Illumina NovaSeq 6000 Sequencing

Version Number: 2.0
Production Start Date: 12/20/17
Version Date: 12/14/20

Summary

The prepared libraries were quantified using KAPA Biosystem's next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. Sequencing of the flow cell was performed on the Illumina NovaSeq sequencer using NovaSeq XP V1.5 Reagent kits, and S2 or S4 flowcell, following a paired end 150 cycle indexed run recipe.

Materials and Reagents

Materials/Reagents/Equipment	**Vendor**	**Product Number**
Disposables		
Easy open 1.7ml microfuge tubes	ISC Bioexpress	C-3293-2
Microfuge tubes with silicone O rings	VWR	89004-298
Pipet Tips, 2-20 ul , LTS, Filter, Low Retention, Red	Rainin	RT-L10FLR
Pipet Tips, 200 ul, LTS, Filter, Green	Rainin	RT-L200F
Pipet Tips, 1000 ul, LTS, Filter, Green	Rainin	RT-L1000F
Pipet Tips Serological, 50ml, Sterile, Individually Wrapped, Corning	Fisher	1367811F
Pipet Tips Serological, 10ml, Sterile, Individually Wrapped, Corning	Fisher	1367811E
Towels, Brown, WypAll L20, Quarter Fold, 2-Ply		7999-52901
Alcohol wipes, Individual		19-014-854
Kimwipes, Large 14X16		799930393

Kimwipes,Small, 4.4X8.4		799930392
Tubes., 15mL, Conical, Sarstedt	Phoenix	SS-4002
Reagents		
NovaSeq 6000 S4 Reagent Kit (300 cycles) V.1.5	Illumina	20028312
NovaSeq Xp 4-Lane Kit V.1.5	Illumina	20028313
TailorMix Dual Indexed PhiX Control Library (Nondenatured)	SeqMatic LLC	TM-502-ND
1N NaOH	Alfa	AA35629-K2
Tris-HCl Buffer, 10mM (pH 8.5)	Bio-World	42020414-1
UltraPure 1M Tris-HCI, pH 8.0	Fisher	15568025
Tween 20, Fisher BioReagents, Poly Bottle; 500mL	Fisher	BP337-500
Sodium Hypochlorite Solution (5% Available Chlorine)	VWR	JT9416-1
Equipment		
NovaSeq 6000 Sequencer	Illumina	
Vortex Genie 2	VWR	G-560
Galaxy MiniStar	VWR	521-2844
Open-side Cart		

EH&S

Personal Protective Equipment (PPE)

Any JGI employee performing this procedure MUST wear safety glasses, a lab coat and gloves.

Satellite Accumulation Areas (SAA)

SAAs for various hazardous chemicals are located throughout the lab. ALL reagents used in these kits and in this SOP MUST be disposed of in the appropriate SAA. If you are not aware of

where the SAAs are located, or how to dispose of the above reagents properly, please contact your supervisor or Area Safety Lead (ASL).

Scheduling

S2

- 1x2x150 cycle run takes 30 hours to complete.
- 2x2x150 cycle run takes 30.5 hours to complete

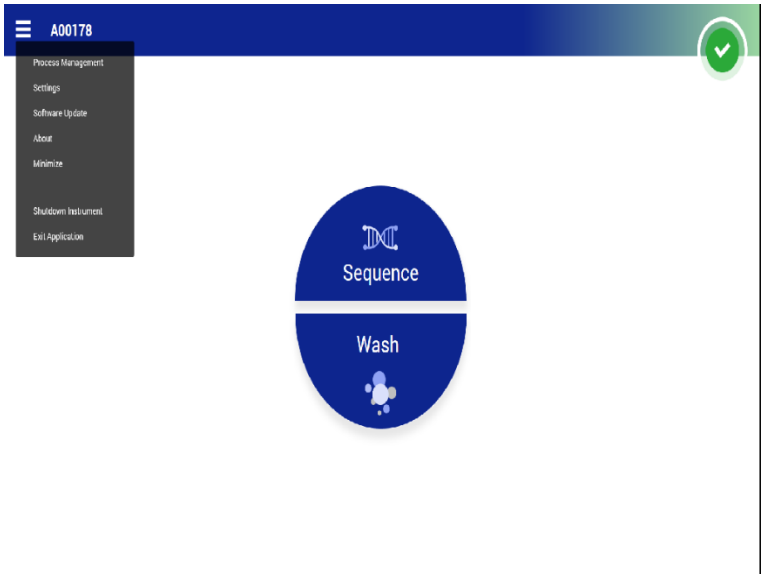
S4

- 1x2x150 cycle run takes 52.5 hours to complete
- 2x2x150 cycle run takes

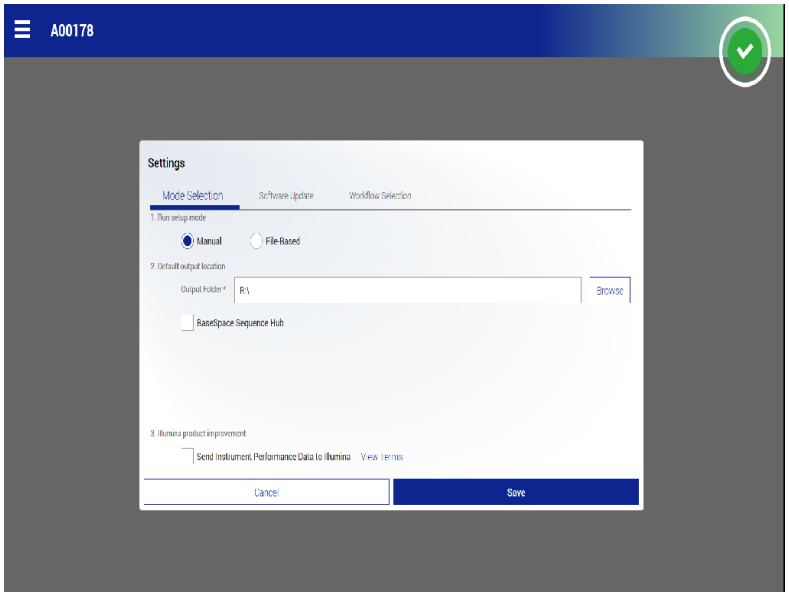
Procedure

NOTE: *All reagents/stock solutions should be prepared prior to the start of the procedure. Samples must be loaded within* ***1 hr*** *after denaturation and ExAmp addition.*

1. **Set up Instrument**
    a. Instrument should be saving files to the network drive
        i. On the top of the Control Software select the tribar
        ii. Select the Settings option

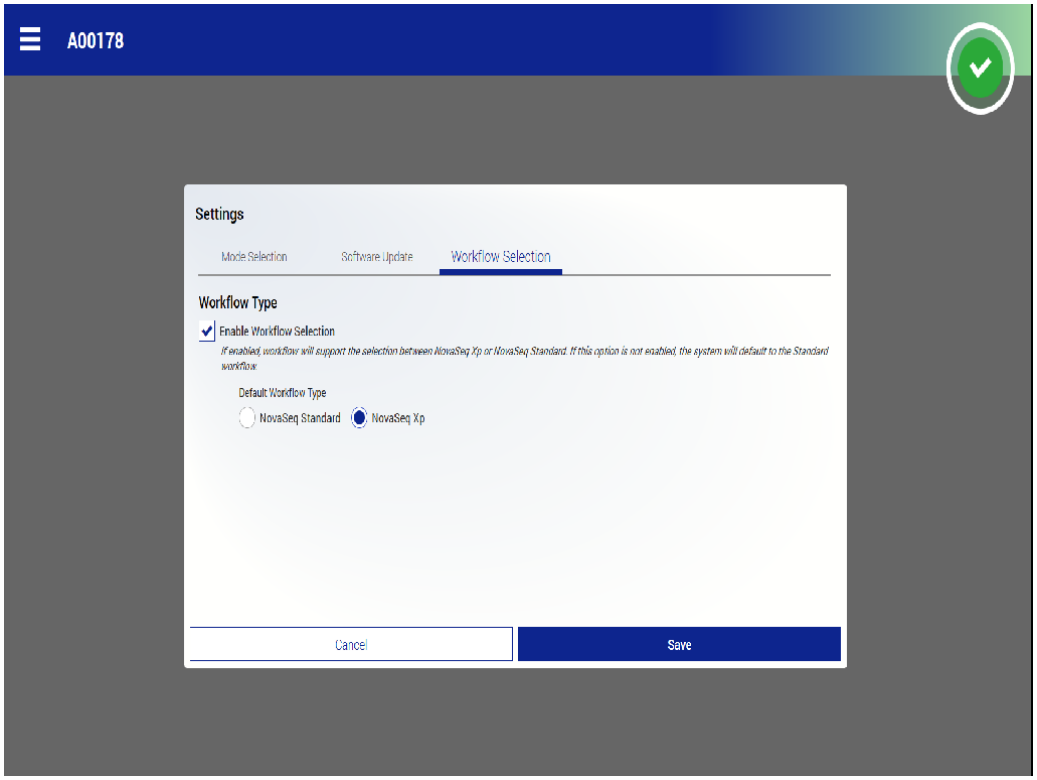


iii. Under Output Folder "R:\" should appear

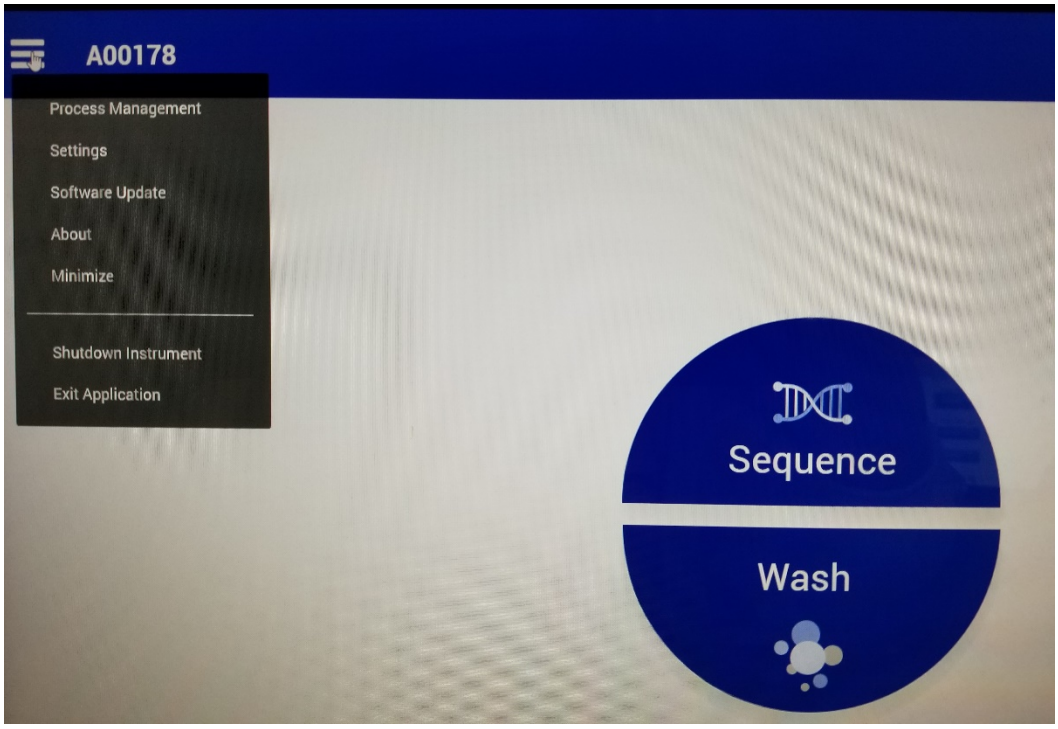


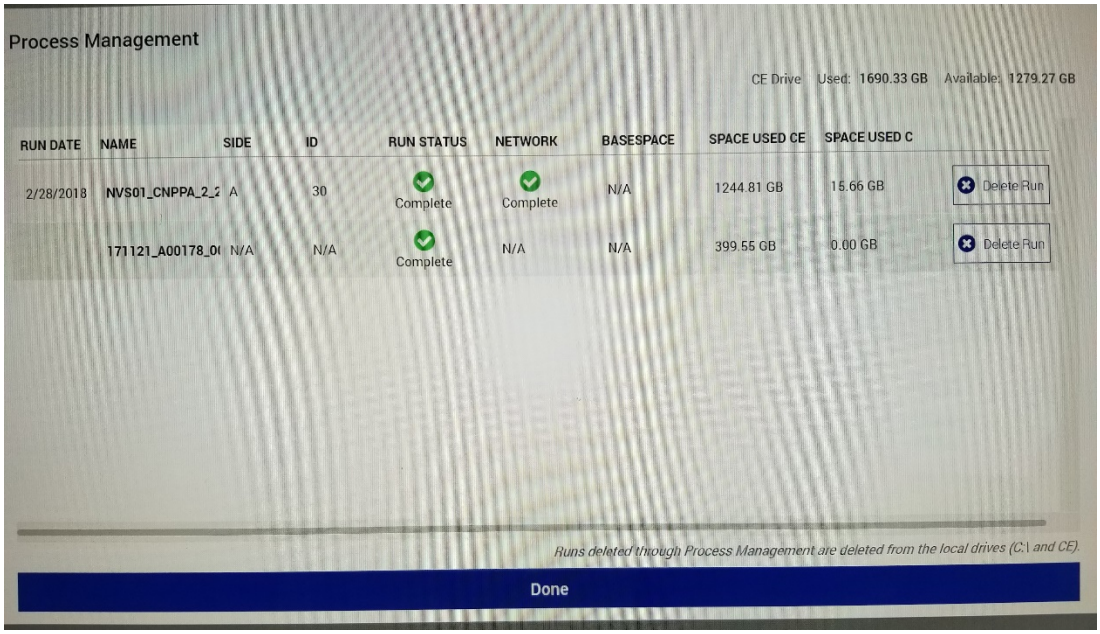
iv. Click **Save**

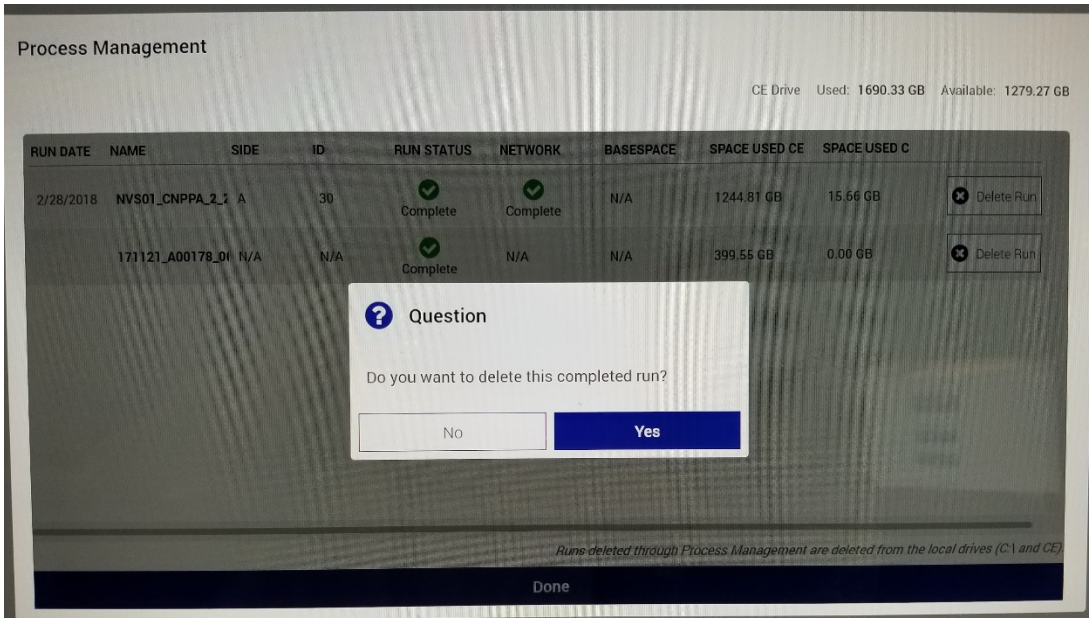
b. Instrument should be set to XP run mode
   i. On the top of the Control Software select the tribar
   ii. Under Workflow Selection make sure NovaSeq XP is selected



c. Previous run files need to be deleted
   i. In Process Management click “Delete Run” for all files that have cleared SDM


A00178
Process Management
Settings
Software Update
About
Minimize
Shutdown Instrument
Exit Application
Sequence
Wash


Process Management
CE Drive Used: 1690.33 GB Available: 1279.27 GB
RUN DATE NAME SIDE ID RUN STATUS NETWORK BASESPACE SPACE USED CE SPACE USED C
2/28/2018 NVS01_CNPPA_2_2 A 30 Complete Complete N/A 1244.81 GB 15.66 GB Delete Run
171121_A00178_0(N/A N/A Complete N/A N/A 399.55 GB 0.00 GB Delete Run
Runs deleted through Process Management are deleted from the local drives (C:\ and CE).
Done



2. Prepare Reagents

a. Check RFID codes on all consumables a day or two before run
   i. Remove SBS kit and Cluster kit from -20°C, flow cell from 4°C, and Buffer kit from room temperature store shelf.
   ii. Open all packages and set up a mock run to check RFID values following step 7 instructions.
   iii. Once all RFID codes are read, replace kits in their packages and place back in the proper storage location from step 2.1a.

b. Defrost Reagents
   i. Remove SBS kit and Cluster kit from -20°C and place in room temperature water bath to defrost for two hours

***NOTE**: Reagents can be refrozen once after complete defrosting or can be stored at 4°C for 24 hours.*

c. Remove flow cell from 4°C once all kits have defrosted, and allow to come to room temperature for ½ hour before loading samples and reagents onto NovaSeq.

3. Prepare Sample

a. Check samples for needed volume and defrost in 4°C
b. Remove dual index Phix from -20°C and defrost in 4°C
c. Make fresh batch of 0.2N NaOH
   i. Add 8ul of 1N NaOH to 32ul of water.
d. Label a colored 1.5mL flip top tube with the library name, lane number, on the top of the tube and the date and your initials on the side of the tube.

e. Each library should have a different color tube. This is to minimize the chance of mix up when vortexing and returning the tubes to the rack.
f. Visually check each tube library name when transferring DNA
g. Keep all tubes closed at all time unless adding a sample/reagent to that tube. Move tubes either horizontally or vertically once a step has been completed in the event you are interrupted.
h. Use Low Retention tips for any reagents that involve DNA.

4. Dilute and Denature Sample and Dual Index PhiX

a. Dilute stock dual index PhiX to either 3nM or loading concentration according to dilution worksheet.
b. Add volume designated in dilution worksheet for PhiX, and each sample to the appropriate tube.
c. Dilute using 10nM TrisHCl pH8.5 according to dilution worksheet to the appropriate tube.
d. Vortex and spin down
e. Add the amount of 0.2N NaOH specified on the dilution worksheet to each tube.
f. Vortex and spin down
g. Incubate at room temperature for 8 minutes.
h. Add specified amount of 400nM TrisHCl pH8 to each tube.
i. Vortex and briefly centrifuge and place on ice.

5. Prepare ExAmp Master Mix

a. Defrost ExAmp kit in 4°C, then keep on ice

NOTE: *If defrosting at room temp make sure it is left out no longer than 10 minutes.*

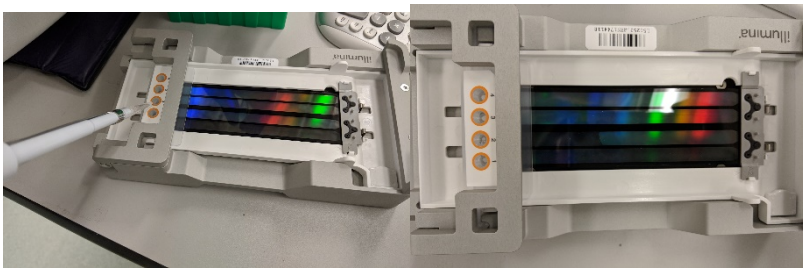
b. Invert, vortex briefly and spin down in centrifuge.
c. Mix together volumes designated in dilution worksheet in the order specified.
d. Vortex for 20-30 seconds and spin down in centrifuge.

NOTE: *For optimal performance immediately proceed to next step. Master mix can be stored for 1 hour on ice.*

6. Load Libraries onto Flow Cell

a. Place room temperature flow cell onto flow cell dock
b. Place manifold over the flow cell and close the clamp.

c. For each tube add the specified amount of master mix designated in the dilution worksheet
    i. Vortex and centrifuge
d. Add 130ul of each tube to the specific lane /well designated on the dilution sheet, waiting 2 minutes for the liquid to reach the outlet end of each lane before loading the next.

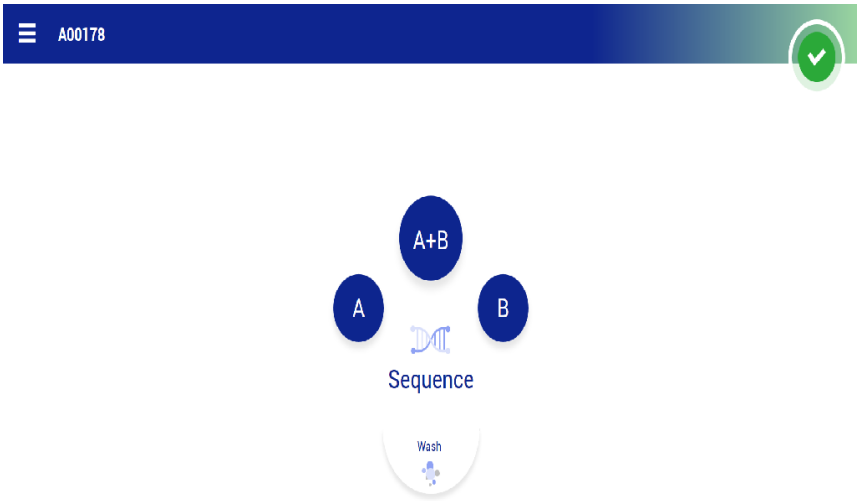


NOTE: *Do not rotate the flow cell, keep steady while liquid is filling the lanes.*

NOTE: *Start the sequencing run within 30 minutes of load the flow cell.*

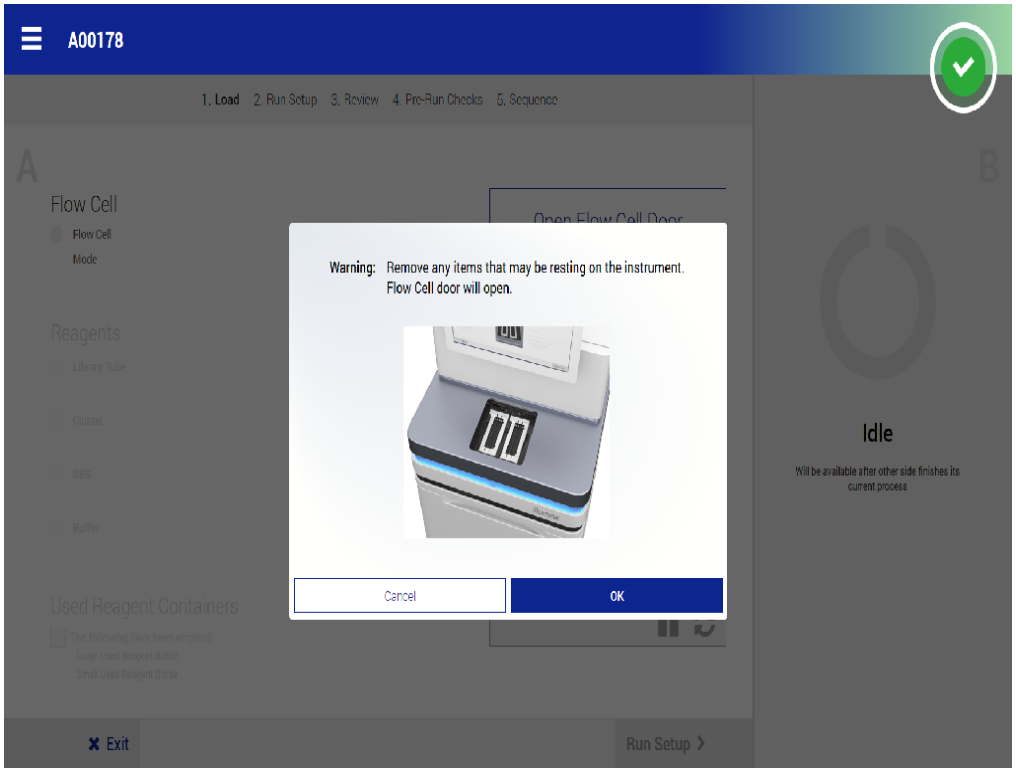
7. Loading the Sequencer

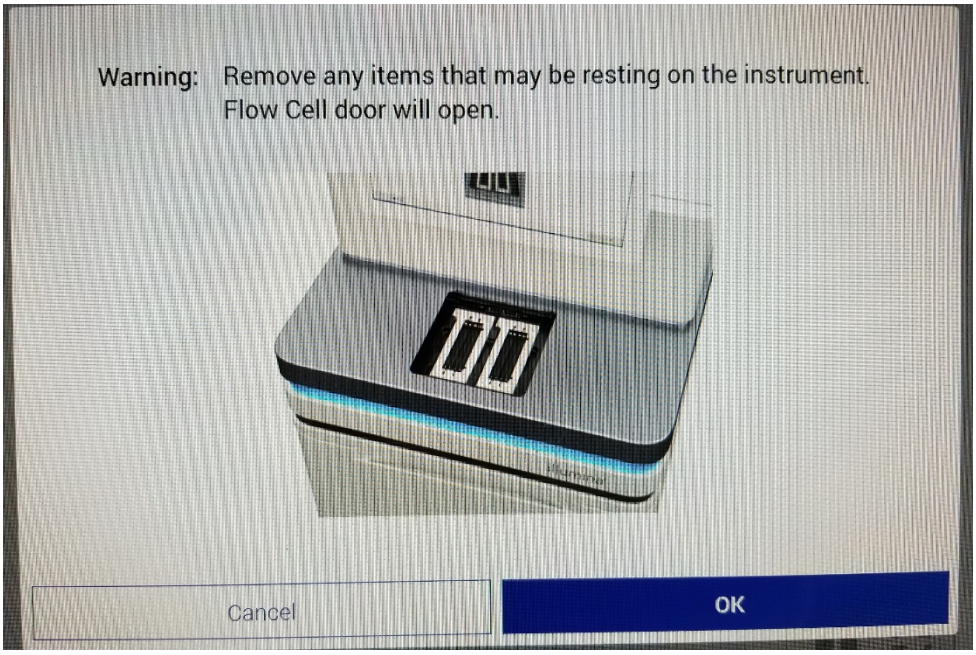
a. On home screen choose to load side **A**, **B** or both **A+B**



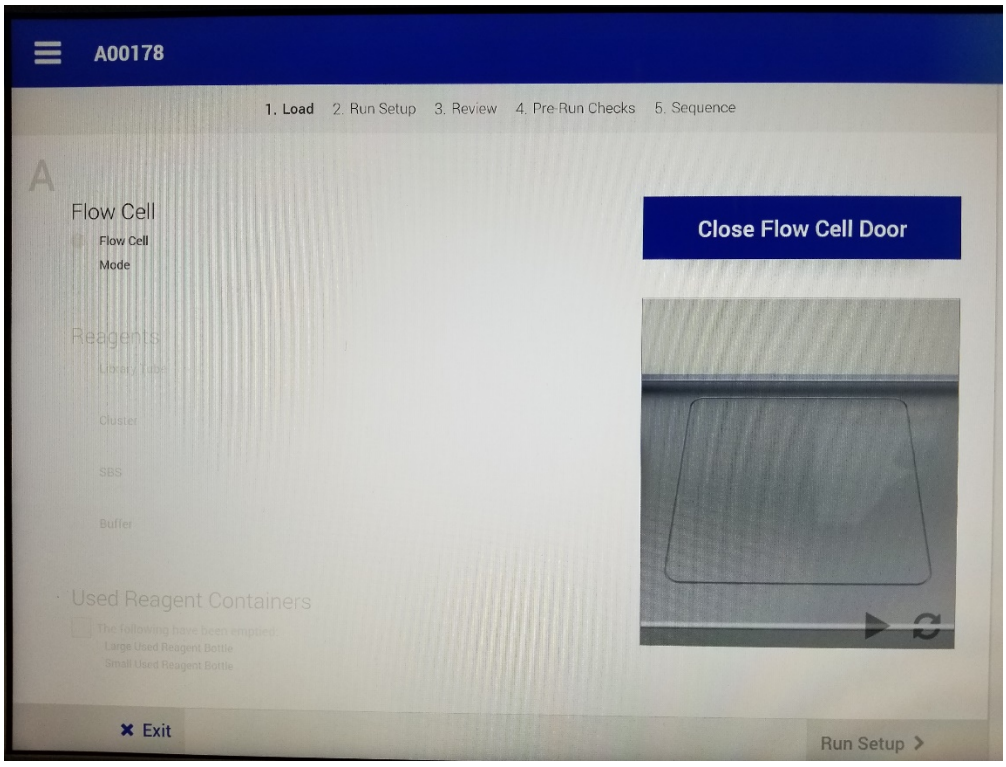
b. On the pop-up window select **OK** to

i. Once initiated the screen will ask you to move anything on the instrument



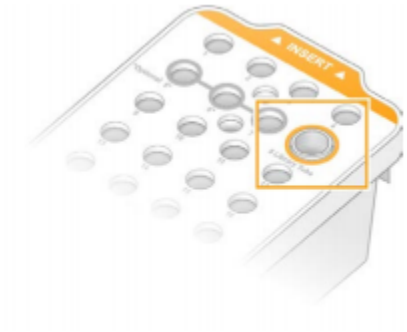


c. Once flow cell door is open insert the flow cell on the flow cell stage and select "**Close Flow Cell Door**"

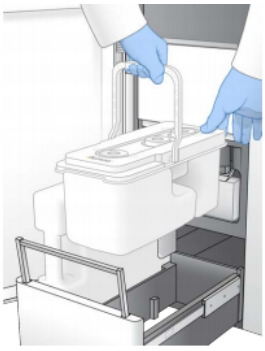


i. It takes the instrument a couple of minutes to read the RFID on the flow cell

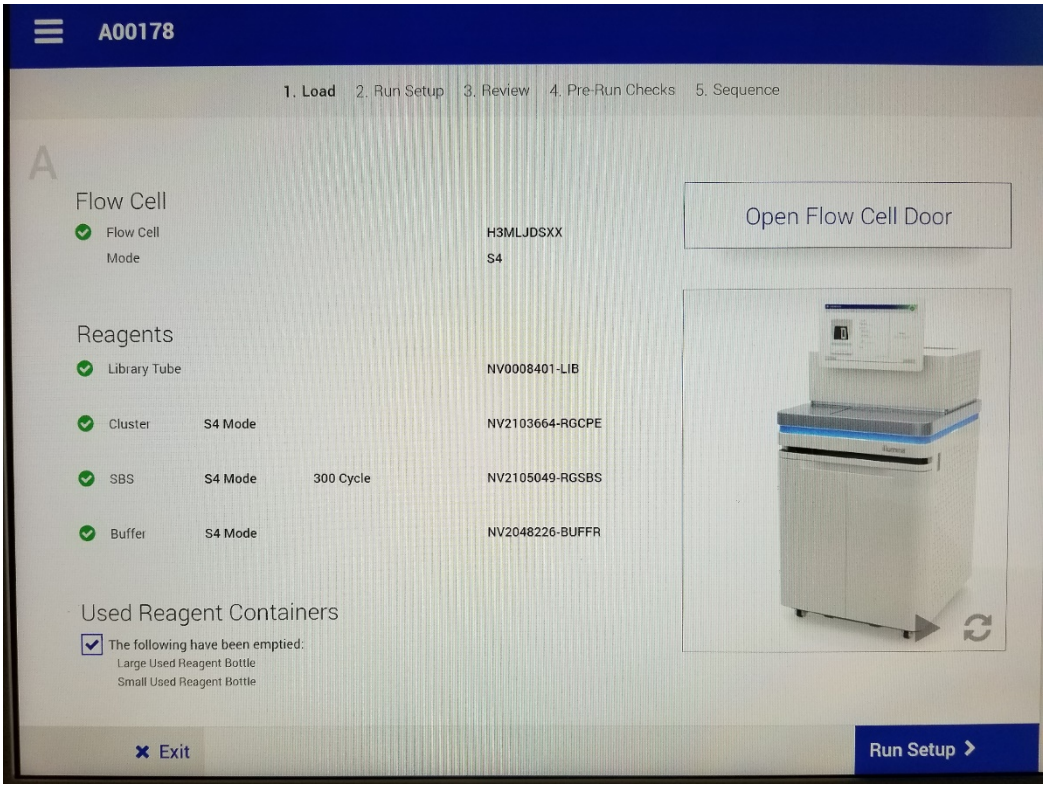
d. Place empty library tube into position #18 on the SBS reagent cartridge



e. Load reagents onto the sequencer.



f. Mark the checkbox stating that used reagent bottles have been emptied



NOTE: *Empty used reagents into the correctly labeled SAA container*

g. Select "**Run Setup**"

8. <u>Run Setup</u>

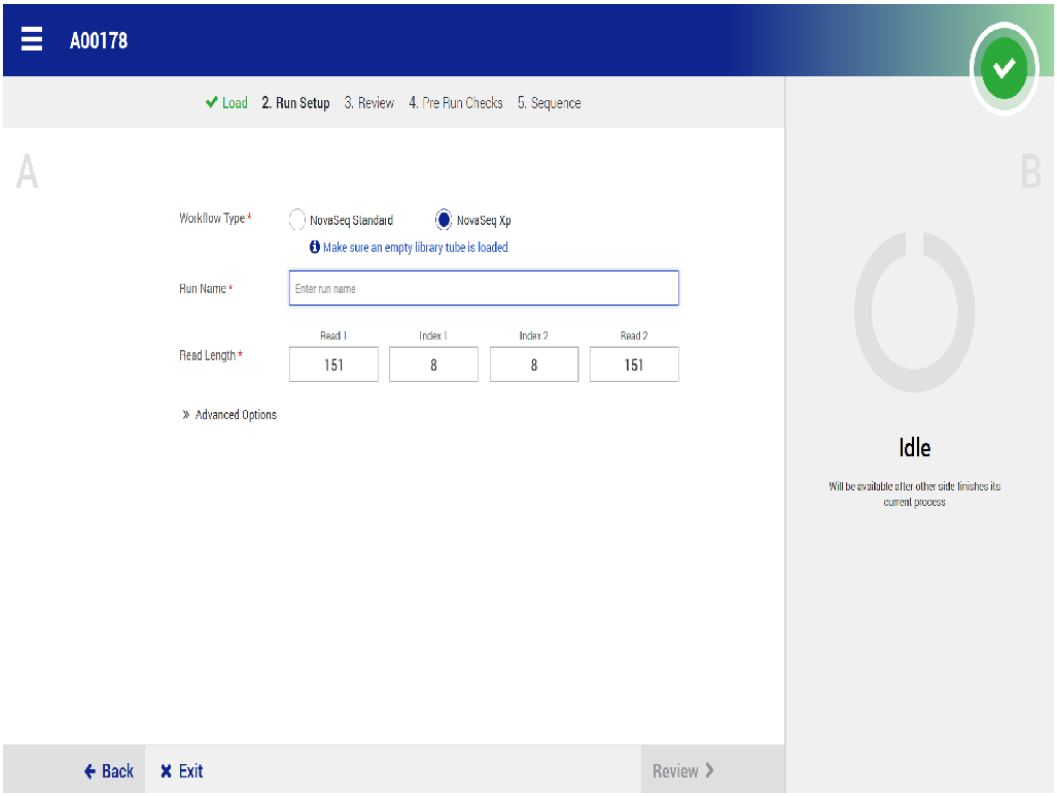
a. Again check to make sure the "**NovaSeq XP**" is selected

b. Enter Run name (example NovaSeq#-Sample in lane 1-MM-DD)

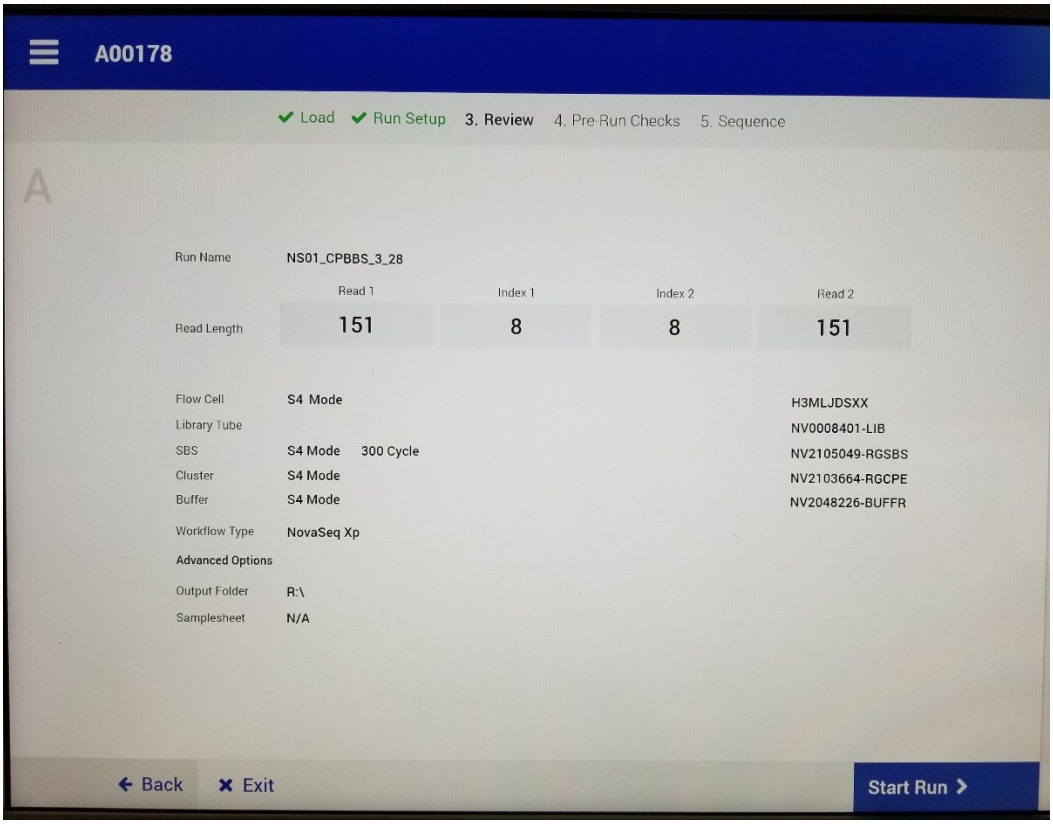
c. A run length of 2X150 with dual index 8 base pairs will be pre-populated. Only change this if the run is custom.

NOTE: *Indexes will be demuliplexed using the full length of each index read.*

d. Click "**Review**"

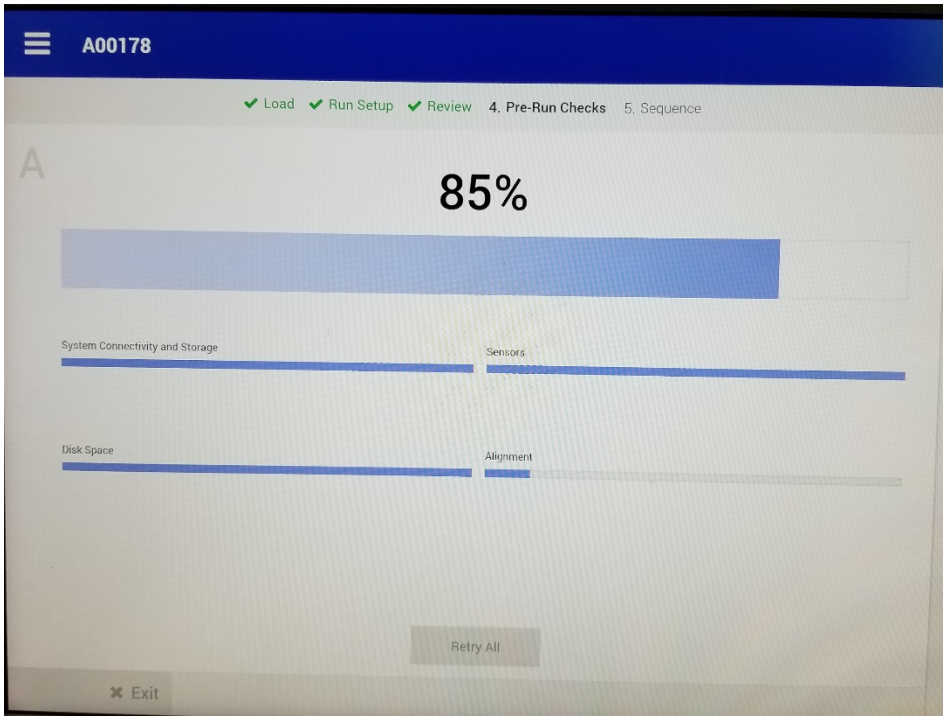


e. Review run parameters



f. If everything looks right select "**Start Run**"

   i. If any of the information is wrong select "**Back**" and enter correct information.



g. Once the instrument finishes its self check the run will start right away.
    i. Display will have Intensity and Q Score values along with clusters passing filter and estimated yield.

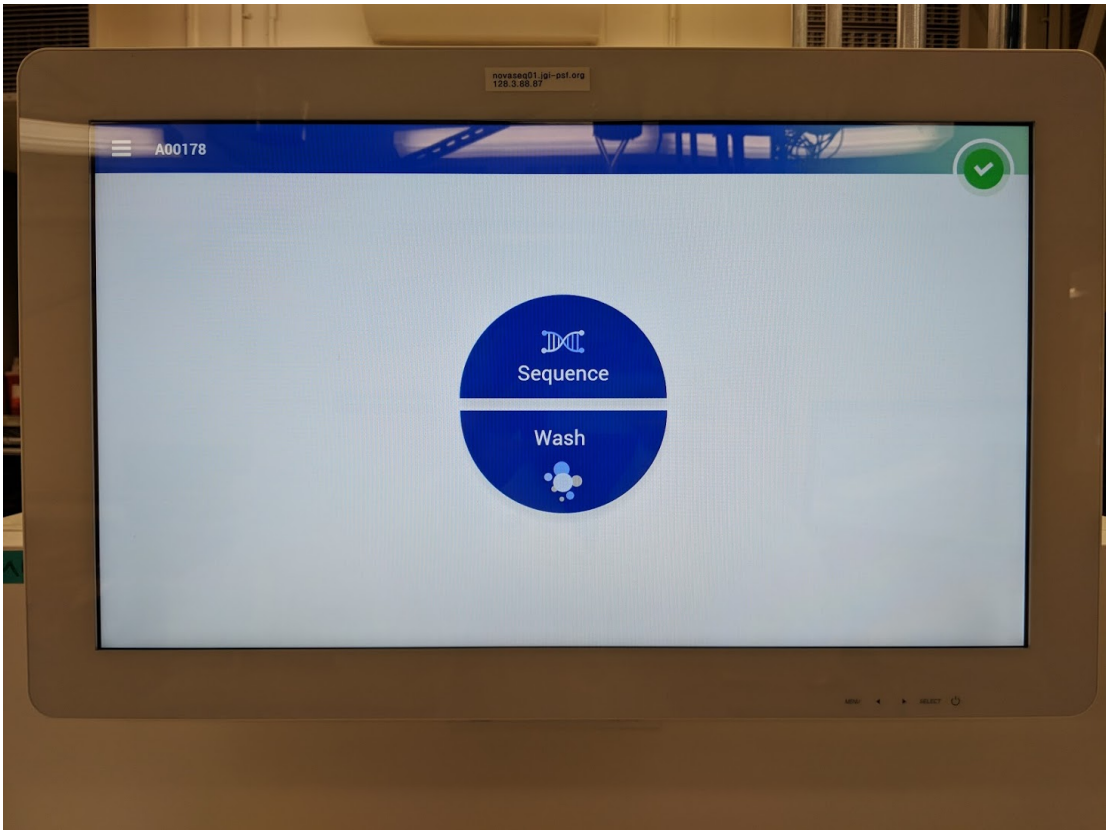
ii.

Instrument Maintenance

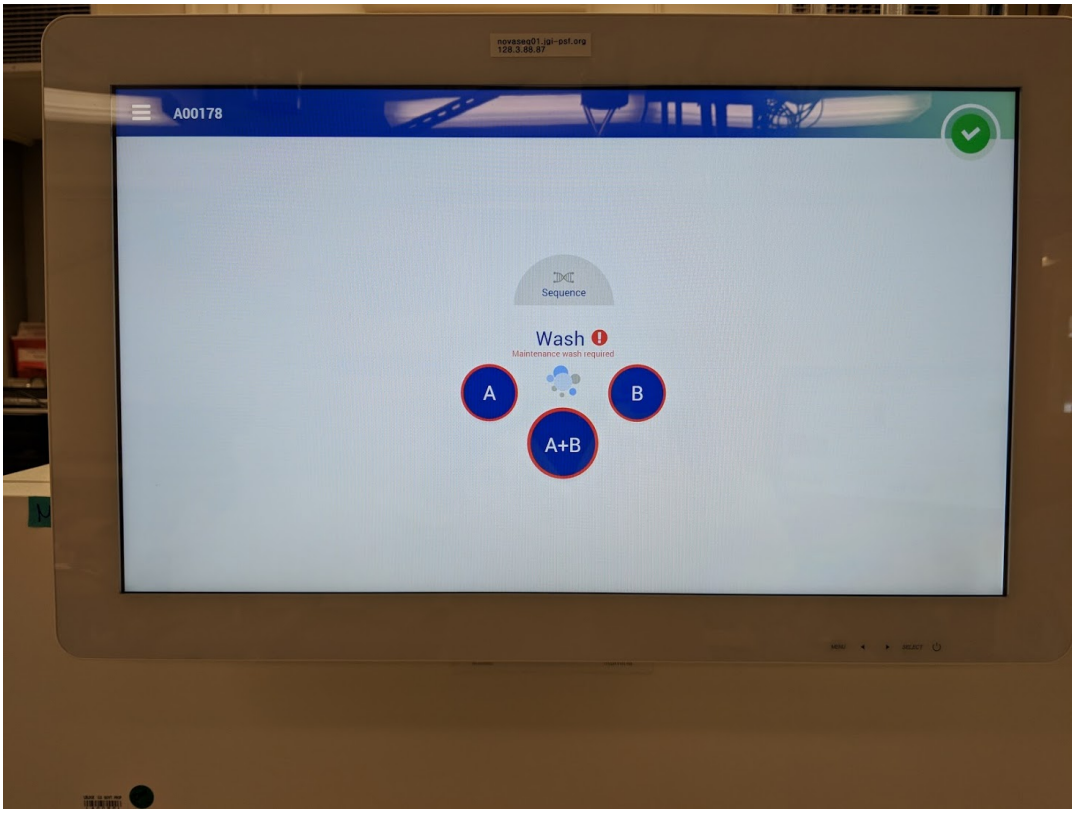
Instrument

The NovaSeq needs to have a maintenance every two weeks that it is idle.

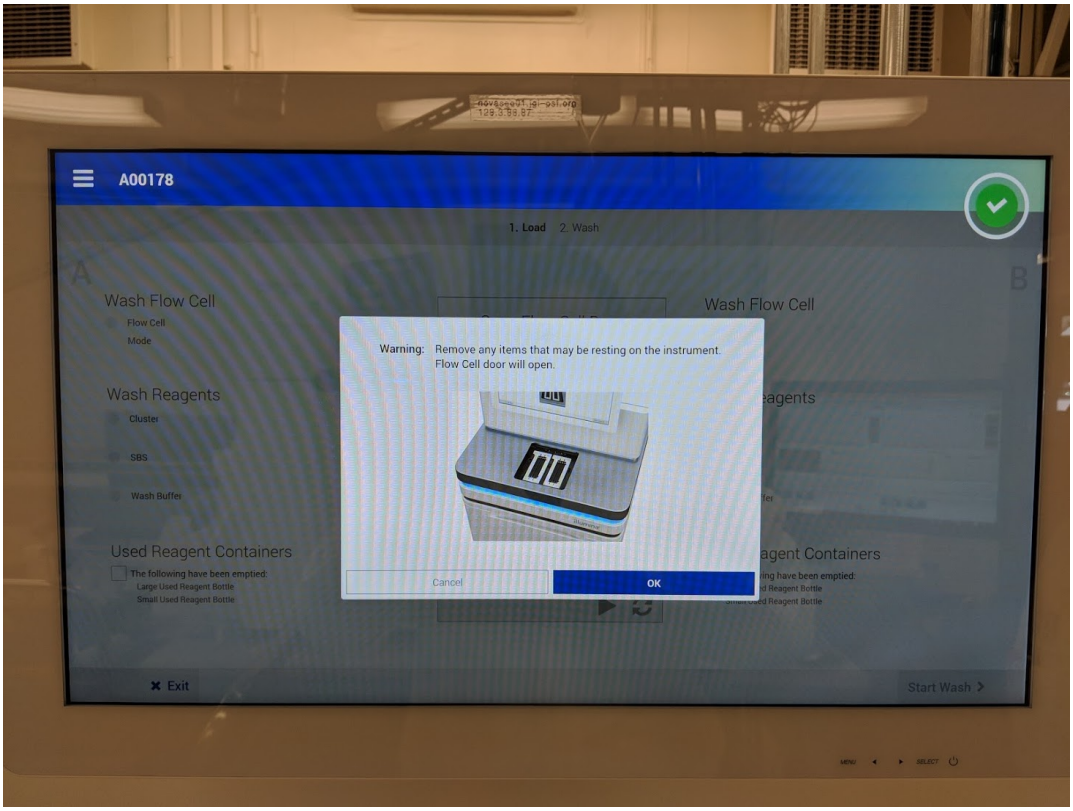
1. **Reagent Preparation**
    a. Add 5ml of 0.25% NaOCl to position #17 of the Cluster wash cartridge.
        i. 500ul of 5% NaOCl added to 9.5mL of H20
    b. Add 400 ml of 0.05% Tween 20 to the center well of the SBS wash cartridge
        i. 100mL of Tween 20 to 1900mL of H20
2. **Set up Instrument**
    a. Select Wash from the main menu of the instrument



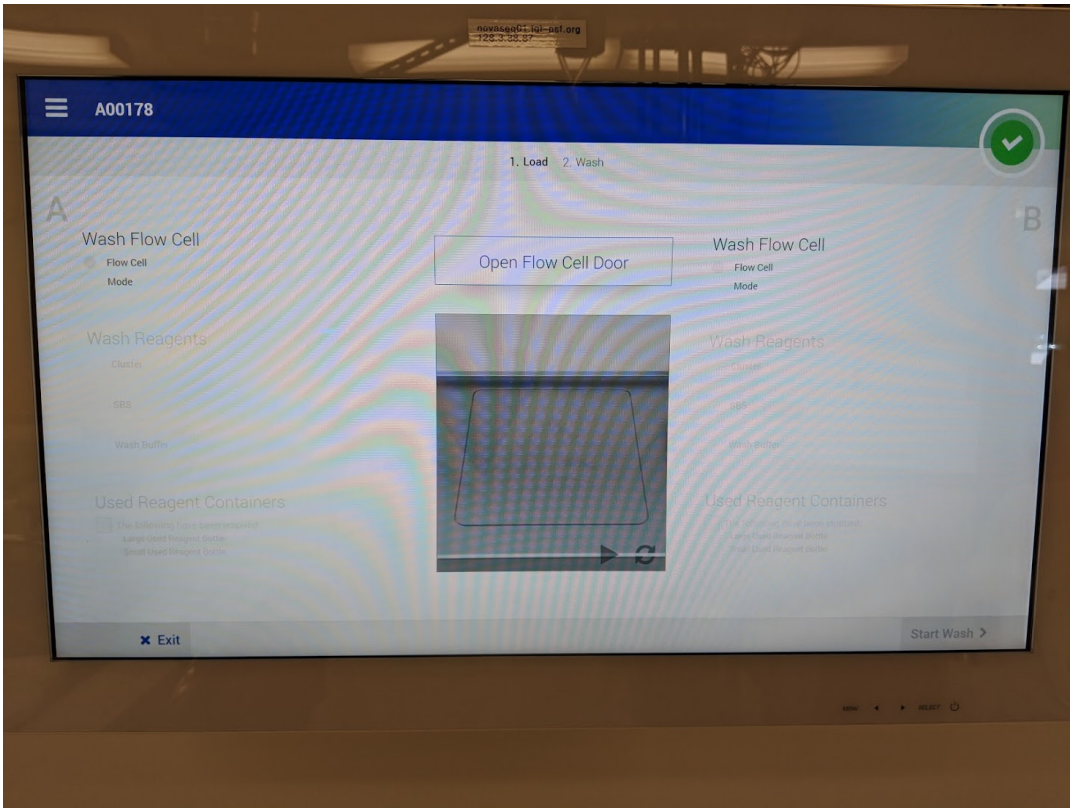
b. Chose to wash “A+B”



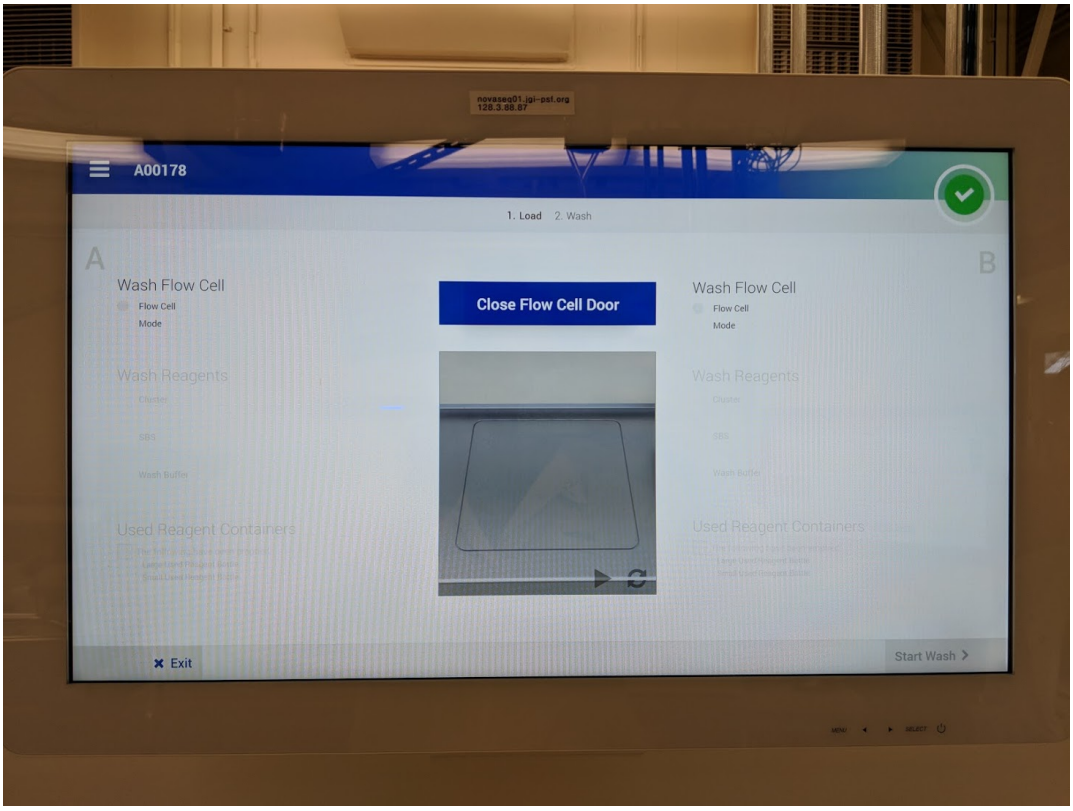
c. Remove any items that are near the flow cell stage. Click “OK”



d. Click the box to “Open Flow Cell Door”



e. Load Wash Flow Cells
f. Clickc “Close Flow Cell Door”

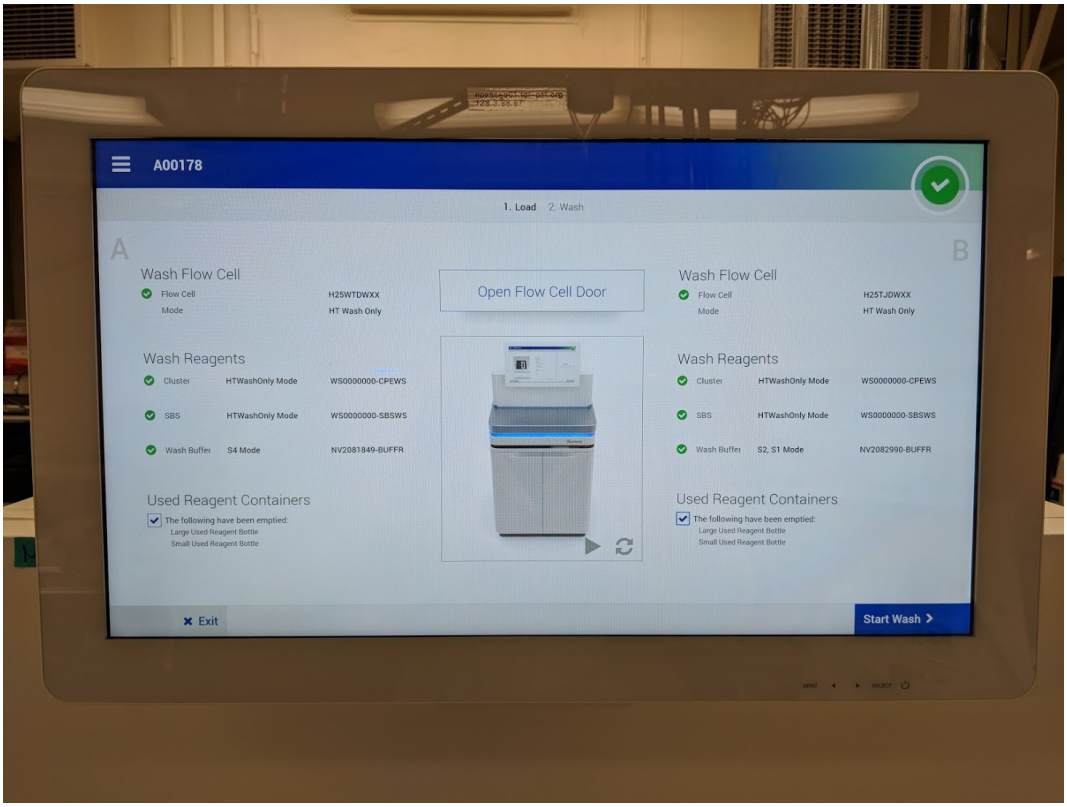


g. Load wash cartridges

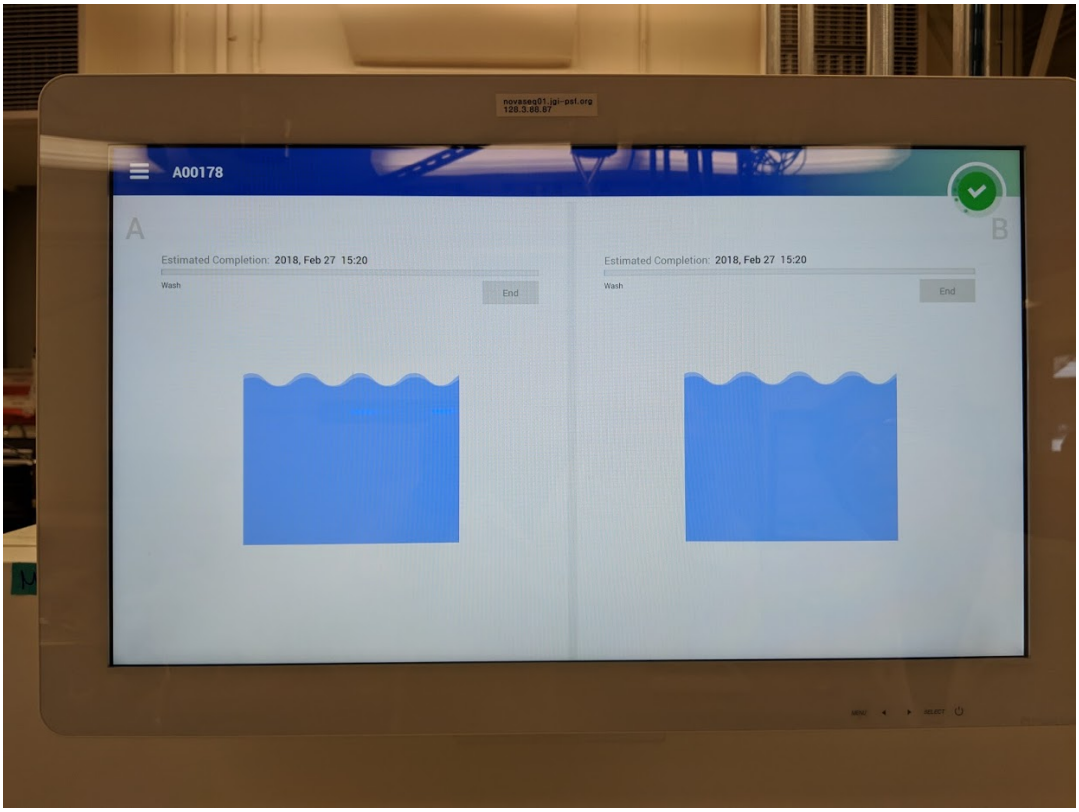
h. Make sure waste bottles have been emptied or if they are empty make sure to open and close the buffer drawer to enable the instrument to read the RFIDs

Note: No wash buffer cartridge is needed, just leave the buffer cartridge from the last run in place.

i. Once the RFID is read, click the box to acknowledge that the waste has been emptied



j. Click “Start wash” on bottom right screen
k. Wash takes about an hour and twenty minutes



Troubleshooting

List Common issues and solutions

SOP Approval

DEPARTMENT	APPROVED BY	DATE
Lab Supervisor		
Research & Development		
Instrumentation		

QC		
Purchasing		
EH & S		
Informatics		
Seq Assessment & Analysis		
Dept Head of Prod Seq		

Appendix

Figures
Tables
Diagrams
Attachments
Contact information for vendors or manufacturers that you want included in the SOP

ADDENDUM TRACKING

AUDIT TRACKING

7/19/18 PCN 174 Illumina.NovaSeq.2018.02.23 full length and one mismatch demultiplexing.