PacBio

Onso™ system cluster generator and short-read sequencer

Operations Guide

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Pacific Biosciences of California, Inc.
1305 O’Brien Dr.

Menlo Park, CA 94025
www.pacificbiosciences.com

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Contact information: Obelis Group

Address: Bd Général Wahis, 53 B-1030 Brussels Belgium. Tel: +3227325954

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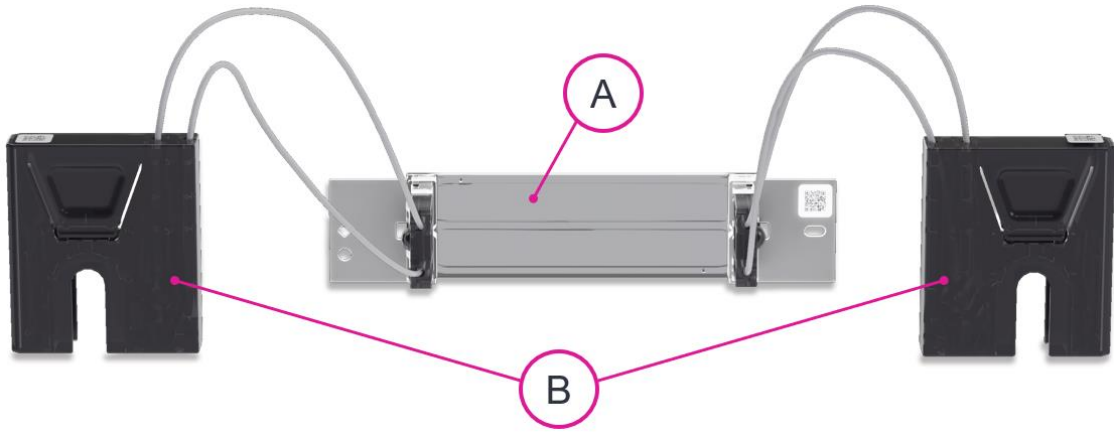
Before you begin

This guide describes the workflow for preparing Sequencing By Binding (SBB™) libraries for cluster generation on the PacBio® Onso™ cluster generator system, as well as preparation for and sequencing of clustered flow cells on the Onso system. Below are descriptions of the main consumables required for both clustering and sequencing procedures.

Flow cell and Main Instrument Connector Assembly (MICA) configuration

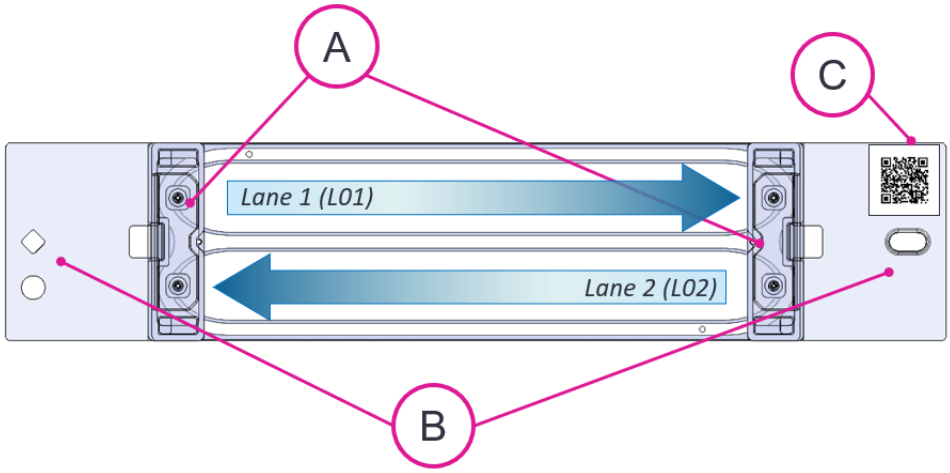
Each clustering and sequencing run requires use of a single flow cell that comes pre-attached to two Main Instrument Connector Assembly (MICA) units as shown below.



The (A) flow cell immobilizes DNA clusters for sequencing and interfaces with the instruments' mechanical, fluidic and optical systems. The flow cell interfaces with the fluidics systems via the (B) pre-attached MICA during clustering and sequencing.

Flow cell configuration

Each flow cell contains two lanes for sequencing libraries, which experience opposing directional flow. Lane 1 (L01) experiences flow of reagents from left to right, while lane 2 (L02) experiences flow from right to left.



(A) Ports on the left- and right-hand side of each flow cell allow for the flow of reagents into and out of the flow cell via the attached MICA. (B) Pinholes on the wings of the flow cell align with metal pins on the cluster generator and sequencing instrument for proper flow cell loading. The left side of the flow cell will have two pinholes, while the right side contains one. A QR code (C) on the upper right corner contains flow cell information and indicates the correct orientation when loading onto the instruments.

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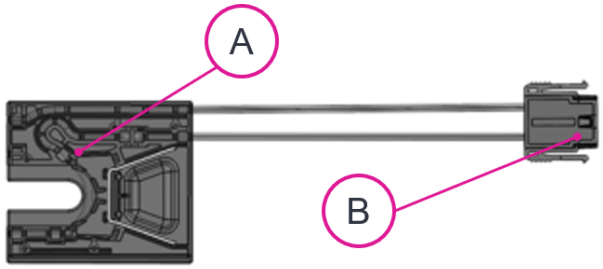
MICA configuration

The MICA comes attached to each flow cell, enabling flow of reagents in/out of the flow cell.



Each MICA contains (A) an instrument connection side, as well as (B) a flow cell connection side.

a) Connecting MICA instrument connection side to instruments

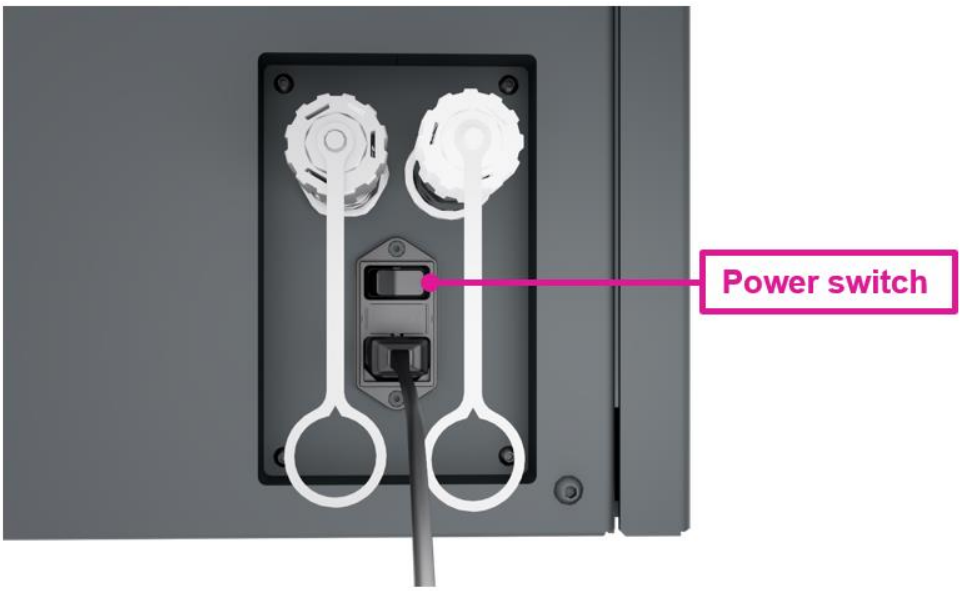
MICA can only be connected in one orientation to the clustering and sequencing instruments. To connect MICA to a cluster generator or the sequencer, align the MICA with the instrument connection slots on the instrument, and gently press down into the slots until an audible click is heard. The audible click indicates that the MICA is connected and engaged. It is encouraged to check MICA engagement by gently pulling the MICA instrument connection side upward – if properly engaged, the MICA should not disconnect from the instrument.

b) Disconnecting MICA instrument connection side from instruments

To release each MICA from a cluster generator or the sequencer, press on the side grooves on both sides of the MICA instrument connector side, and pull up.

Powering Instruments On

Both the cluster generator and sequencer need to be powered ON to operate. The power switch on the cluster generator can be found in the back of the instrument while the power switch on the sequencer can be found on the right side of the instrument. Flip the power switch to turn the instruments on. For the sequencer, wait



approximately 30 seconds to then power on the computer by pressing the power button on the PC.

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Safety guidelines

Please contact the Environmental Health and Safety (EHS) Manager to understand appropriate disposal of reagents and materials used in this operations guide as guidelines may differ per user site.

Refer to SDS (102-855-900) and hazard insert (103-033-900) for reagent specific hazard ratings for the Onso clustering reagent plate.

Refer to SDS (102-850-900) and hazard insert (103-032-600 and 103-032-800) for reagent specific hazard ratings for the sequencing reagent pack.

For all other reagents, refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

General best practices

DNA library input

DNA libraries should contain Onso indexed adapters.

DNA libraries should be accurately quantitated with the Onso Library quant kit (see Procedure & checklist - qPCR Quantification of Onso libraries) or a comparable qPCR quant kit using appropriate primers. There should be **at least 16 µL of starting dsDNA library available for use at a concentration of ≥500 pM per lane of a flow cell before clustering**.

Multiplexing

When multiplexing samples, a minimum of 4 samples is advised for pooling prior to clustering if users do not choose to use the Library QC (LQC) spike-in. Using fewer than 4 samples in the absence of an LQC spike-in will result in too low sample diversity during sequencing.

If multiplexing samples in the absence of LQC spike-in, a minimum of 8 Onso indexed adapters are required to supply sufficient index diversity.

For recommendations around appropriate LQC spike-in amounts for multiplexing purposes, refer to the section below titled "**Library loading concentration and control spike-in guidelines**".

Library loading concentration and control spike-in guidelines

Optimal loading concentration

Determining the best loading concentration to achieve optimal cluster density will depend upon several factors, such as the starting material of your library, average library size, and library prep method. Over-clustering will compromise overall sequencing run performance, whereas under-clustering will not maximize potential data output. Therefore, striking a balance between over-clustering and under-clustering will increase the likelihood of obtaining optimal sequencing data quality, while reducing the amount of sequencing that may be required to achieve target Gb or fold coverage.

To determine the optimal loading concentration for your library, performing a library input titration is advised for each new library type being clustered.

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Library QC (LQC) control spike-in

A control library (LQC) can be added to the DNA library before clustering to increase sample diversity for difficult to sequence low-diversity libraries. LQC should not be used if the sample library shares sequence similarities with Lambda or *E. coli*, as distinguishing between sample reads and control library reads will be challenging.

We recommend a 5% LQC spike-in amount, however, upwards of 5% can be used if samples contain low diversity. In cases of low diversity, we recommend using an LQC spike-in amount of approximately 20%.

Reagent and sample handling

Room temperature is defined as any temperature in the range of **18-23°C** for this protocol.

All reagents and samples should be brought to room temperature and properly mixed before use. Proper mixing includes inversion and pipette mixing when applicable. Differences in recommended mixing methods are specified at each appropriate step.

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Clustering on the cluster generator

This section describes the workflow for flow cell clustering of Onso compatible libraries in preparation for sequencing on the Onso sequencing system. After completing this section of the guide, a clustered flow cell can either be sequenced immediately, by following the Onso sequencing section of this guide, or can be stored at 4°C for up to 1 week.

Each clustering reagent plate supports clustering of 2 lanes on a single flow cell.

For recommendations on sample multiplexing, refer to "General Best Practices" and "Library loading concentration and control spike-in guidelines" sections above.

During run setup, users are able to select a sample sheet prepared and saved on a drive accessible by the PC. For sample sheet information, templates, and guidance, refer to the PacBio document titled "Obc2fastq Reference guide".

Users should perform theoretical calculations with the appropriate procedure in section 3 (either 3a or 3b) to first determine the library input volume required per lane **BEFORE** diluting library input concentrations to 500 pM to ensure that minimum volume requirements are met.

Cluster generator

The (A) workspace door lifts upward to allow for loading of the (B) flow cell with attached MICA. Users can operate the cluster generator by way of the (C) cluster generator touchscreen. The (A) workspace door and (D) reagent drawer will remain locked in the closed position prior to initiating the clustering software, and during a clustering run. When initiating the clustering software by clicking "**Get started**" (see section 5 below), the reagent drawer can be prompted by the user to open/close by way of the software. The reagent drawer opens towards the user, and houses the (E) clustering reagent plate, (F) waste container, and contains (G) storage locations for wash cartridges. Users are advised to not push closed or pull open the reagent drawer. Clustering reagent plates are single-use and contain a QR code which should be visible from the right-hand side when the drawer is open and when the reagent plate is properly loaded on the clustering instrument. The waste container should be emptied and returned to its correct position within the instrument prior to starting a clustering run. The (H) magnetic flow cell clamp and metal guide pins are seated within the workspace door, allowing the user to properly place and secure the flow cell. The flow cell clamp lifts upward, hinging away from the user.

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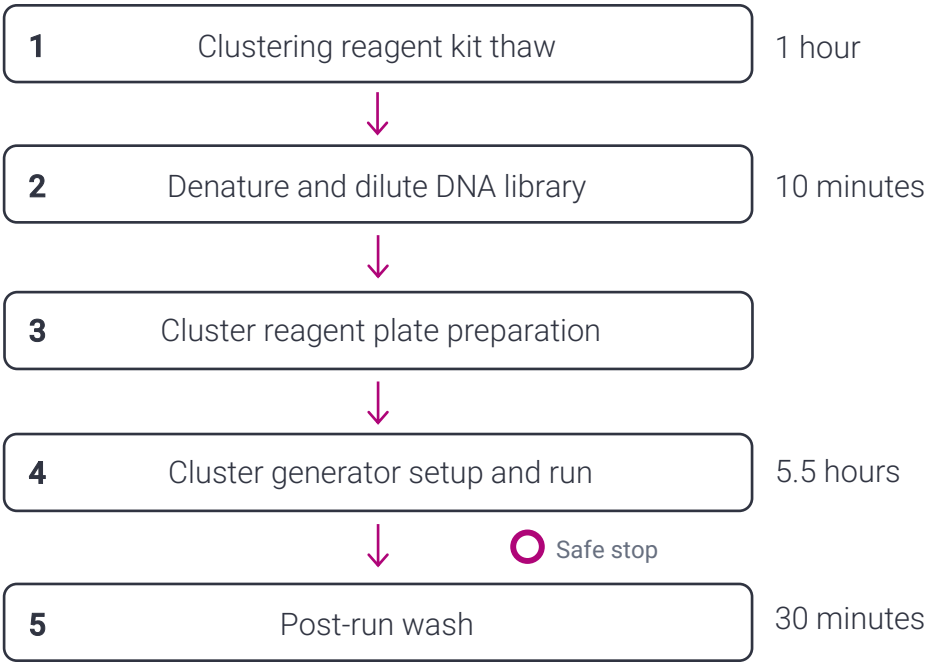
Clustering workflow



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Required materials and equipment

Clustering Materials	Supplier/Manufacturer
Onso™ clustering reagent kit	PacBio® 102-855-900
Onso™ clustering reagent plate	PacBio® 102-752-200
Library dilution buffer (DIL buffer)	PacBio® 102-386-600
Onso™ flow cell kit	PacBio® 103-064-700
Wash cartridge set	PacBio® 102-932-300
Empty troughs	provided in accessory kit (PacBio® 102-673-800)
Onso™ clustering instrument wash plate	PacBio® 102-673-600
Onso™ cluster generator system (instrument)	PacBio® 102-837-100
Nuclease-free water, molecular biology grade	Any Major Lab Supplier (MLS)
Sodium Hydroxide (NaOH), ≥ 1N	Any MLS
Low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	Any MLS
DNA low-bind tubes	Any MLS
Bleach (NaClO), ≥ 5%	Any MLS
Tween-20, molecular biology grade, prepared to a 10% (w/v)	Any MLS
100X TE Buffer, molecular biology grade	Any MLS
Vortexer	Any MLS
Microcentrifuge	Any MLS
Pipettes, 2-1000 µL pipetting range	Any MLS
Pipette tips, 2-1000 µL	Any MLS

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Clustering procedure

1. Thawing clustering reagents

Each clustering reagent plate supports clustering of up to 2 lanes on a single flow cell.

✔	Step	Instructions
	1.1	Remove the Onso clustering reagent kit from -20°C storage, unbox and remove clustering reagent plate from mylar bag. Discard mylar bag.
	1.2	Thaw contents of the Onso clustering reagent kit: • Place the DIL at room temperature to thaw. DIL can be stored at 4°C after thaw for future use. • Allow clustering reagents plate to thaw for 1 hour in a room temperature (18-25°C) water bath. Water should not pass more than halfway up the clustering plate (1.5 in.). Thawing of DIL and clustering reagent plate can be conducted simultaneously.
	1.3	After 1 hour thawing, remove the reagent plate from the water bath. Wipe the clustering reagent plate top and underside dry.
	1.4	Mix reagents in the clustering reagent plate by inverting the reagent pack slowly 10 times.
	1.5	Gently tap the reagent plate on the benchtop 10 times to consolidate any reagent liquid to the bottom.

2. Preparing instrument wash reagent

The instrument wash reagent detailed here can be stored up to 2 weeks at 4°C. The preparation procedure detailed below is for a final volume of 1 liter and makes use of a 10% (w/v) Tween-20 solution.

The final formulation of wash buffer contains 0.05% Tween-20, 10 mM Tris, 1 mM EDTA, and has a pH of 8.0.

The instrument wash reagent is required during the cluster generator pre-run checks, cluster generator post-run wash, as well as when the cluster generator instrument requires a standby wash, or maintenance.

✔	Step	Instructions
	2.1	In a 1000 mL bottle, add 985 mL of molecular biology grade water.
	2.2	Add 5 mL of a prepared 10% (w/v) Tween-20 solution to the bottle for a final concentration of 0.05% Tween-20.
	2.3	Add 10 mL of 100X TE buffer into the bottle.
	2.4	Cap and invert the bottle at least 10 times to mix.

3. Denaturing and diluting the DNA library

Optimal loading concentration of your library for cluster generation will depend on your sample type and library prep method. If not using a control library (LQC) spike-in, proceed with Step 3a.1. If using an LQC spike-in, proceed with Step 3b.1. Refer to "Library loading concentration and control spike-in guidelines" under the section "General best practices" for recommendations on loading concentrations and LQC spike-in percentages.

Combined volume of library and 0.1N NaOH should not exceed 32 µL to minimize the concentration of NaOH carried over into the clustering reaction. If the desired loading concentration requires a library volume > 16 µL, it is recommended to dilute your library to an input concentration > 500 pM.

All steps below should be duplicated to prepare both lanes of a single flow cell for clustering. Alternatively, if clustering only one lane of the flow cell, perform instructions below for the 1 lane which will contain clustered

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library, while skipping forward to step 3a.8 or 3b.11 guidance for the empty lane. Note that once a flow cell is clustered once, it should not be re-clustered.

Users should perform theoretical calculations with the appropriate procedure in section 3 (either 3a or 3b) to first determine the library input volume required per lane **BEFORE** diluting library input concentrations to 500 pM to ensure that minimum volume requirements are met.

✔	Step	3a. Denature and dilute DNA library **WITHOUT LQC control library spike-in**
	3a.1	Retrieve a 1 mL tube of DIL buffer from 4°C storage.
	3a.2	Calculate the library input volume required per lane as follows:

Calculating library input volume (per lane)

d = (a * c)/b		User input
a	Desired total loading concentration (pM)	
b	Starting library input concentration (pM)*	500
c	Final library volume (µL)	380
d	Volume of starting library (µL)	

* **Note:** If the "Desired total loading concentration" (a) requires a "Volume of starting library" (d) > 16 µL, it is recommended to change "Starting library input concentration (pM)" (b) to > 500pM.

	3a.3	Dilute the library to an input concentration of 500 pM using 10 mM Tris, 0.1 mM EDTA, pH8.0 for the required volume (calculated in Step 3a.2-line **d**) per lane. Pipette mix and spin down. If the "Desired total loading concentration" requires a library volume > 16 µL, it is recommended to change "Starting library input concentration (pM)" (b) to > 500pM.
	3a.4	Prepare 100 µL of fresh 0.1N sodium hydroxide (NaOH).
	3a.5	For each lane to be clustered, obtain a new DNA low-bind tube, and add the required volume of starting library (calculated in Step 3a.2 line **d**) for each respective lane. If using the same preparation in both lanes of the flow cell, the reactions can be pooled into one tube.
	3a.6	Add an equivalent volume of fresh 0.1N NaOH as calculated in Step 3a.5 above to the respective tube containing library. Pipette mix up and down at least 10 times and spin down. Do not vortex. The total volume of library and 0.1N NaOH should not exceed 32 µL. (e.g. Add 10 µL of fresh 0.1N NaOH to 10 µL of library.)
	3a.7	Incubate the library and NaOH mixture for 5 minutes to allow for proper denaturation.
	3a.8	Add DIL buffer to the library and NaOH mixture to reach a final volume of 380 µL. Pipette mix and spin down. **Immediately** proceed to **section 4** titled "Onso clustering reagent plate preparation". If clustering a flow cell with an empty lane, simply add 380 µL of DIL buffer to a DNA low-bind tube designated for the empty lane. **Immediately** proceed to **section 4** titled "Onso Clustering reagent plate preparation"

OR (see next page)

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✔	Step	3b. Denature and dilute DNA library WITH LQC control library spike-in
	3b.1	Retrieve a 1 mL tube of DIL buffer from 4°C storage.

Calculate the LQC concentration required per lane as follows in the table below.
For libraries with low sample diversity, it is advised to increase the percent spike-in of LQC up to 20%, whereas otherwise a standard 5% spike-in amount is suitable.

Refer to the section "Library loading concentration and control spike-in guidelines" under "General best practices" for sample library and LQC loading concentration recommendations.

3b.2

Calculating LQC spike-in concentration (per lane)		
	c = a * (b/100)	User input
a	Desired total library loading concentration (pM)	
b	LQC spike-in percent (%)	
c	LQC spike-in concentration (pM)	

3b.3

Calculate the LQC input volume required per lane as follows:

Calculating LQC input volume (per lane)		
	f = (c * e)/d	User input
c	LQC spike-in concentration (pM)	
d	LQC starting concentration (pM)	2000
e	Final library volume (µL)	380
f	Volume of starting LQC library (µL)	

It is recommended to have an LQC input concentration of at least 500 pM, but this value can be increased if the volume of LQC + DNA library > 16 µL.

3b.4

Calculate the sample library concentration required per lane as follows:

Calculating sample library concentration (per lane)		
	g = a - c	User input
a	Desired total loading concentration (pM)	
c	LQC spike-in concentration (pM)	
g	Final sample library concentration (pM)	

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3b.5 Calculate the sample library volume required per lane as follows:

Calculating sample library volume (per lane)		
	i = (g * e)/h	User input
g	Final sample library concentration (pM)	
h	Starting sample library concentration (pM)*	500
e	Final library volume (µL)	380
i	Volume of starting sample library (µL)	

*Note: It is recommended to have sample library input concentration (h) of at least 500 pM, but this value can be increased if the volume of LQC + DNA library > 16 µL.

3b.6 Dilute the sample library to a starting concentration of 500 pM using Low TE buffer for the required volume (calculated in Step 3b.5) per lane. Vortex to mix and spin down.

If volume of starting sample library (from Step 3b.5) + volume of starting LQC library (from Step 3b.3) exceeds 16 µL, it is recommended to adjust starting sample and/or LQC concentrations to be > 500 pM.

3b.7 Prepare 100 µL of fresh 0.1N sodium hydroxide (NaOH).

3b.8 In a DNA low-bind tube, add the volume of starting sample library (**i**) and volume of starting LQC library (**f**), per lane.

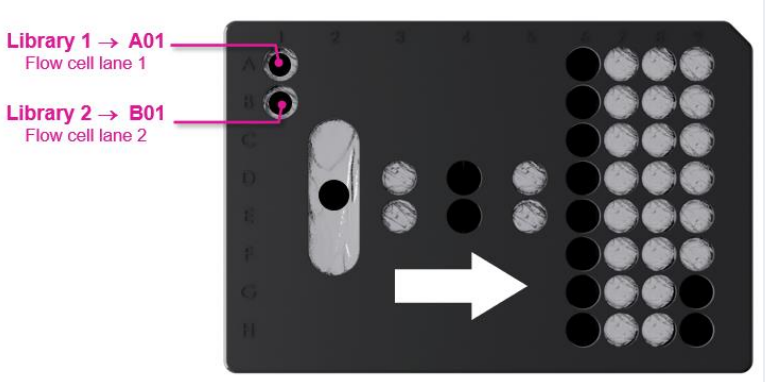
3b.9 Add an equivalent volume of fresh 0.1N NaOH as calculated in Step 3b.8 to the same tube. Gently pipette mix up and down at least 10 times and spin down. Do not vortex. The volume of starting sample library (**i**) + volume of starting LQC library (**f**) + 0.1N NaOH should not exceed 32 µL per lane (e.g., Add 10 µL of fresh 0.1N NaOH to 10 µL of combined (sample + LQC) libraries.)

3b.10 Incubate the library and NaOH mixture for 5 minutes at room temperature to allow for proper denaturation.

3b.11 Add DIL buffer to the library, LQC, and NaOH mixture to reach a final volume of 380 µL. Pipette mix and spin down. **Immediately** proceed to **section 4** titled "Clustering reagent plate preparation".

If clustering a flow cell with an empty lane, simply add 380 µL of DIL buffer to a DNA low-bind tube designated for the empty lane. **Immediately** proceed to **section 4** titled "Clustering reagent plate preparation"

4. Onso clustering reagent plate preparation

✔	Step	Instructions
	4.1	Puncture the foil seal in positions A01 and B01 with a sterile pipette tip and discard the used tip.
	4.2	Puncture the oval foil seal in column 2 with a sterile pipette tip and discard the used tip.
	4.3	Add 375 µL of diluted library to position A01 or B01 (A01=Lane 1, B01=Lane 2). ⚠ Leave the oval trough in column 2 empty until after the clustering run has completed (Refer to section 5 "Post-run wash"). 

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5. Cluster generator setup and run

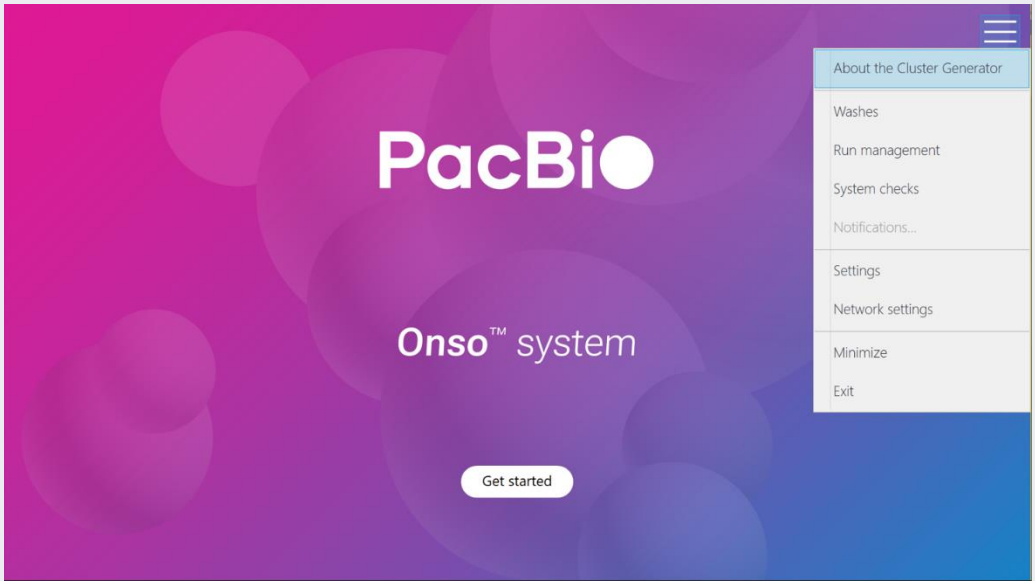
✔	Step	Instructions

Check the status of the cluster generator instrument.

- If the instrument is off, refer to the section above titled "Before you begin" for instructions on how to power on the instrument.
- If the instrument is on, and at the home screen, select "**Get started**" to begin.

5.1

- If selecting "**Get started**" on the home screen does not advance, click on the hamburger menu at the top right and select "**Washes**". Refer to the section titled "**Instrument washes**" on how to perform a clustering wash and then proceed to step 5.2 below.



5.2 In the *Load Consumables* screen, select "**Open workspaces**".

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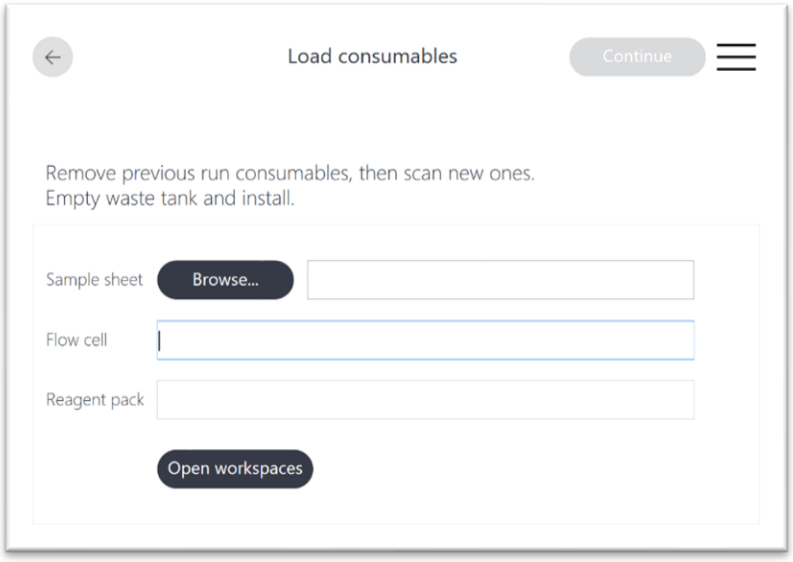


At the top of the cluster generator, open the workspace door by lifting the cover upwards.

5.3 Unload previous run consumables:

- Remove the wash cartridges if loaded, by gently pinching the sides of the wash cartridges and lifting up and out of the instrument slots.
- Place the wash cartridges in the wash cartridge storage locations in the instrument reagent drawer.

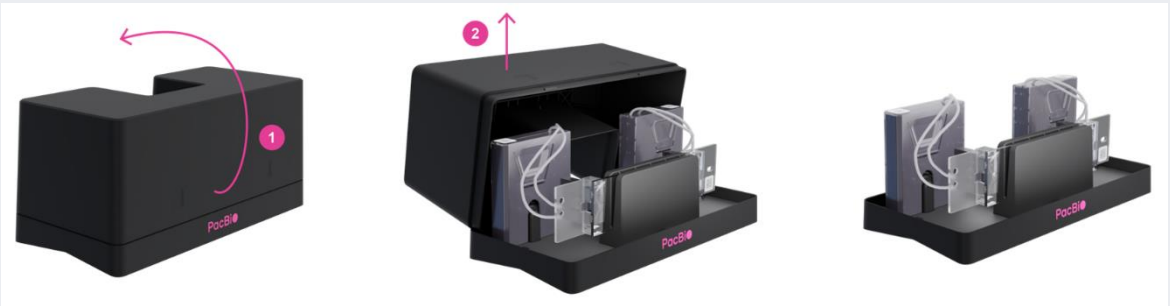
Wash cartridges are safe to use for up to 30 days from initial use.

- If a previous clustering reagent plate is found loaded in the instrument reagent drawer, dispose of the previous clustering reagent plate and its contents in the appropriate waste stream.
- If a clustering instrument wash plate and troughs are found loaded in the instrument reagent drawer, remove and clean the troughs by rinsing with DI water and drying with a lint-free wipe. Store the plate and troughs upside down or in a bag to prevent dust accumulation between uses.

5.4 Lift the flow cell clamp upwards to allow for loading of a new flow cell.

5.5 Open the Onso flow cell kit:

1. Hold the base of the flow cell kit in one hand with the PacBio logo forward-facing. Lift and roll the top-front of the box towards the back.
2. Lift the lid of the flow cell kit upward and set aside.



5.6 Load the flow cell kit onto the cluster generator, by placing the bottom base of the opened flow cell kit onto the cluster generator workspace area, such that the PacBio logo on the base of the flow cell kit is facing the user and the front of the flow cell kit base is flush with the front of the workspace area. This will cause the base of the flow cell kit to sit against the flow cell clamp hinge.

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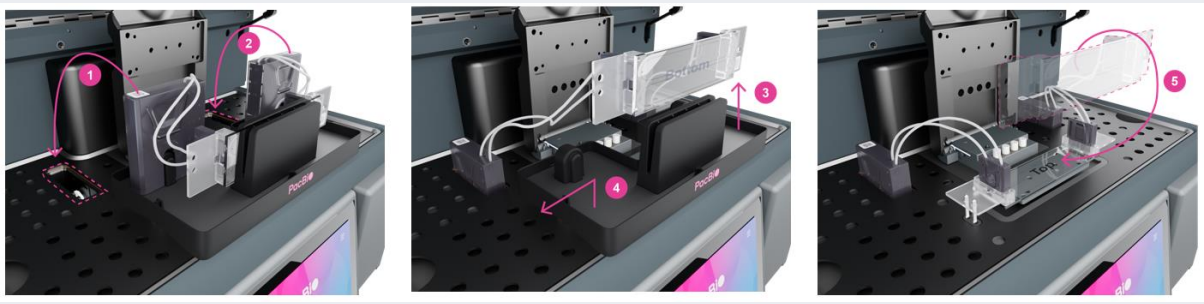
Load the flow cell in the cluster generator instrument workspace:

1. Remove the left MICA from the flow cell kit base and align the MICA instrument connector side with the instrument connection slots such that the fluidic tubing is closest to the user. Connect the left MICA by gently pushing down into the slot until an audible click is heard. The audible click indicates that the MICA has been engaged.
2. Repeat step 1 above with the right MICA instrument connector side, aligning and connecting it to the instrument connection slot with the fluidic tubing closest to the user.
   It is encouraged to verify MICA engagement by gently pulling the MICA instrument connectors upward – if properly installed, the MICA instrument connectors should not easily disconnect from the cluster generator.
3. Holding either the left or right wing of the flow cell, lift the flow cell directly upward out of the kit base. In this orientation, the bottom of the flow cell is facing the user.
4. With the flow cell still lifted, remove the flow cell kit base from the workspace area by lifting upward slightly and moving out to the side until the kit base is no longer seated on the instrument.

5.7

5. Seat the lifted flow cell onto the workspace area with the bottom of the flow cell facing down. Ensure that the flow cell is seated properly by aligning the pinholes on the flow cell with the metal guide pins on the cluster generator loading stage of the workspace area. The flow cell wing with two pinholes (circle and diamond) will be positioned on the left wing and the wing containing one pinhole (oval) will be positioned on the right.
   Users can also verify correct orientation of the flow cell in the instrument workspace if the QR code on the flow cell is legible on the top right corner of the flow cell, away from the user.



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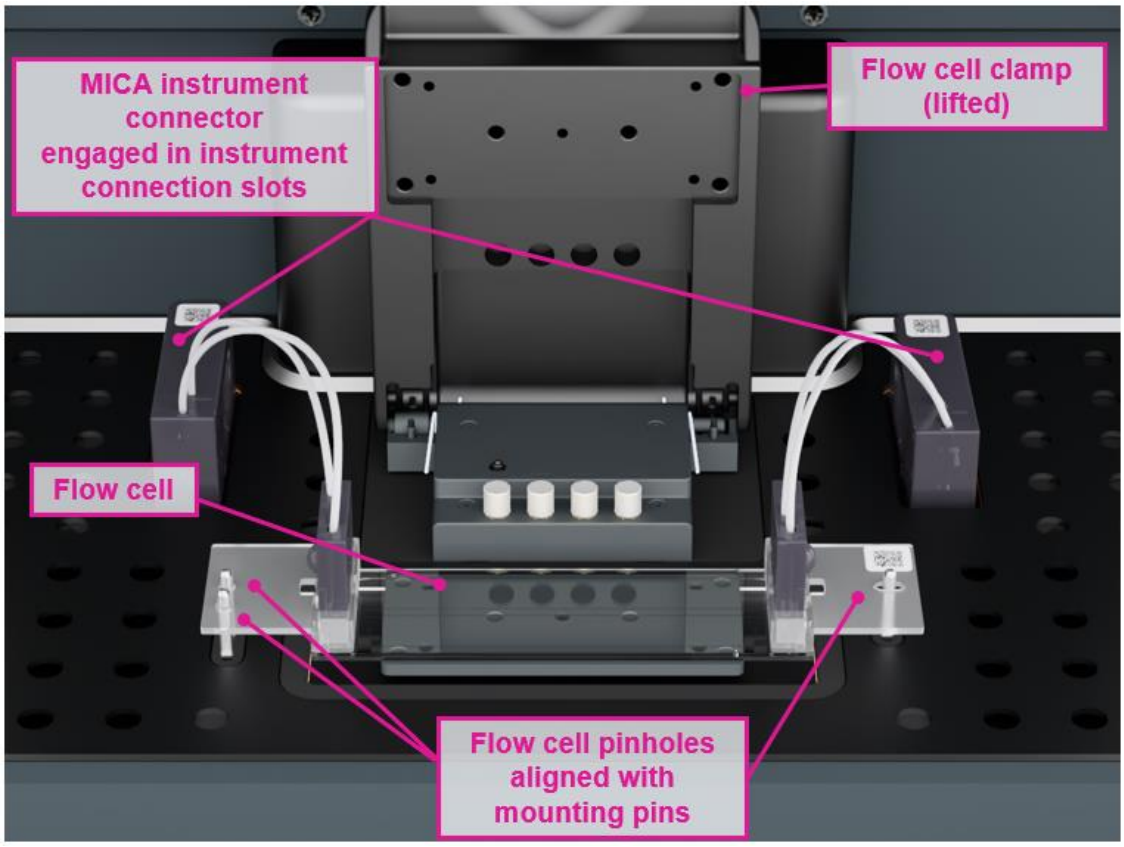
5.8



Gently and slowly close the flow cell clamp to ensure that the flow cell is properly secured.

5.9 Reconnect the lid to the flow cell kit base in reverse manner in which it was opened (refer to step 5.5). Retain the flow cell kit for flow cell removal and storage after clustering is complete.

Load the clustering reagent plate into the cluster generator instrument drawer:

1. Hold the clustering reagent plate with the white arrow on the top of the plate pointing towards the instrument. Lower one side of the plate, angled such that the white arrow points down into the instrument drawer. Ensure the downward angled side of the plate is seated within the loading tray.
2. Lower down the upward-facing edge (closest to the user) to meet the surface of the loading tray. The clustering plate should now be level with the loading tray.

5.10

If placed properly, the clustering reagent plate QR code should be visible from the right side of the instrument to scan into the software.

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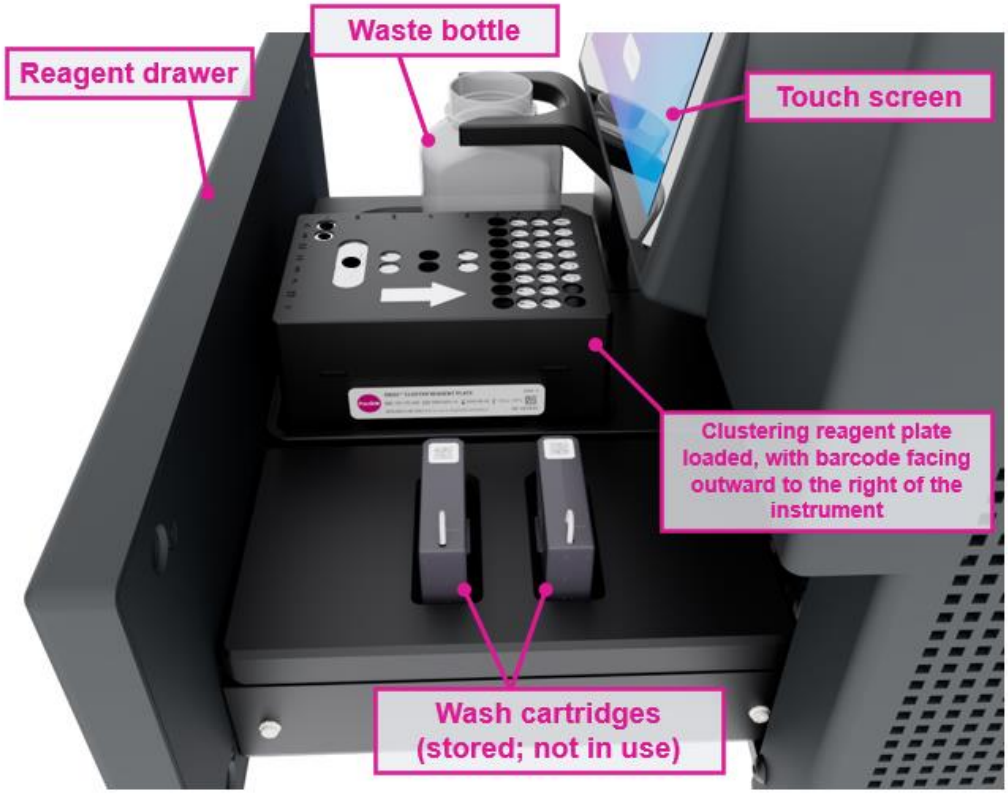


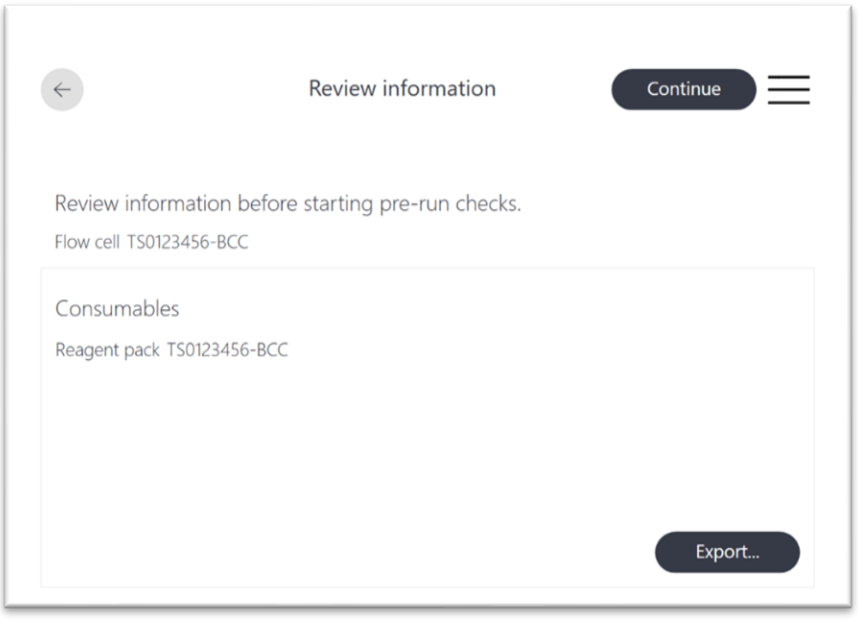
5.11 Remove the waste container from the instrument and empty the contents into the appropriate waste stream. Ensure the emptied bottle is placed back into the correct position in the instrument.

5.12 Using a barcode scanner, scan in the flow cell and clustering reagent plate QR codes into the respective fields on the *Load consumables* software screen.

5.13 Close the cluster generator workspace door and click "**Close workspaces**" on the touchscreen. Once the cluster generator drawer is closed, select "**Continue**" to advance.

⚠ DO NOT push or manually force instrument drawer to close.

5.14 Verify that the consumables entered are correct and select "**Continue**" to advance. If changes need to be made, click the back arrow at the top left to make the necessary changes.



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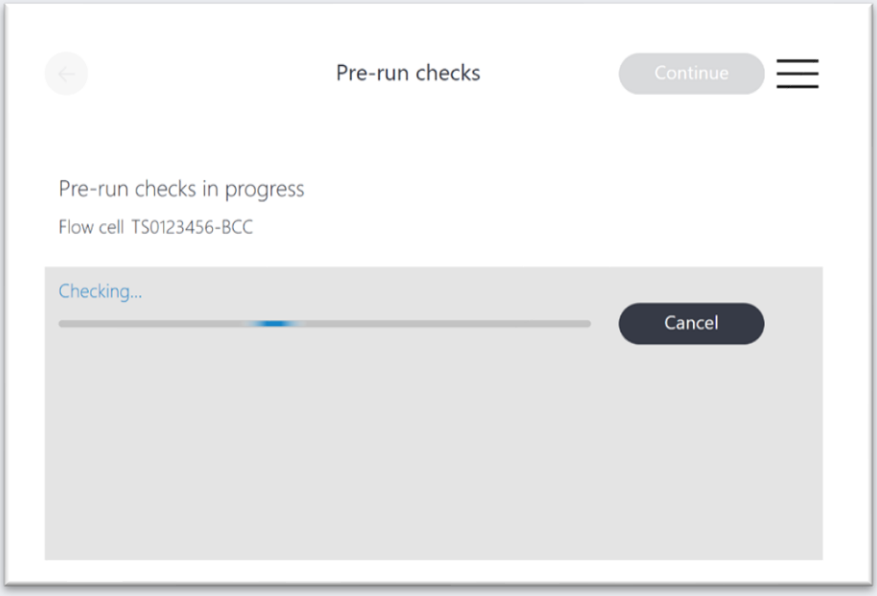
The cluster generator will then perform a series of pre-run checks over a 10-minute period. If pre-run checks complete successfully, the instrument will commence to clustering.

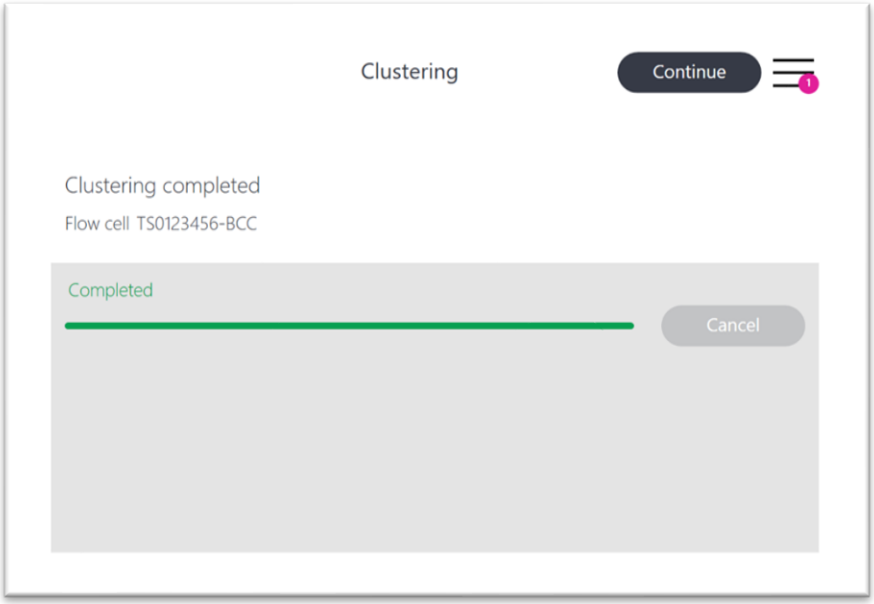
Users are advised to check the instrument after 10 minutes to ensure pre-run checks completed successfully and that clustering has commenced. If an error is encountered, refer to the Troubleshooting section at the end of the Operations guide.

5.15



The clustering workflow will last approximately 5.5 hours. Flow cells can remain on the instrument overnight and moved to storage at 4°C the following morning, or user can proceed to section 6 below ("Post-run wash") as soon as clustering is complete.

5.16



SAFE STOPPING POINT

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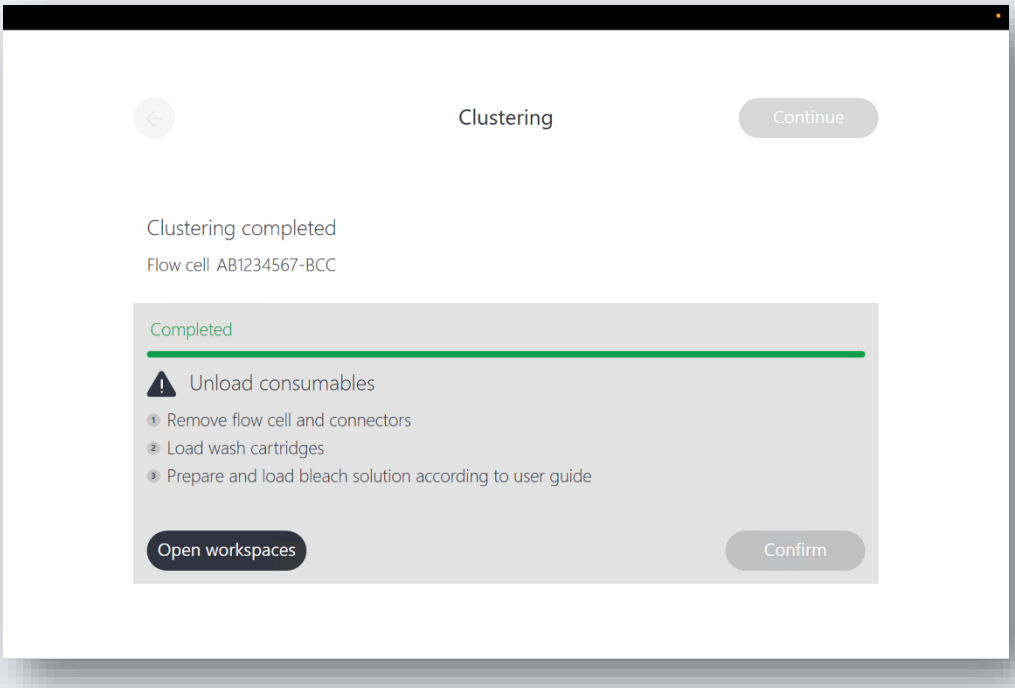
6. Post-run wash

✔	Step	Instructions
	6.1	After cluster generation is complete, the screen below will appear, prompting the user to unload the clustered flow cell with attached MICA, load the wash cartridges, and add 0.1% bleach solution to the clustering reagent plate for the post-run wash.
	6.2	Lift the workspace door open. Gently lift the magnetic flow cell clamp.
	6.3	Retrieve and open the flow cell kit previously used to load the flow cell. Review step 5.5 for instructions on how to open the flow cell kit if stored closed.
	6.4	Unload the clustered flow cell: 1. Lift the flow cell vertically until it is no longer secured by the metal guides pins on the cluster generator loading stage. Rotate the flow cell such that the top of the flow cell faces the instrument and away from the user. 2. With the flow cell still lifted, slide the flow cell kit base to the center of the workspace area, and set the base down on the workspace area with the PacBio logo facing the user. 3. Gently reseat the flow cell directly down into the flow cell holding slot within the kit base. In this orientation, the bottom of the flow cell is facing the user. 4. Disengage the right MICA instrument connector side by pinching the side grooves of the MICA and lifting upward out of the instrument connection slot. Seat the right MICA into the flow cell kit base with the QR code facing outward. 5. Repeat step 4 above with the left MICA instrument connector side to disengage from the instrument and seat within the flow cell kit base.



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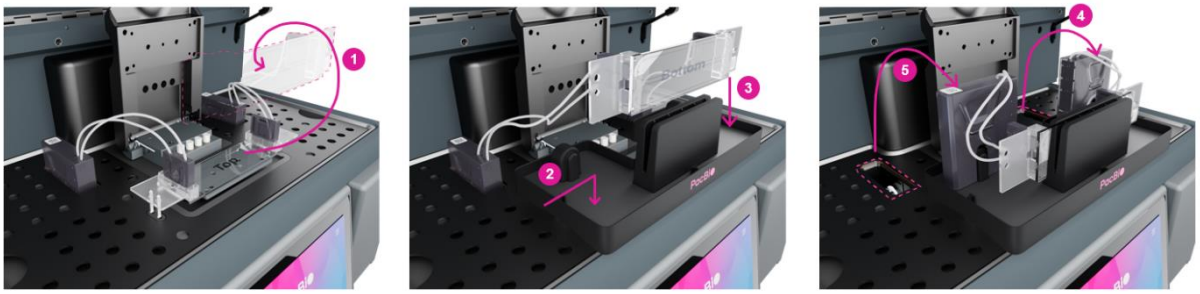

Remove the flow cell kit base from the instrument workspace. Retrieve the flow cell kit lid. Close the flow cell kit lid:

1. Hold the base of the flow cell kit in one hand with the PacBio logo forward-facing. Seat the back of the flow cell kit lid onto the base.
2. Roll the top-front of the flow cell kit lid towards the PacBio logo.
3. Press down to secure, being careful not to pinch any fluidics tubing between the kit lid and base.

6.5

Retain the clustered flow cell in the flow cell kit– this flow cell is ready for sequencing on the sequencer. If not sequenced immediately, it can be stored at 4°C for up to 1 week.

Select "Open workspaces" on the software screen. Retrieve the wash cartridges from within the cluster generator drawer and align one on each side with the instrument connector slots in the cluster generator workspace. Press down to engage wash cartridges until an audible click is heard.

6.6

It is encouraged to verify wash cartridge engagement by gently pulling the wash cartridges upward. If properly installed, the wash cartridges should not easily disconnect from the cluster generator.

6.7 Lower the magnetic flow cell clamp and close the workspace door.

6.8 Prepare at least 30 mL of fresh 0.1% hypochlorite (bleach) solution.

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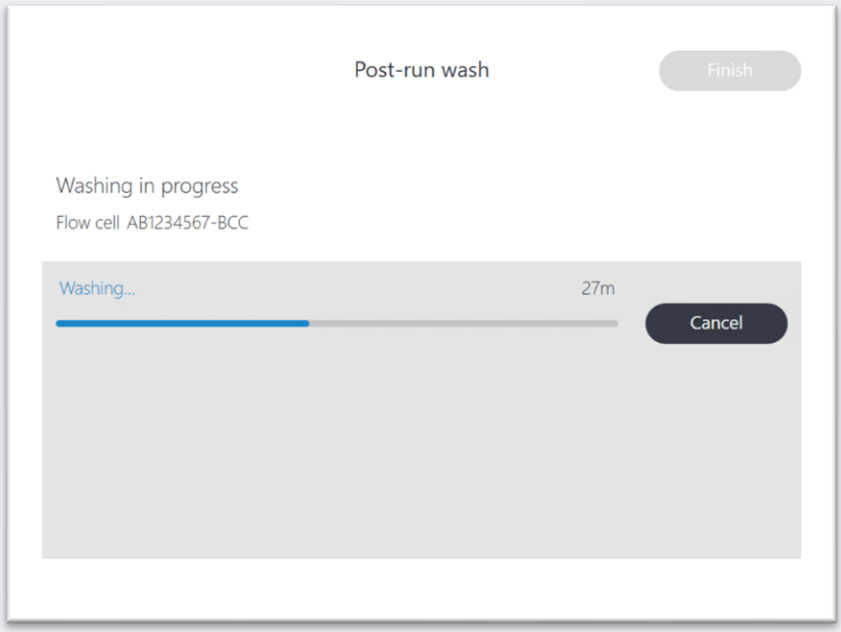
6.9 With the clustering reagent plate still housed in the reagent drawer, use a fresh serological pipette tip to add 25 mL of the 0.1% hypochlorite solution into the previously punctured center oval in column 2 of the clustering reagent plate.

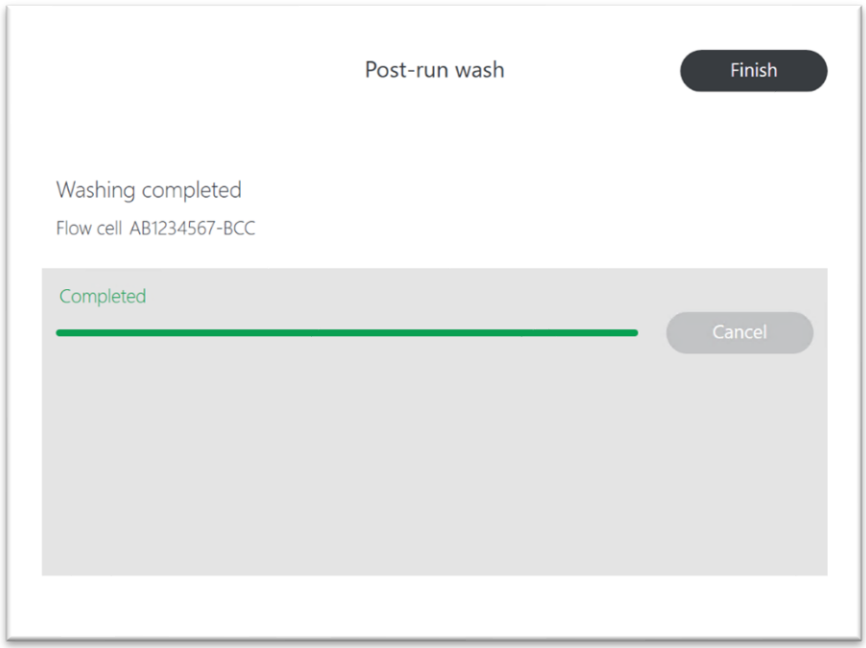
6.10 Click "**Close workspaces**" for the cluster generator drawer to close shut. Select "**Confirm**" to confirm the tasks listed in the software screen were completed.

⚠ DO NOT push or manually force reagent drawer to close.

6.11 Select "Continue" on the software to initiate the 30-minute post-run wash.



6.12 Following the wash, select "**Finish**" on the completed screen to bring the software back to the home screen.



6.13 When the post-run wash is complete, the user will be allowed to access and dispose of the clustering reagent plate, if necessary.
Wash cartridges should stay connected to the instrument inside the workspace door until the next run is loaded but can be retained at room temperature for up to 30 days after initial use.

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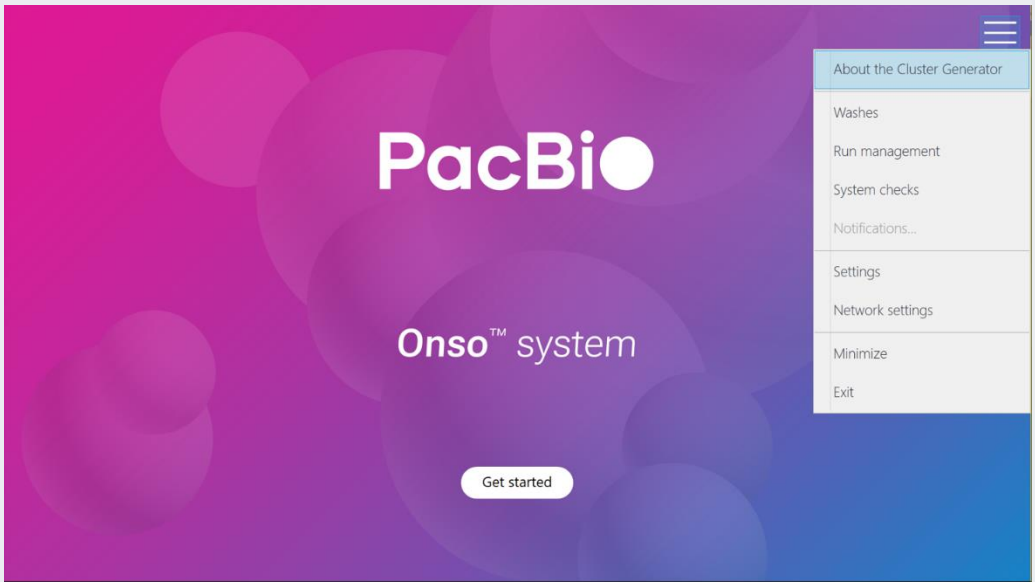
7. Instrument washes

There are two types of instrument washes outside of the standard clustering workflow: 1) standby wash, and 2) maintenance wash. A standby wash is required if the instrument is idle for ≥5 days and lasts approximately 30 minutes. A maintenance wash is required if the instrument is idle for ≥10 days and lasts approximately 60 minutes. Both types of washes require the use of instrument wash reagents, which are to be prepared as described in the section titled "Prepare instrument wash reagent" above. To perform an instrument wash, a clustering instrument wash plate is required along with four empty troughs supplied in the accessory pack.

The cluster generator will require a standby wash in one or more of the following scenarios: 1) if pre-run checks fail and 2) if the instrument is idle for ≥5 days.

The cluster generator will require a maintenance wash in one or more of the following scenarios: 1) used routinely every 7 days, 2) if a run is cancelled, and 3) if the instrument is idle for ≥10 days thus preparing for long-term shutdown.

✔	Step	Instructions
	7.1	Rinse the clustering instrument wash plate and four empty troughs with DI water prior to use and dry with a lint-free towel.
	7.2	Load the four empty troughs onto the plate and ensure they are pressed firmly down until the tip of each trough meets the plate.
	7.3	On the clustering instrument screen, click on the top right hamburger menu and select "Washes". 

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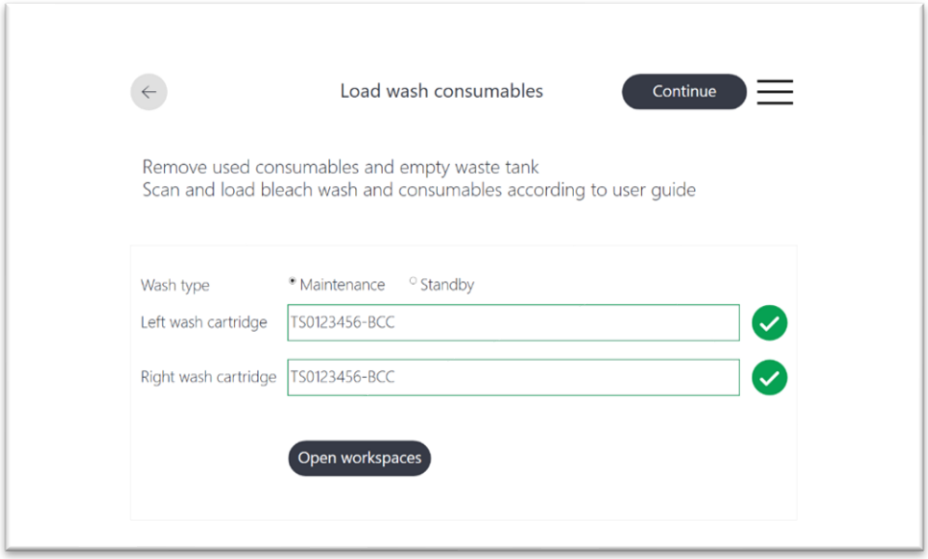
For “**Wash type**” select either “**Maintenance**” or “**Standby**” in the following screen. To open the reagent drawer, select “**Open workspaces**”. Lift open the workspace door.

7.4



7.5 If a previous clustering reagent plate is housed in the reagent drawer, remove and dispose in the appropriate waste streams.
If the wash cartridges are not loaded in the instrument connection slots within the workspace door, retrieve the wash cartridges from within the cluster generator drawer, and align one on each side with the instrument connector slots in the cluster generator workspace. Press down to engage wash cartridges until an audible click is heard.

It is encouraged to verify wash cartridge engagement by gently pulling the wash cartridges upward. If properly installed, the wash cartridges should not easily disconnect from the cluster generator.

7.6 Remove the waste container from the instrument and empty the contents into the appropriate waste stream. Ensure the emptied bottle is placed back into the correct position in the instrument.

7.7 Load the instrument wash plate and fill with appropriate reagents while on-instrument:

1. Load the clustering instrument wash plate with the four empty troughs onto the instrument. Refer to **step 5.10** for instructions on how to properly load the plate onto the instrument. Visually inspect to ensure the plate is loaded correctly and is aligned flat within the instrument.
2. With the plate loaded, use a serological pipette to add 28 mL of the instrument wash reagent to the three troughs furthest away from the user, facing the interior of the instrument.
3. Add 28 mL of user prepared 0.1% NaOCl (Bleach) solution to the first trough.

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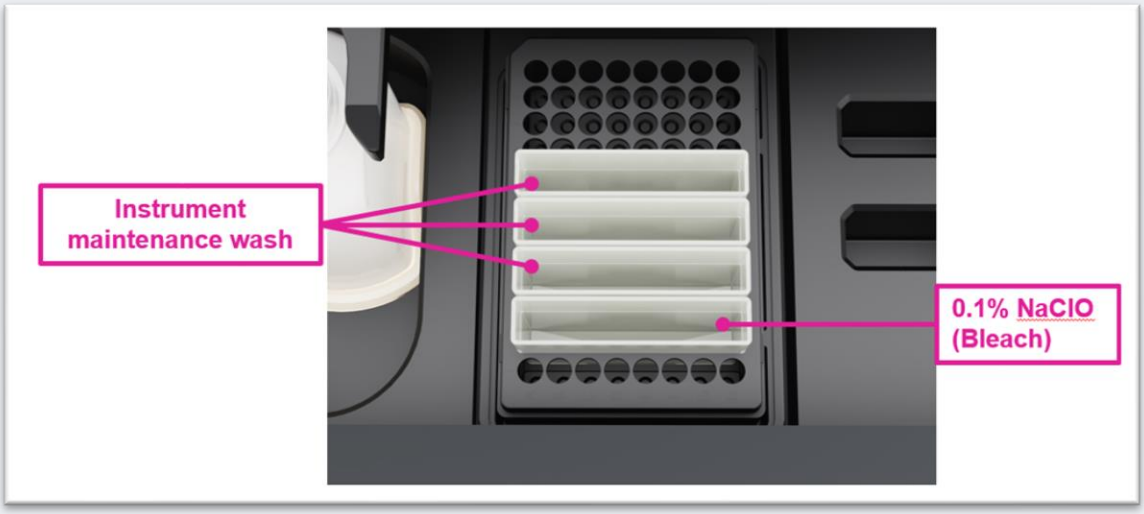
The same serological pipette can be used for step 2 and 3, when performed in the respective order.



	7.8	Scan in the wash cartridge QR codes in the prompted areas of the software screen.
	7.9	Close the flow cell workspace door.
	7.10	Select "**Close drawer**" on the software screen. Select "**Continue**" to advance the software.
	7.11	The cluster generator will then perform a series of pre-run checks over a 10-minute period. If pre-run checks complete successfully, the instrument will commence the wash. ⚠ Users are advised to check the instrument after 10 minutes to ensure pre-run checks completed successfully and that the maintenance wash has commenced. If an error is encountered, refer to the Troubleshooting section at the end of the Operations guide. Once complete, consumabes can remain on the instrument until next use.

8. Long-term shutdown

When expecting the instrument to sit idle for more than ≥10 days, prepare the instrument for a long-term shutdown by performing a maintenance wash.

✔	Step	Instructions
		Preparing clustering instrument for long-term shutdown
	8.1	Perform an instrument maintenance wash as described in the section above, titled "Instrument washes".
	8.2	Once complete, leave consumables and wash pack loaded on the instrument.
	8.3	If shutdown is expected to be longer than 10 days, power off the instrument by flipping the power switch located at the back of the instrument. See "Before you begin" section titled "Powering ON instrument" to identify power switch location.
		Preparing clustering instrument for use after long-term shutdown
	8.4	If the clustering instrument was powered off for long-term shutdown, power on instrument. See "Before you begin" section titled "Powering ON instrument" to identify power switch location.
	8.5	Using fresh wash reagents, perform an instrument maintenance wash as described in the section above, titled "Instrument washes".
	8.6	Once the instrument maintenance wash is complete, the instrument is ready to use.

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Sequencing

This section describes the workflow for sequencing an Onso clustered flow cell on the Onso sequencing system.

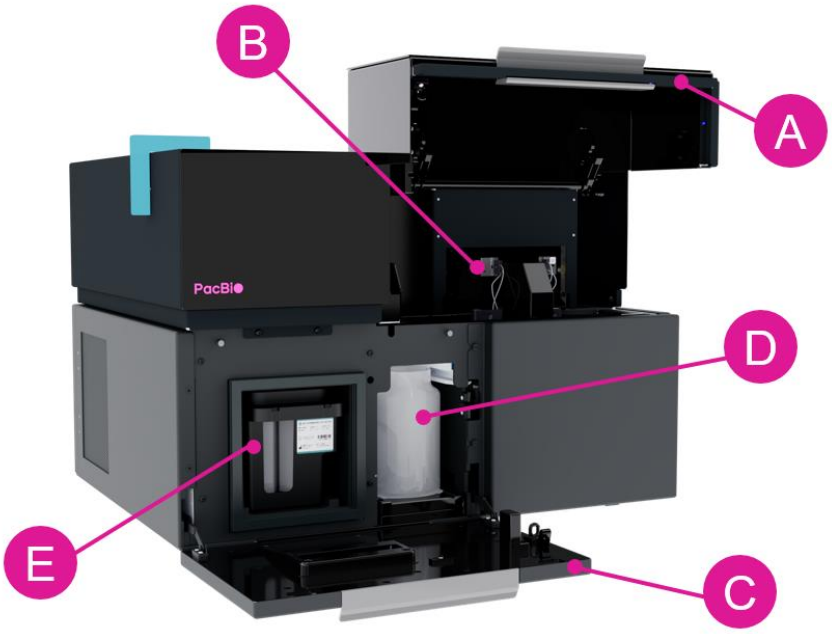
Each clustered flow cell and each sequencing reagent pack only support a single sequencing run. The number of cycles supported per reagent pack is indicated by the specific reagent pack being used (e.g., 200-cycle reagent pack can support either 1x200 or 2x100 cycle run; 300-cycle reagent pack can support either 1x200, 2x100 or 2x150 cycle runs).

The maximum number of cycles per index read is 14. For the number of cycles per insert read, users are to add 2 cycles to the desired run configuration (e.g., 100 cycles should be entered as 102; 150 cycles should be entered as 152).

Sequencing converted pre-existing P5/P7 libraries that have been clustered will require use of custom sequencing primer reagents. Instructions on the use of custom primers can be found in the section titled "Optional custom primer addition" in the Onso sequencing section of this guide.

During run setup, users are able to select a sample sheet prepared and saved on a drive accessible by the PC. For sample sheet information, templates, and guidance, refer to the PacBio document titled "Obc2fastq Reference guide".

Onso system short-read sequencer



The (A) workspace door lifts upward to allow for loading of the flow cell with attached MICA on the (B) flow cell loading mounts or pins. The workspace door will remain locked in the closed position prior to initiating the sequencing software, and during a sequencing run. By clicking "**Get started**" on the software *Home* screen (see section 3a below), the software will proceed to the *Load consumables* screen where the user can prompt the software to unlock the (C) reagent door, which opens toward the user. The (D) waste container and (E) sequencing reagent pack are housed within the reagent door. The waste container should be emptied and returned to its correct position within the instrument prior to starting a sequencing run, and during washes. Sequencing reagent packs are single-use and contain a barcode which should be visible when the reagent pack is properly loaded on the sequencing instrument.

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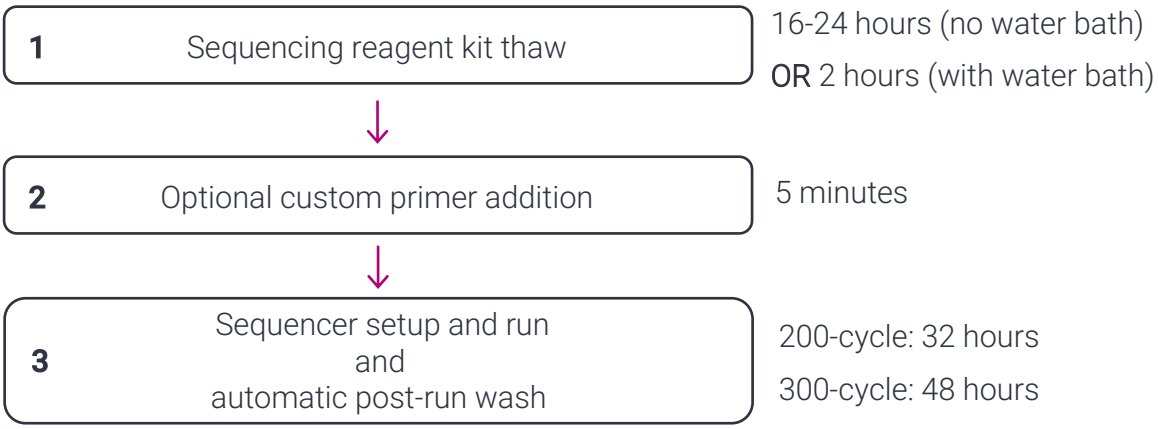
Sequencing workflow



Required materials and equipment

Sequencing Materials	Supplier/Manufacturer
Onso™ sequencing system	PacBio® 102-837-000
Clustered flow cell with attached MICA	Prepared by user
Onso™ sequencing reagent kit	200-cycle kit: PacBio® 102-656-400
	300-cycle kit: PacBio® 102-700-700
Reagent pack removal tools	PacBio® provided in accessory kit (PacBio® 102-512-502)
Onso™ sequencer instrument wash pack	PacBio® provided in accessory kit
Instrument wash reagent	Prepared by user (see section 2 of "Onso clustering")
Bleach (NaClO), ≥5%	Any Major Lab Supplier (MLS)
70% Isopropyl Alcohol	Any MLS
Lint-free wipes	Kimtech or other MLS
Serological Pipettor	Any MLS
Serological Pipettes (10 mL, 25 mL, 50 mL, or 100 mL)	Any MLS

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Sequencing procedure

1. Thawing sequencing reagent pack thaw

An Onso sequencing reagent pack can be removed from the box and mylar bag prior to thaw, as described below.

✔	Step	Instructions
	1.1	Remove the sequencing reagent pack from -20°C storage. Remove the reagent pack from packaging and the mylar bag. Discard the packaging and mylar bag.
	1.2	Check that the foil seals of each reagent bottle are intact by visual inspection of the top of the reagent pack. If not, dispose of the reagent pack in accordance with your site chemical waste disposal practices and obtain a new reagent pack.
	1.3	Thaw the sequencing reagent pack in one of two ways: • Thaw at room temperature (18-25°C) for 16 to 24 hours prior to sequencing. • Thaw the reagent pack in a room temperature (18-25°C) water bath with circulating water set to 25°C for 2 hours and remove promptly. Water bath depth should be 4-6 inches when the reagent pack is in the water bath. Ensure thawing in the water bath does not exceed more than 2 hours.
	1.4	Dry the thawed reagent pack with a towel before proceeding. Be sure to wipe any condensation or water off the bottles in addition to the exterior of the reagent pack plastic clamshell.
	1.5	Firmly grip the ends of the reagent pack with both hands. Thoroughly mix the contents by inverting the sequencing reagent pack at least 10 times. Visually inspect to confirm the reagent pack is fully thawed and mixed.

2. Optional custom primer addition

Custom primers should be added to the thawed reagent pack when sequencing converted libraries. If not using custom primers for sequencing, then proceed to section 3 to set up sequencing. Tube numbers indicated in magenta refer to the reagent pack position in the table below.

2a. Custom primer addition for sequencing pre-existing P5/P7 libraries clustered with Onso indexed library control spike-in

Description Primer	Custom Primer to add	Reagent Pack Position
Insert Read 1 Primer	Custom Insert Read 1 Primer	8
Index 1 Primer (downstream of the insert)	Custom Index Read 1 Primer	7
Insert Read 2 Primer	Custom Insert Read 2 Primer	6
Index 2 Primer (upstream of the insert)	Custom Index Read 2 Primer	5

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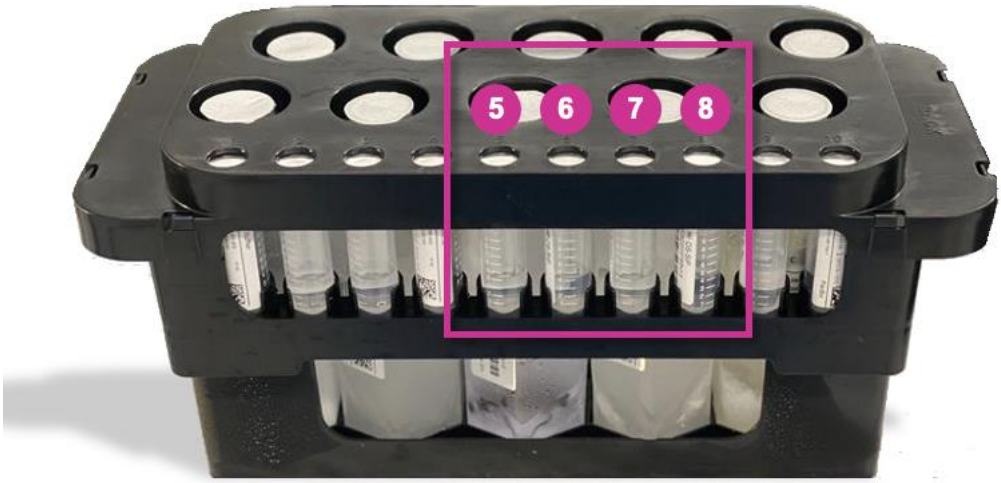

✔	Step	Instructions
	2.1	Ensure the sequencing reagent pack is thoroughly mixed. Rotate the custom primer addition tubes in the reagent pack (positions 5-8 as denoted in the image at the start of the section) such that the liquid level in the tube is visible from the side facing the user.
	2.2	Using a fresh P1000 pipette tip for each tube, puncture the foil seals of the custom primer tubes in positions 5-8 of the reagent pack. Discard the tips after each use.
	2.3	Using a P200 pipette, spike in 20 µL of primer solution into the appropriate position of the reagent pack as indicated in the table at the start of the section, using the following guidelines: o Combine 20 µL of each of the appropriate 100 µM custom primer to the appropriate position defined above. o Visually inspect the side of the custom primer tubes to ensure the primer is added directly to the buffer in the tube by submerging the pipette tip in the buffer solution during primer addition. Users are required to purchase custom sequencing primers. Please contact Support@pacb.com for primer sequences
	2.4	Using a P1000 pipette set to 1000 µL, pipette mix each tube at least 10 times after primer addition. Be sure to change pipette tips between tubes. Visually inspect the side of the custom primer tubes during mixing to ensure the pipette tip is submerged in the solution during mixing.

Note: if setting up in this configuration DO NOT select custom primers during run setup.

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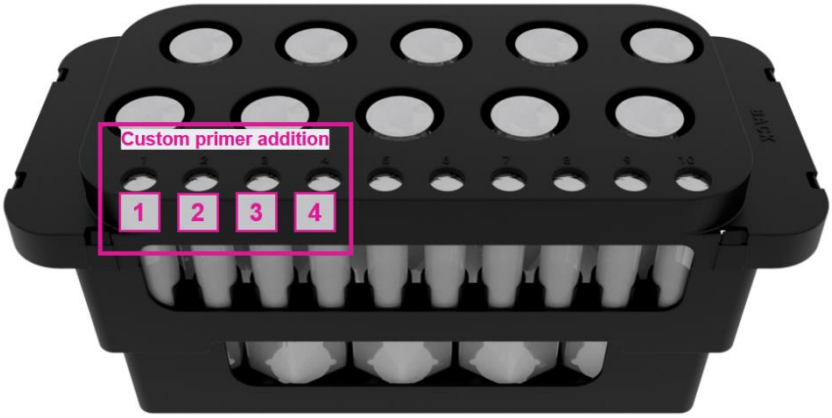
2b. Custom primer addition for sequencing pre-existing P5/P7 libraries clustered with NO Onso indexed library control spike-in

Description of Custom Primer	Reagent Pack Position
Custom **Insert Read 1 Primer**	4
Custom **Index 1 Primer** (downstream of the insert)	3
Custom **Insert Read 2 Primer**	2
Custom **Index 2 Primer** (upstream of the insert)	1



✔	Step	Instructions
	2.1	Ensure the sequencing reagent pack is thoroughly mixed. Rotate the custom primer addition tubes in the reagent pack (positions 1-4 as denoted in the image at the start of the section) such that the liquid level in the tube is visible from the side facing the user.
	2.2	Using a fresh P1000 pipette tip for each tube, puncture the foil seals of the custom primer tubes in positions 1-4 of the reagent pack. Discard the tips after each use.
	2.3	Using a P200 pipette, spike in 20 µL of primer solution into the appropriate position of the reagent pack as indicated in the table at the start of the section, using the following guidelines: o Spike in 20 µL of the appropriate 100 µM primer stock solution for each custom primer into the appropriate reagent pack position. o Visually inspect the side of the custom primer tubes to ensure the primer is added directly to the buffer in the tube by submerging the pipette tip in the buffer solution during primer addition.
	2.4	Using a P1000 pipette set to 1000 µL, pipette mix each tube at least 10 times after primer addition. Be sure to change pipette tips between tubes. Visually inspect the side of the custom primer tubes during mixing to ensure the pipette tip is submerged in the solution during mixing.

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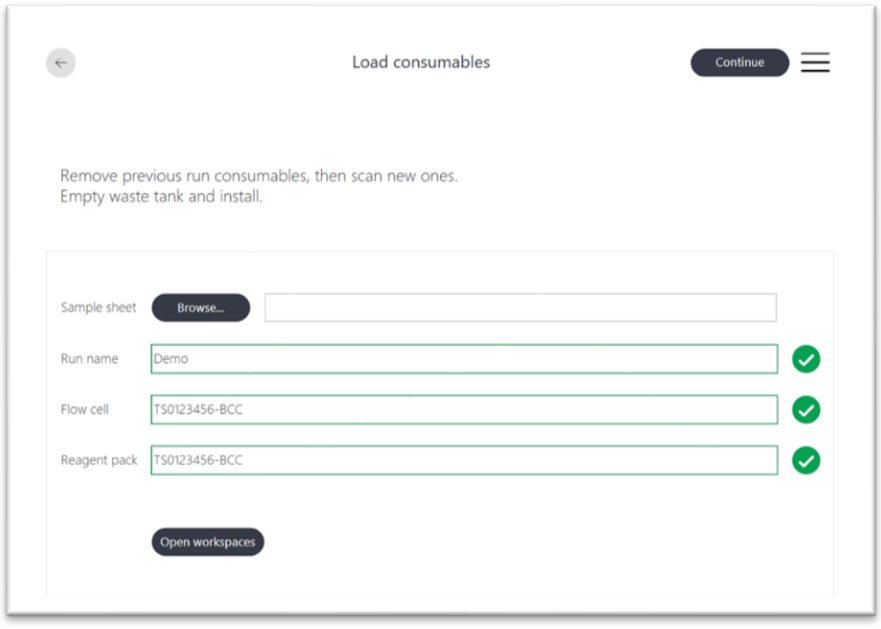
3. Sequencing set up

✔	Step	Instructions
	3.1	Check the status of the sequencing instrument. • If the instrument and/or the PC is off, refer to the section above titled "Before you begin" for instructions on how to power on the instrument and PC. • If the instrument is on, and at the home screen, select "**Get Started**" to begin. 
	3.2	In the *Load Consumables* screen, select "**Open workspaces**". The instrument will unlock the doors. Open the reagent door and workspace door. 
	3.3	Unload previous run consumables: • If a previous flow cell is found loaded on the instrument, gently pinch the sides of the MICA and lift up and out of the MICA instrument slots. Gently remove the flow cell from the instrument stage being careful to slide the flow cell off the metal guide pins. Discard the flow cell and attached MICA in the appropriate waste stream.

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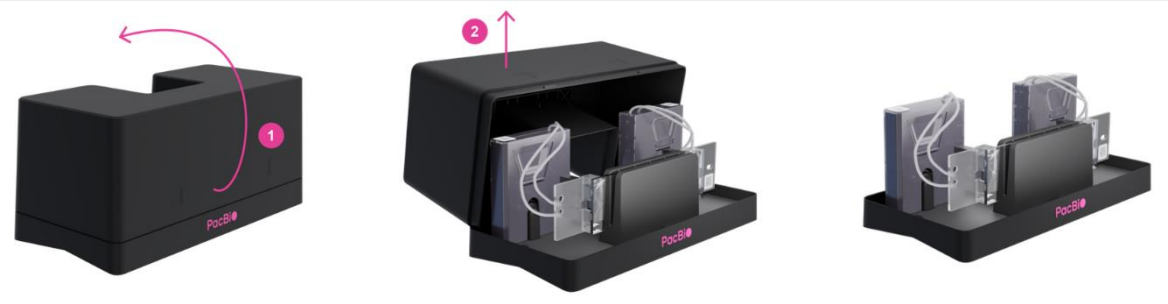
- If wash cartridges are found loaded, remove them by gently pinching the sides of the wash cartridges and lifting up and out of the instrument slots. Place the wash cartridges in a plastic bag for future use.

  Wash cartridges are safe to use for up to 30 days from initial use.
- If a previous sequencing reagent pack is found loaded in the instrument reagent door, dispose of the previous sequencing reagent pack and its contents in the appropriate waste stream. Refer to the section titled "Dekitting and Reagent Discard" for detailed instructions.
- If a sequencing instrument wash pack is found loaded in the instrument reagent door, remove and clean the wash pack by rinsing with DI water and drying by inverting and tapping gently upside down. Dry the exterior of the wash pack with a lint-free wipe. Store the wash pack upside down or in a bag to prevent dust accumulation between uses.

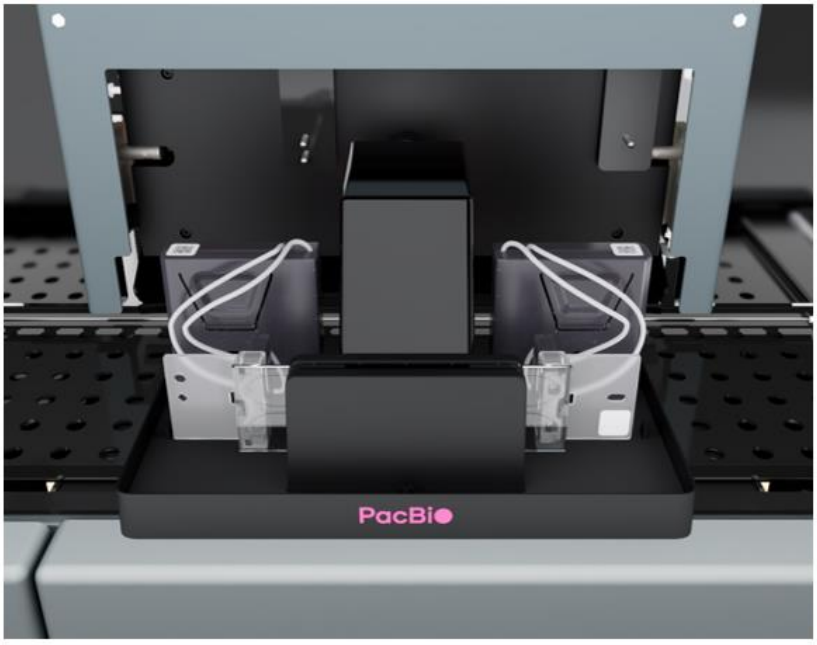
3.4 Empty the internal waste container and return the container to the instrument. Ensure that the waste container is loaded correctly under the waste line spout. See section "Dekitting and Reagent Discard" for detailed instructions.

3.5 Open the flow cell kit containing the clustered flow cell:

1. Hold the base of the flow cell kit in one hand with the PacBio logo forward-facing. Lift and roll the top-front of the box towards the back.
2. Lift the lid of the flow cell kit upward and set aside.



3.6 Load the flow cell kit containing the clustered flow cell onto the sequencer, by placing the bottom base of the opened flow cell kit onto the sequencing instrument workspace area, such that the PacBio logo on the base of the flow cell kit is facing the user. The cutout in the flow cell kit base should wrap around the preload cover.



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3.7 Obtain a lint-free wipe saturated in 70% isopropyl alcohol.

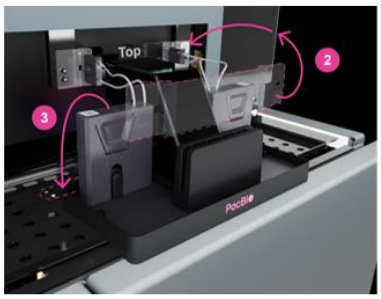
Load the flow cell in the sequencer instrument workspace:

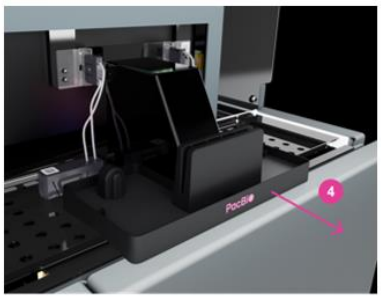
1. Lift the flow cell out of the kit base and wipe clean with isopropyl alcohol:
    a. Holding either the left or right wing of the flow cell, lift the flow cell directly upward out of the kit base. In this orientation, the bottom of the flow cell is facing the user.
    b. Wipe clean the front and back of the flow cell surface using a lint-free wipe saturated with 70% isopropyl alcohol.
    c. Allow the 70% isopropyl alcohol to dry from the flow cell surface or wipe dry with a new lint-free wipe.
2. Mount the flow cell onto the instrument metal guide pins:
    a. Rotate the flow cell away from the user and over the preload cover. The QR code must be in the upper right-hand corner, and the top of the flow cell facing the user once rotated.
    b. Align the pinholes on the flow cell with the metal guide pins on the sequencer in the workspace area. The flow cell wing with two pinholes (circle and diamond) will be positioned on the left wing and the wing containing one pinhole (oval) will be positioned on the right.
    c. Slide the flow cell onto the guide pins, away from the user.
3. Engage the left and right MICA:

3.8
    a. Remove the left and right MICA from the flow cell kit base and align the MICA instrument connector sides with the instrument connection slots such that the fluidic tubing is towards the center of the instrument, while the QR code is pointed towards the left and right sides of the instrument, respectively.
    b. Connect both left and right MICA by gently pushing each down into their respective slots until an audible click is heard for each. The audible click indicates that the MICA has been engaged.

It is encouraged to verify MICA engagement by gently pulling the MICA instrument connectors upward – if properly installed, the MICA instrument connectors should not easily disconnect from the cluster generator.

4. Remove the flow cell kit base from the workspace area by sliding the base towards the user until the kit base is no longer seated on the instrument. Users can either retain the flow cell kit lid and base or dispose in appropriate waste streams.





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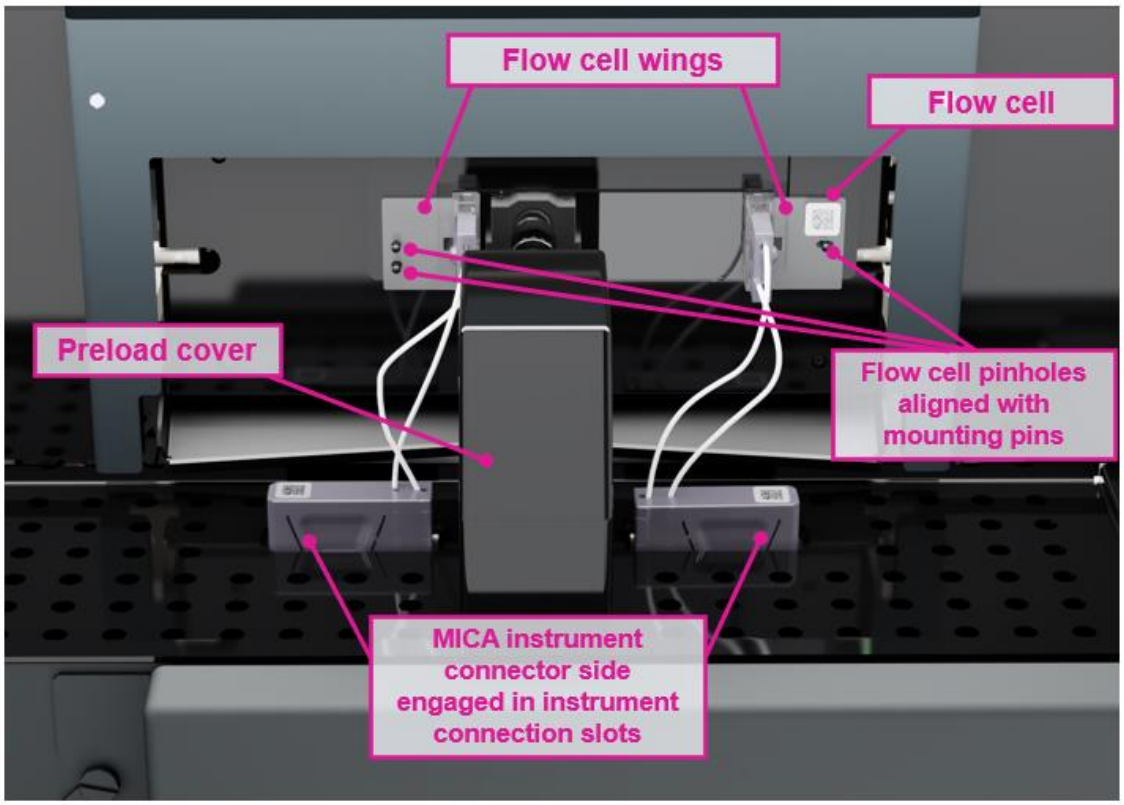

3.9 Load the prepared sequencing reagent pack into the instrument by sliding the reagent pack into the internal compartment of the sequencing instrument reagent door. The reagent pack barcode should be facing the user. Gently push the reagent pack all the way to the rear of the reagent door internal compartment.

On the *Load consumables* software screen, users can either select a prepared sample sheet for the sequencing run or enter fields manually.

See PacBio document "obc2fastq reference guide" for detailed instructions on how to prepare a sample sheet, if desired.

3.10
- "Run name" – if using a sample sheet, this field will populate from the selected sample sheet, otherwise, users are to manually enter a run name identifier.
- "Flow cell" – use the barcode scanner to scan in the flow cell QR code. If the scanned flow cell ID differs from that in the sample sheet, a warning will be issued to notify the user. The software will use the scanned field as ground truth if user chooses to proceed.
- "Reagent pack" – use the barcode scanner to scan in the reagent pack.

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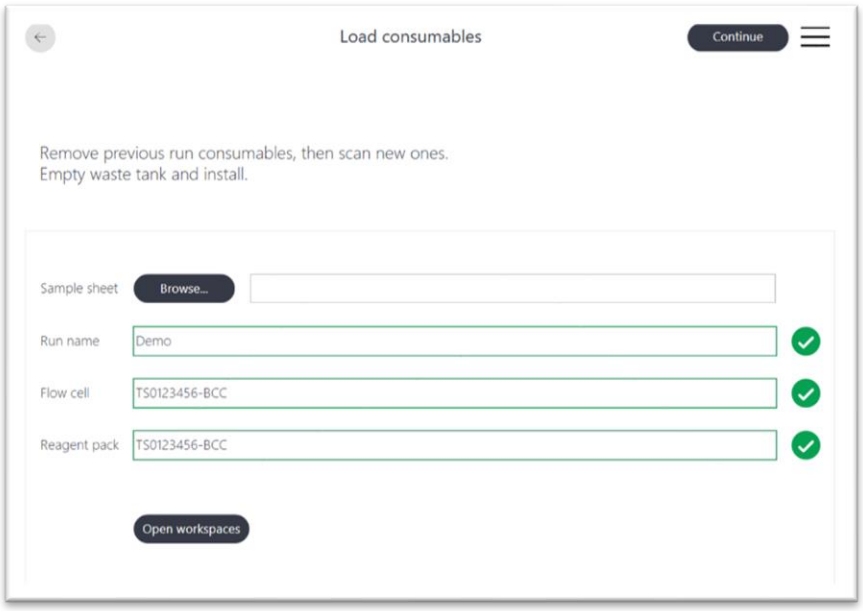
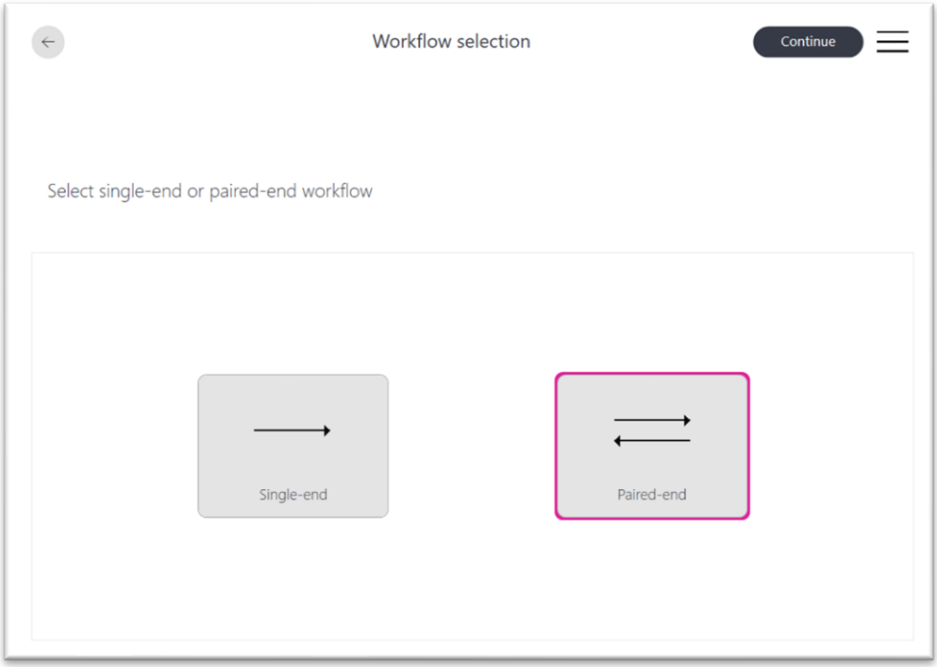


3.11 Close the sequencer workspace door and reagent door. Once closed, select "**Continue**" to advance.

3.12 Select the appropriate run configuration (single-end or paired-end) on the software screen. Select "**Continue**" to proceed onto the next screen.



3.13 On the *Run parameters* software screen, users can either complete fields manually, or use the prepared and selected sample sheet for the sequencing run. For the fields below, if a discrepancy is found between the sample sheet and user input directly on the software screen, the software will run as indicated on the software screen.

- "Insert read 1" & "Insert read 2" -- users must add 2 cycles to the number of desired insert cycles. (e.g. for 100 base pair insert read 1, enter "102" in the "Insert read 1" field.
- "Index 1" & "Index 2" – input the number of index reads up to 14 base pairs each.

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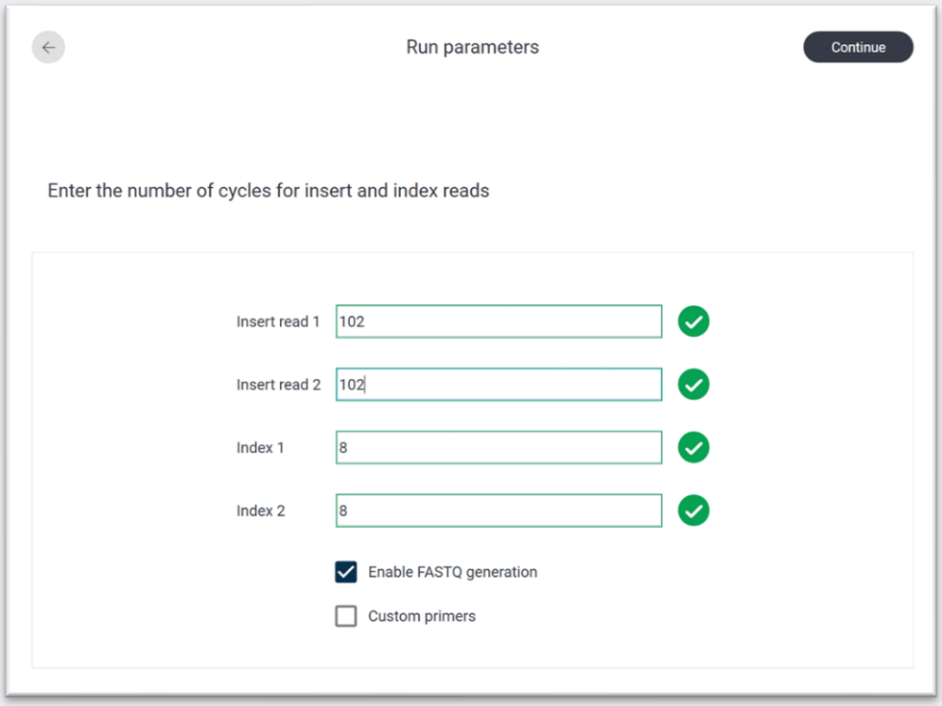
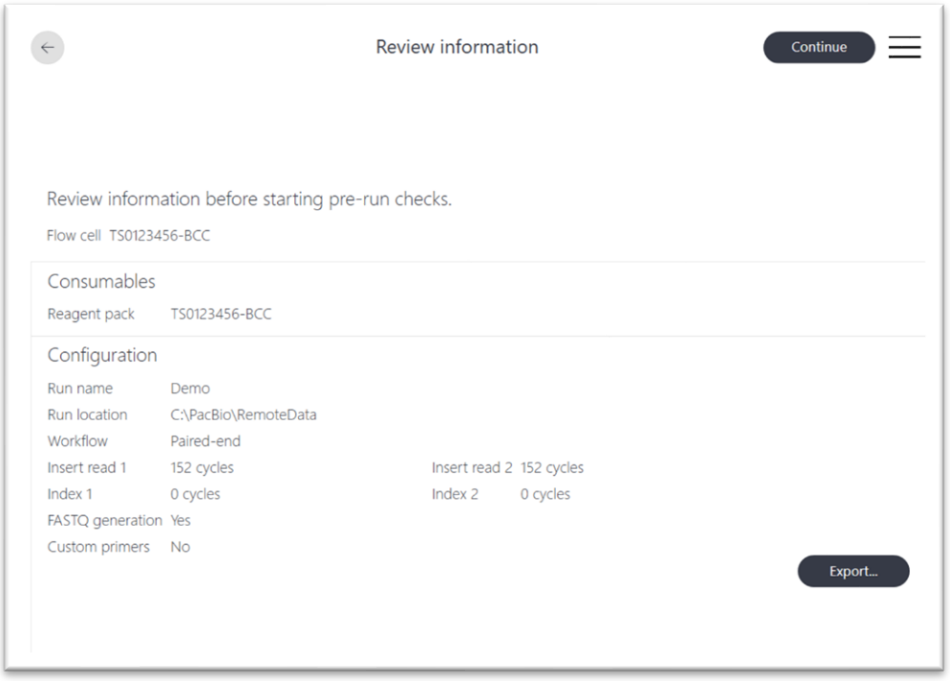
- "Enable FASTQ generation" – select if outputs preferred in FASTQ format.
- "Custom primers" – select if custom primers are to be used. Refer to section 2 of "Onso sequencing" of this guide.

The example shown below is for a 2x100 sequencing run for native Onso libraries. Native Onso libraries contain 8 base pair index sequences, and do not require the use of custom primers.



Confirm field entries are correct. Select "Continue" to advance.
Verify that the fields in the *Review information* screen are correct. If changes need to be made, click the back arrow at the top left to make the necessary changes. Select "Continue" to advance.

3.14



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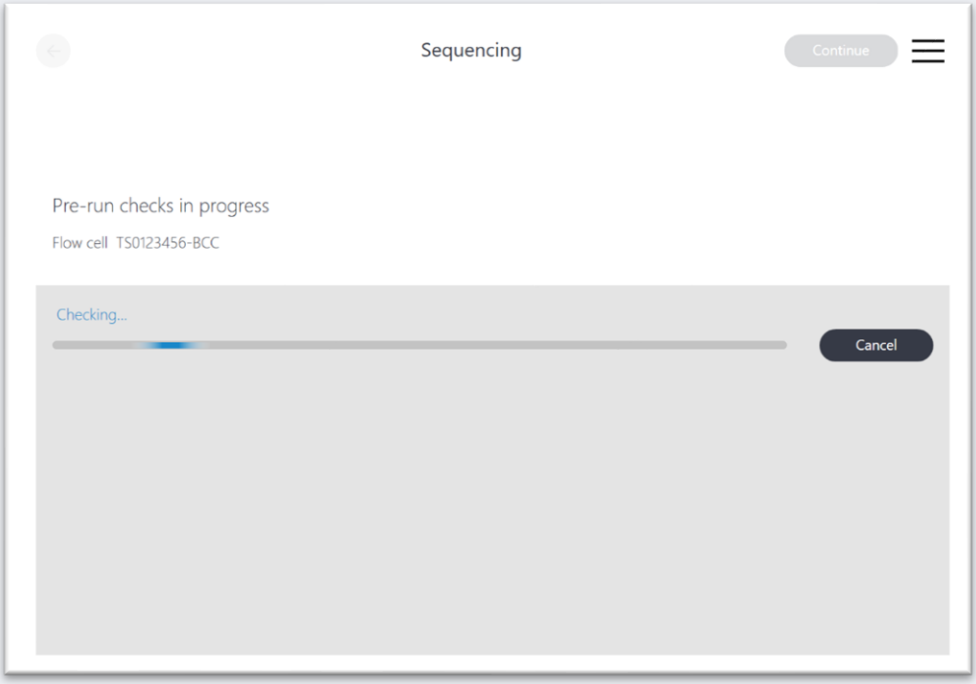
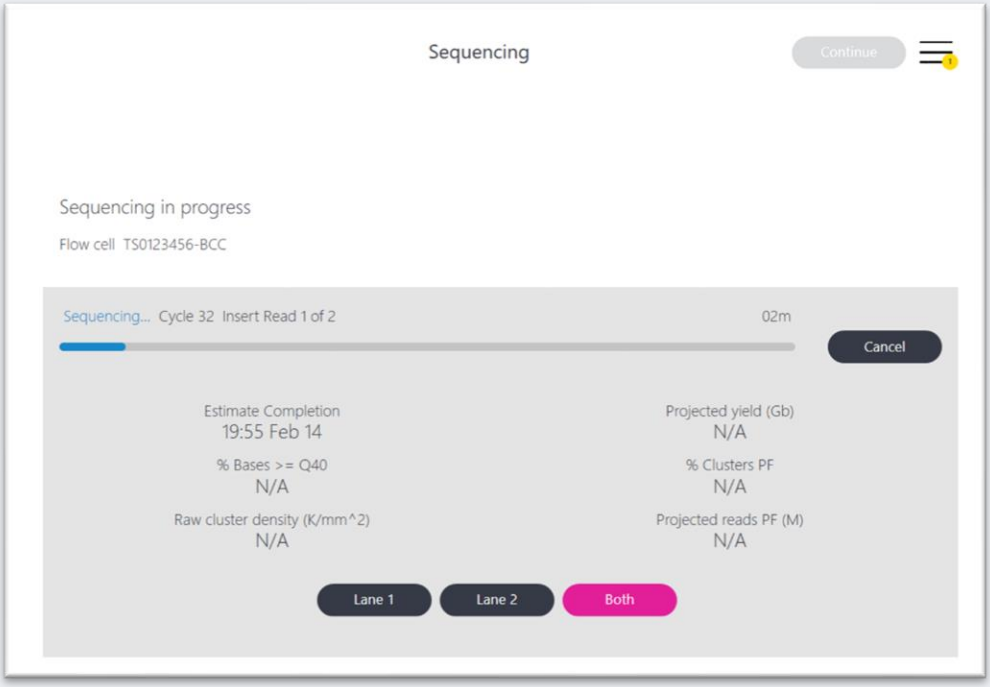
The sequencer will then perform a series of pre-run checks. The pre-run checks are designed to check disk space availability, preload engagement, and fluidic path with the flow cell and MICA connected. If any of the steps fail, refer to the "Troubleshooting" section of the guide.



3.15 Once the pre-run check has been successfully completed, the sequencing run will automatically begin. Run progress can be monitored on the sequencing screen.



3.16 A sequencing run can be terminated by selecting the "**Cancel**" button on the screen. The run will stop and end after the completion of the current step. If a run is cancelled, a maintenance wash must be performed to prepare the instrument for the next run. Additional instructions can be found in the "Instrument washes" section.

3.17 When all sequencing cycles have completed, the software will automatically begin a post-run wash to prepare the instrument for the next run. Once completed, click "**Continue**" in the top corner of the user interface.

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Used consumables, including the flow cell, MICA, and reagent pack are safe to leave on the instrument. When unloading the instrument, proceed to the section "Unloading the sequencing instrument".

If the next sequencing run is not expected to be performed within 5 days, it is recommended to perform a standby wash. If the instrument is expected to be idle for at least 10 days, perform a maintenance wash and prepare the instrument for long-term shutdown. For standby and maintenance washes proceed to section "Instrument washes" and/or section "Long-term shutdown" for detailed instructions.

4. Unloading the sequencing instrument

✔	Step	Instructions
	4.1	Once post-run wash is complete, select "Finish" to return to the *Home* screen.
	4.2	Select "Get started" to enter the *Load consumables* screen. Select "Open workspaces" to unlock the reagent door and workspace door.
	4.3	Remove the used reagent pack from the internal compartment of the reagent door. Discard unused reagents in accordance with your site chemical waste disposal practices. Refer to "De-kitting and Reagent Discard" for detailed instructions.
	4.4	Remove the previous flow cell and MICA by gently pinching the grooves of the MICA and lifting them up and out of the MICA connection slots. Pull the flow cell of the instrument stage, being careful to unload them off the metal guide pins. Refer to section "De-kitting and reagent discard" for instructions on disposal of the flow cell and MICA.
	4.5	 It is recommended to load and engage the instrument wash cartridges into the instrument connection slots when no flow cell or MICA are loaded on the instrument. Retrieve the wash cartridges and align one on each side with the instrument connector slots in the sequencer workspace. Press down to engage wash cartridges until an audible click is heard. It is encouraged to verify wash cartridge engagement by gently pulling the wash cartridges upward. If properly installed, the wash cartridges should not easily disconnect from the sequencing instrument.
	4.6	Close the reagent door and workspace area.
	4.7	Select the back arrow at the top left of the screen to return to the *Home* screen.

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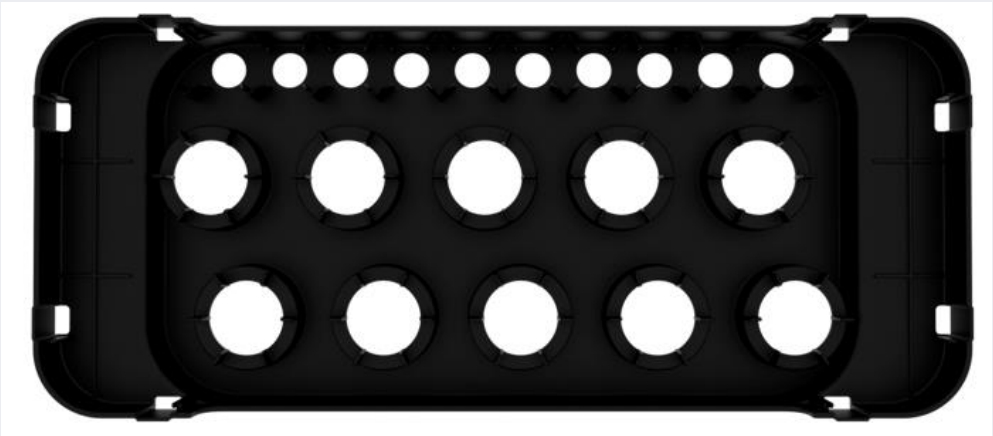
5. De-kitting and discarding the reagent pack

To dispose of an unused or used reagent pack, the reagent pack clamshell can be opened with the use of 2 reusable reagent pack removal tools, provided in the accessory kit. This allows for disposal of the individual bottles separately and separate from the plastic pack.

✔	Step	Instructions
	5.1	• Align a reagent pack removal tool along each of the short sides of the reagent pack, such that the tabs of the tools fit securely over the handles of the reagent pack. • Press down the edges of the reagent pack removal tools until the clips engage underneath with an audible click. The tabs of the tool will push out the clips from the reagent pack. • Rotate the reagent pack such that both handles are accessible at the same time. Lift up on the two reagent pack removal tools simultaneously to separate the top and bottom clamshell components of the reagent pack.
	5.2	Grasp under the reagent pack handles. Lift and remove the lid of the reagent pack, placing the top of the lid face down.
	5.3	Identify the two clips per reagent pack removal tool that are engaged on the reagent pack lid. Release the reagent pack removal tools from the reagent pack lid by gently bending the clips outward and allowing the tools to disengage from the reagent pack handles.  The reagent pack removal tools should be saved and stored for future use.
	5.4	Remove the bottles from the pack and discard the bottle and its contents in accordance with your site chemical waste disposal practices. Refer to the SDS for information that will help you determine how to meet your site disposal practices for each bottle and its contents. Note: The plastic reagent pack clamshell is made of high-density polyethylene (HDPE) with talc.
	5.5	If liquids will be poured from their bottles as part of disposal, widen the hole of each punctured reagent bottle and primer tubes with a serological pipette. All bottles have threading, so they can be securely closed with a cap if necessary for disposal. Two caps are provided in each kit for your use.
	5.6	Remove the reagent waste container from the instrument and dispose the contents in accordance with your site chemical waste disposal practices. Ensure that the empty internal waste container is returned to the instrument and is aligned correctly under the waste line spout.
	5.7	Separate the MICA from the flow cell by pressing the circular knob on the flow cell connector and pulling away from the flow cell. Used flow cells can be discarded in a properly labelled sharps or broken glass disposal. For disposal of used MICA, check with local EH&S for appropriate disposal recommendations.

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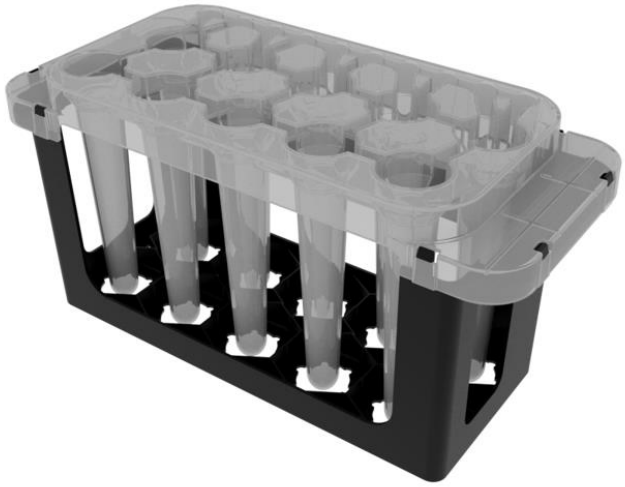
6. Instrument washes

There are two types of instrument washes outside of the standard sequencing workflow: 1) standby wash, and 2) maintenance wash. Both types of washes require the use of the sequencing instrument wash pack and contains two wash steps requiring user interaction between them. The first wash step is performed with diluted bleach (prepared as described below), and the second wash step is performed using instrument wash reagent (prepared as described in section 2 of the Onso clustering section of this guide).

The Onso sequencing instrument will require a standby wash in one or more of the following scenarios: 1) if pre-run checks fail and 2) if the instrument is idle for ≥5 days. For a standby wash, the first wash step using diluted bleach lasts approximately 20 minutes, and the second wash step using instrument wash reagent lasts approximately 20 minutes.

The Onso sequencing instrument will require a maintenance wash in one or more of the following scenarios: 1) used routinely every 7 days, 2) if a run is cancelled, and 3) if the instrument is idle for ≥10 days thus preparing for long-term shutdown. For a maintenance wash, the first wash step using diluted bleach lasts approximately 20 minutes, and the second wash step using instrument wash reagent lasts approximately 2 hours.



✔	Step	Instructions
	6.1	Prepare 1 L of 0.1% NaClO (Bleach) solution, and 1 L of instrument wash reagent. See section 2 of Onso clustering, titled "Prepare instrument wash reagent".
	6.2	Rinse the sequencer instrument wash pack with DI water prior to use. Dry the instrument wash pack by inverting and tapping gently upside down and dry the exterior with a lint-free wipe.
	6.3	Add approximately 1 L of 0.1% NaClO (Bleach) solution to the instrument wash pack up to the fill lines.
	6.4	On the software *Home* screen, click on the hamburger menu at the top right, to select "Washes".

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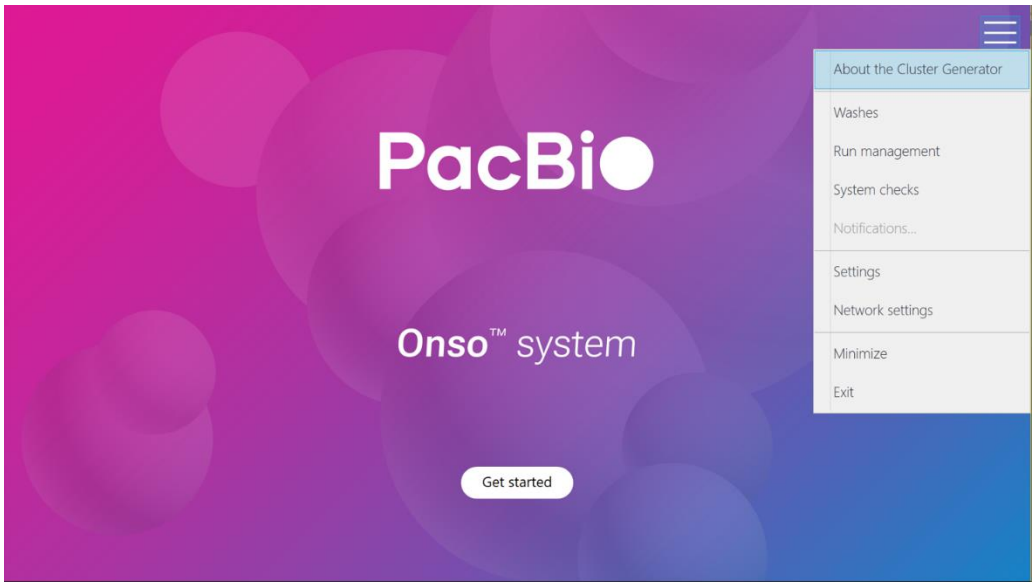
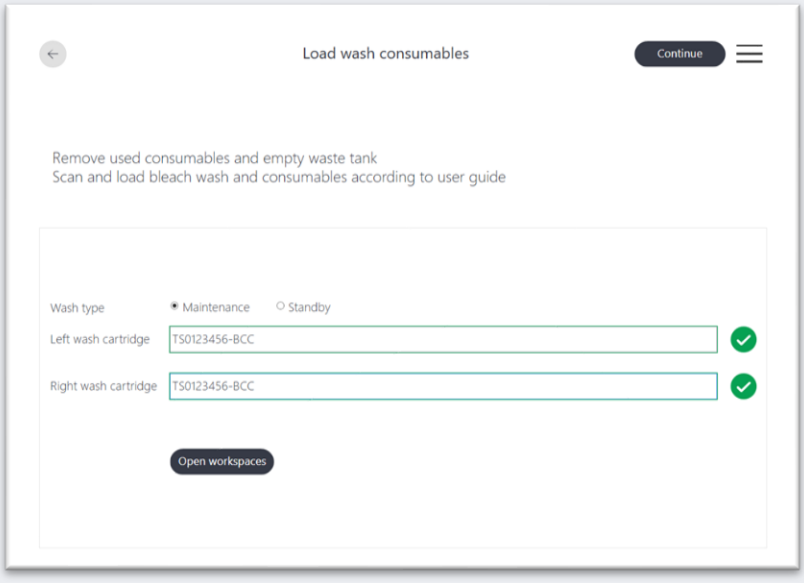


6.5	Click "**Open workspaces**" on the *Load wash consumables* screen to unlock the workspace door and reagent door. For "**Wash type**" select "**Maintenance**" or "**Standby**".
6.6	Remove the waste container from the instrument and empty the contents into the appropriate waste stream. Ensure the emptied bottle is placed back into the correct position in the instrument.
6.7	Load the instrument wash pack in the interior compartment of the reagent door.
6.8	If previously used sequencing run consumables and reagents are found on the instrument, refer to the section above titled "Unloading the sequencing instrument". Connect the wash cartridges by aligning one on each side with the MICA instrument connector slot. Press down to engage, and check engagement by tugging gently upward to see that it does not lift out.
6.9	Scan in the left and right Onso wash cartridge QR codes.  Close the reagent door and the workspace door. Select "**Continue**" to enter the pre-wash checks screen.
6.10	The instrument will proceed to perform pre-wash checks. If any steps fail, refer to the Troubleshooting section of the guide. Once pre-wash checks complete successfully, the wash will automatically begin and will last approximately 20 minutes.

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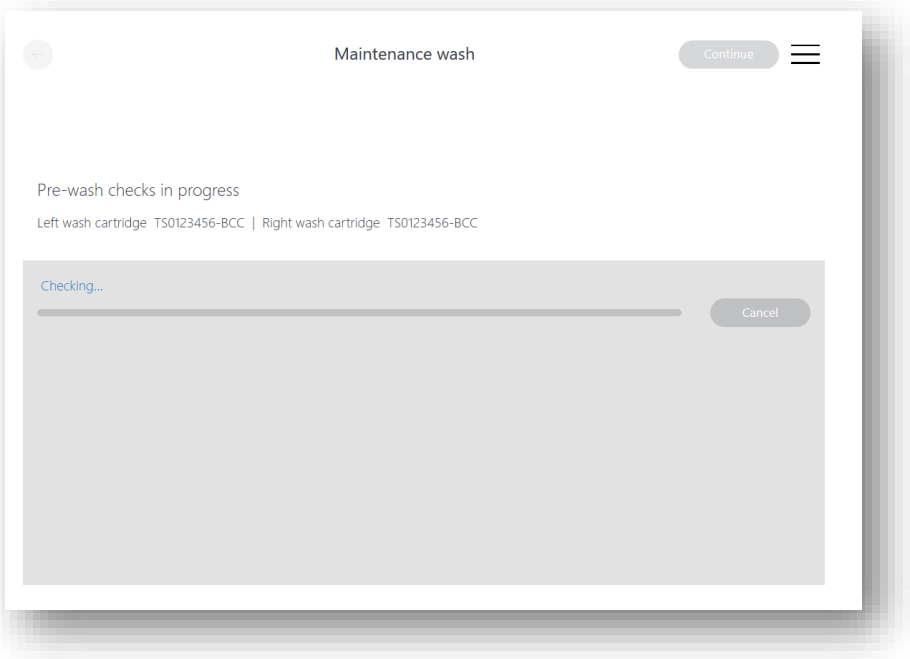

6.11	When the bleach wash portion of the two-part wash completes, select "**Continue**" to advance.
6.12	Select "**Open workspaces**" to unlock the reagent door.
6.13	Remove the waste container from the instrument and empty the contents into the appropriate waste stream. Ensure the emptied bottle is placed back into the correct position in the instrument.
6.14	Remove the instrument wash pack from the interior compartment of the reagent door. Discard the remaining bleach solution from the wash pack in the appropriate waste stream.
6.15	Rinse the sequencer instrument wash pack with DI water prior to use. Dry the instrument wash pack by inverting and tapping gently upside down and dry the exterior with a lint-free wipe.
6.16	Add approximately 1 L of instrument wash reagent to the instrument wash pack up to the fill lines.
6.17	Load the instrument wash pack in the interior compartment of the reagent door. Close the reagent door and select "**Continue**" to advance.
6.18	The instrument will proceed to perform pre-wash checks. If any steps fail, refer to the Troubleshooting section of the guide. Once pre-wash checks complete successfully, the wash will automatically begin and will last approximately 20 minutes for standby wash, or 2 hours for maintenance wash. Once complete, consumables can remain on the instrument until next use.

7. Long-term shutdown

When expecting the instrument to sit idle for more than ≥10 days, prepare the instrument for a long-term shutdown by performing a maintenance wash. If planned to be idle for ≥10 days, the instrument and PC are to be powered off.

✔	Step	Instructions
		Preparing sequencing instrument for long-term shutdown
	7.1	Perform an instrument maintenance wash as described in the section above, titled "Instrument washes".
	7.2	Once complete, leave consumables and wash pack loaded on the instrument.
	7.3	If shutdown is expected to be longer than 10 days, power off the instrument by flipping the power switch located at the back of the instrument. See "Before you begin" section titled "Powering ON instrument" to identify power switch location.

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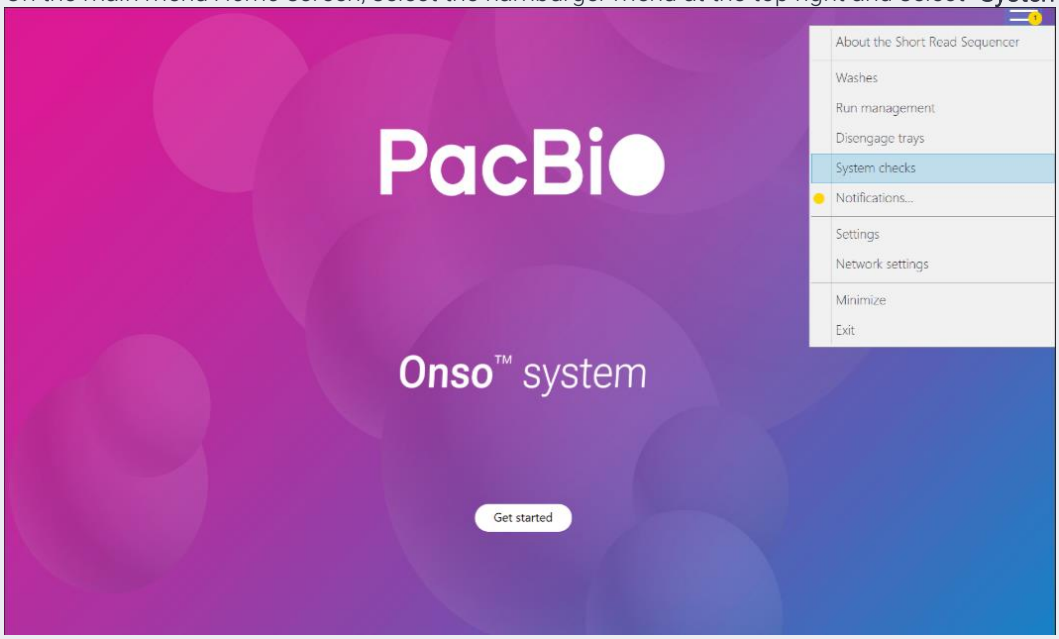
	Preparing sequencing instrument for use after long-term shutdown
7.4	If the sequencing instrument and PC were powered off for long-term shutdown, power on the instrument, wait 30 seconds, and power on the PC. See "Before you begin" section titled "Powering ON instrument" to identify power switch location.
7.5	Using fresh wash reagents, perform an instrument maintenance wash as described in the section above, titled "Instrument washes".
7.6	Once the instrument maintenance wash is complete, the instrument is ready to use.

8. Run management

✔	Step	Instructions
	8.1	On the main menu *Home* screen, select the hamburger menu at the top right and select "Run management".
	8.2	Check the amount of space currently available. Sequencing runs take a lot of space, and it is recommended to delete runs that have a Copy Status noted as "Complete" to clear space for the next run. ⚠ Do not delete runs that do not have a Copy status as "Complete", or data will be lost.

9. System checks

For troubleshooting purposes, a PacBio Technical Support representative may request that the user perform system checks to collect instrument health data. System checks should only be performed with guidance from a PacBio Technical Support representative. Navigate to the hamburger menu and select System checks to open the System checks menu to select the desired system check tests. Once system checks complete, test results can be found in D:\PacBio\Metrics\PreRun to be shared with PacBio Technical Support.

✔	Step	Instructions
	9.1	On the main menu *Home* screen, select the hamburger menu at the top right and select "System checks". 

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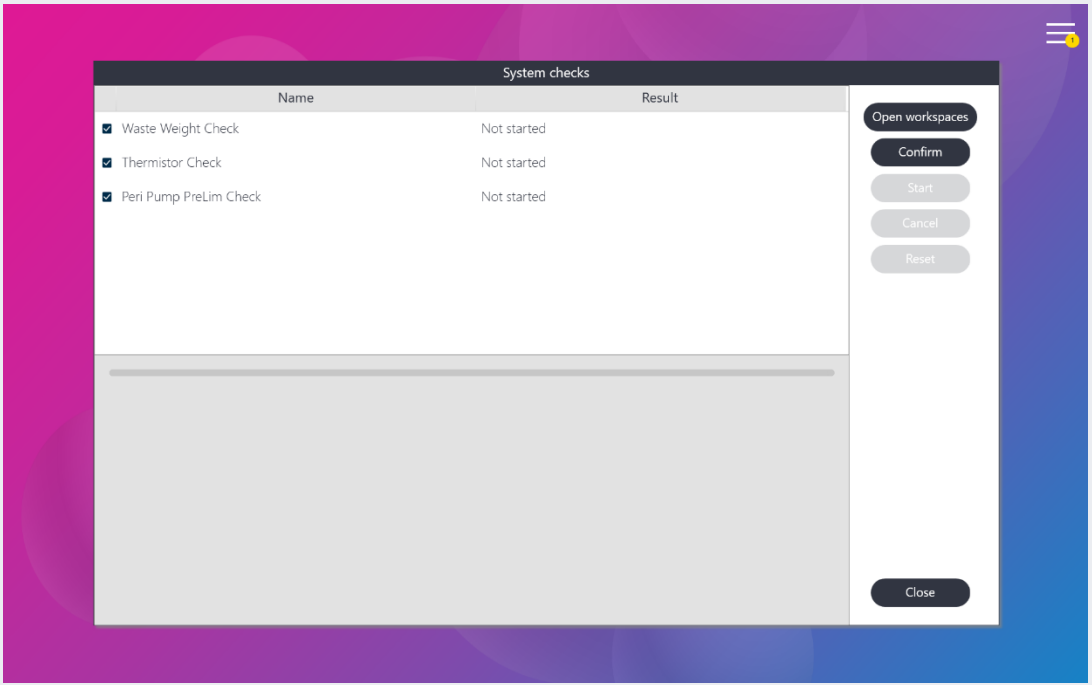

Waste weight check and/or thermistor check tests

9.2	Select "**Open workspaces**". Load an empty waste bottle and empty instrument wash pack into the system.
9.3	Close both workspaces and select "**Confirm**".
9.4	Select "**Start**" to begin the system check tests.
9.5	Once tests complete, results can be found in D:\PacBio\Metrics\PreRun
9.6	When finished, select "**Close**" to go back to the *Home* screen.

Peri pump prelim check test

9.7	Select "**Open workspaces**". Load an empty waste bottle and wash pack filled with 1L of instrument wash reagent into the system.
9.8	Load the wash cartridges onto the system and check the engagement by gently pulling upward on both cartridges to verify they do not lift out of the system.
9.9	Close both workspaces and select "**Confirm**".
9.10	Select "**Start**" to begin the system check test.
9.11	Once the test is complete, results can be found in D:\PacBio\Metrics\PreRun
9.12	When finished, select "**Close**" to go back to the *Home* screen.

Troubleshooting

Error	Troubleshooting Instructions
Sequencing instrument does not connect to software	Check to ensure a wired connection exists between the instrument and PC. Power off the sequencer and exit the sequencing software. Exiting the software will cause the PC to shut down. Restart the sequencer, wait 30 seconds, then power on the sequencer.

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	If trouble persists, contact your PacBio field service and support.
Preload does not engage/disengage	If the preload does not engage during pre-run checks, re-initialize the pre-run checks. If the preload does not disengage after a run has completed, restart both the instrument and PC. If the problem persists, contact your PacBio field service and support.
Pre-run check failure	Check the MICA or wash cartridge connection by pinching the grooves on the MICA or wash cartridge and gently lifting up to release. Reseat the MICA or wash cartridges into the instrument ports and retry the pre-run check. If reconnecting the MICA does not resolve the issue, unload the flow cell and MICA and perform a maintenance wash. If the maintenance wash completes, retry loading the flow cell consumable. If the maintenance wash fails, contact your PacBio field service and support.
Sequencing reagent pack not detected	Ensure that the reagent pack is pushed all the way into the internal compartment of the reagent door. The reagent door must be able to close without obstruction. If the reagent pack does not fully fit into the reagent door internal compartment, remove the sequencing reagent pack and check for any unexpected obstructions in the drawer. If no obstruction is detected, re-insert the reagent pack, ensuring that the primers are oriented towards the right-hand side, and the sequencing reagent pack barcode is facing the user when inserted into the instrument. Gently push the reagent pack all the way to into the internal compartment of the reagent door and close the reagent door.
Low Disk Space	See section titled "Run management" for instruction. If trouble persists, contact your PacBio field service and support.

Revision history (description)	Version	Date
Initial release.	01	AUG 2023
Updated screenshots referencing "workspaces" door.	02	AUG 2023
Updated maintenance wash to 7 days instead of 14 days. Other minor updates throughout.	03	DEC 2023
Updated software screens, addition of standby wash, and other minor updates throughout.	04	APR 2024

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