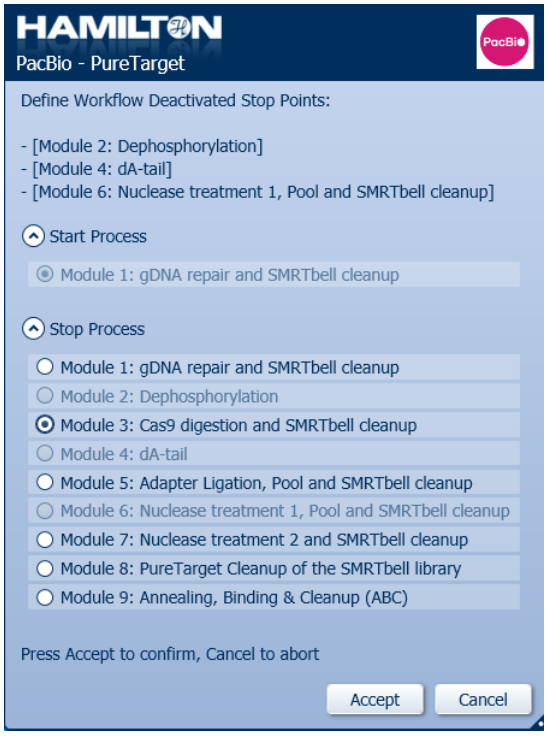
3. Start the PacBio method "**PacBio_PureTarget96_v3.2.7**".

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

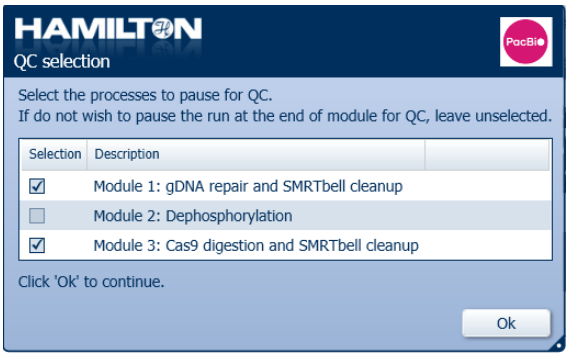
5. **Define workflow.** Select the start process at "**Module 1: gDNA Repair and SMRTbell cleanup**" and click "Accept". Select the stop process at "**Module 3: Cas9 digestion and SMRTbell cleanup**" and click "Accept".

Note: Only designated safe Starting and Stopping points can be selected.



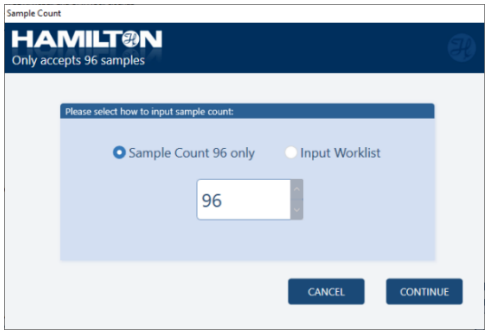


6. **QC selection.** Quality checks are optional. Select the checkbox for modules where you would like the run to pause for quality control checks. Modules that are not designated as safe stopping points will appear disabled and cannot be selected. If any of the boxes are left unselected, the method will continue directly to the next module without pausing.



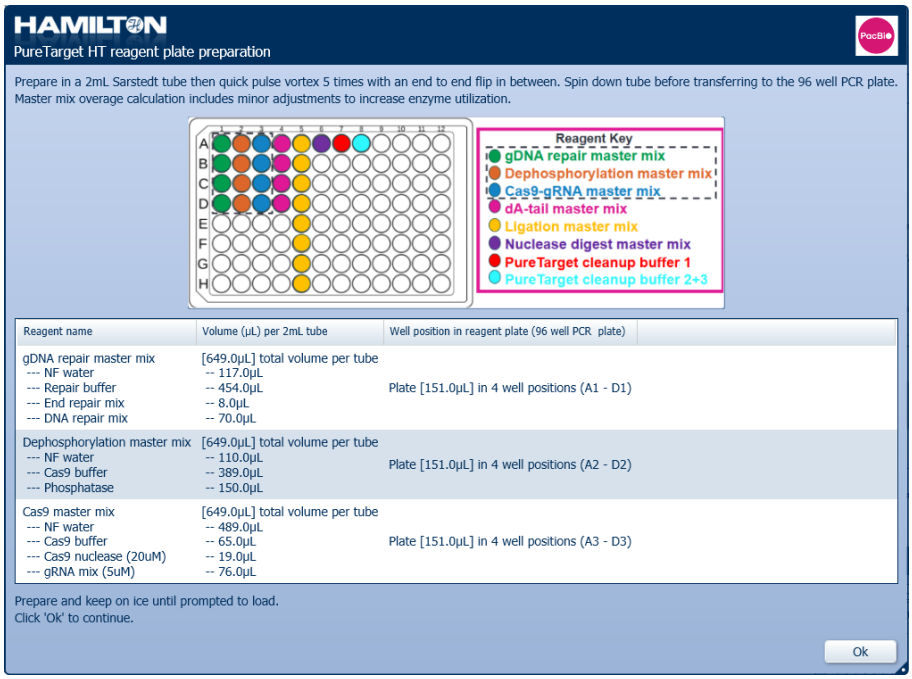
7. **Sample count selection.** There are two ways to enter the sample count:

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



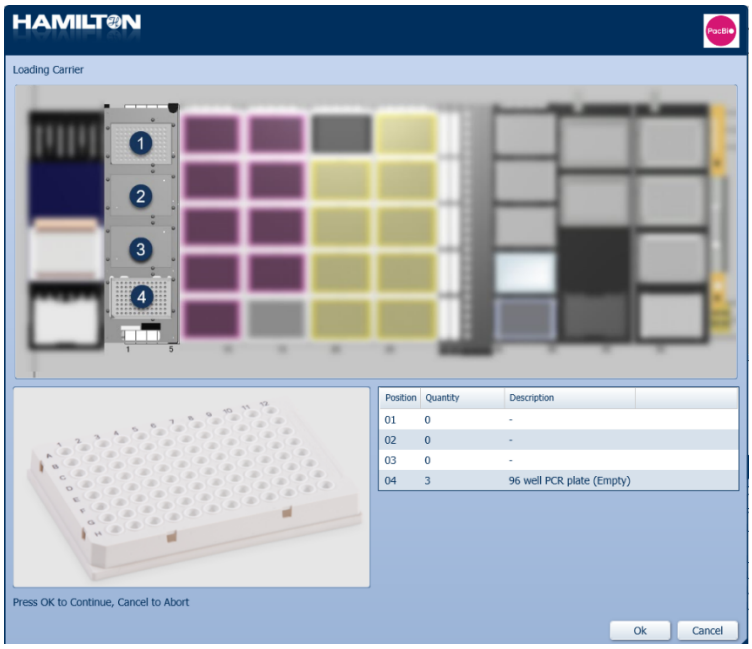
8. **Prepare and plate reagents.** Prepare the reagents according to the provided instructions in 2 mL tubes. For master mixes, pulse vortex 5 times. Spin down the tube, then transfer the contents to the reagent plate in the positions specified in the reagent chart.

   Plate the reagents specified in the chart into a 96-well PCR plate (Bio-Rad, HSP9601). Seal the plate using a clear adhesive PCR film and spin down the plate. Keep the reagent plate on ice until ready to load onto the instrument.



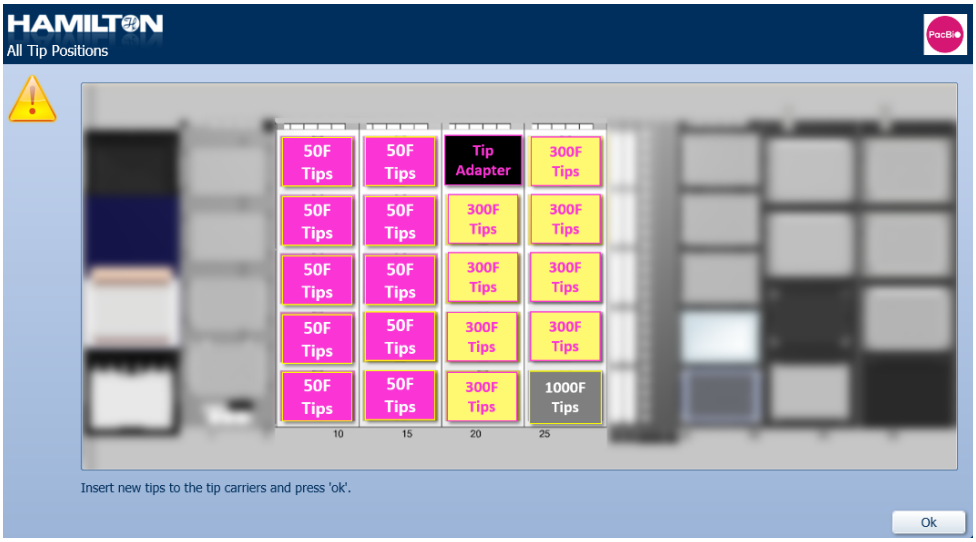
9. **Stack and load the Comfort Lids.** Place clean Comfort Lids on the On Deck Thermal Cycler (ODTC) lid parking position 3. If the quantity is more than 1, stack them neatly on top of each other. Make sure position A1 is in the upper left-hand corner.

10. **Stack and load plates.** Place new 96 well PCR plates onto the plate stacker in position 4. When the quantity is more than 1, stack them neatly on top of each other. Make sure position A1 is in the upper left-hand corner.



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

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



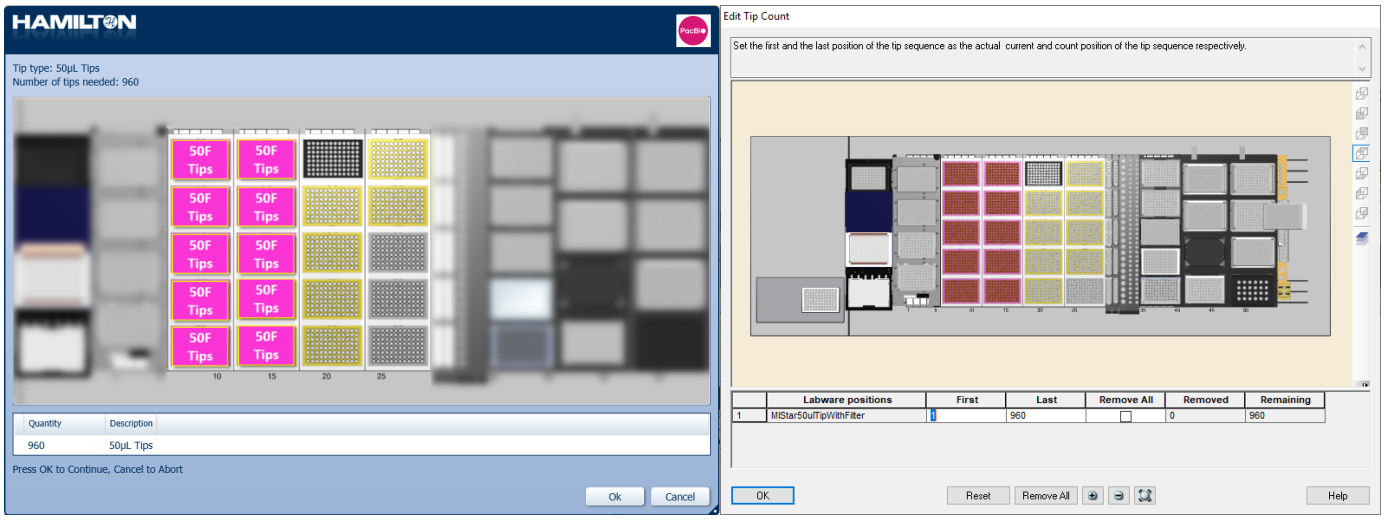
13. **Load the tips and ensure the tip count matches what is on the deck.** Load the 50 µL, 300 µL, and 1000µL tips into their designated positions on the instrument. The tip type to load is under "**Description**" and the number of required tips is under "**Quantity**".

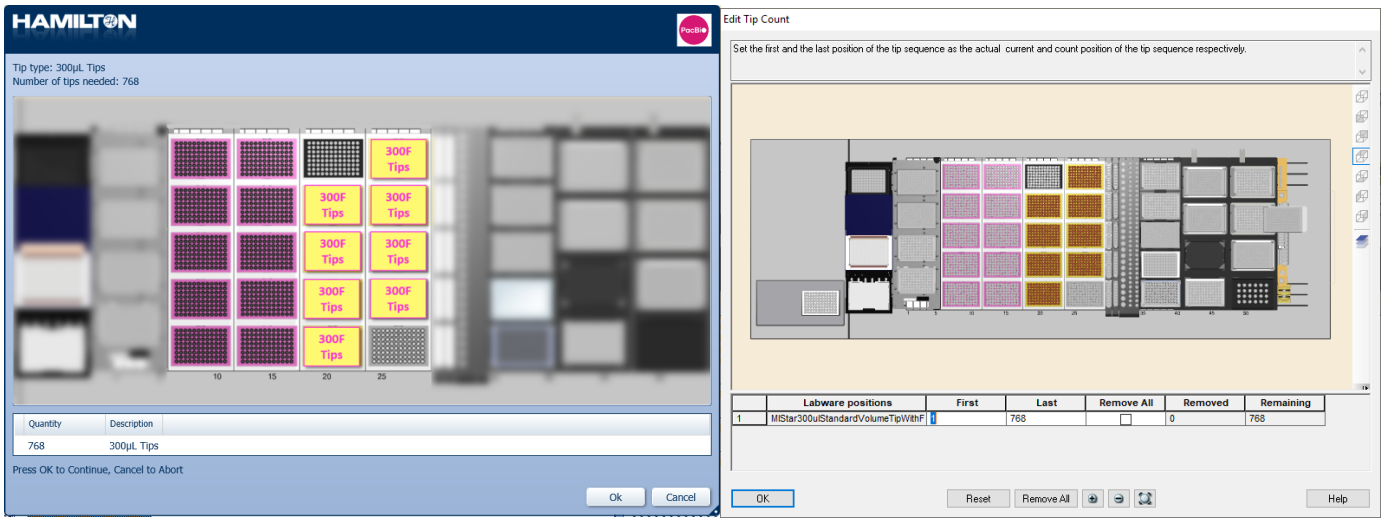
    Click 'Ok" to continue to tip deck matching display. To match tips to the deck, click on the corresponding tip positions so they are highlighted. Once the selections match the deck layout, click "OK" to continue.

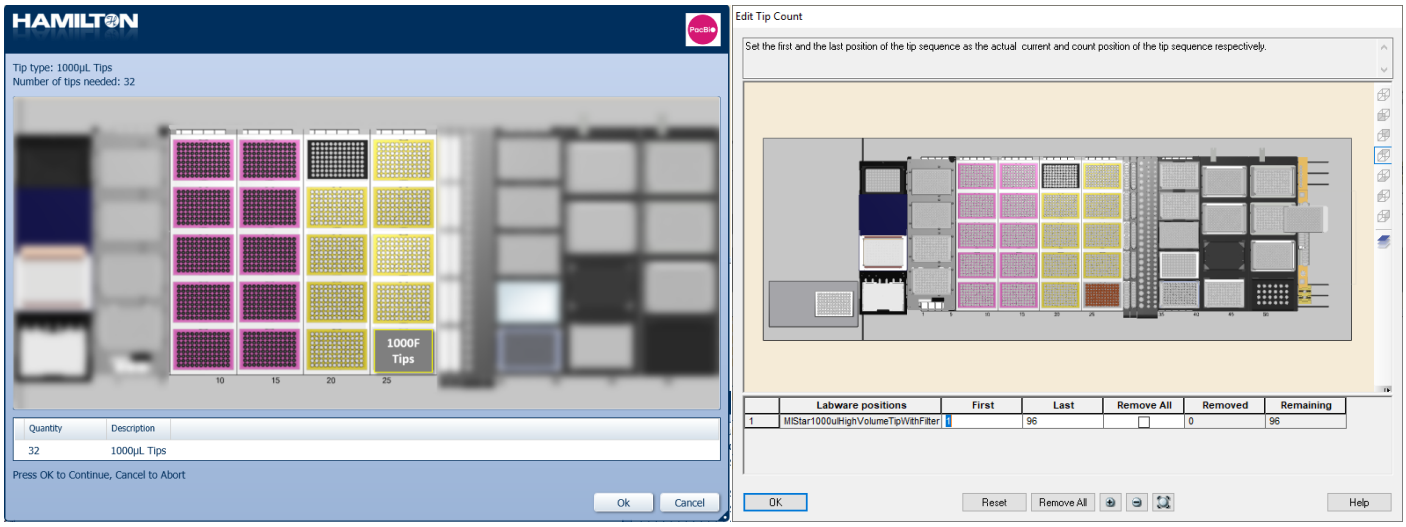
Note: If tips run out during the run, the system will pause and prompt for a refill.

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

Labware	Quantity
50 µL filtered conductive tips	960
300 µL filtered conductive tips	768
1000 µL filtered conductive tips	32







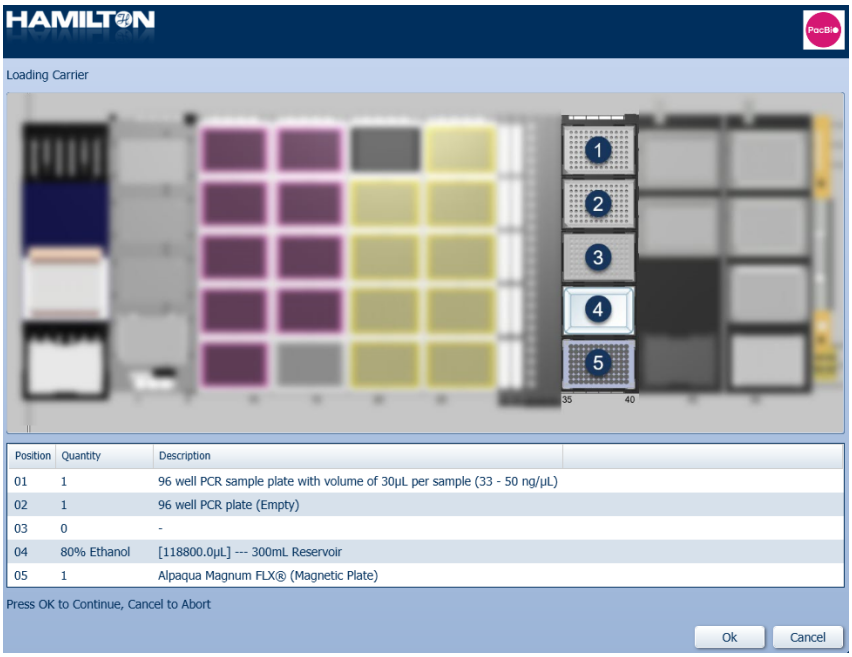
14. **Load 20 mL reservoirs in the reagent trough carrier.** Place the 20 mL reservoirs containing their respective reagents into the reagent trough carrier located in track 31. Refer to the table below for the SMRTbell cleanup bead and Elution buffer volumes to load onto the instrument. SMRTbell cleanup beads should be fully resuspended before adding to the reservoir.

Reagent	Reagent amount (µL)
SMRTbell Cleanup beads	10,148
Elution buffer	9,902

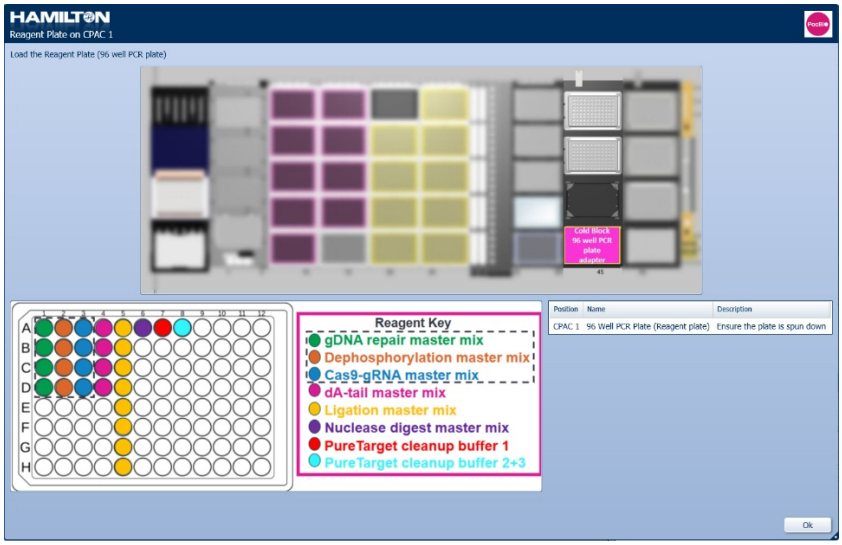
15. **Load the plate processing carrier.** Load the sample input plate, a new 96-well PCR plate for elution, and an 80% ethanol reservoir onto the carrier in the positions shown in the loading prompt. Fill the 300 mL reservoir with the volume of 80% ethanol specified in the table. Ensure the sample input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

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

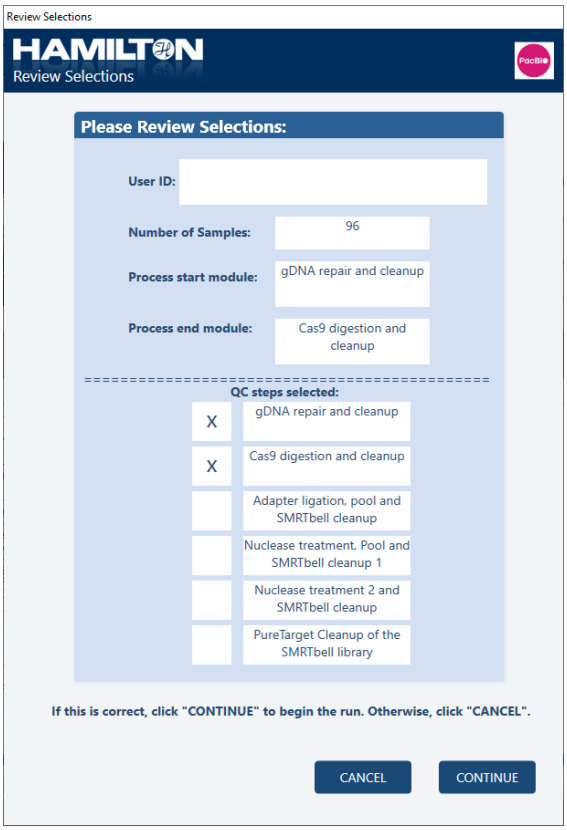
Reagent	Reagent amount (µL)
80% ethanol	118,800



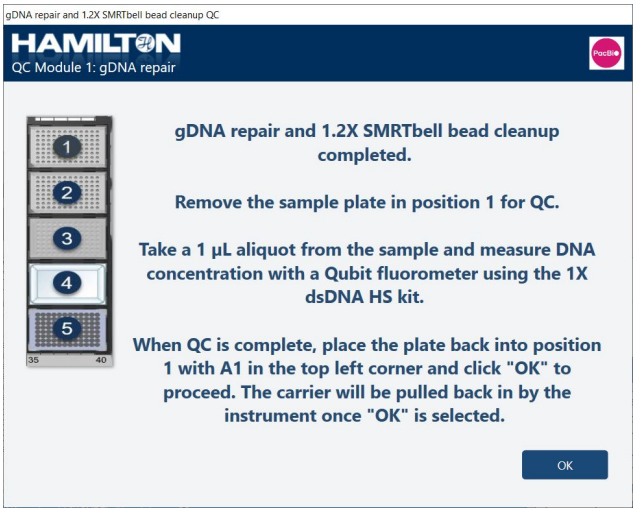
16. **Load the reagent plate.** Spin down the reagent plate to ensure there are no bubbles and place it on CPAC 1 once it has reached 4°C.



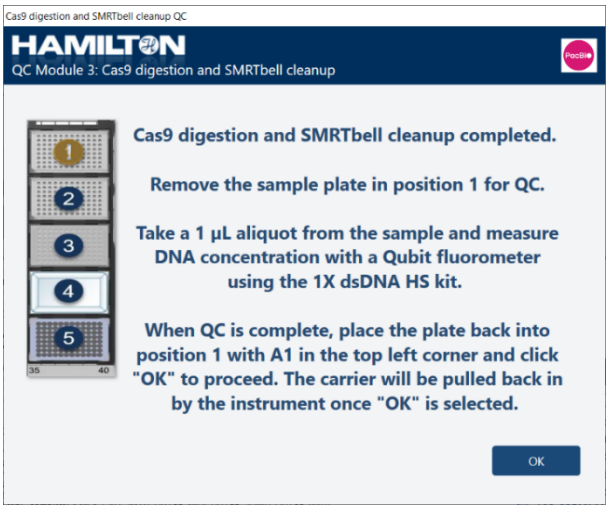
17. **Review selections.** The example prompt is configured for the recommended Day 1 workflow (see *Workflow Overview*). Click CONTINUE to begin the run at Module 1: gDNA repair and SMRTbell cleanup. If a module is selected for QC, an "X" will appear in the box next to it and the run will pause at the end of the selected modules.



18. **Optional quality checks.** In Day 1, if the quality check for modules 1 or module 3 were selected, quant samples with the Qubit 1x dsDNA HS assay. For guidance refer to the section Quality Checks.



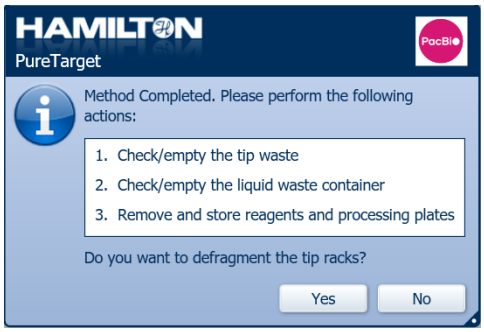
QC prompt for gDNA repair & SMRTbell cleanup



QC prompt for Cas9 digestion & SMRTbell cleanup

19. **Cas9 digestion and SMRTbell cleanup complete.** At the end of Cas9 digestion and SMRTbell cleanup, the sample output plate is in position 1 on the plate carrier.

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



Day 2: dA-tail to PureTarget cleanup of the SMRTbell library & ABC

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

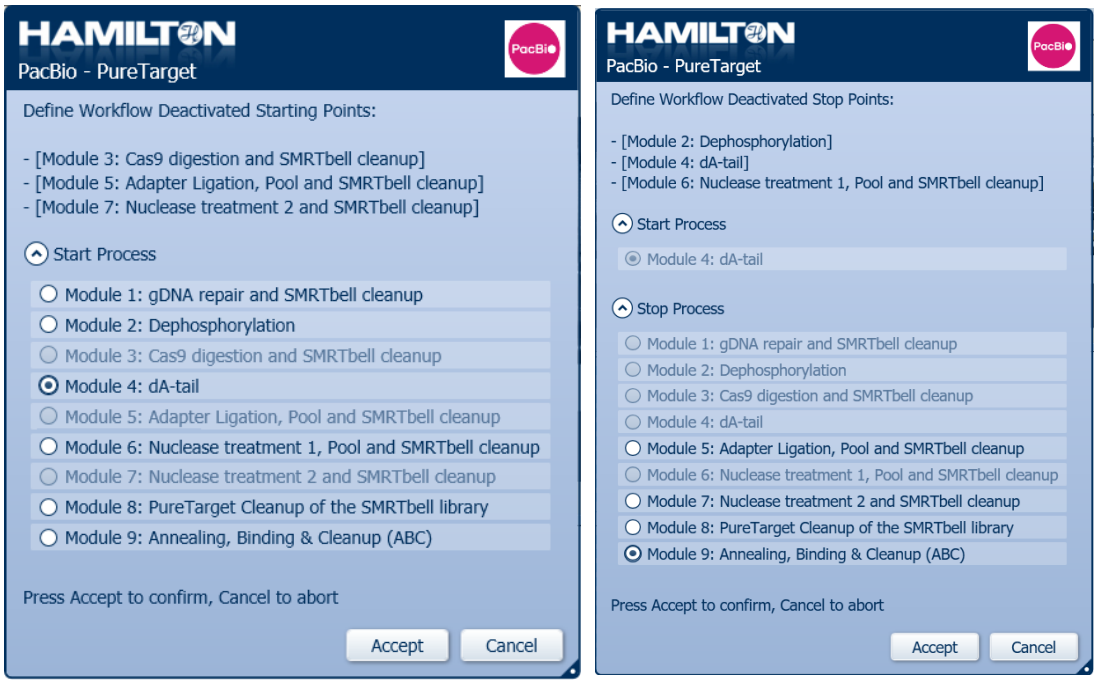
Component	Labware
Sample input plate	96 well PCR plate
Reagent plate	96 well PCR plate
1 empty plate	96 well PCR plate
Empty deep well plate	96 Well 0.8 mL Polypropylene Deepwell Storage Plate
SMRTbell cleanup beads	20 mL reservoir & 2 mL Sarstedt tube
Elution buffer	20 mL reservoir
80% ethanol	300 mL reservoir
SMRTbell adapter index plate	96 well PCR plate
dA-tail master mix	2 mL Sarstedt tube – for master mix prep only
Ligation master mix	2 mL Sarstedt tube – for master mix prep only
Nuclease treatment master mix	2 mL Sarstedt tube – for master mix prep only
PT cleanup bead binding master mix	2 mL Sarstedt tube
PT cleanup wash buffer	2 mL Sarstedt tube
Loading buffer	2 mL amber tube
Centrifugation tube	1.5 mL LoBind tube
4 ODTC lids stacked	Comfort Lid

2. Start the PacBio method "**PacBio_PureTarget96_v3.2.7**".

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

4. **Define workflow.** Select the start process at **"Module 4: dA-tail"** and click "Accept." Select the stop process at **"Module 9: ABC"** and click "Accept."

   Note: Only designated safe Starting and Stopping points can be selected.

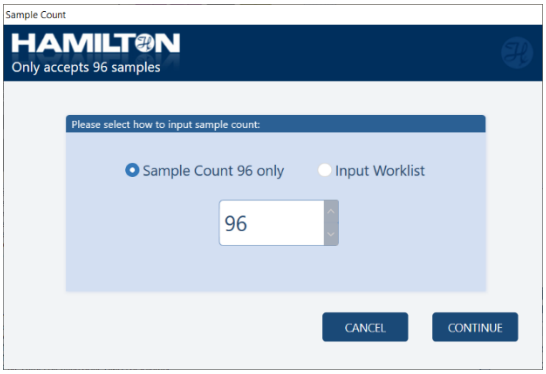


5. **QC selection.** Quality checks are optional. Select the checkbox next to any modules where you would like the run to pause for quality control checks. Modules that are not designated as safe stopping points will appear disabled and cannot be selected. If any of the boxes are left unselected, the method will continue directly to the next module without pausing.

6. **Sample count selection.** There are two ways to enter the sample count:

   (1) In “Sample count input” enter 96. Only 96 samples are compatible with this workflow.

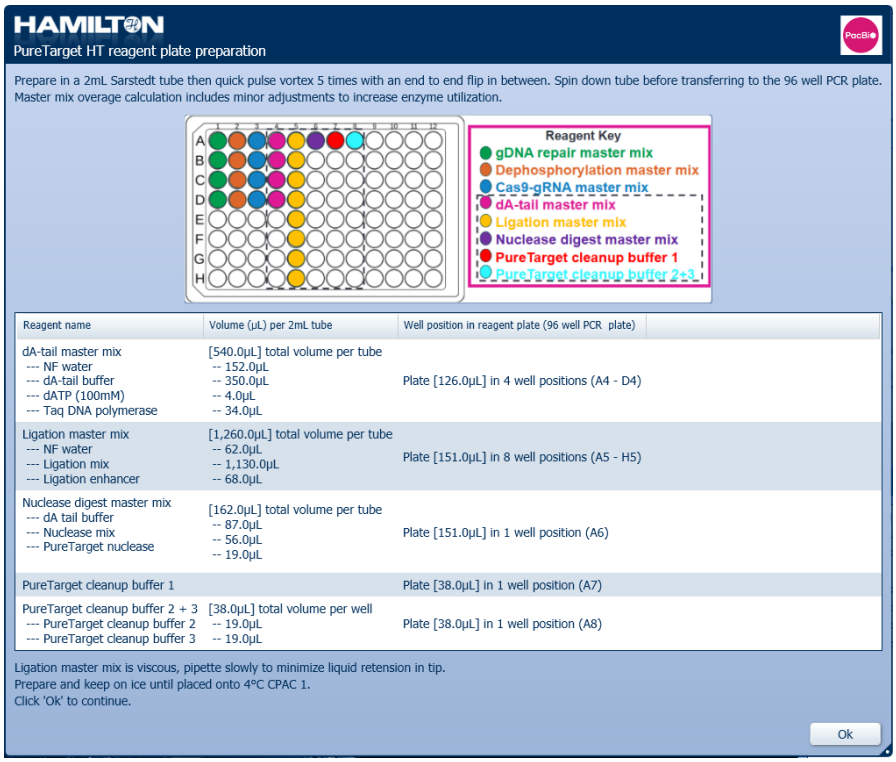
   (2) In “Worklist input,” a worklist input file can be uploaded for sample tracking. See the Appendix for instructions.



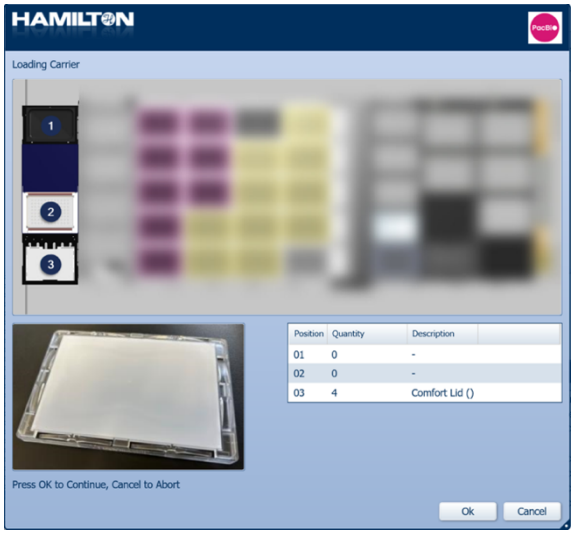
7. **Prepare and plate reagents.** Prepare the reagents according to the provided instructions in 2 mL tubes. For master mixes, pulse vortex 5 times. Spin down the tube and transfer the contents to the reagent plate in the positions specified in the reagent chart.

   Plate the reagents specified in the chart into a new 96-well PCR plate. Seal the plate using a clear PCR adhesive film and spin down the plate. Keep the reagent plate on ice until ready to load onto the instrument.

   Note: ABC reagents are prepared at the start of ABC. Do not prepare and store ABC reagents beforehand.

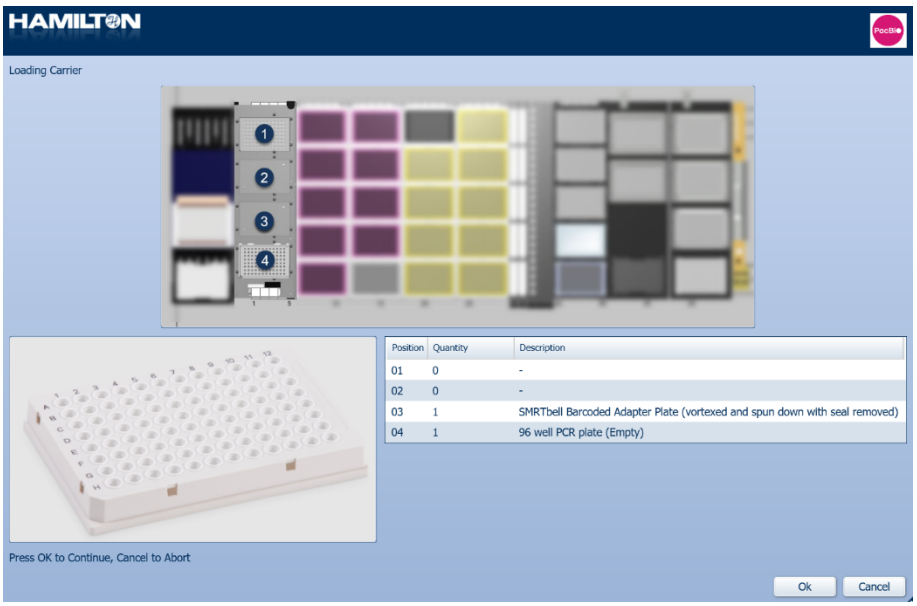


8. **Stack and load the Comfort Lids.** Place clean Comfort Lids in the On Deck Thermal Cycler (ODTC) lid parking position 3. If the quantity is more than 1, stack them neatly on top of each other. Make sure position A1 is in the upper left-hand corner.



9. **Stack and load plates.** Place new 96 well PCR plates into the plate stacker in position 4. When the quantity is more than 1, stack them neatly on top of each other. Ensure that position A1 is in the upper left-hand corner. **Vortex and spin down the SMRTbell barcoded adapter plate**. Unseal and place into position 3.

Note: After the SMRTbell barcoded adapter plate is used the instrument places it back into the stacker.



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

11. **Tip deck layout.** A prompt displaying the tip positions will appear, including the tip support adapter position. There are four 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 1000 µL filtered conductive tips.

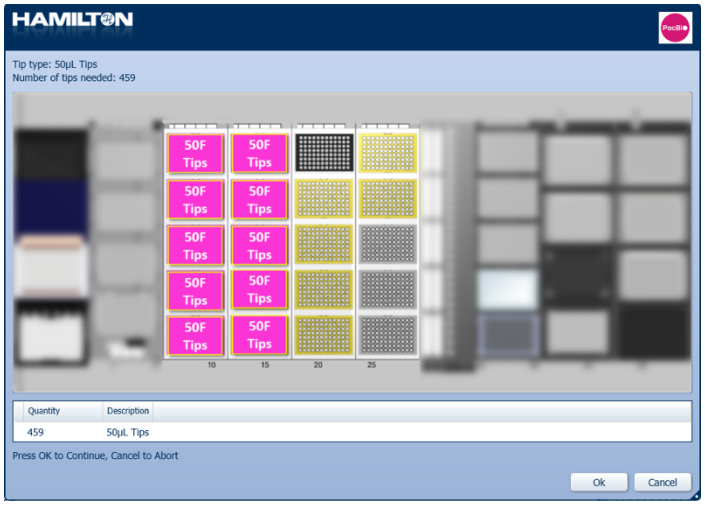
12. **Load the tips and ensure the tip count matches what is on the deck.** Load the 50 µL, 300 µL, and 1000µL tips into their designated positions on the instrument. The tip type to load is under "**Description**" and the number of required tips is under "**Quantity**".

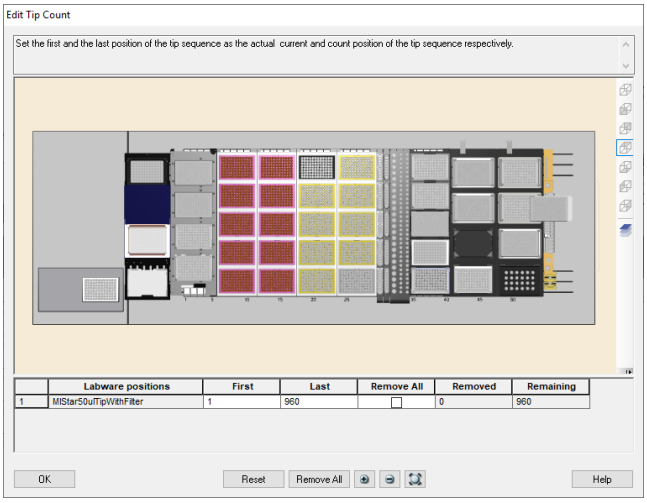
    Click 'Ok" to continue to tip deck matching display. To match tips to the deck, click on the corresponding tip positions so they are highlighted. Once the selections match the deck layout, click "OK" to continue.

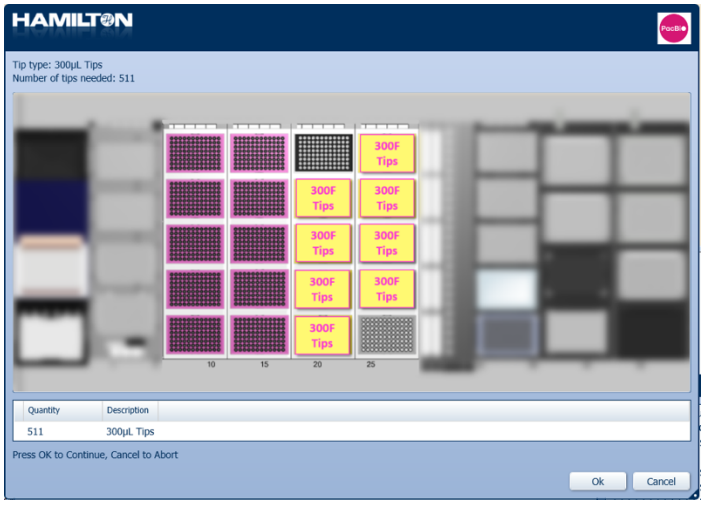
Note: If tips run out during the run, the system will pause and prompt for a refill.

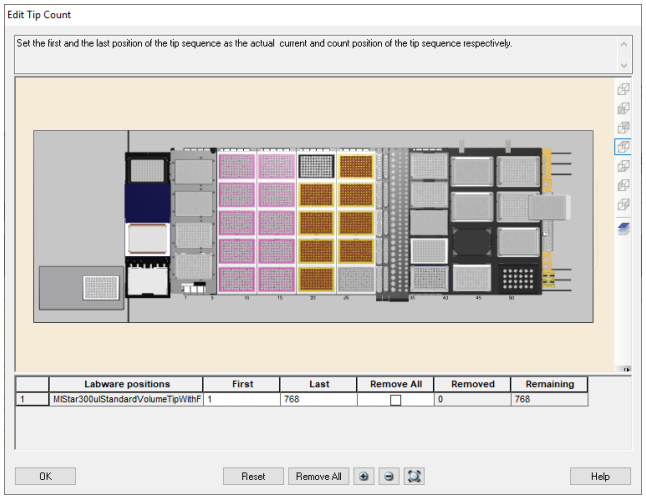
Note: It is critical that these selections match exactly what is loaded on the deck. Inaccurate tip selections may cause collisions or sample failure.

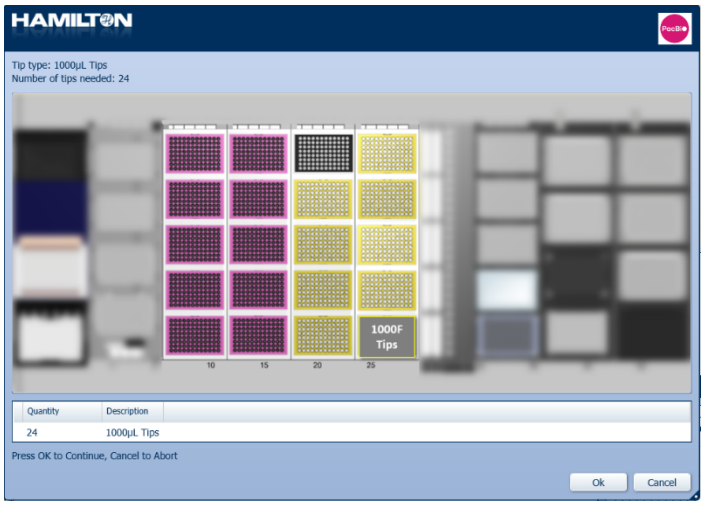
Labware	Quantity
50 µL filtered conductive tips	550
300 µL filtered conductive tips	416
1000 µL filtered conductive tips	24

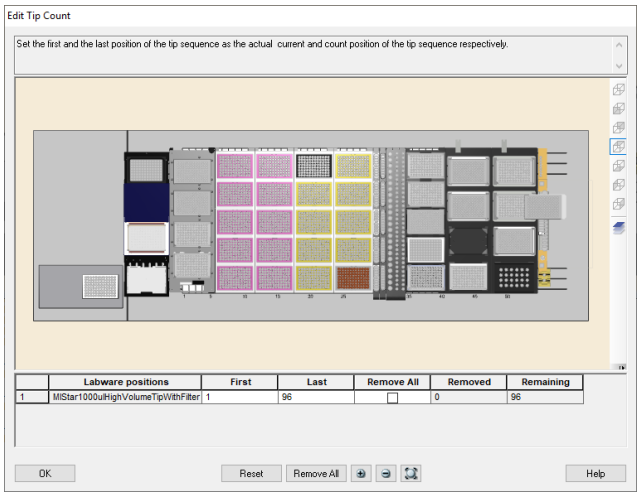






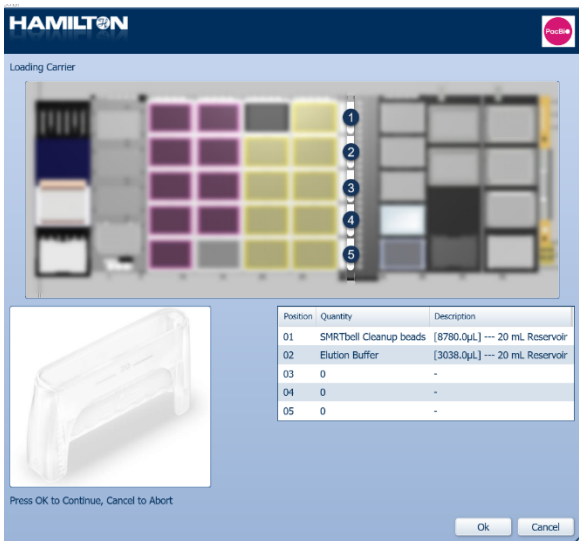




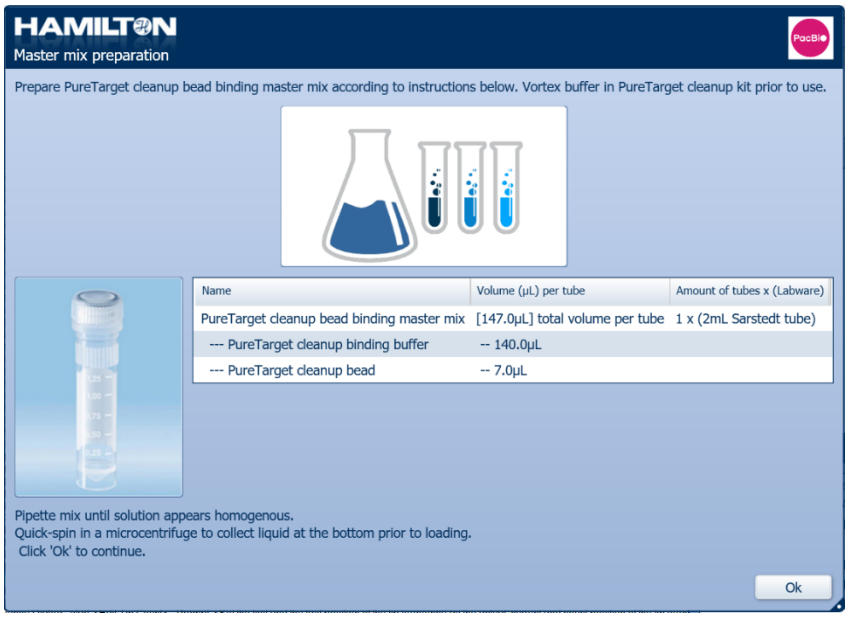


13. **Load 20 mL reservoirs in the reagent trough carrier.** Place the 20 mL reservoirs containing their respective reagents into the reagent trough carrier located in track 31. Refer to the table below for the SMRTbell cleanup bead and Elution buffer volumes to load onto the instrument. SMRTbell cleanup beads should be fully resuspended before adding to the reservoir.

Reagent	Reagent amount (µL)
SMRTbell Cleanup beads	8,780
Elution buffer	3,038

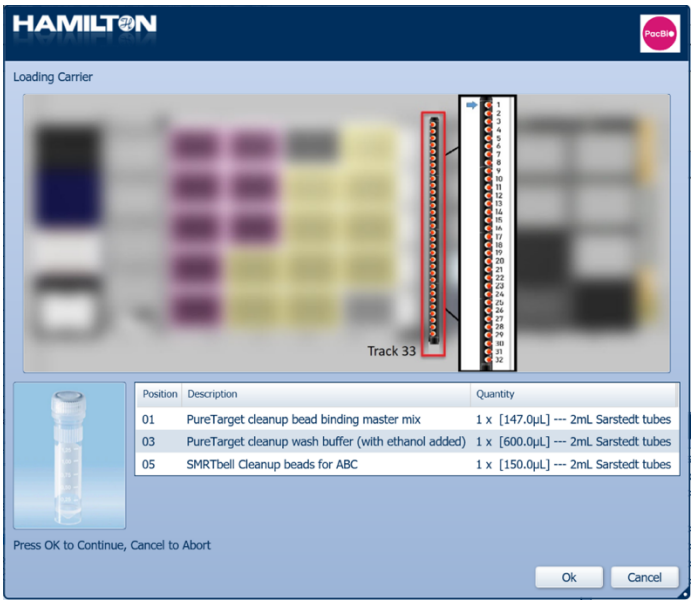


14. **Load PureTarget cleanup buffer reagents.** Prepare the PureTarget cleanup bead binding master mix in a 2 mL Sarstedt tube. Pulse vortex and quick spin. Remove the cap and load the 2 mL Sarstedt tube into the tube carrier in track 33.



Note: When opening a new PureTarget cleanup kit add 15 mL of 200 proof ethanol to the wash buffer and mix well.

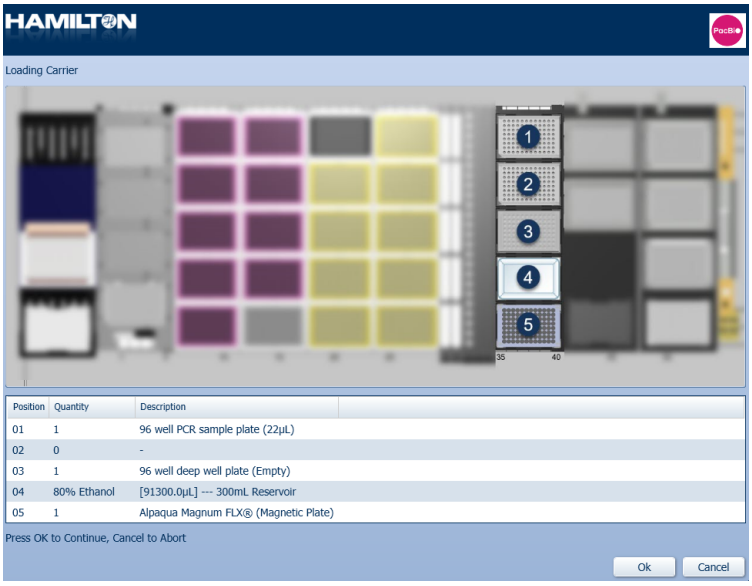
Reagent	Reagent amount (µL)
PureTarget cleanup bead binding master mix	147
PureTarget cleanup wash buffer (with ethanol added)	600
SMRTbell cleanup beads for ABC	150



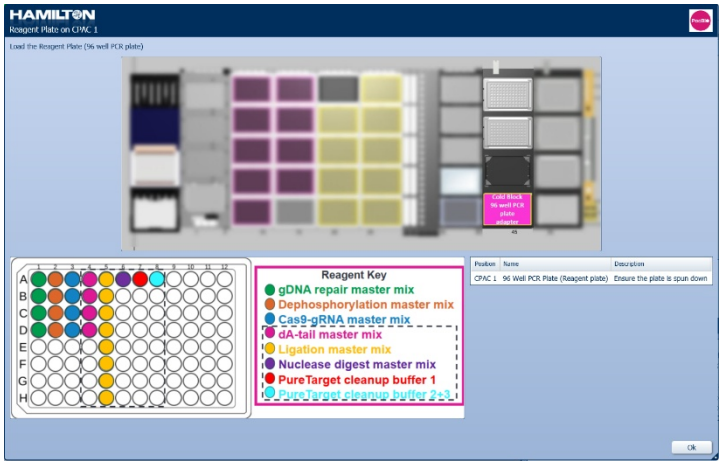
15. **Load the plate processing carrier.** Load the sample input plate from Day 1, a new 96 deep well plate and 80% ethanol reservoir onto the carrier into the positions shown in the loading prompt. Fill the 300 mL reservoir with 80% ethanol in the amount shown in the table.

    Note: The Alpaqua Magnum FLX magnetic plate is in position 5.

Reagent	Reagent amount (µL)
80% ethanol	91,300

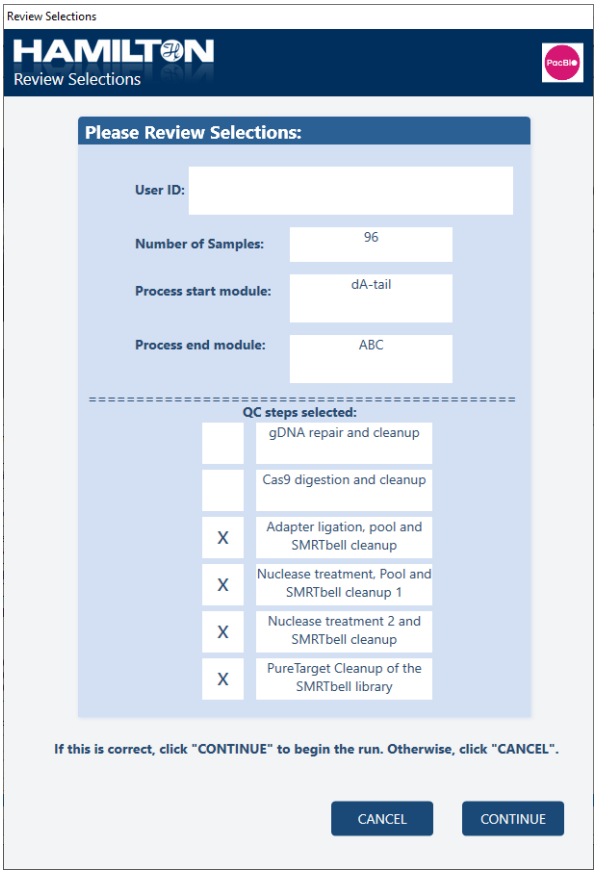


16. **Load the reagent plate.** Spin down and place the reagent plate on CPAC 1 once it reached 4°C.

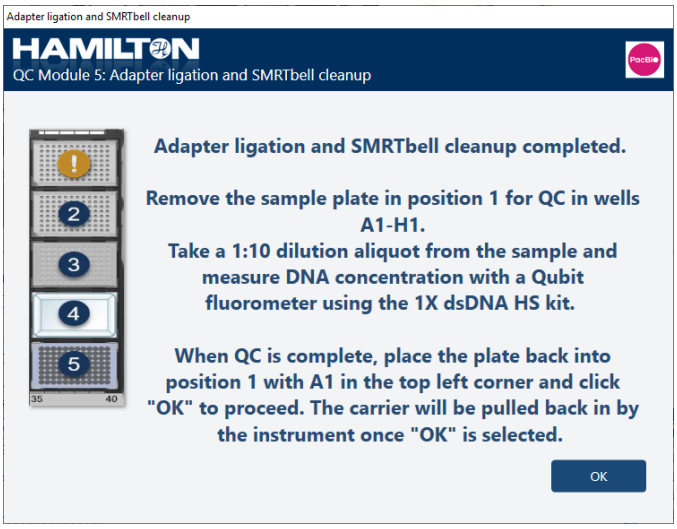


17. **Review selections.** The example prompt is configured for the recommended Day 2 workflow (see *Workflow Overview*). Click CONTINUE to begin the run at dA-tail. If a module is selected to for QC, an "X" will appear in the box next to it and the run will pause at the end of the selected modules.

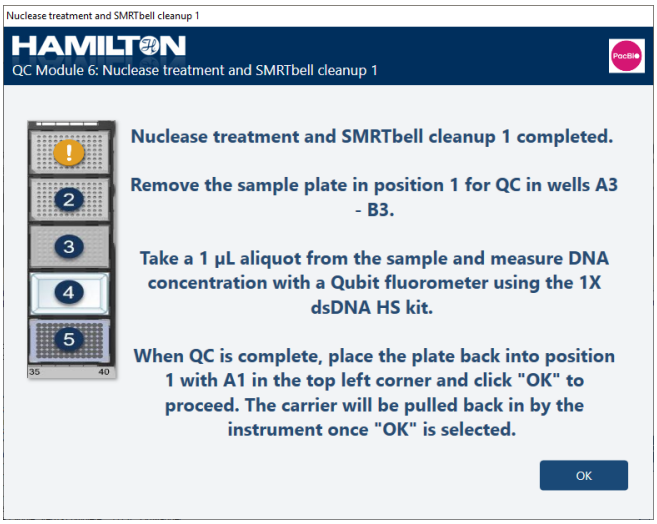
18.



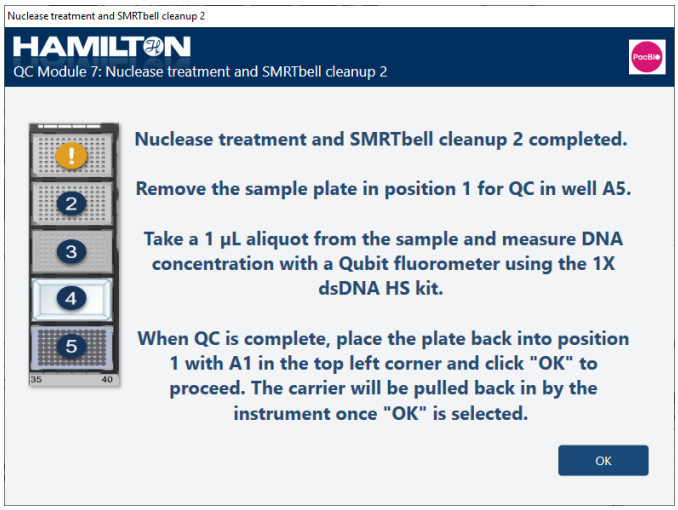
19. **Optional quality checks.** In Day 2, if the quality check for modules 5, 6, 7 or 8 were selected, quant samples with the Qubit 1x dsDNA HS assay. For guidance refer to the Quality Checks.



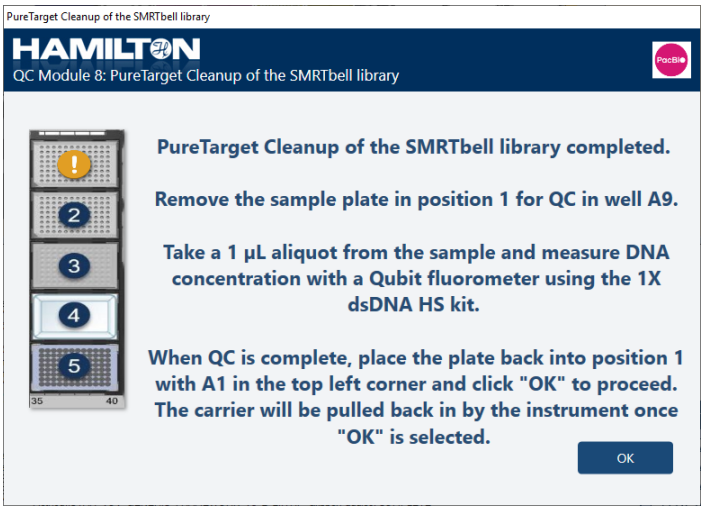
QC prompt for Adapter ligation & SMRTbell cleanup



QC prompt for Nuclease treatment & SMRTbell cleanup 1



QC prompt for Nuclease treatment & SMRTbell cleanup 2

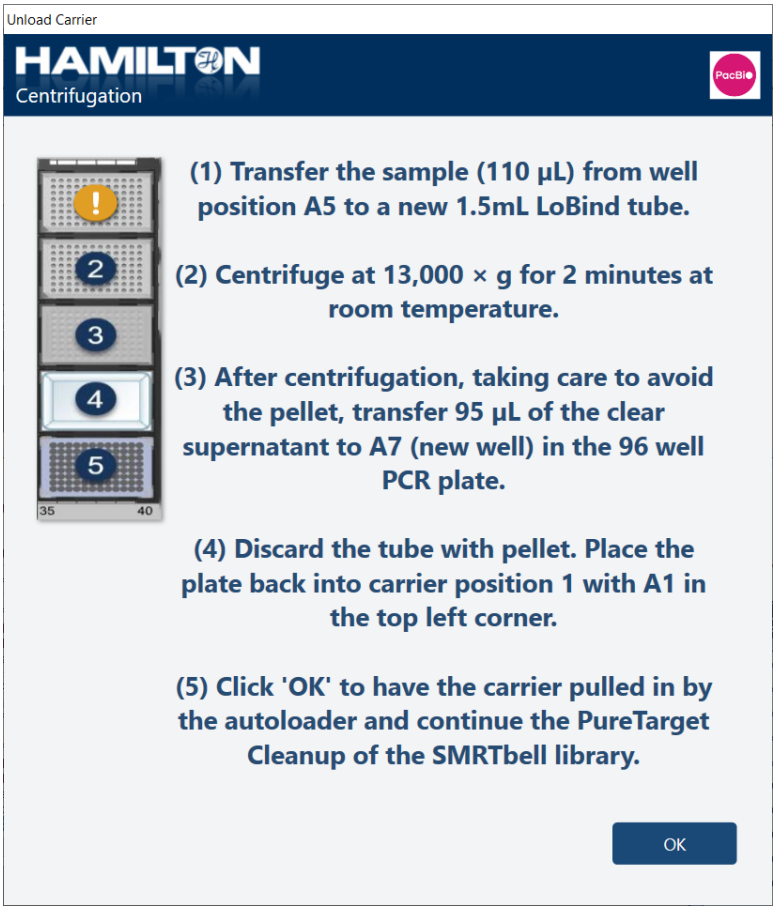


QC prompt for PureTarget cleanup of the SMRTbell library

20. **PureTarget library cleanup centrifugation.** The plate carrier will automatically move out. Follow the directions specified in the prompt for off-deck centrifugation.

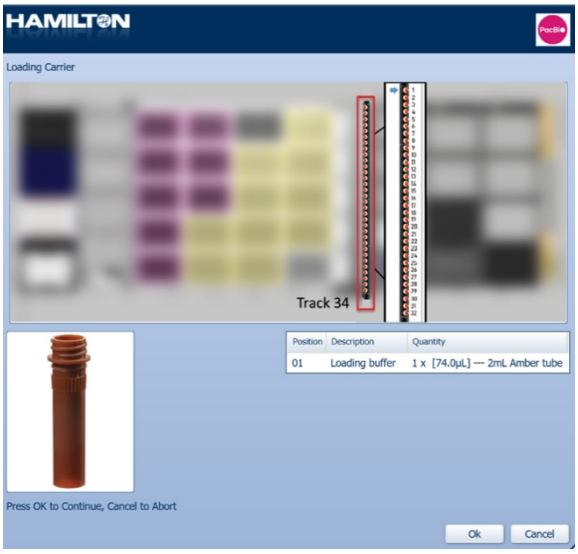
Note: It is critical when removing the supernatant to not touch or grab the pellet with the tip.

21. Transfer supernatant to new well location A7 of the SAME plate.

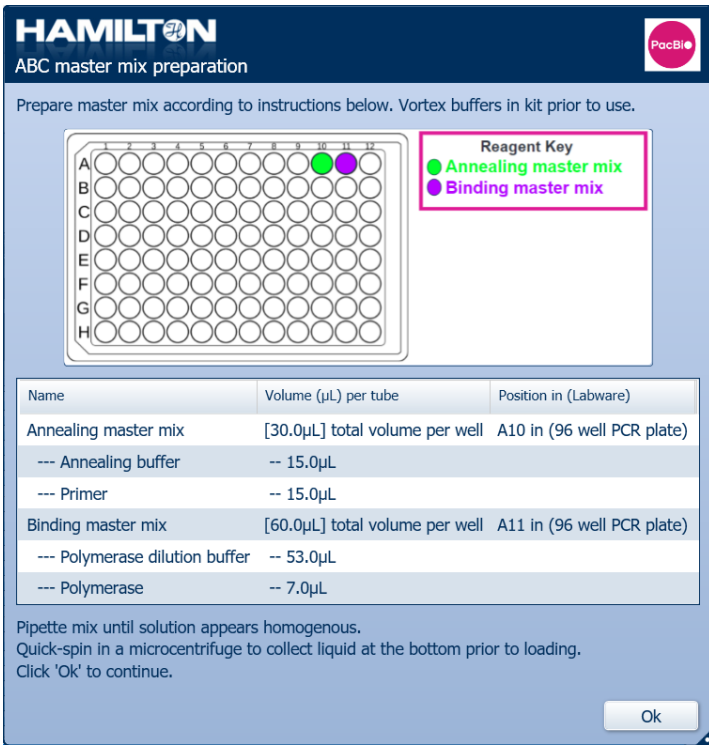


22. **Module 9: ABC – When the run reaches module 9, load the Loading buffer to tube carrier in track position 34**. Once the loading buffer has been thawed, transfer 74 µL to an amber tube. Spin down and un-cap before loading into position 1 of the tube carrier.

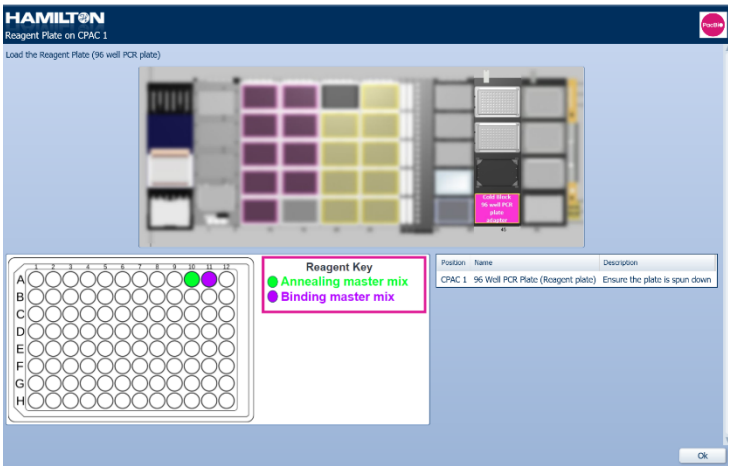
Note: The ABC loading buffer for sequencing is light-sensitive and needs to be in the amber tube.



23. **Prepare the ABC master mixes in wells A10 and A11 in a 96 well PCR plate.** Keep the reagent plate on ice until loading the instrument.



24. **Load the ABC reagent plate.** Spin down and place the reagent plate on CPAC 1 once it reached 4°C.



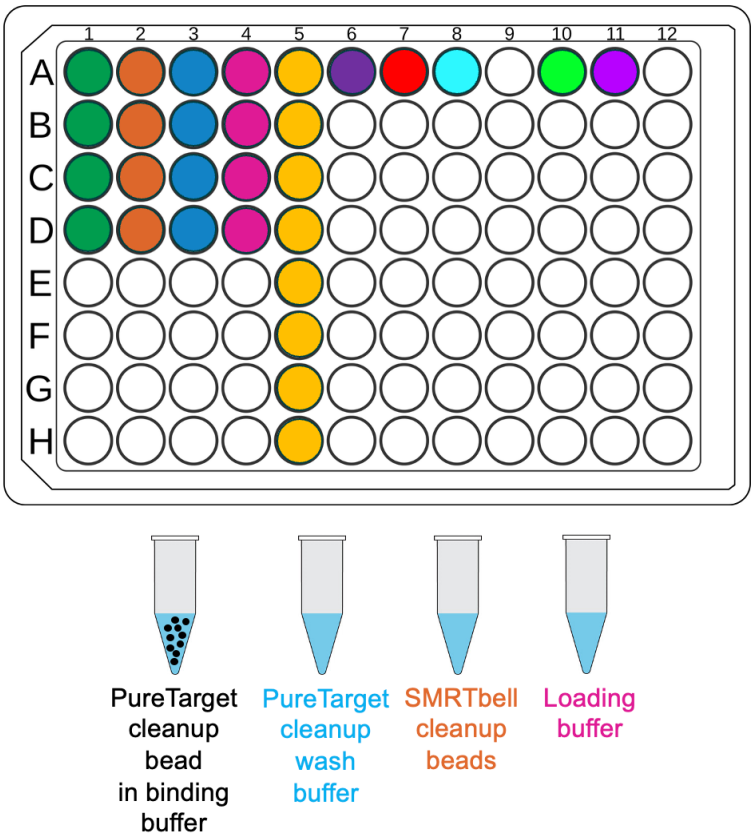
25. **ABC complete.** Protect from light. Continue to sequencing using "Procedure & Checklist – Generating PureTarget libraries with PureTarget kit 96 – automation protocol."

26. **Perform the following actions.** Empty the tip waste, check the liquid waste container, then remove and store reagents and processing plates. Select "No" to close the run software. Select "Yes" to have the instrument re-rack tips.

Appendix

Quick guide - reagent master mix layout and preparation for automation:

Reagent master mix plate layout –plate positions of master mixes for gDNA repair, dephosphorylation, Cas9-gRNA digestion, dA tailing, adapter ligation, nuclease digestion, PureTarget cleanup, and ABC.



Reagent Key

- gDNA repair master mix
- Dephosphorylation master mix
- Cas9-gRNA master mix
- dA-tail master mix
- Ligation master mix
- Nuclease digest master mix
- PureTarget cleanup buffer 1
- PureTarget cleanup buffer 2+3
- Annealing master mix
- Binding master mix

Quick guide - reagent master mix preparation for automation:

- Quick-pulse vortex master mixes 5 times with an end-to-end flip in between, and spin down.
- When pipetting the master mix into the well, only dispense to the 1st stop.
- Ligation master mix is viscous, pipette slowly to minimize liquid retention in the tip.
- PureTarget cleanup bead is heavy and requires thorough resuspension by vortexing.
- Master mix overage calculation includes minor adjustments to increase enzyme utilization.

Day1			
DNA repair (5 µL/rxn)	1X	96X+overage	Plate 151 µL each well to A1-D1
Nuclease free water	0.94	117	
Repair buffer M96	3.5	454	
End repair mix M96	0.06	8	
DNA repair mix M96	0.5	70	

Dephosphorylation (5 µL/rxn)	1X	96X+overage	Plate 151 µL each well to A2-D2
Nuclease free water	0.9	110	
Cas9 buffer	3	389	
Phosphatase	1.1	150	

Cas9-gRNA (5 µL/rxn)	1X	96X+overage	Plate 151 µL each well to A3-D3
Nuclease free water	3.75	489	
Cas9 buffer	0.5	65	
Cas9 nuclease (20 µM)	0.15	19	
gRNA Panel	0.6	76	

Day2			
dA tailing (4 µL/rxn)	1X	96X+overage	Plate 126 µL each well to A4-D4
Nuclease free water	1.125	152	
dA tail buffer	2.6	350	
dATP (100 mM)	0.025	4	
Taq DNA pol	0.25	34	

Adapter ligation (10 µL/rxn)	1X	96X+overage	Plate 151 µL each well to A5-H5
Nuclease free water	0.5	62	
Ligation mix M96*	9.0	565 µL twice	
Ligation enhancer M96	0.5	62	

Nuclease digestion (13 µL/rxn)	1X	10X+overage	Plate 151 µL to A6
dA tail buffer	7	87	
Nuclease mix M96	4.5	56	
PureTarget nuclease	1.5	19	

Move master mix plate to RT, after 2nd nuclease incubation			
PureTarget cleanup	1X	1X+overage	Plate 38 µL directly into A7
PureTarget cleanup buffer 1	28	38	

PureTarget cleanup buffer 2+3	1X	1X+overage	Plate 38 µL directly into A8
PureTarget cleanup buffer 2	14	19	
PureTarget cleanup buffer 3	14	19	

PureTarget cleanup bead binding master mix (105 µL /rxn)	1X	1X+overage	
PureTarget cleanup bead	5	7	Add to a 2 mL tube and load 147 µL
PureTarget cleanup binding buffer	100	140	
PureTarget cleanup wash buffer (EtOH added)	400	600	Add to a 2 mL tube and load 600 µL

Annealing master mix (25 µL/rxn)	1X	1X+overage	
Annealing buffer	12.5	15	Plate 30 µL directly into A10
Standard sequencing primer	12.5	15	

Binding master mix (50 µL/rxn)	1X	1X+overage	
Polymerase dilution buffer	44	53	Plate 30 µL directly into A11
Sequencing polymerase	6	7	
Loading buffer	23	74	Add to a 2 mL amber tube and load 74 µL

Input file:

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

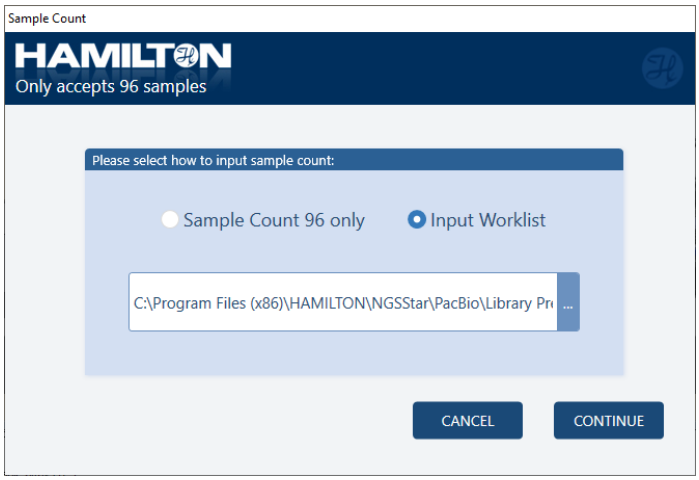
```
C:\Program Files (x86)\HAMILTON\NGSStar\PacBio\PureTarget\Files\Example Worklists
```

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

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

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

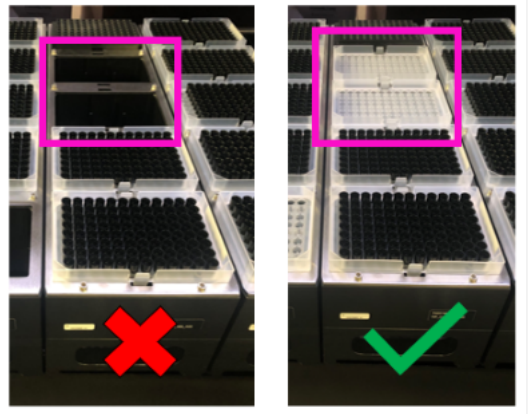
When sample count is prompted at start-up, navigate to edited file with updated sample information and select.



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

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

- ❌ *What not to do*
- ✅ *What to do*



Sample location:

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

Sample location	Safe Start	Safe stop	Input plate location & input volume	Output plate location & output volume
Module 1: gDNA repair and SMRTbell cleanup	x	x	Plate carrier position 1 (A1–H12), 30µL	Plate carrier position 1 (A1–H12), 26µL
Module 2: Dephosphorylation	x		Plate carrier position 1 (A1–H12), 26 µL	Plate carrier position 1 (A1–H12), 35 µL
Module 3: Cas9 digestion and SMRTbell cleanup		x	Plate carrier position 1 (A1–H12), 35 µL	Plate carrier position 1 (A1–H12), 22 µL
Module 4: dA-tail	x		Plate carrier position 1 (A1–H12), 22 µL	Plate carrier position 1 (A1–H12), 26 µL
Module 5: Adapter Ligation, Pool and SMRTbell cleanup		x	Plate carrier position 1 (A1–H12), 26 µL	Plate carrier position 1 (A1–H1), 62 µL
Module 6: Nuclease treatment 1, Pool and SMRTbell cleanup	x		Plate carrier position 1 (A1–H1), 62 µL	Plate carrier position 1 (A3–B3), 62 µL
Module 7: Nuclease treatment 2 and SMRTbell cleanup		x*	Plate carrier position 1 (A3–B3), 62 µL	Plate carrier position 1 (A5), 55 µL
Module 8: PureTarget cleanup of the SMRTbell library	x	x	Plate carrier position 1 (A5), 55 µL	Plate carrier position 1 (A9), 25 µL
Module 9: Annealing, binding and cleanup (ABC)	x	x	Plate carrier position 1 (A9), 25 µL	Plate carrier position 1 (A10), 23 µL

*Module 7 safe stop store plate at -20C

Revision history (description)	Version	Date
Initial release	01	September 2025
Updated DIN to >8	02	October 2025
Included additional sample guidance for DNA input	03	January 2026
Updated for Revio SPRQ-Nx	04	May 2026
Updated for Vega SPRQ-Nx	05	August 2026

PN 103-740-900 REV05 AUG2026