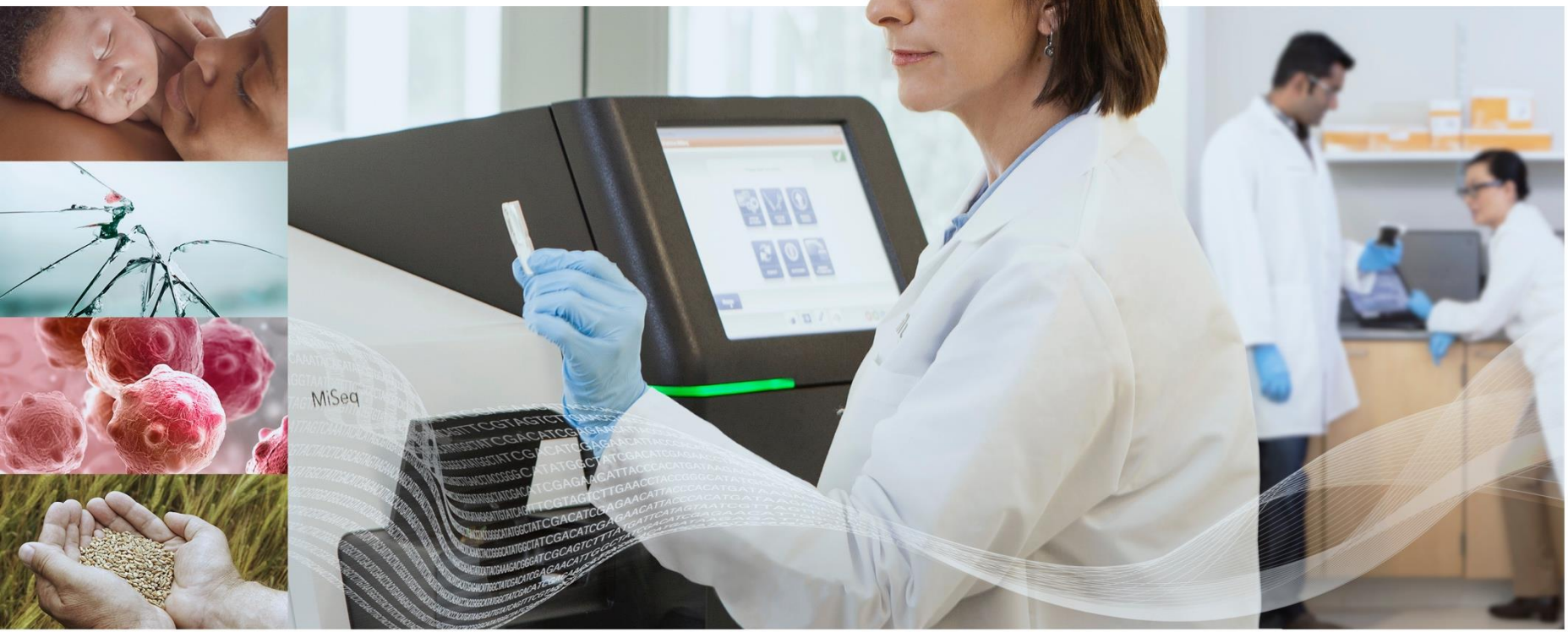
Introduction to loading the MiSeq™ and the NextSeq™

Rayne Lawrence, FAS



Starting a Run Using the NextSeq System

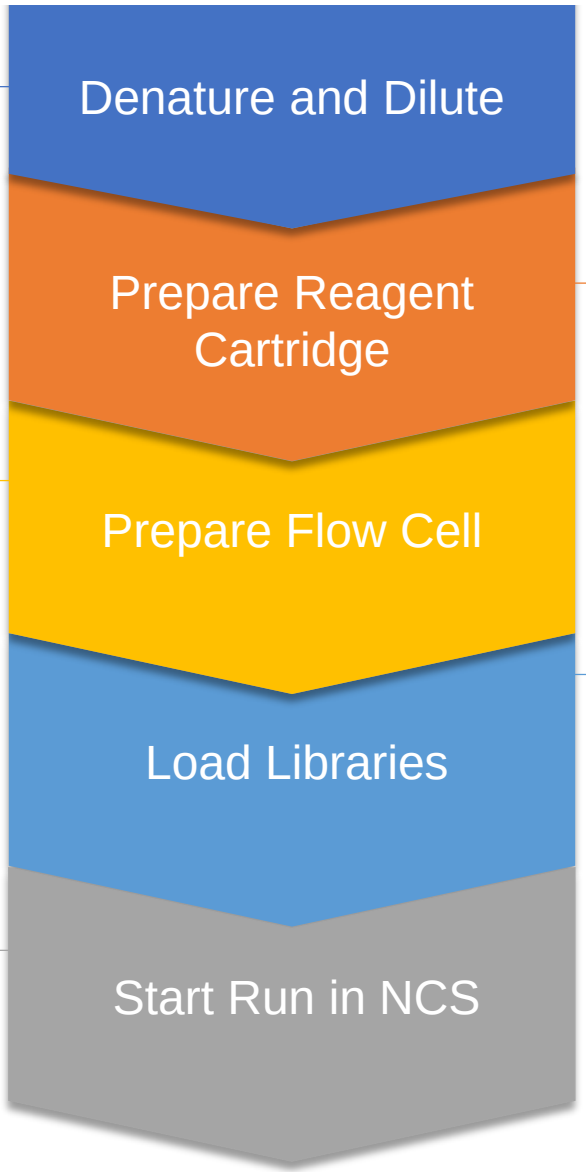
How to Start a Run

NextSeq workflow

Denature and dilute your library samples

Inspect and prepare flow cell

Start sequencing run in NextSeq Control Software

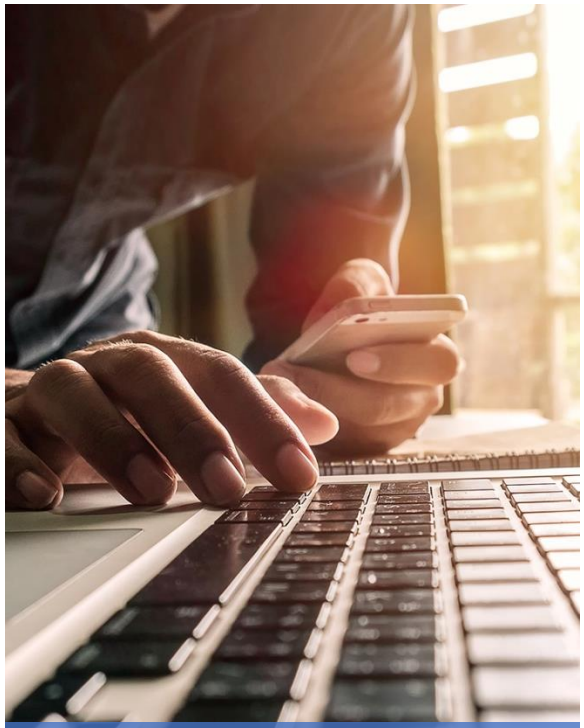


Thaw and prepare reagent cartridge which contains clustering and sequencing reagents

Load library and optional custom primers

Setting up a Sequencing Run

Run Modes



Local Run Manager *

Data saved to specified output folder for automatic analysis in local run manager

Manual*

Data saved to specified output folder for later analysis off the instrument

BaseSpace™ (optional)

Requires BaseSpace Sequence Hub account, internet connection, sample sheet for integrated analysis

*For analysis purposes, BaseSpace Sequence Hub can pair with either run mode. When the run mode is Local Run Manager and BaseSpace Sequence Hub is configured, both applications analyze the data

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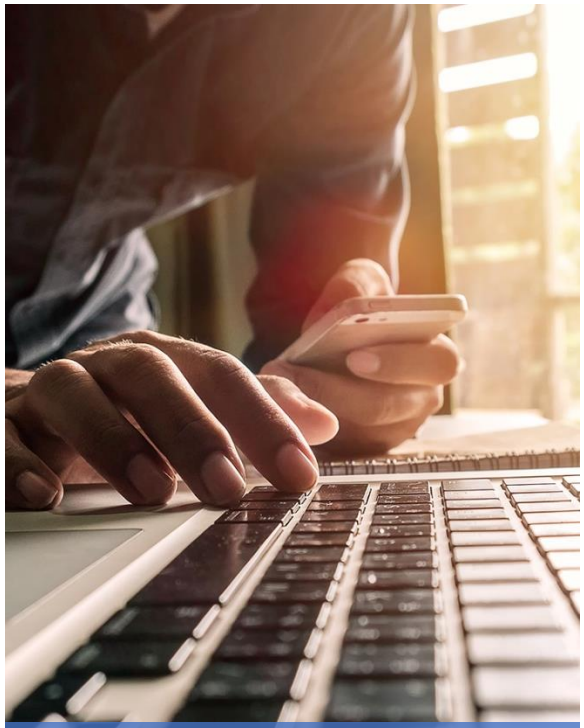
Setting up a Sequencing Run

Run Modes



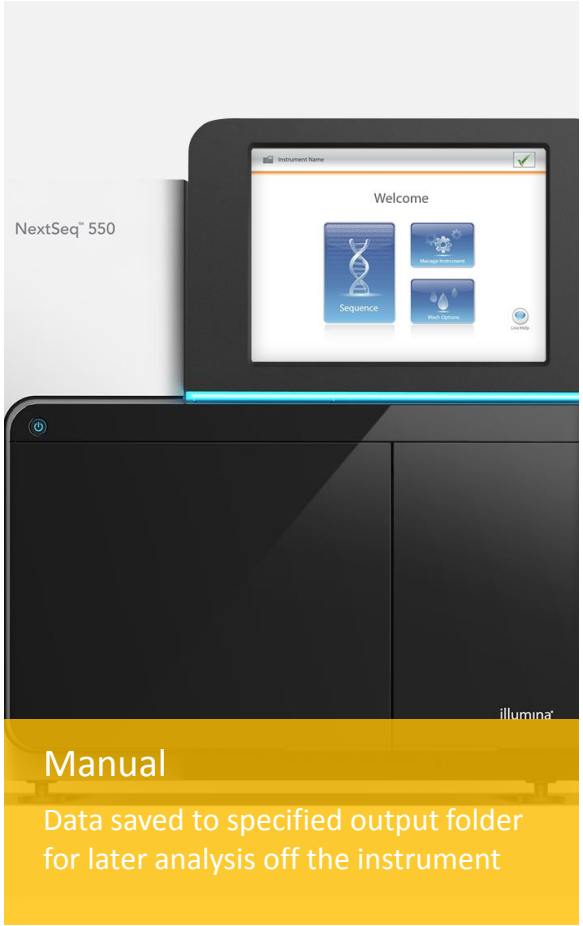
Local Run Manager

Data saved to specified output folder for automatic analysis in local run manager

Local Run Manager is an integrated software for recording samples for a run, specifying run parameters, monitoring status, analyzing sequencing data, and viewing results

Setting up a Sequencing Run

Run Modes



Manual

Data saved to specified output folder for later analysis off the instrument

Determine the run and analysis parameters prior to library prep and consumable prep, so that there is no delay when starting your sequencing run

Create Run

- Perform the Create Run step to define the following parameters of your sequencing run:

Establish an index scheme	• How many samples do I want to multiplex?
Identify the pooling strategy	• Which libraries will I sequence together?
Define sequencing read length	• How long to my reads need to be? • How much data do I need?

Setting up a Sequencing Run

Run Modes

When setting up a sequencing run, you can select one of the following BaseSpace Sequence Hub options

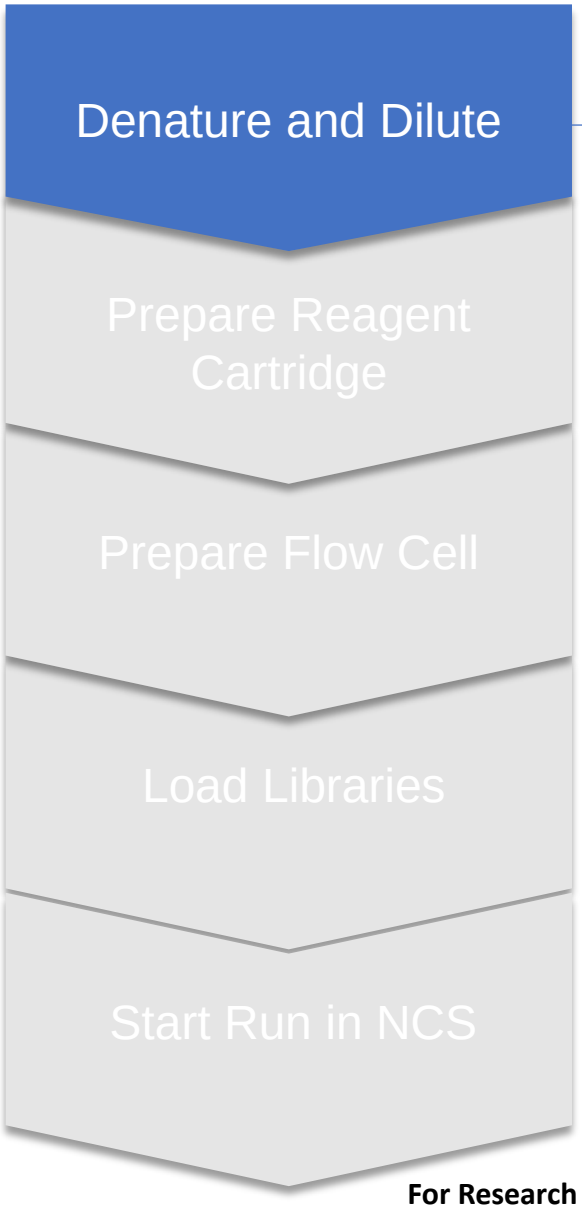
- **Run Monitoring and Storage**
  - Send InterOp files, log files, and run data to BaseSpace Sequence Hub for remote monitoring and analysis. Requires a BaseSpace Sequence Hub account, an internet connection, and a sample sheet
- **Run Monitoring Only**
  - Send InterOp and log files to BaseSpace Sequence Hub for remote run monitoring. This option is the default. Requires a BaseSpace Sequence Hub account and an internet connection

BaseSpace (optional)

Requires BaseSpace Sequence Hub account, internet connection, sample sheet for integrated analysis

How to Start a Run

Denature and Dilute



- Denature and dilute your sample
  - Target cluster densities (170 – 220 K clusters/mm2)
  - Denature and dilution for the NextSeq requires 200 mM Tris-HCl, pH7, a user-supplied consumable
    - 200 mM Tris-HCl, pH7 ensures NaOH is fully hydrolyzed in the final solution
- Denature and dilute PhiX 1.8 pM
- Mix sample library and PhiX control to result in a 1% PhiX control volume ratio
- A total of 1.3 mL of denatured and diluted sample is needed for loading into the reagent cartridge

How to Start a Run

Prepare Reagent Cartridge

Denature and Dilute

Prepare Reagent Cartridge

Prepare Flow Cell

Load Libraries

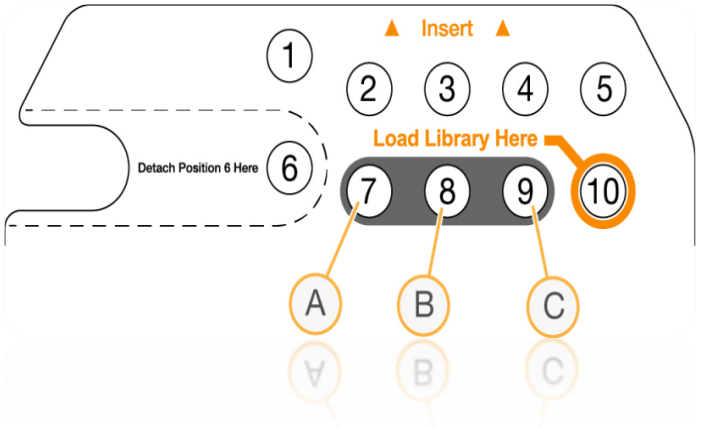
Start Run in NCS

- Thaw cartridge in room temperature water bath for approximately 60 minutes
  - Do not submerge above lower lid edge
  - Ensure all reagents are fully thawed
- Gently dry the base of the cartridge
- Manually invert the cartridge 5 times to mix the thawed reagents
- Gently tap the cartridge on the bench to reduce air bubbles

How to Start a Run

NextSeq workflow

- Denature and Dilute
- **Load Custom Primers**
- Prepare Flow Cell
- Load Libraries
- Start Run in NCS



A. Custom Read 1 primer

B. Custom Read 2 primer

C. Custom Index 1 and Index 2 primers

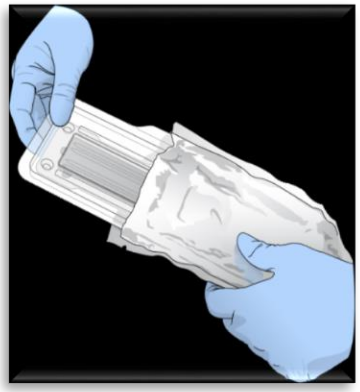
How to Start a Run

Prepare Flow Cell

- Denature and Dilute
- Load Custom Primers
- **Prepare Flow Cell**
- Load Libraries
- Start Run in NCS

- Set the flow cell package aside at room temperature for 30 minutes
- Remove the foil packaging and then remove the flow cell cartridge from the plastic clamshell casing
- Clean the glass surface of the flow cell using an alcohol wipe
- Dry the glass with a lint-free tissue or lens paper

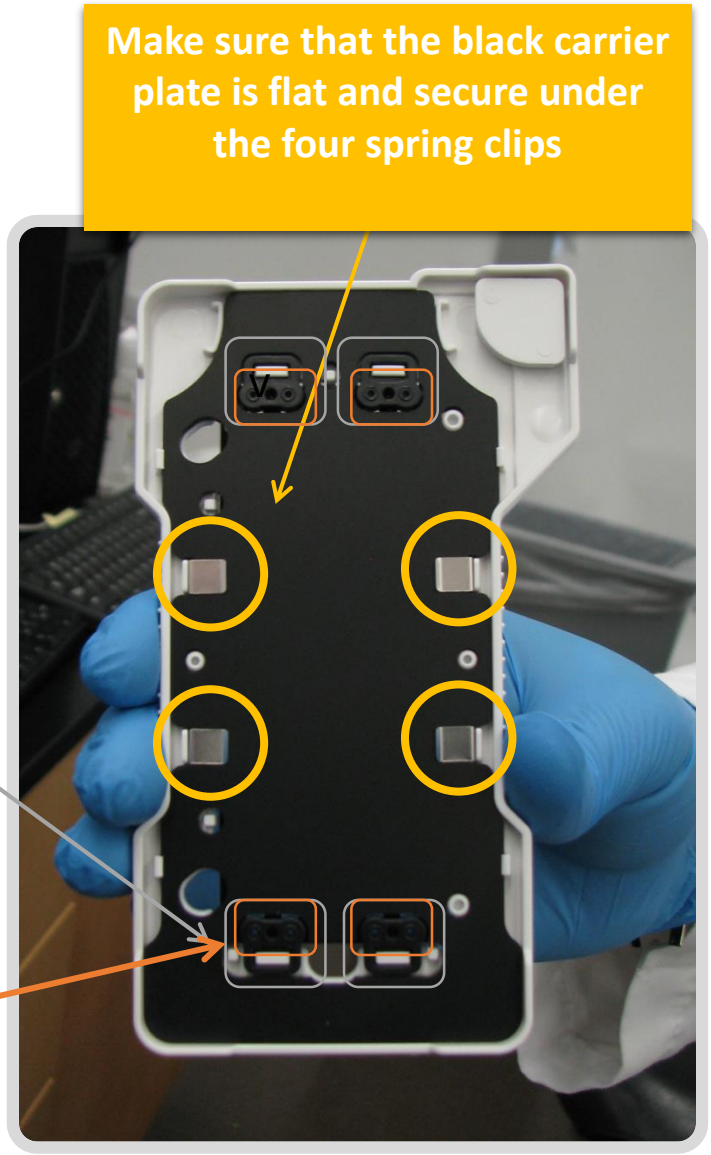
Note: After the foil packaging has been opened, use the flow cell within the next 12 hours



How to Start a Run

Inspect the flow cell

- Inspect the four metal spring clips on both the top and back sides of the flow cell
- On the back side of the flow cell, visually inspect the flow cell ports, gaskets, and carrier plate



How to Start a Run

Load Libraries

Denature and Dilute

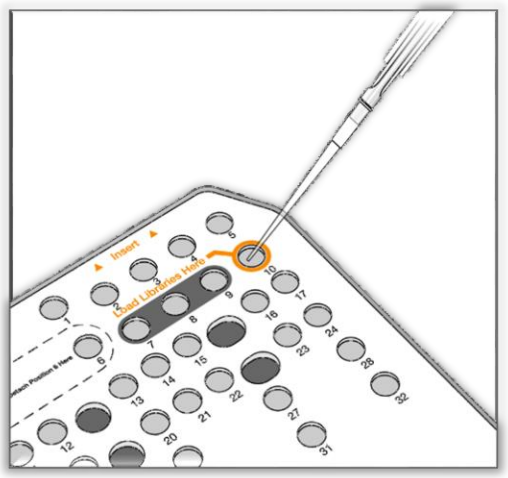
Load Custom Primers

Prepare Flow Cell

Load Libraries

Start Run in NCS

- **Load sample in highlighted position on reagent cartridge**
  - Pierce foil with clean pipette tip
  - Add library by pipetting to bottom of well
    - Load 1.3 ml of library
  - Avoid adding library with air bubbles



How to Start a Run

Start Run in NextSeq Control Software

Denature and Dilute

Load Custom Primers

Prepare Flow Cell

Load Libraries

Start Run in NCS

- From the Home screen, select **Experiment**
  - Oversized buttons make on-screen navigation easy
- On the Select Assay screen, select **Sequence**
  - The Sequence command opens the imaging compartment door, release consumables from a previous run, and opens the series of run setup screens

How to Start a Run

Setting up a Sequencing Run

Denature and Dilute

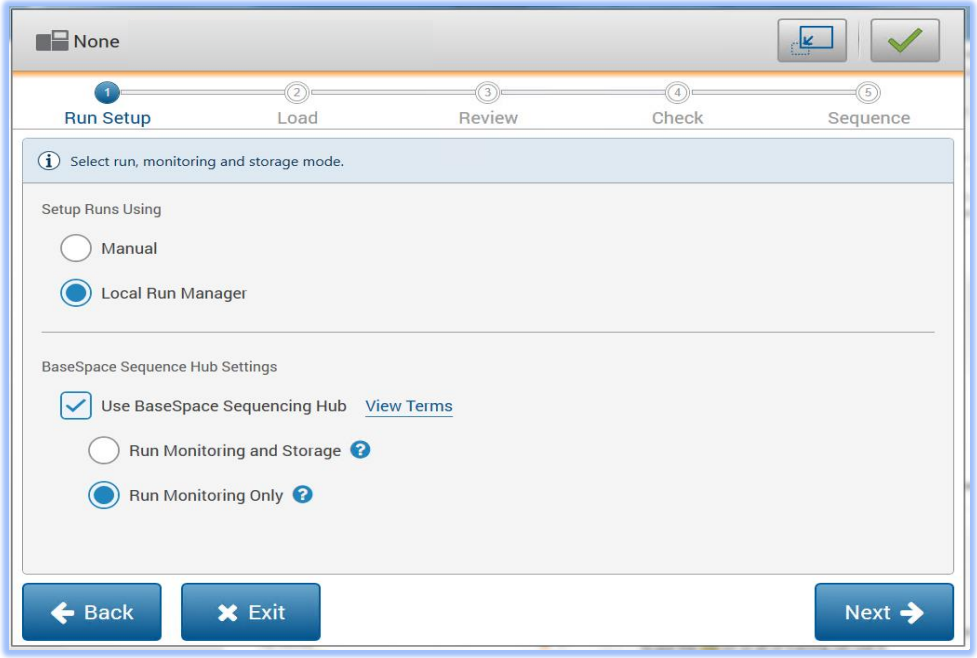
Load Custom Primers

Prepare Flow Cell

Load Libraries

Select Run Mode

- Select the sequencing run mode
  - Run mode selection will determine where to enter run information and how to analyze data
- Local Run Manager is the default run mode and provides streamline workflow where separate sample sheet and analysis applications are not required



How to Start a Run

Setting up a Sequencing Run

Denature and Dilute

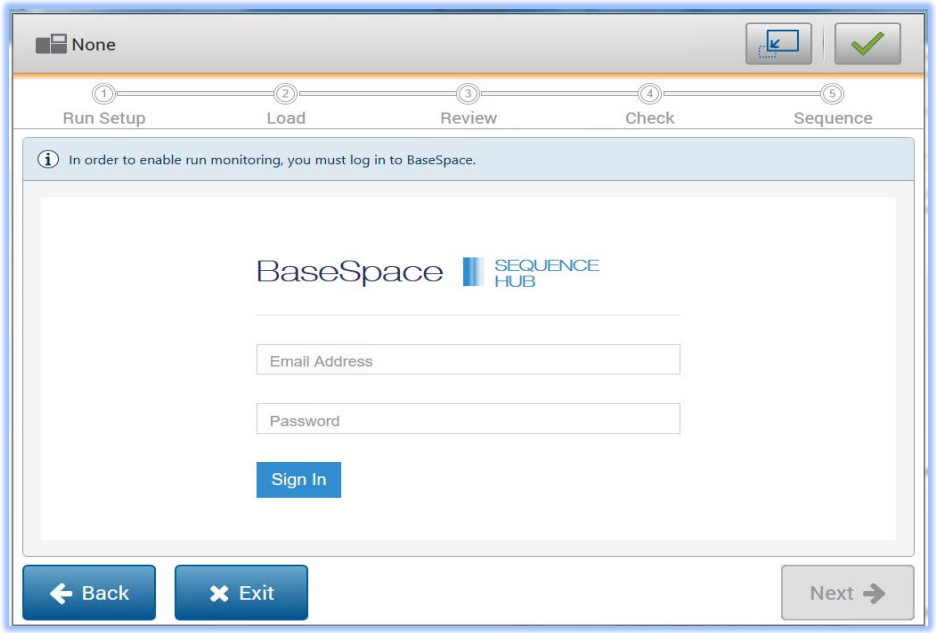
Load Custom Primers

Prepare Flow Cell

Load Libraries

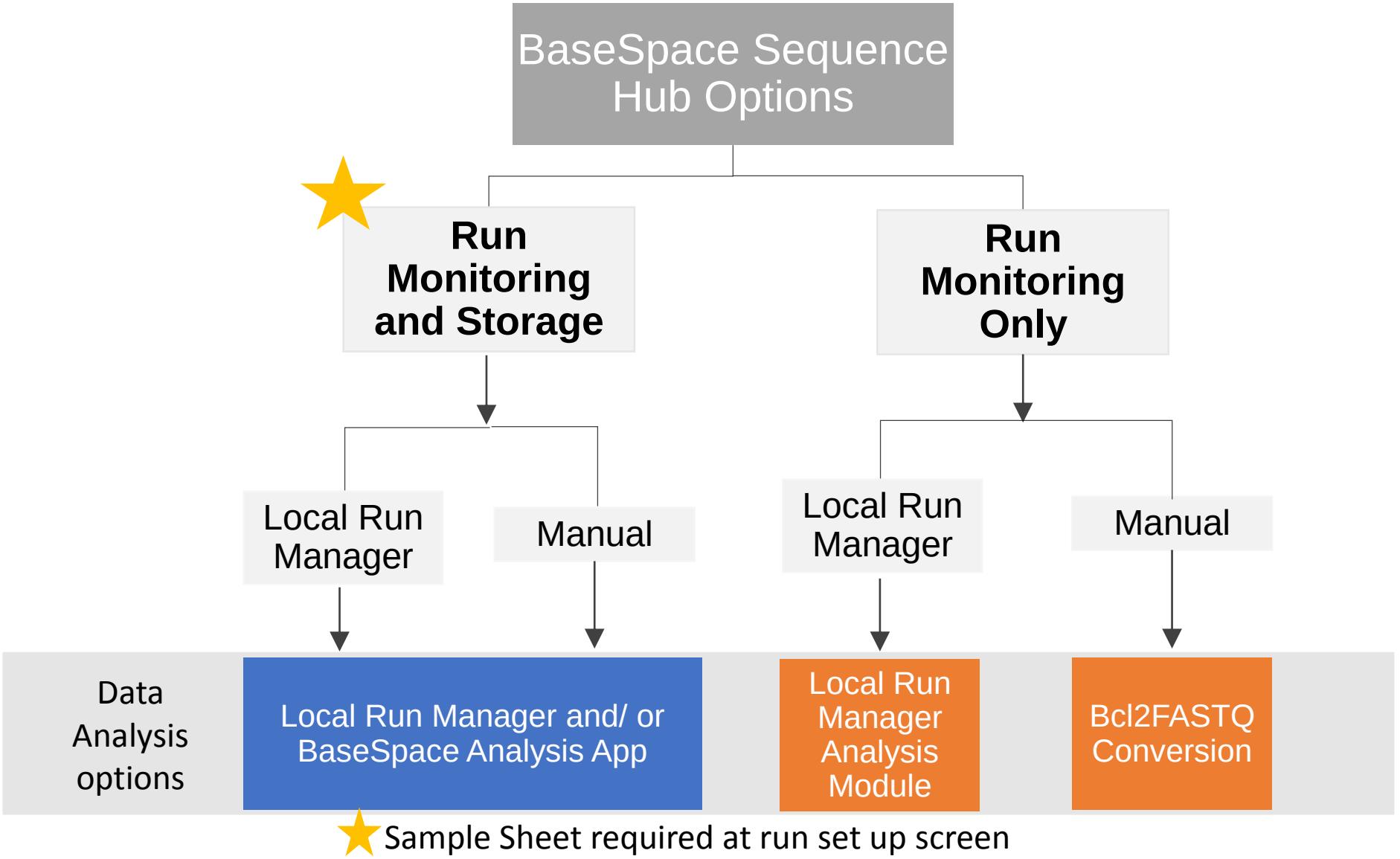
BaseSpace Settings

- BaseSpace Connectivity
  - Log in credentials are required when these options are selected
  - Available Workgroups become available after log in



Run Mode Selection

with BaseSpace Connectivity



How to Start a Run

Setting up a Sequencing Run

Denature and Dilute

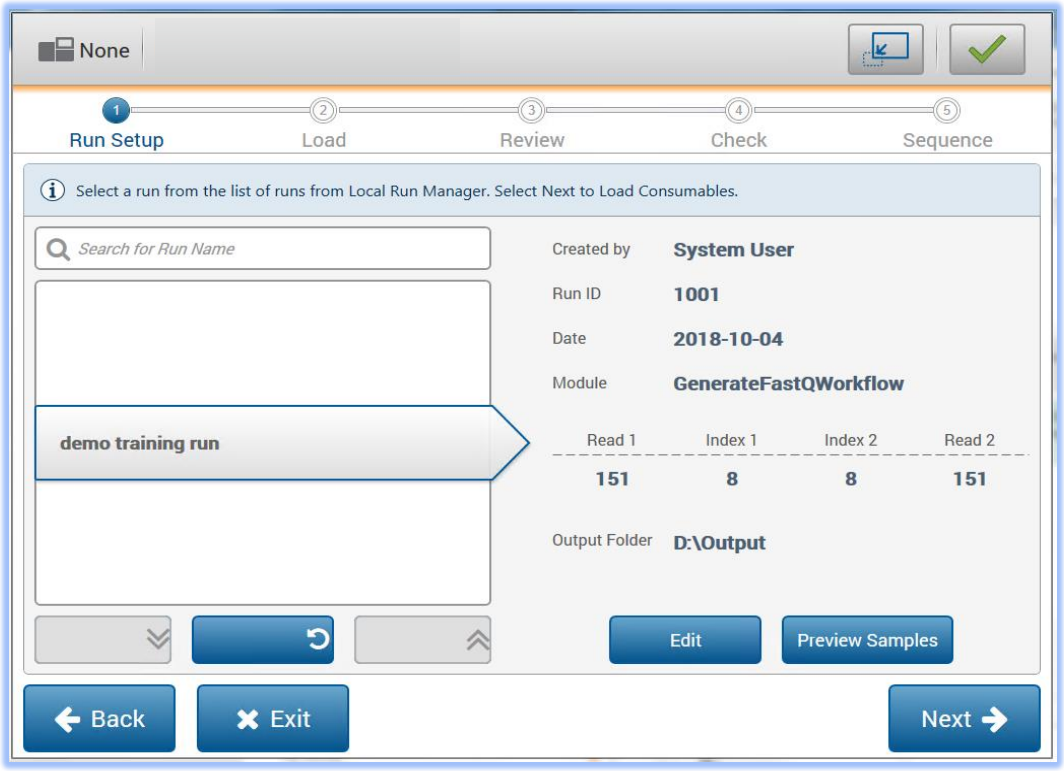
Load Custom Primers

Prepare Flow Cell

Load Libraries

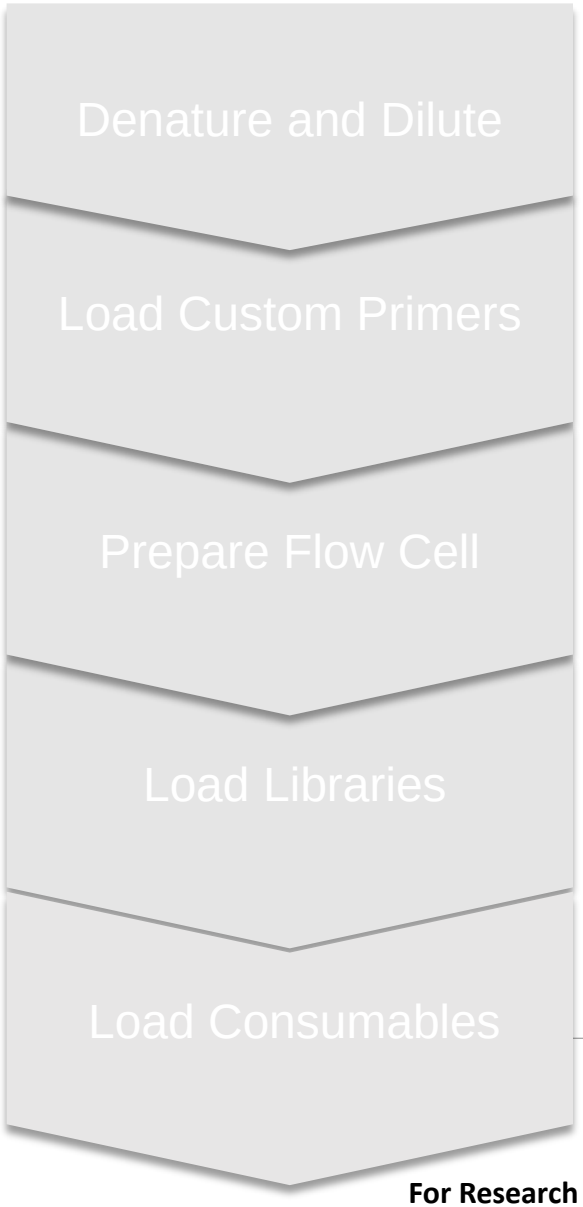
Local Run Manager

- Select a run name from the list of available runs
  - Confirm run parameters
  - Option available to edit and preview sample list



How to Start a Run

Setting up a Sequencing Run



Each consumable loaded into NextSeq contains a unique Radio Frequency ID, or RFID

The RFID is recognized by the NCS, allowing the software to automatically populate the barcode numbers of each consumable

Note: A short delay after loading each NextSeq consumable is normal

Starting a Run in Manual Run Mode

Start the Run in NCS

Manual Run Mode

Denature and Dilute

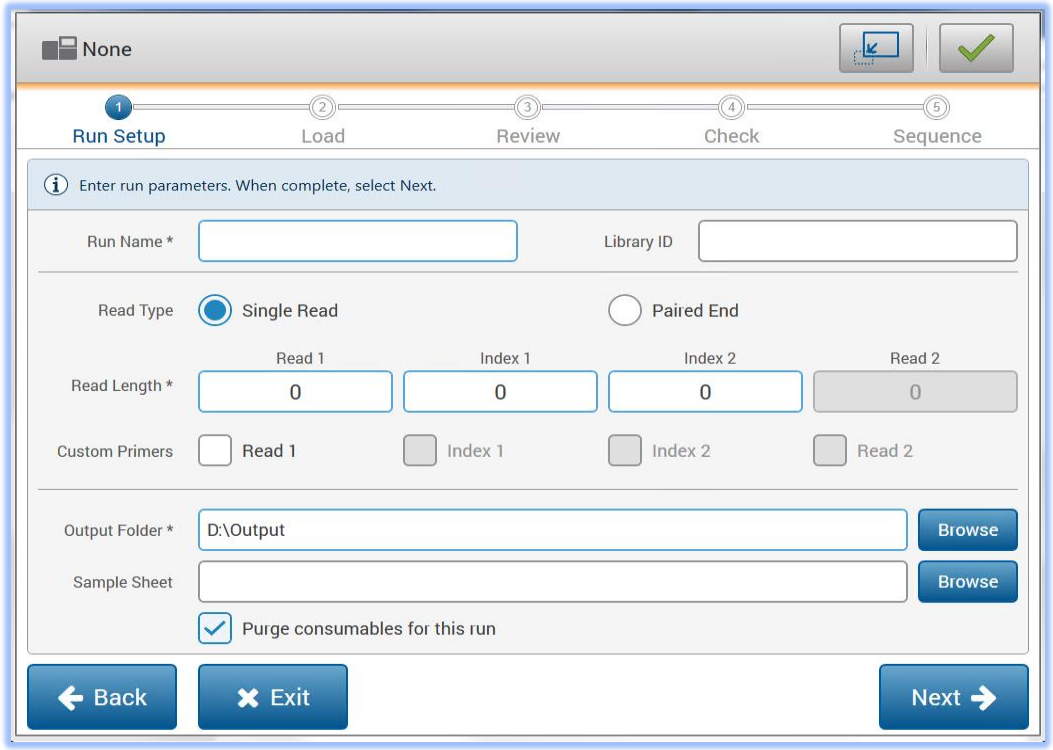
Load Custom Primers

Prepare Flow Cell

Load Libraries

Start Run in NCS

- Simple Manual Run Setup through NCS
  - Enter run name and parameters
  - Browse to navigate to a network location



Save run to a network drive

How to Start a Run

Start Run in NextSeq Control Software

Denature and Dilute

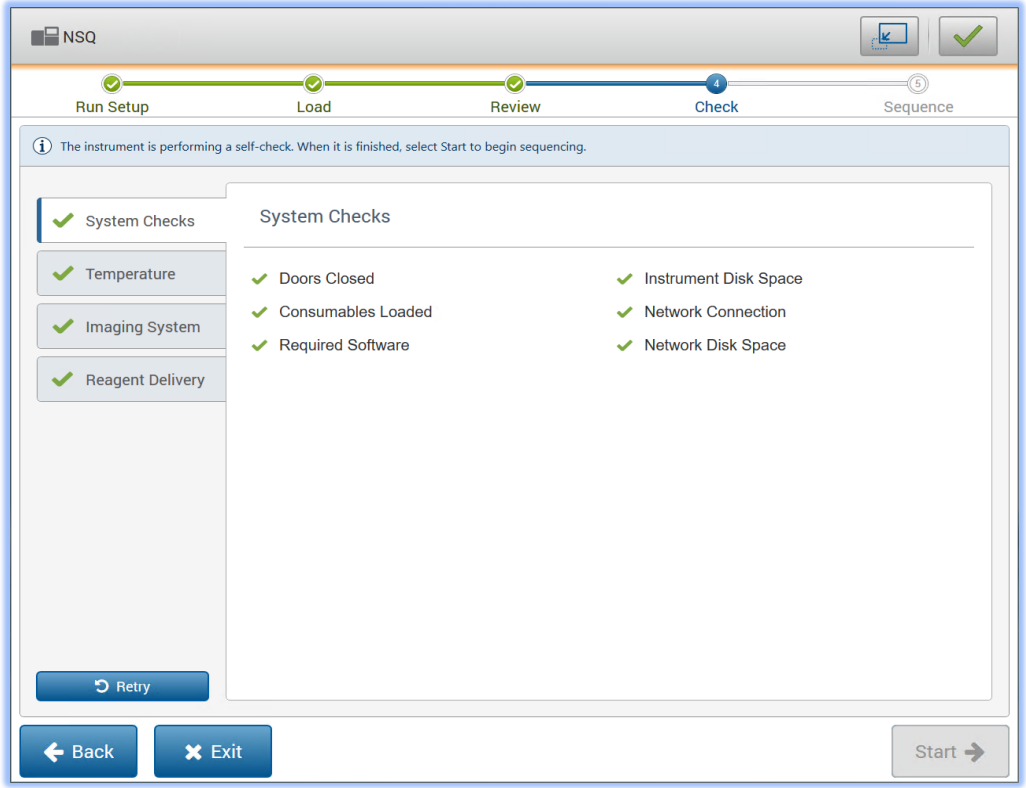
Load Custom Primers

Prepare Flow Cell

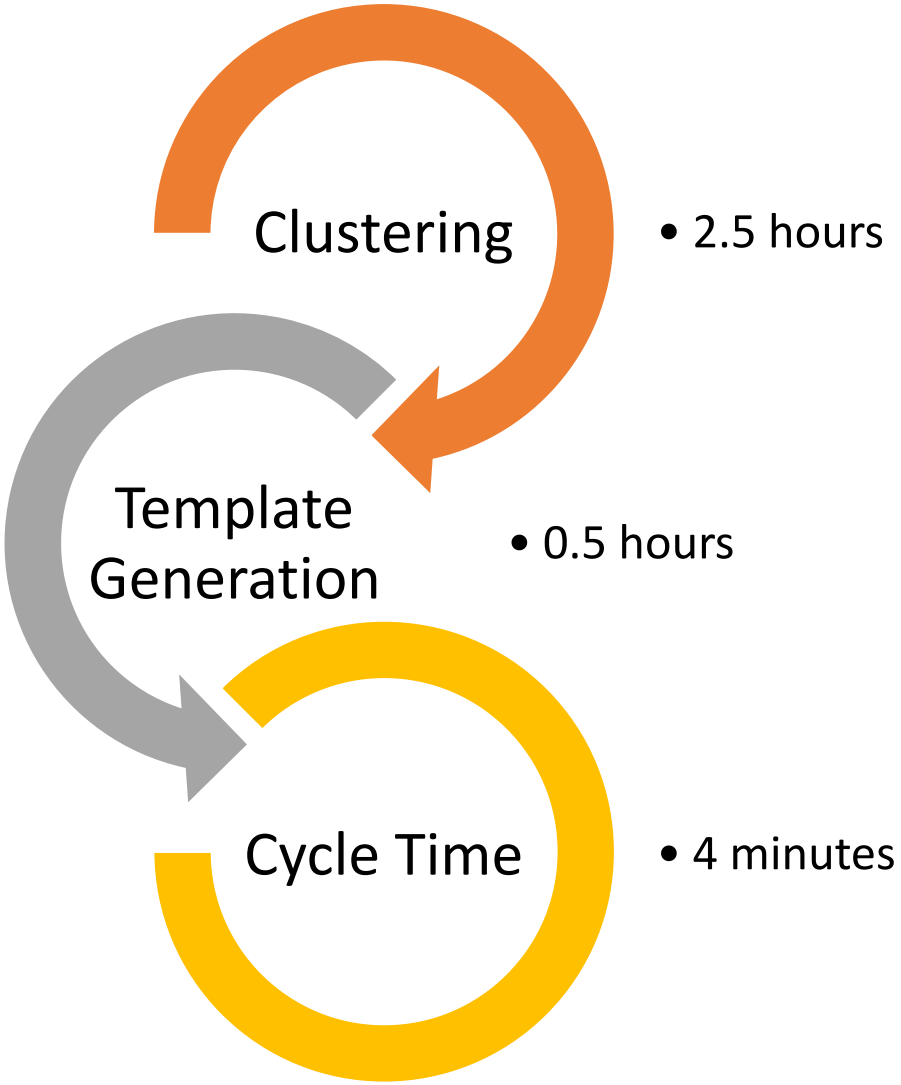
Load Libraries

Automated Pre Run Check

- The software performs an automated check of the system.
- When the automated check is complete, select Start. The sequencing run begins



Timing on NextSeq System



Starting a Run Using the MiSeq System

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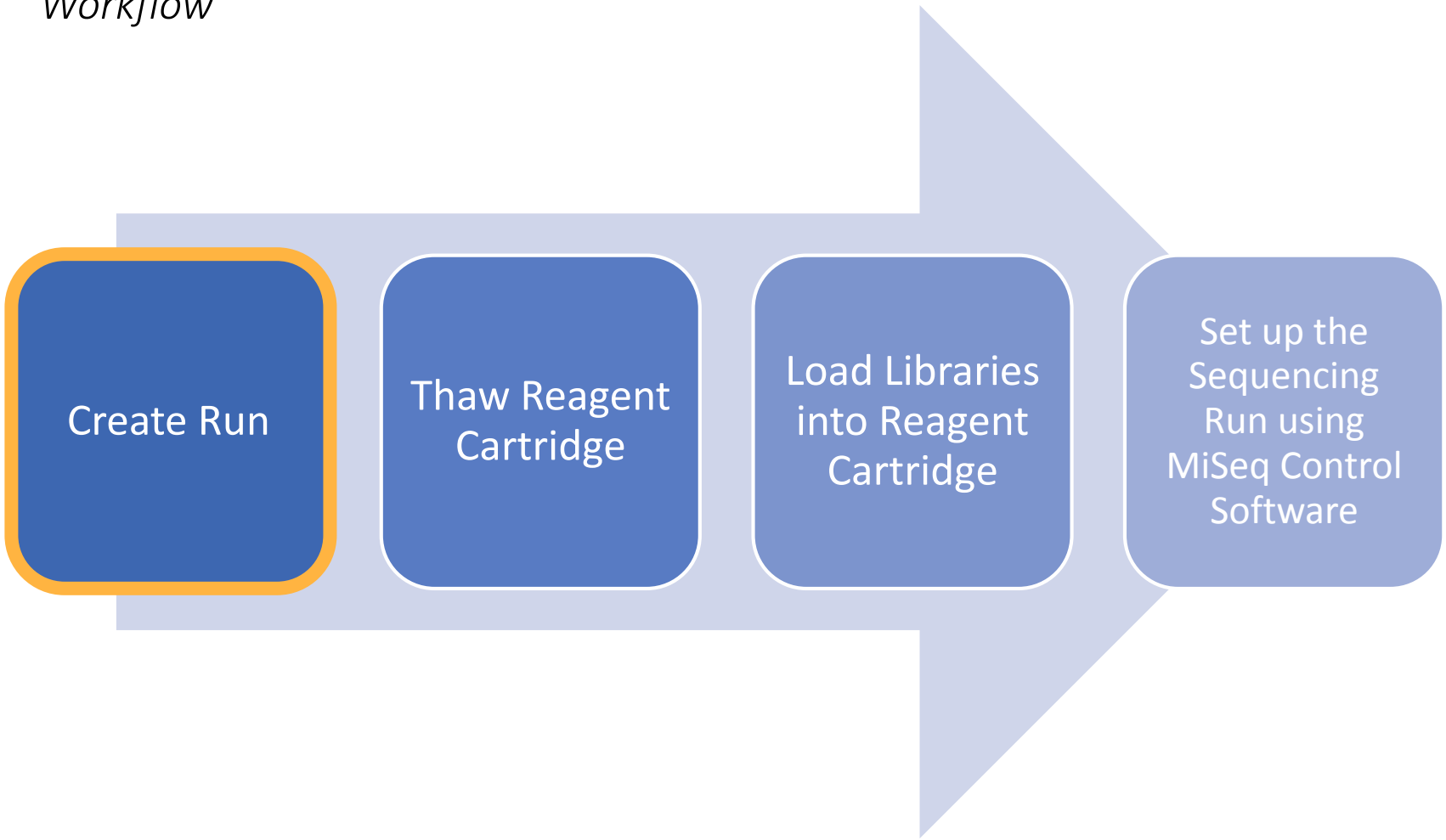
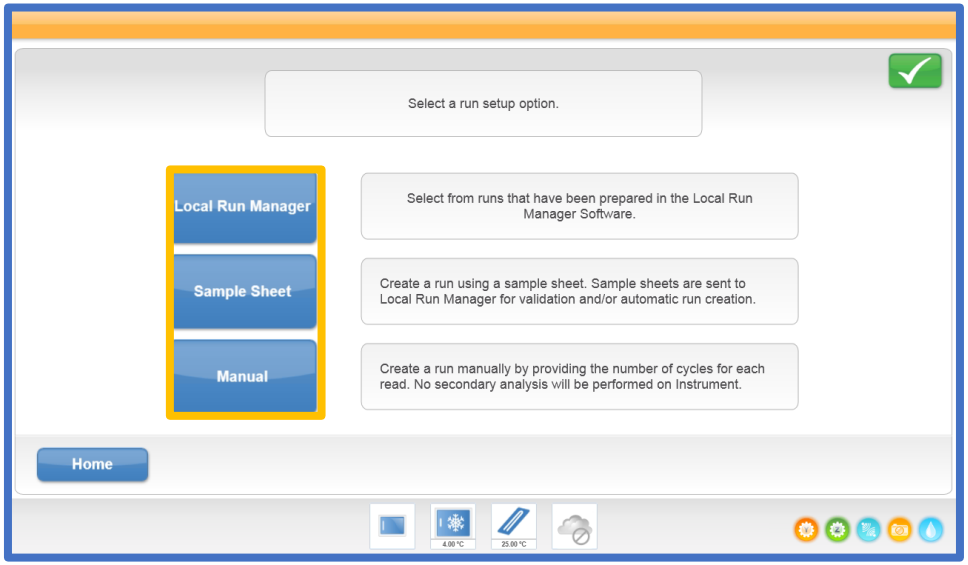
How to Start a Run

Workflow



Create Run

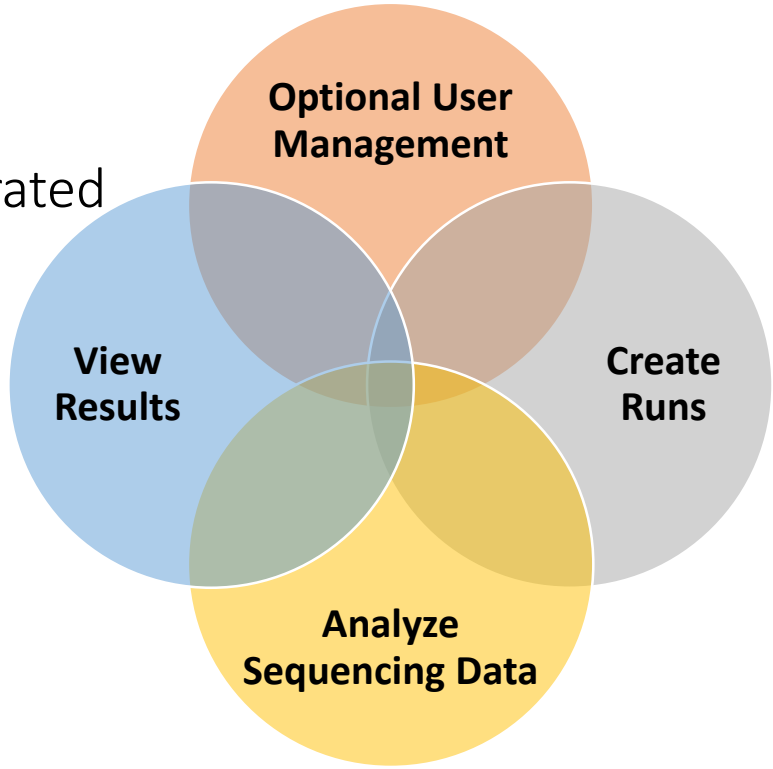
- There are multiple options for completing the Create Run step

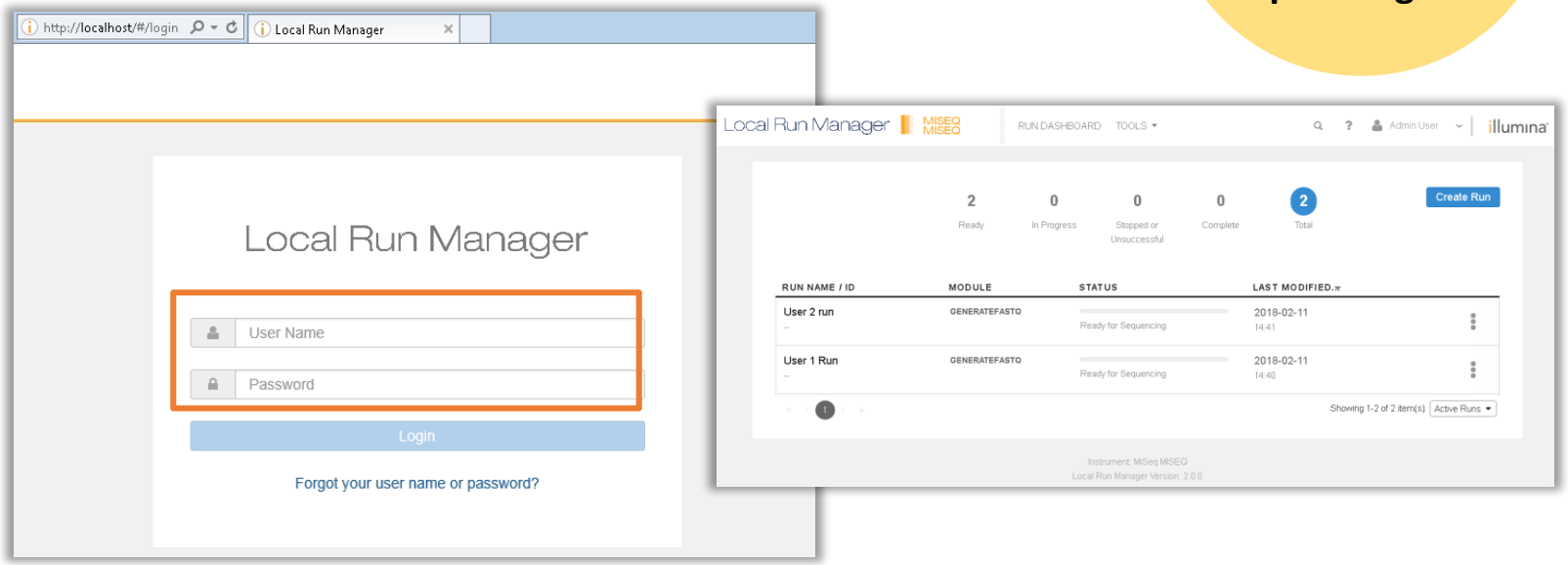


Create Run: Local Run Manager

- Local Run Manager provides a fully integrated solution for the sequencing workflow

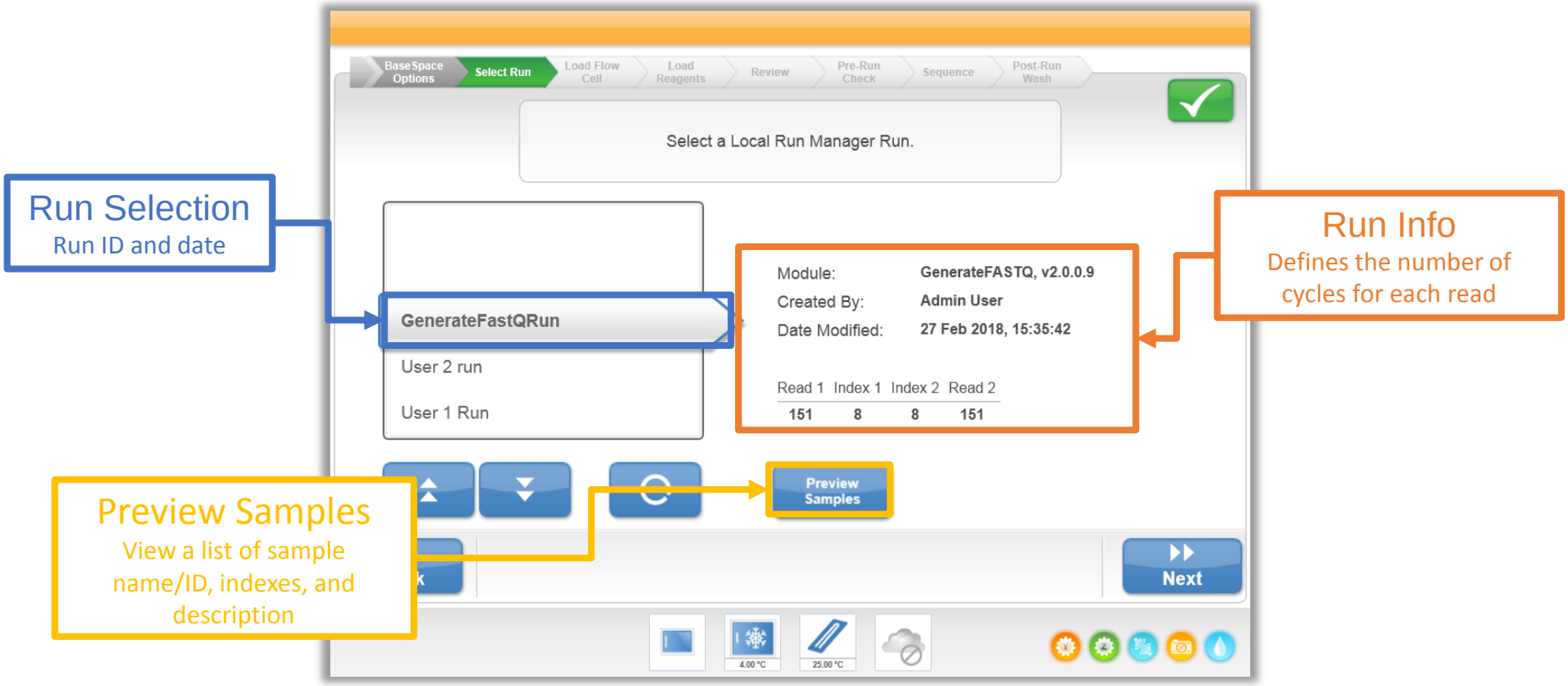
- Accessed on or off instrument via a web browser





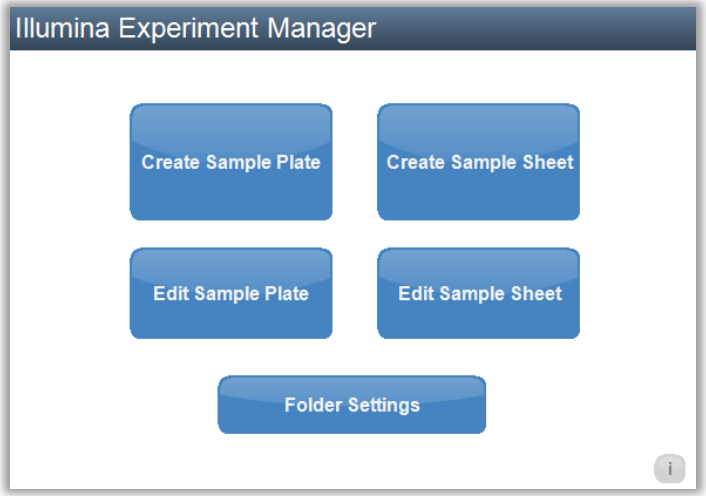
Create Run: Local Run Manager

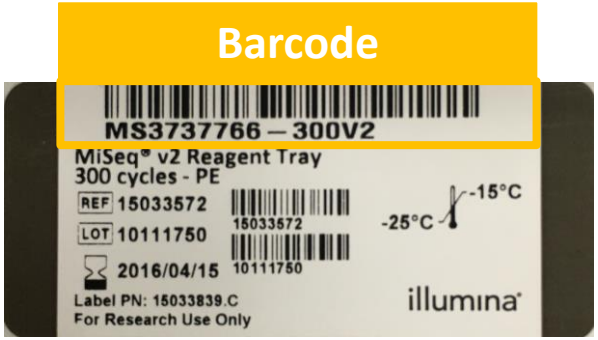
- Select Run
  - Preview samples
  - Edit the run in Local Run Manager



Create Run: Illumina Experiment Manager

- Illumina Experiment Manager (IEM) is an application to create and edit sample plates and sample sheets
- Sample plates store information regarding:
  - Assay type
  - Plate name
  - Sample indexes
- Sample sheets are .CSV files that store information to perform and analyze a sequencing runs
- Sample sheet file name can be the reagent cartridge barcode followed by *.CSV





Use IEM to create a sample sheet **before starting library preparation**

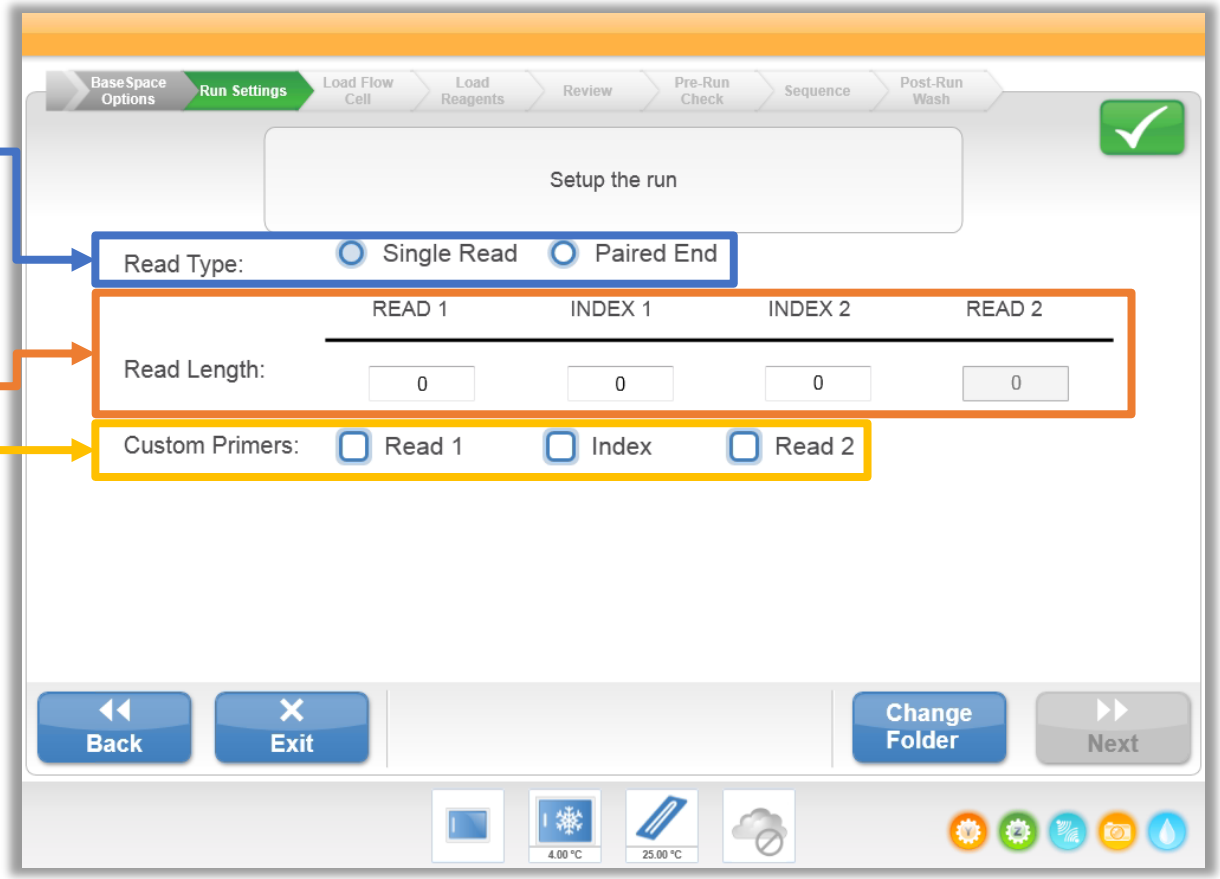
Create Run: Manual

- Minimal parameters required to begin a sequencing run
  - This feature only available beginning with MiSeq Control Software version 3.0

Read Type
Determines whether the run will perform a single or paired-end run

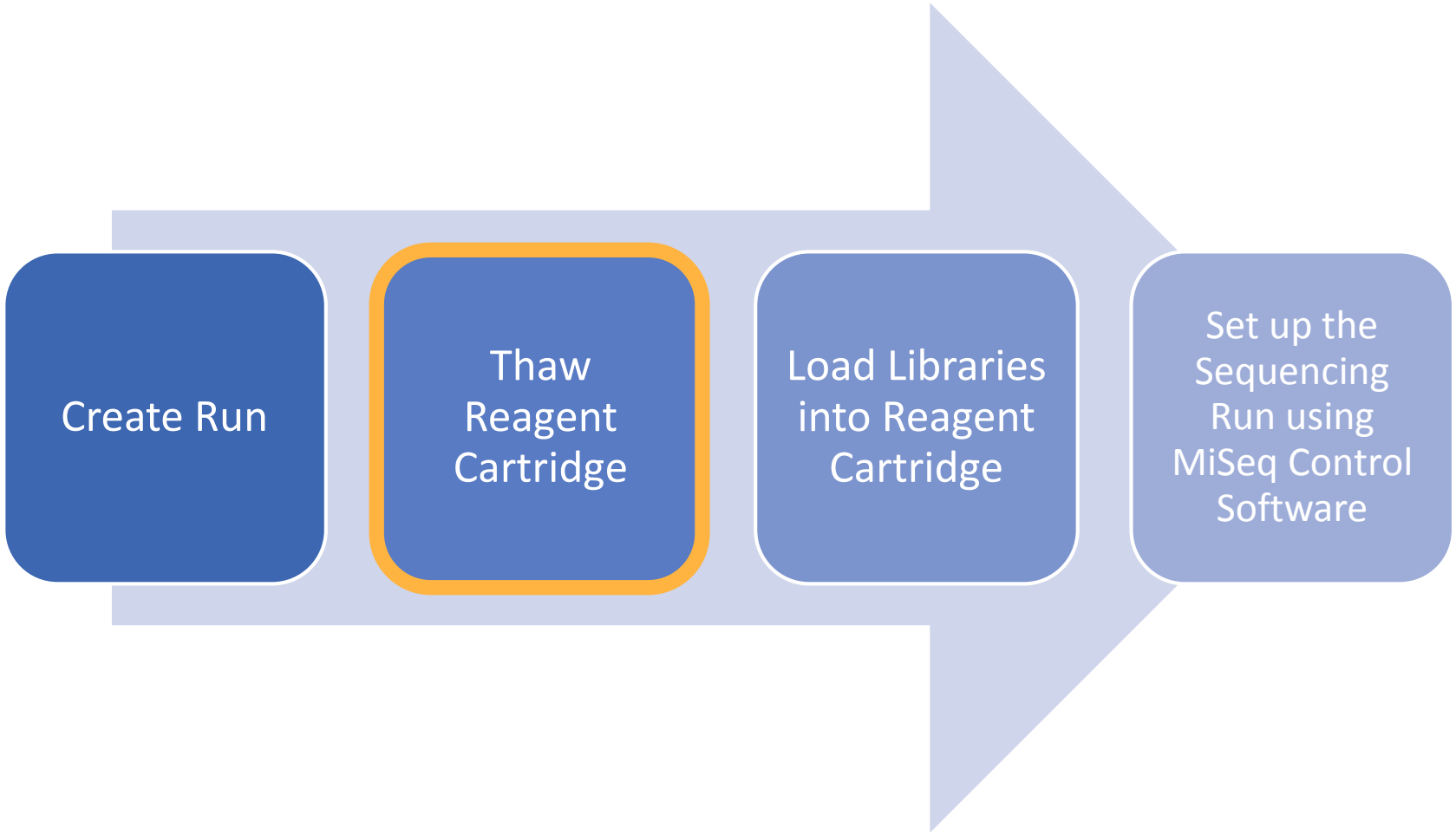
Read Length
Defines the number of cycles for each read

Custom Primers
Select whether the use of customer primers are desired for a read
Note: custom primers for Index Read 2 not available



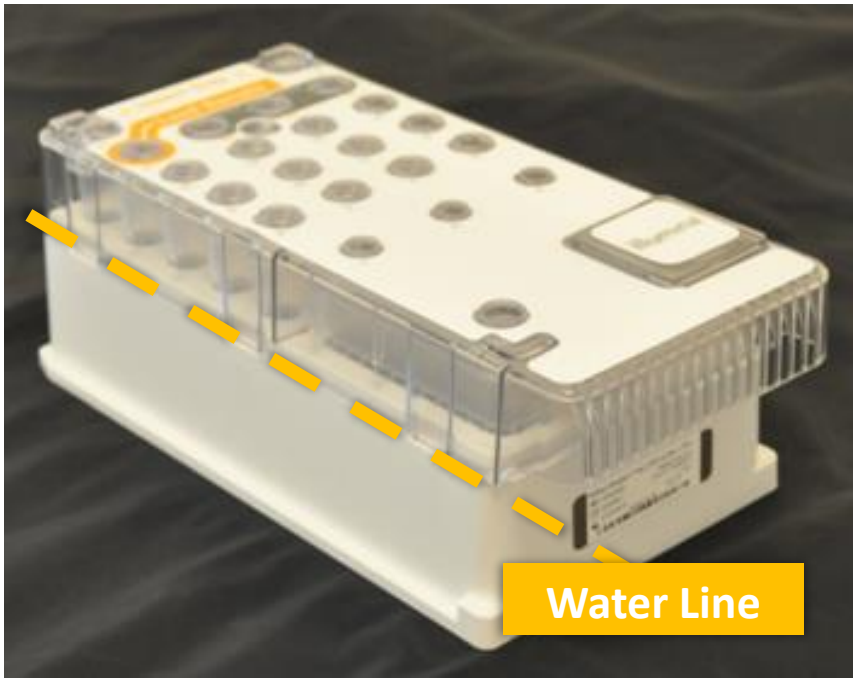
How to Start a Run

Workflow



MiSeq Reagent Cartridge Preparation

- Thaw cartridge in room temperature water bath for 60-90 min
  - Do not submerge above lower lid edge
  - Be sure all reagents are fully thawed
- Manually invert cartridge 10X
- Tap cartridge on hard surface to remove bubbles and dislodge water from cartridge base
- Place cartridge on ice until starting run



MiSeq Reagent Kits

Each Kit is Single use and Contains:

- **1 Tube of HT1**
- **1 Reagent Cartridge (V2 or V3)**
- **1 PR2 bottle (V2 or V3)**
- **1 Flow cell from list below:**

Kit	Imaging	Cycles in Kit*	Catalog Number
MiSeq Reagent Kit v3	19 tiles, dual surface (38 tiles total)	600 150	MS-102-3003 MS-102-3001
MiSeq Reagent Kit v2	14 tiles, dual surface (28 tiles total)	500 300 50	MS-102-2003 MS-102-2002 MS-102-2001
MiSeq Reagent Micro Kit v2	4 tiles, dual surface (8 tiles total)	300	MS-103-1002
MiSeq Reagent Nano Kit v2	2 tiles, single surface (2 tiles total)	500 300	MS-103-1003 MS-103-1001

* Each kit contains 25 additional cycles to allow two eight-cycle index reads and the 7 dark cycles required for paired end turn around.

Flow Cell Output

Standard Flow Cells

MISEQ REAGENT KIT V2	
READ LENGTH	OUTPUT
1 × 36 bp	540-610 Mb
2 × 25 bp	750-850 Mb
2 × 150 bp	4.5-5.1 Gb
2 × 250 bp	7.5-8.5 Gb

MISEQ REAGENT KIT V3	
READ LENGTH	OUTPUT
2 × 75 bp	3.3-3.8 Gb
2 × 300 bp	13.2-15 Gb

Reads Passing Filter	V2	V3
Single reads	12-15 M	22-25 M
Paired- End Reads	24-30 M	44-50 M

Low Output Applications

Flow cell	# of Reads	Read length	2 x 75 Output	2 x 150 Output	2 x 250 Output
Micro FC	4 M	Up to 2 x 150	600 Mb	1.2 Gb	----
Nano FC	1 M	Up to 2 x 250	150 Mb	300 Mb	500 Mb

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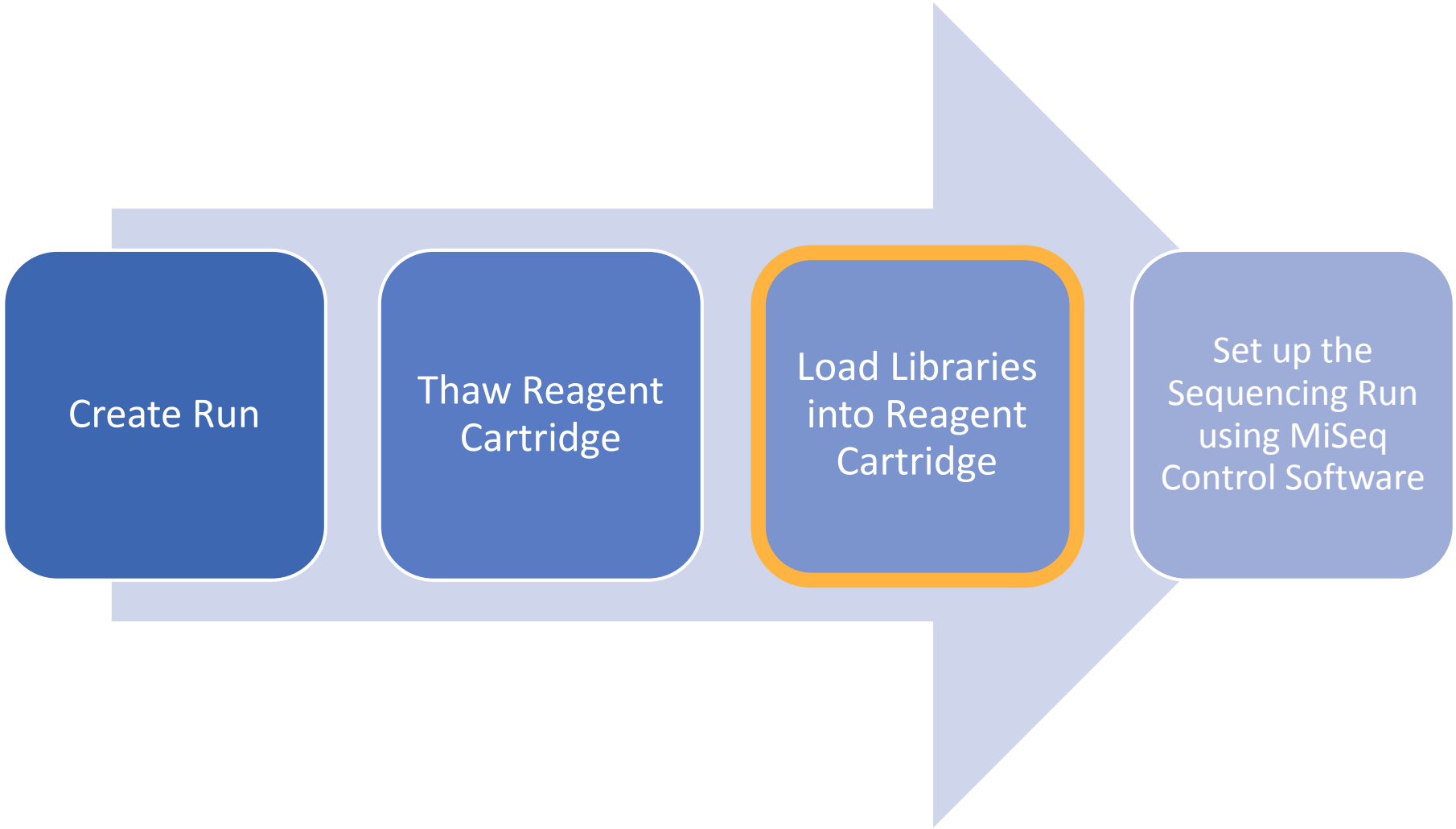
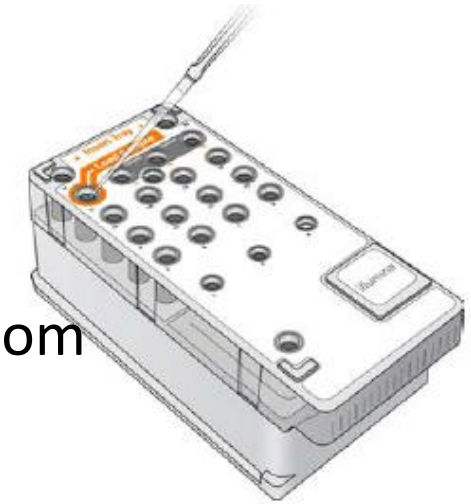
How to Start a Run

Workflow



Prepare and Load Libraries into Cartridge

- Libraries must be single stranded and diluted prior to loading onto the MiSeq cartridge
- Prepare your libraries for sequencing according to the MiSeq Denature and Dilute Libraries guide
  - Recommended: Add denatured and diluted PhiX control library to your libraries
- Load libraries in highlighted position on reagent cartridge
  - Pierce foil
  - Add libraries
  - Avoid air bubbles
  - Gently tap cartridge so libraries go to bottom

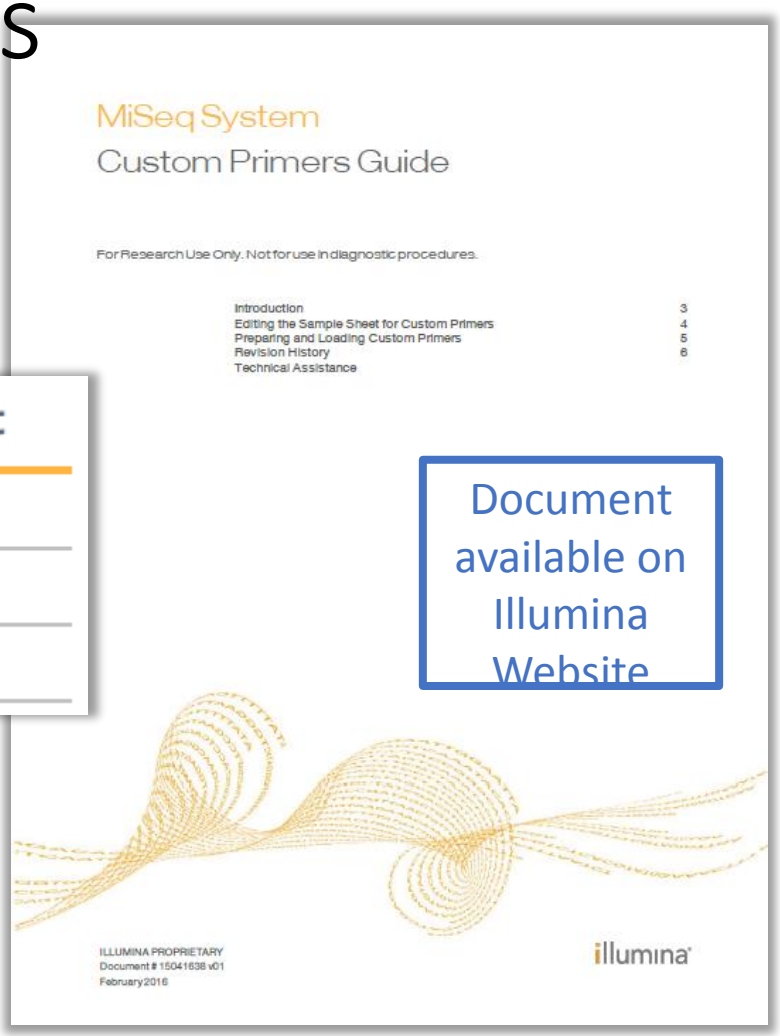


Using Custom Primers

- Use Ports 18 -20 on MiSeq reagent cartridge

Read	Port
Custom Primer for Read 1	18
Custom Primer for the Index Read	19
Custom Primer for Read 2	20

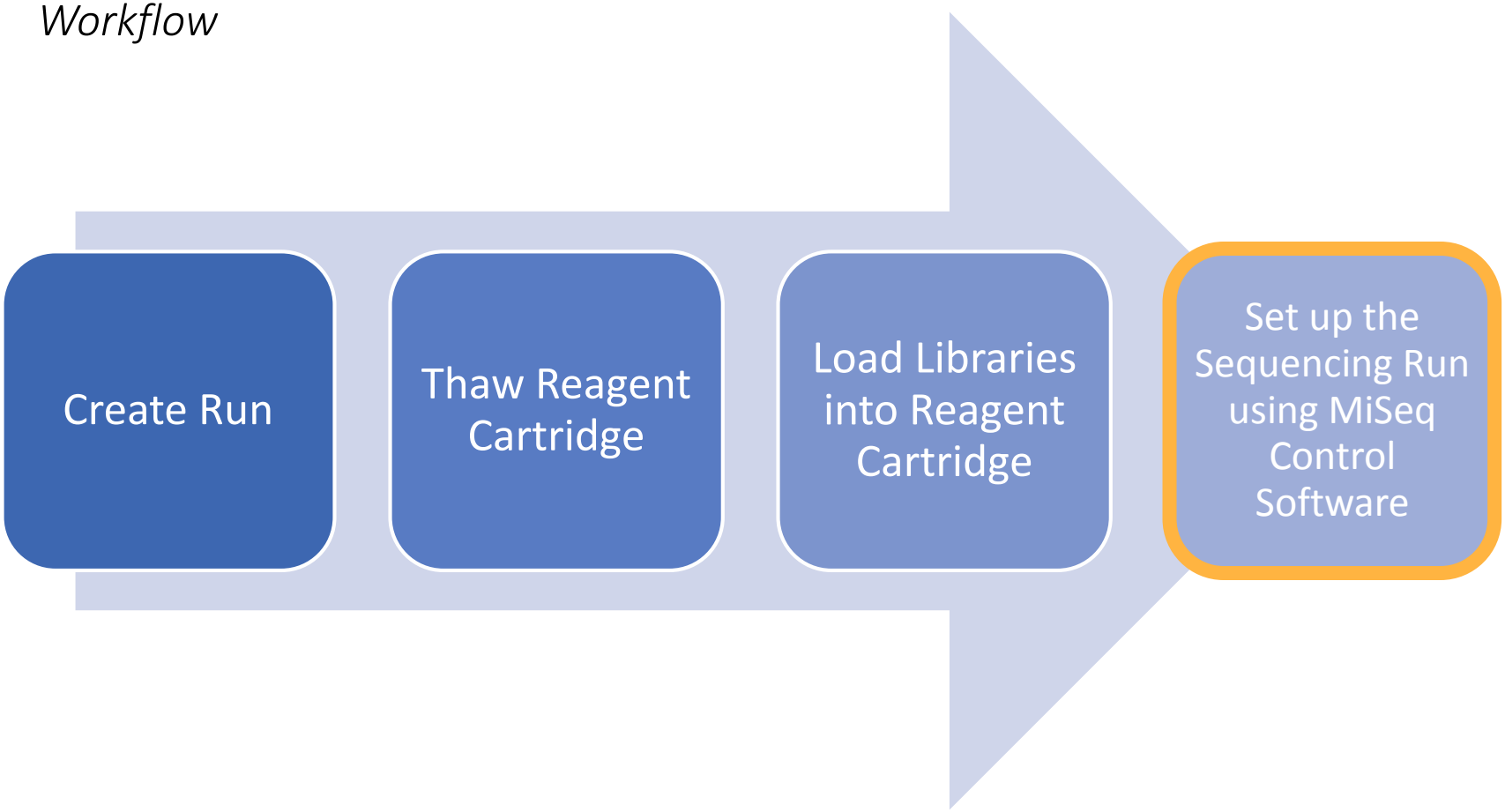
- A combination of Custom and Illumina primers can be used
- Using a custom primer for the second index read is not possible on the MiSeq platform



Document available on Illumina Website

How to Start a Run

Workflow



MiSeq Control Software and Radio Frequency

Identification (RFID)

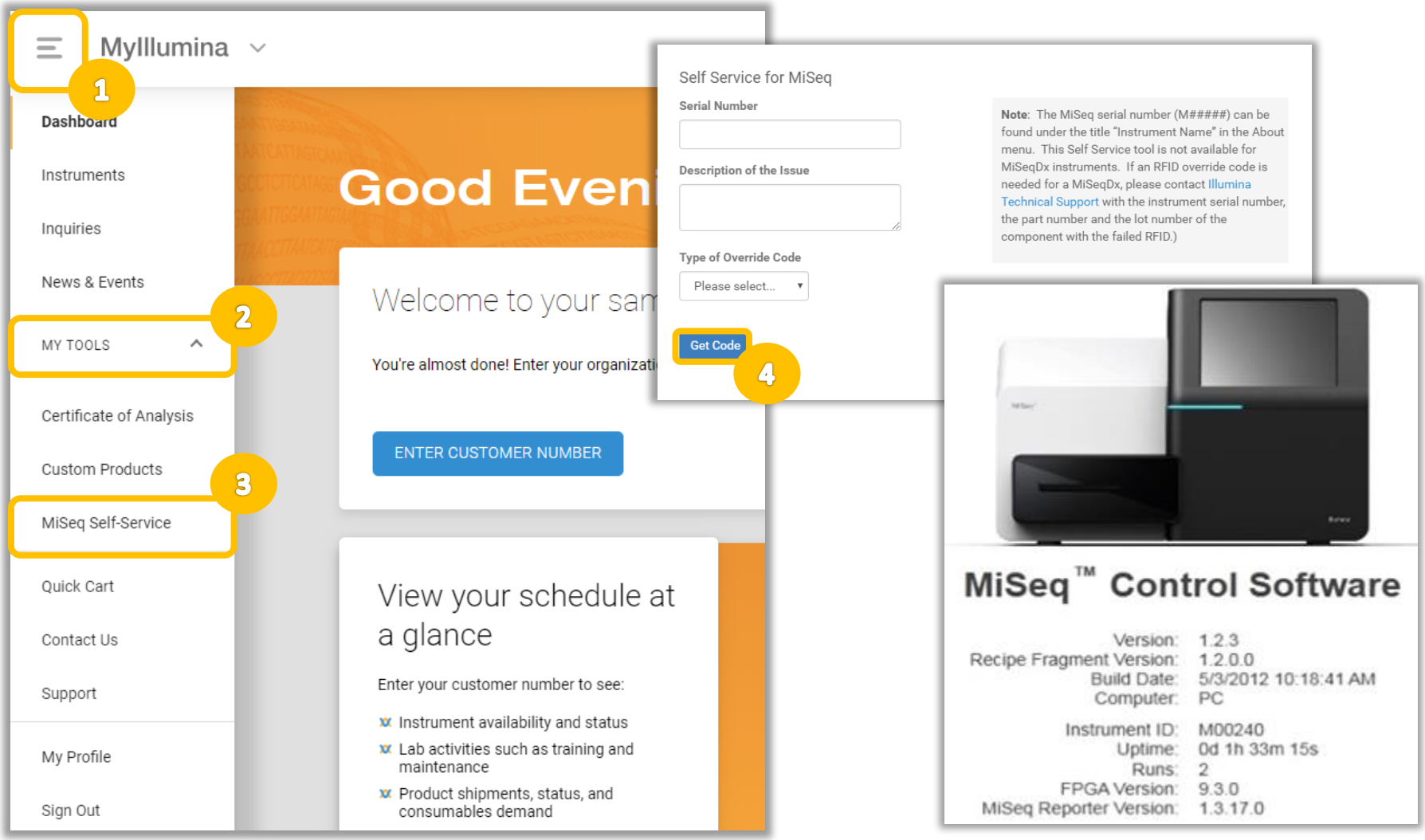
Each consumable loaded onto the MiSeq System contains a unique Radio Frequency ID, or RFID

The software recognizes the RFID and automatically populates the barcode numbers of each consumable

Obtain a temporary bypass code from the Illumina website if the system cannot read the consumable RFID

MyIllumina

How to obtain a temporary bypass code



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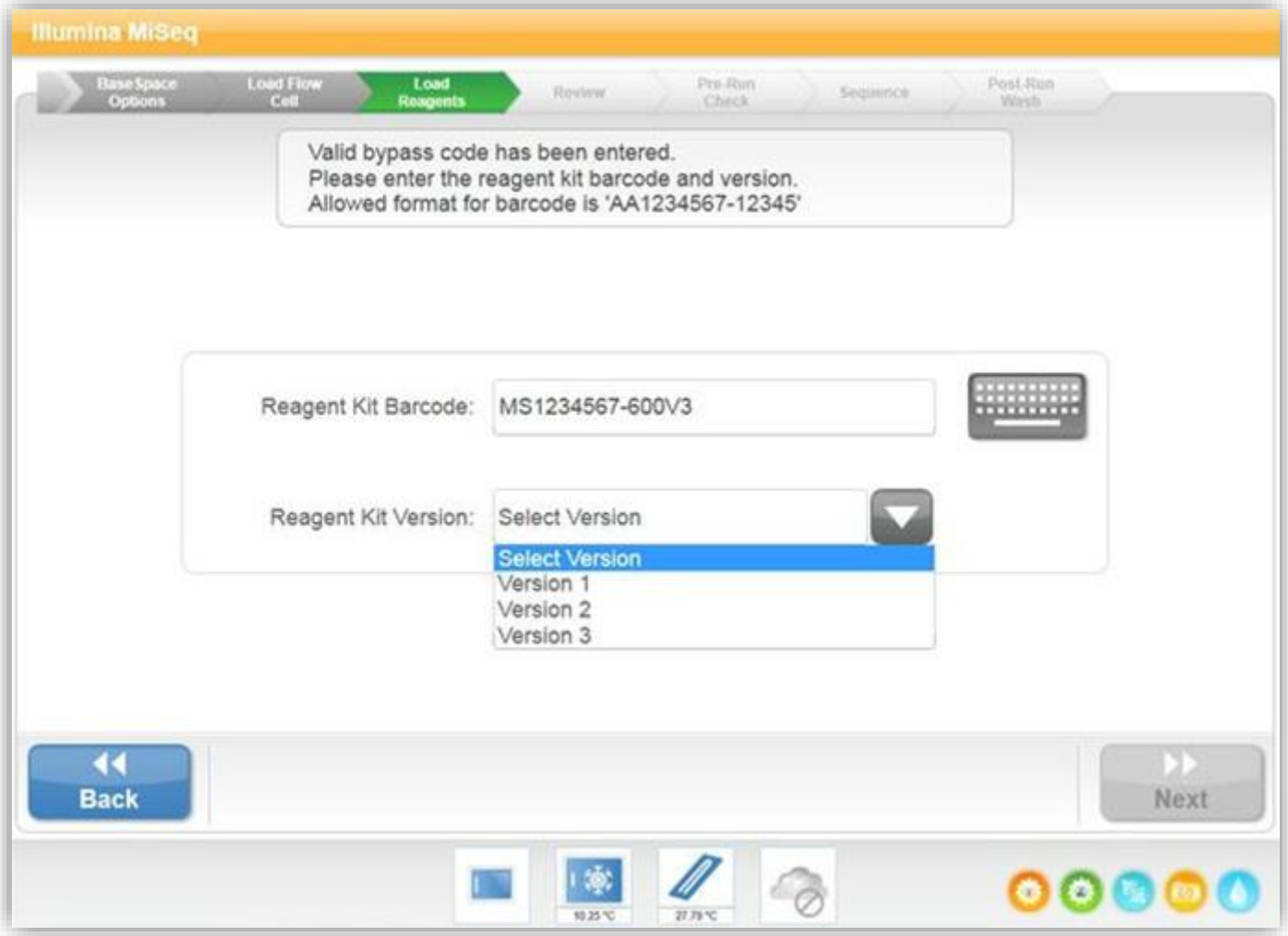
MyIllumina
Input Bypass Code



How to Calculate the Estimated Run Duration on a MiSeq