



# cBot and HiSeq Systems Best Practice and Maintenance

Presented by:

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# Session Objectives

- ▶ By the end of this training, you will be able to:
  - List the best practice steps for cluster generation and sequencing
  - Describe cBot and HiSeq maintenance steps

# Best Practice



## Cluster Generation



1. Lab Tracking Form
2. Confirm Reagent Delivery

## Sequencing



1. Reagents Tracking
2. First Base Report
3. Real Time Metrics
4. Thumbnails

# Lab Tracking Form Location

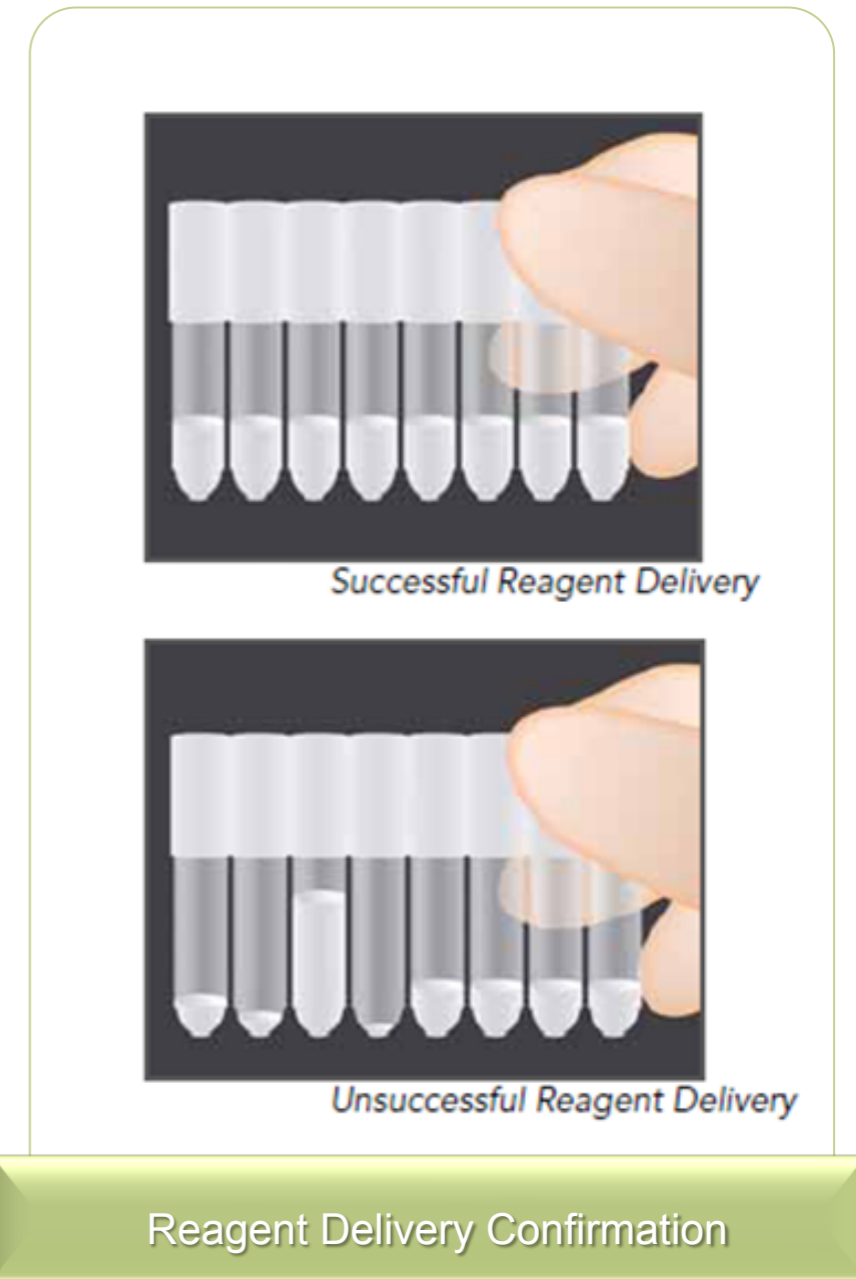
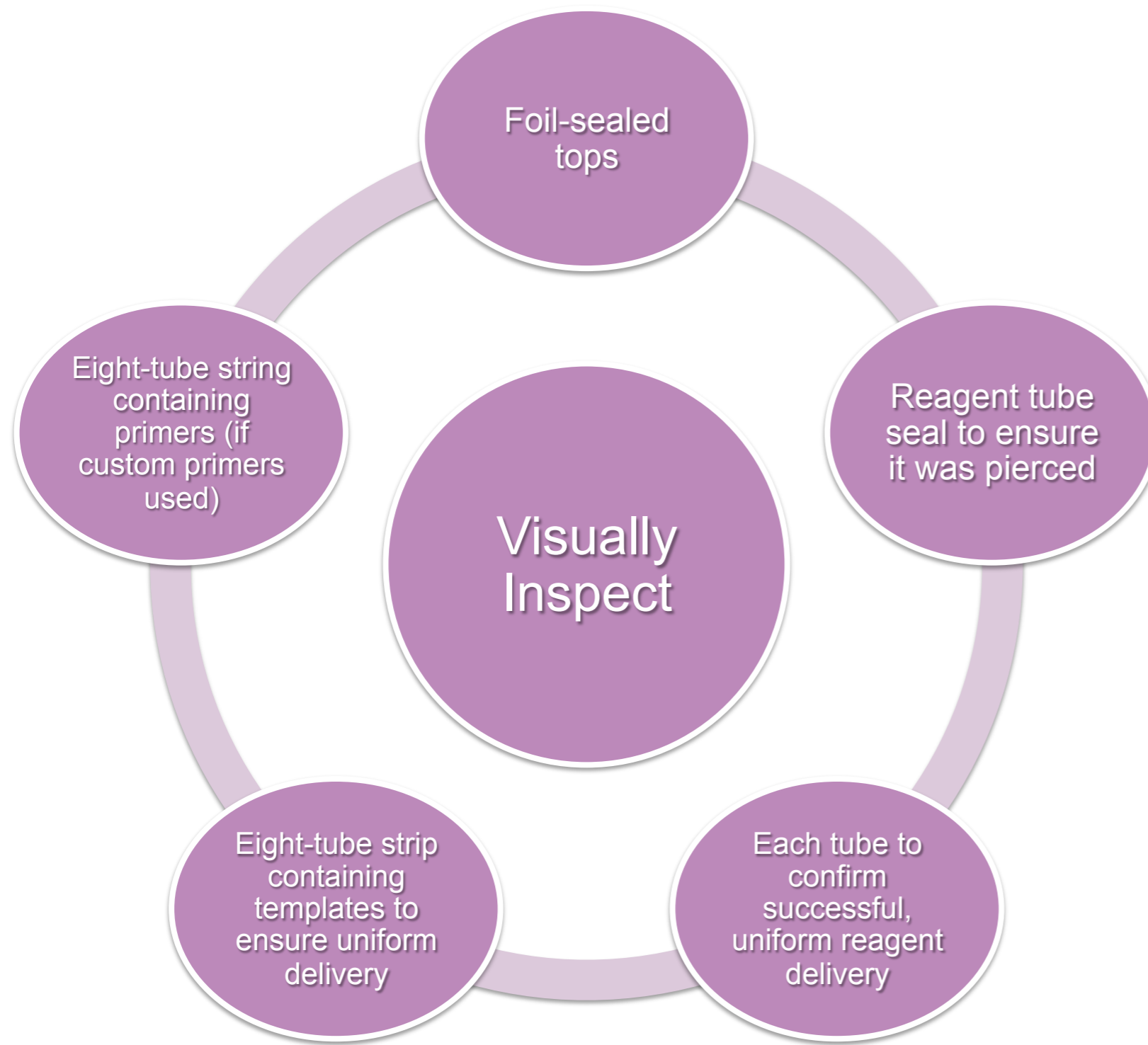
## Purpose of LTF

- Good lab practice
- Allows reagent tracking (reagent lots numbers, etc.)
- Serves as troubleshooting tool

The screenshot shows the Illumina website's support page for the cBot. The navigation menu includes 'APPLICATIONS', 'SYSTEMS', 'SERVICES', 'SCIENCE', and 'SUPPORT'. The breadcrumb trail is 'Support / Sequencing / Sequencing Instruments / cBot / Documentation & Literature'. A 'cBot Support' section features an image of the cBot machine and a 'Download User Guide' button. A 'What's New' section lists recent updates. A 'Documentation' table lists various resources, with the 'cBot Lab Tracking Form' highlighted in a purple box.

Description	Document Type	File Info
<a href="#">cBot Lab Tracking Form</a>	Lab Tracking Form	PDF (< 1 MB)
<a href="#">cBot Quick Reference Guide</a>	Quick Reference Guide	PDF (2 MB)
<a href="#">cBot Rehyb Kit Insert</a>	Kit Insert	PDF (< 1 MB)
<a href="#">cBot Safety and Compliance Guide</a>	Safety and Compliance Guide	PDF (< 1 MB)
<a href="#">cBot User Guide</a>	User Guide	PDF (16 MB)
<a href="#">TruSeq Cluster Kit v3 (cBot-HS) What's New in Cluster Kit v3</a>	Product Insert	PDF (< 1 MB)
<a href="#">TruSeq PE Cluster Kit v2 (cBot) Reagent Preparation Guide</a>	Reagent Prep Guide	PDF (< 1 MB)
<a href="#">TruSeq PE Cluster Kit v3 (cBot-HS) Reagent Preparation Guide</a>	Reagent Prep Guide	PDF (< 1 MB)
<a href="#">TruSeq SR Cluster Kit v2 (cBot) Reagent Preparation Guide</a>	Reagent Preparation Guide	PDF (< 1 MB)
<a href="#">TruSeq SR Cluster Kit v3 (cBot-HS) Reagent Preparation Guide</a>	Reagent Prep Guide	PDF (< 1 MB)
<a href="#">Version Compatibility Reference</a>	WebHelp	WebHelp html

# cBot Best Practice



# Best Practice



## Cluster Generation



1. Lab Tracking Form
2. Confirm Reagent Delivery

## Sequencing



1. Reagents Tracking
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# Lab Tracking Form Location

## Purpose of LTF

- Good lab practice
- Allows reagent tracking (reagent lots numbers, etc.)
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The screenshot shows the Illumina website's support page for the HiSeq 2000. The navigation menu includes 'APPLICATIONS', 'SYSTEMS', 'SERVICES', 'SCIENCE', and 'SUPPORT'. The 'SUPPORT' menu is active, and the breadcrumb trail reads 'Support / Sequencing / Sequencing Instruments / HiSeq 2000 / Documentation & Literature'. The main content area features a 'HiSeq 2000 Support' section with a 'Download User Guide' button. A 'What's New' section lists recent updates. A sidebar on the left contains a 'Documentation & Literature' section, which is highlighted with a purple box. Below this, a table of documentation is displayed, with the 'HiSeq Lab Tracking Form' entry highlighted by a purple border.

Description	Document Type	File Info
<a href="#">Changing Manifold Gaskets on the HiSeq or HiScanSQ</a>	Product Insert	PDF (< 1 MB)
<a href="#">Experiment Manager User Guide</a>	User Guide	PDF (< 1 MB)
<a href="#">HiSeq 2000 Quick Reference Guide</a>	Quick Reference Guide	PDF (1.2 MB)
<a href="#">HiSeq 2000 User Guide</a>	User Guide	PDF (6.3 MB)
<a href="#">HiSeq Lab Tracking Form</a>	Lab Tracking Form	PDF (< 1 MB)
<a href="#">HiSeq Safety and Compliance Guide</a>	Safety and Compliance Booklet	PDF (< 1 MB)
<a href="#">HiSeq Site Preparation Guide</a>	Site Prep Guide	PDF (< 1 MB)
<a href="#">Illumina Adapter Sequences</a>	Letter	PDF (< 1 MB)
<a href="#">Multiplexed Sequencing Primers and PhiX Control Kit v2 Reagent Preparation Guide</a>	Reagent Preparation Guide	PDF (< 1 MB)
<a href="#">Sequencing Nextera Libraries on the HiSeq</a>	User Guide	PDF (< 1 MB)
<a href="#">TruSeq SBS Kit v3 - HS (50 Cycles) Reagent Preparation Guide</a>	Reagent Prep Guide	PDF (< 1 MB)
<a href="#">TruSeq SBS Kit v3 (HS) What's New in SBS Kit v3</a>	Product Insert	PDF (< 1 MB)
<a href="#">TruSeq SBS Kit v3 - 200 Cycles (HS) Reagent Preparation Guide</a>	Reagent Prep Guide	PDF (< 1 MB)



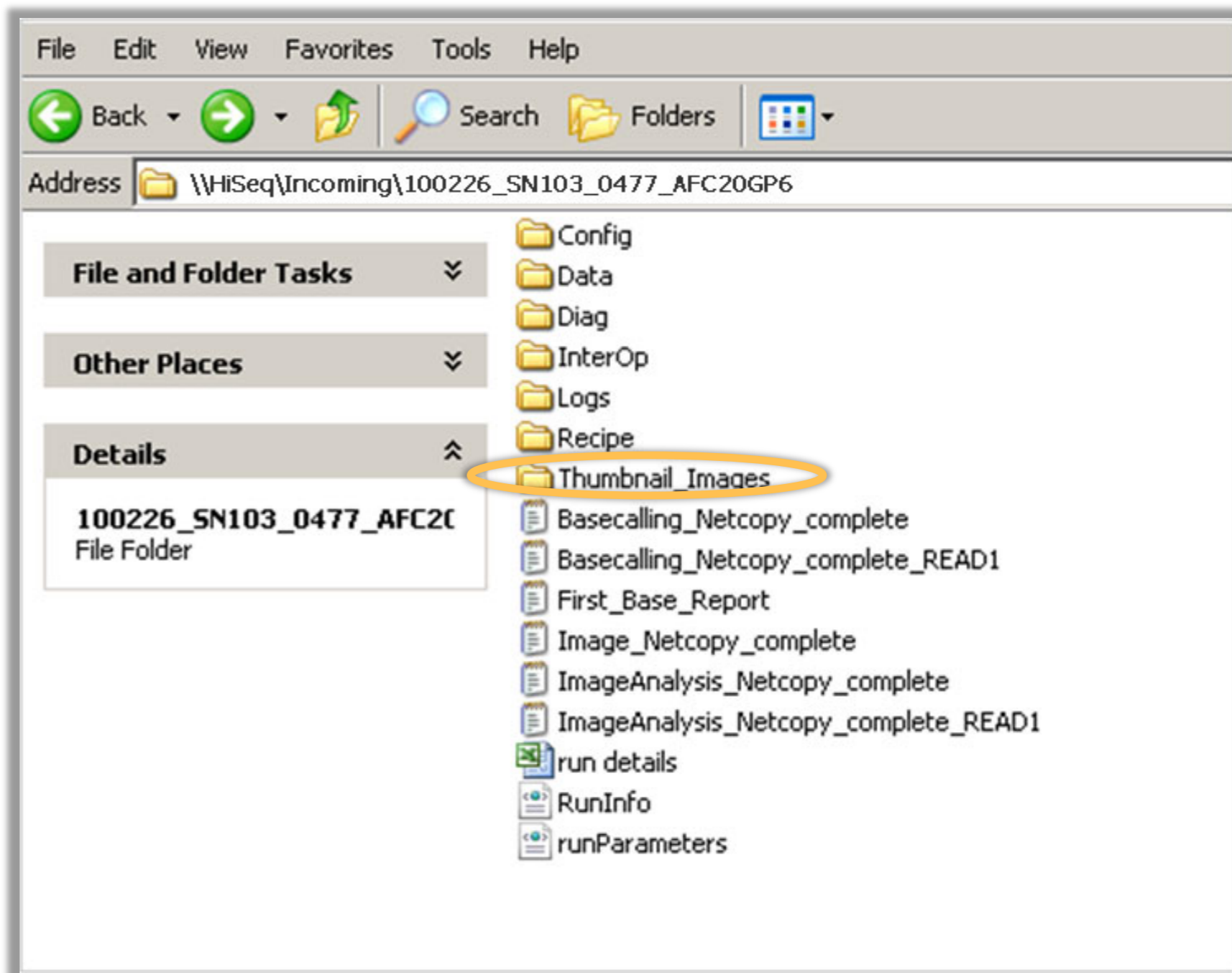
# Useful Files and Folders



# Thumbnails

## Thumbnails

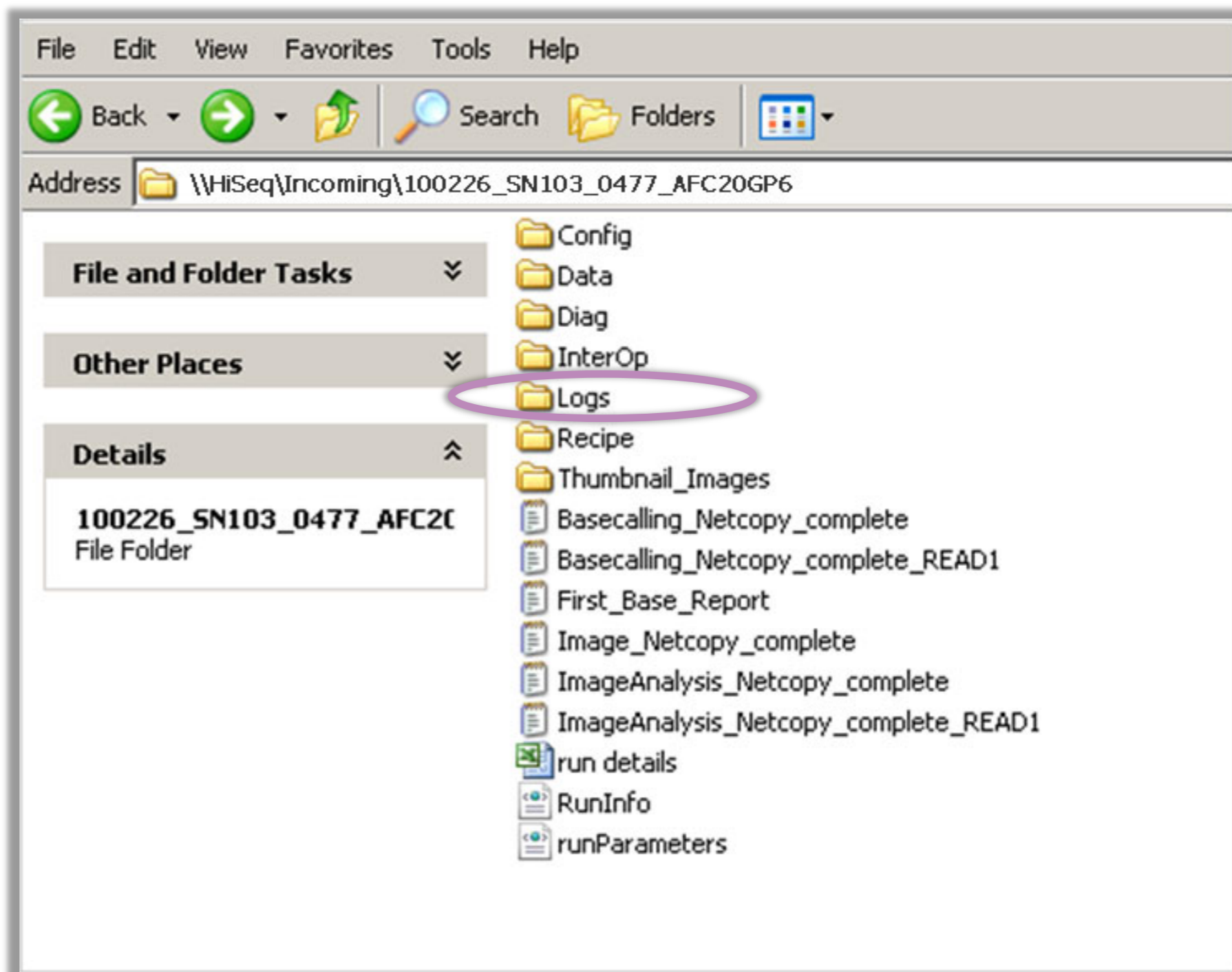
- Saved as .jpeg files
- Organized by lane, then by cycle



# Logs

## Logs

- Contains run logs
- Organized by cycle



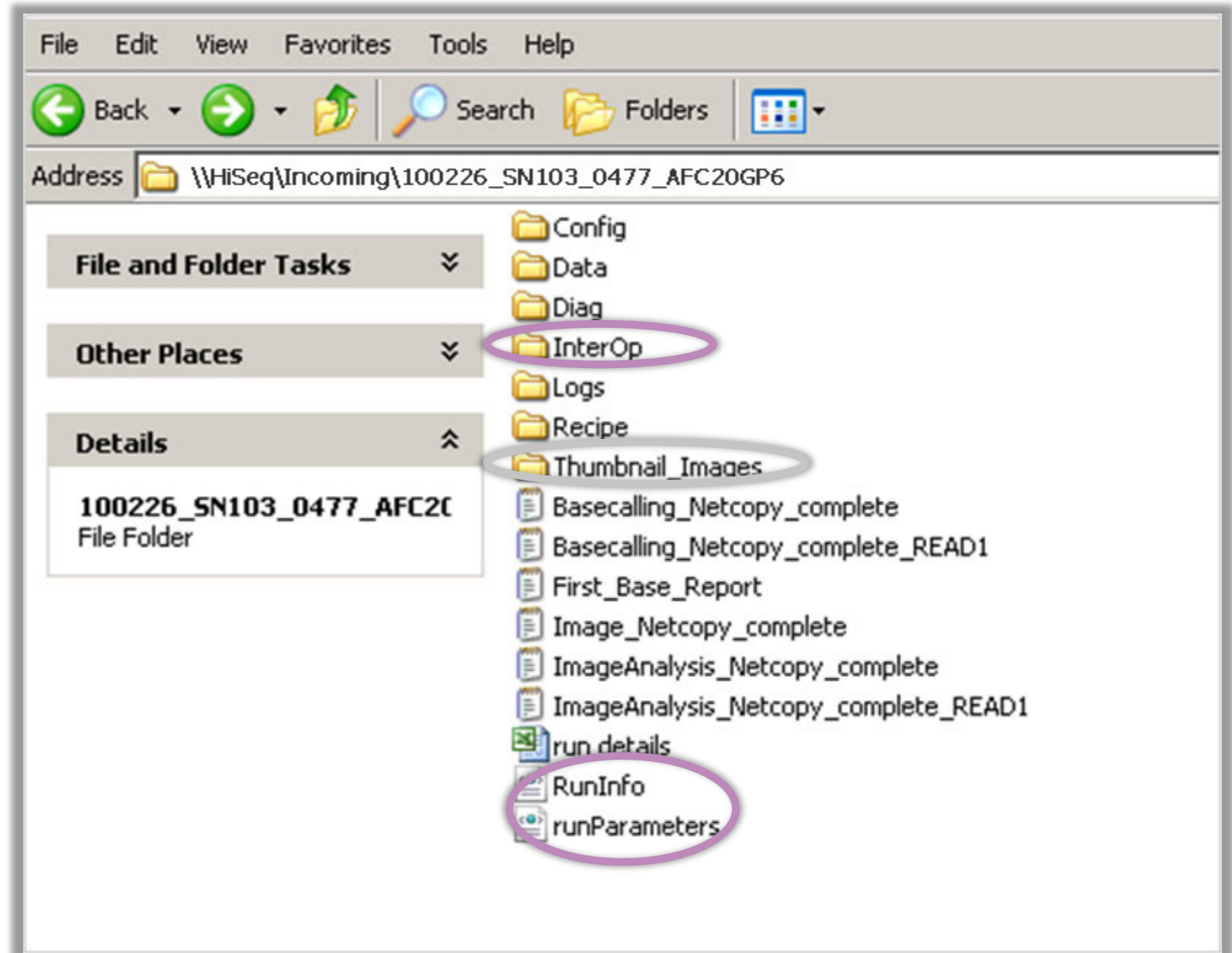
# Viewing real-time metrics using “Sequencing Analysis Viewer” (SAV)

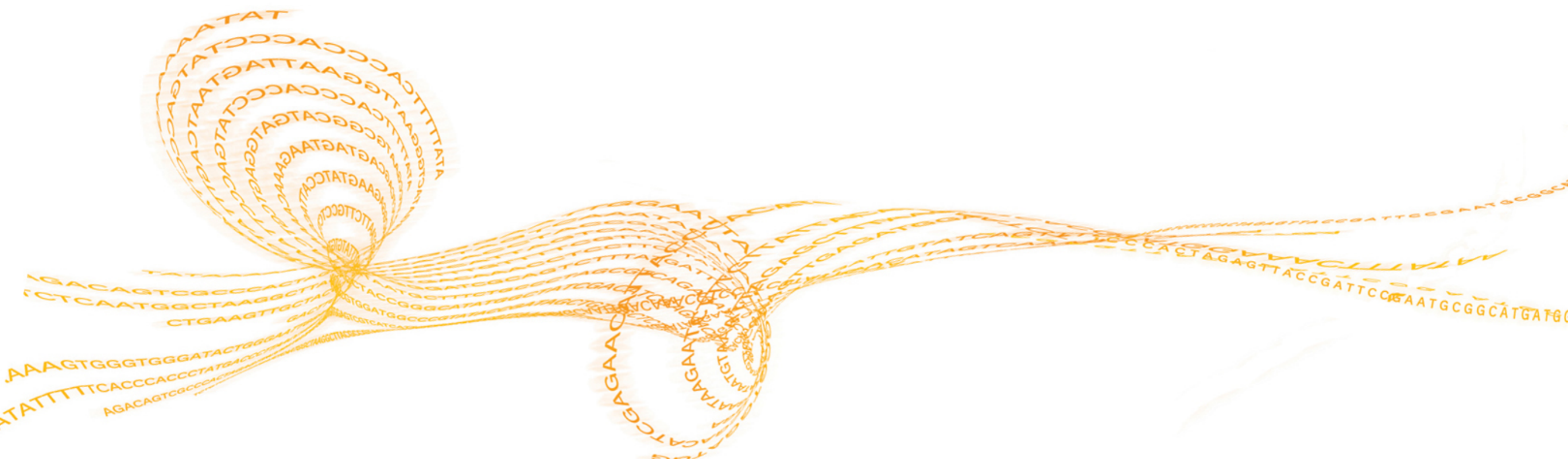
## Files/Folders needed to review real-time metrics using SAV

- RunInfo.xml
- runParameters.xml
- InterOp folder

### Optional:

- Thumbnail Images





# Instrument Maintenance

# cBot Maintenance

## cBot



1. Pre-run Wash
2. Post-run Wash
3. Monthly Wash
4. Monthly Coolant Level Check

## HiSeq Sequencing System



1. Water wash
2. Maintenance wash
3. HiSeq idling/powering down
4. Fluidic care
5. Flow cell plate care

# cBot Maintenance

## Post-Run Wash

- Always perform a post-run wash after each run to clear salts and enzymes from the instrument hardware and prevent clogs

## Pre-Run Wash

- A pre-run wash is recommended if the instrument has been idle for more than 24 hours

## Monthly Maintenance Wash

- Perform a monthly maintenance wash once each month



Figure 30 Fill the Wash Reservoir



Figure 31 Dry the Wash Reservoir

# cBot Monthly Maintenance Procedures

## *Maintenance Wash*



### Monthly Maintenance Wash Procedure

- Run manual wash with deionized water
- Run manual wash with 5% DECON solution
- Use deionized water and lint-free cleaning tissue to remove all maintenance wash solution from the wash reservoir
- Run manual wash with deionized water two (2) times

# cBot Monthly Maintenance Procedures

## Check Coolant Level

Low coolant reservoir



### Monthly Coolant Level Check Procedure

- Ensure green coolant is visible through coolant window (rear instrument panel)
- If coolant is low, use flat-head screwdriver to remove coolant reservoir cap and refill reservoir to just below the cap
- Use only Illumina-supplied coolant (Part number: 1003709)

Properly refilled coolant reservoir





# HiSeq Sequencing System Maintenance

## cBot



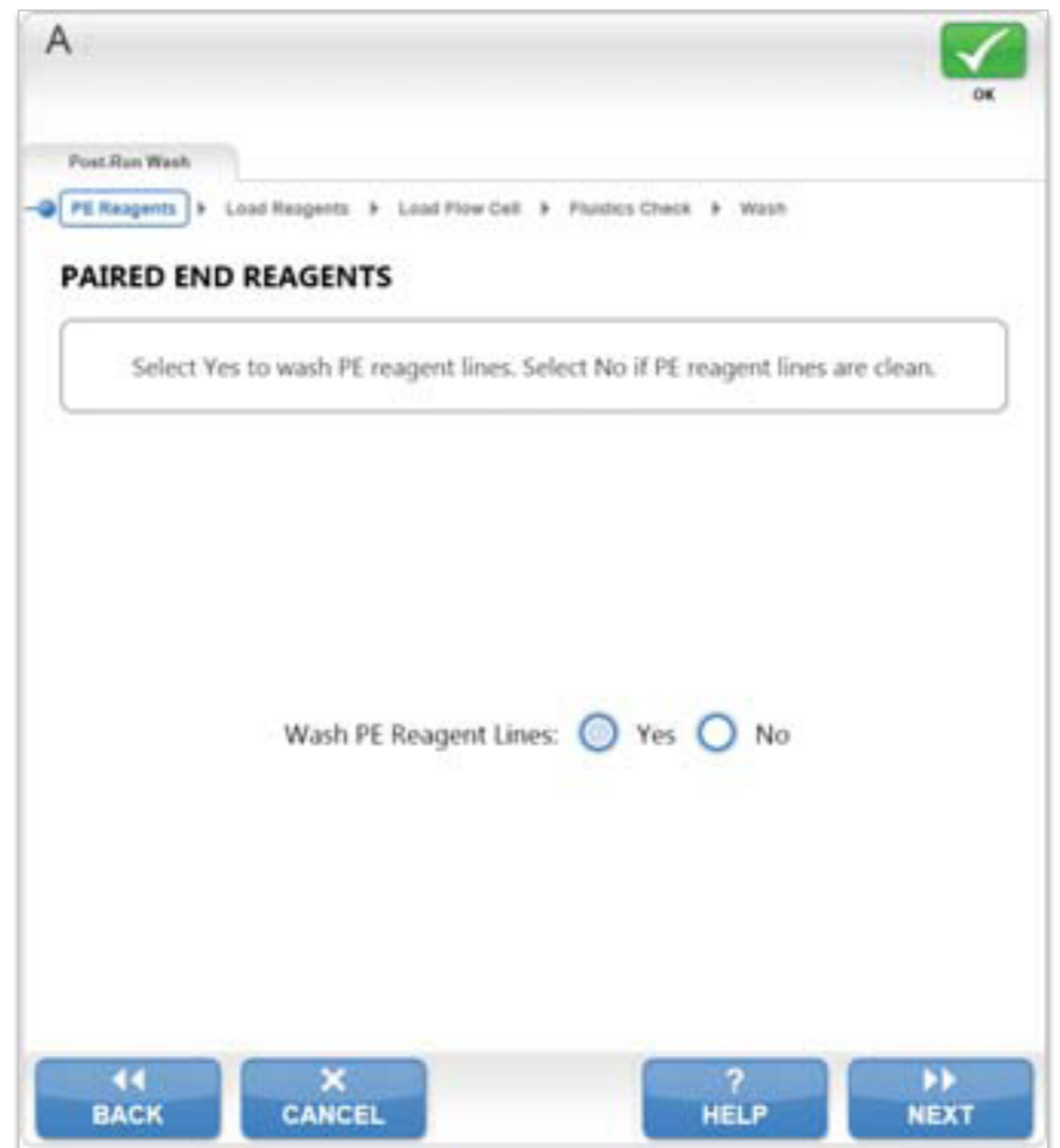
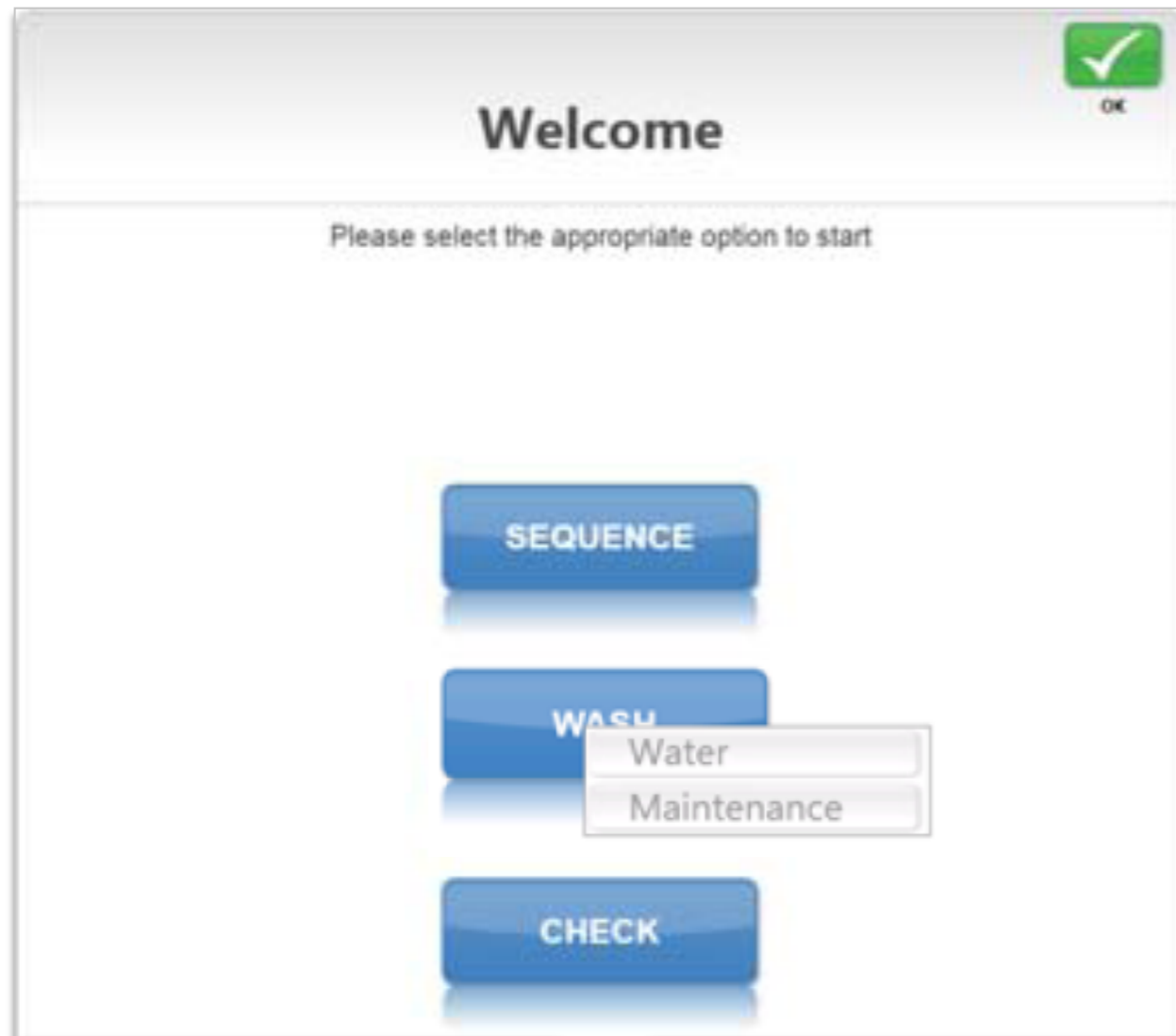
1. Pre-run Wash
2. Post-run Wash
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## HiSeq Sequencing System



1. Water wash
2. Maintenance wash
3. HiSeq idling/powering down
4. Fluidic care
5. Flow cell plate care

# HiSeq Sequencing System Wash



Water System Wash

Maintenance System Wash

SBS lines only

SBS and PE lines

# HiSeq Sequencing System: Water Wash

Flushes lines with lab-grade water  
(20 mL per bottle)

- Recommended before a run if:
- instrument has been idle for more than one day
  - performing a fluidics check to do a water wash

If performing a subsequent run using remaining reagents, perform instrument wash between sequencing kits

	Expected Wash Deliveries	Approximate Run Time Per Step
<i>Positions</i>	<i>Delivered Volume</i>	
8 SBS positions	32 mL	20 minutes
8 SBS positions and 10 paired-end positions	72 mL	60 minutes

# HiSeq Sequencing System Maintenance Wash

Perform full maintenance wash after each run

Number wash bottles to ensure they are always loaded in the same position

Fill bottle for the Water Wash 1 and Water Wash 2 to the top to ensure sippers are rinsed of reagents

Dispose of water used for Wash Wash 1 – DO NOT re-use

Check delivery volumes to ensure wash completed successfully

Positions	Delivery Volumes
Water Wash 1	72 mL
NaOH Wash	36 mL
Water Wash 2	72 mL

# HiSeq Idling

Prepare HiSeq for idling if the instrument will be used within 10 days

- Perform maintenance wash at any unused flow cell position
- Leave flow cell loaded with manifolds up
- Leave reagent sippers in water
- Do not turn off the instrument

Using instrument after idling

- Perform a water wash and confirm delivery volumes before starting a run

# HiSeq Powering Down

Power HiSeq down if instrument will not be used for more than 10 days

- Perform a maintenance wash on both flow cell positions
- Remove flow cells from stages and clean flow cell stages
- Load empty reagent bottles under sippers
- Turn off instrument

When ready to restart the instrument

- Load water at all positions
- Turn on instrument
- Perform a water wash at both flow cell positions and PE positions, and confirm delivery volumes

# Fluidics Care

Inspect sipper tubes and guides for damage before each run

Remove any reagent build-up from gasket seating area

Wipe out excess condensation inside reagent chiller after each post-wash

Inspect pump syringes for leakage or excess bubbles during wash



# Flow Cell Plate Care

Heating surface must be cleaned before each run with non-abrasive material

Peltier plates are optically flat and must not be chipped or damaged to ensure flow cell is seated properly

Vacuum seal groove must be free of debris for seal to hold







Questions?