

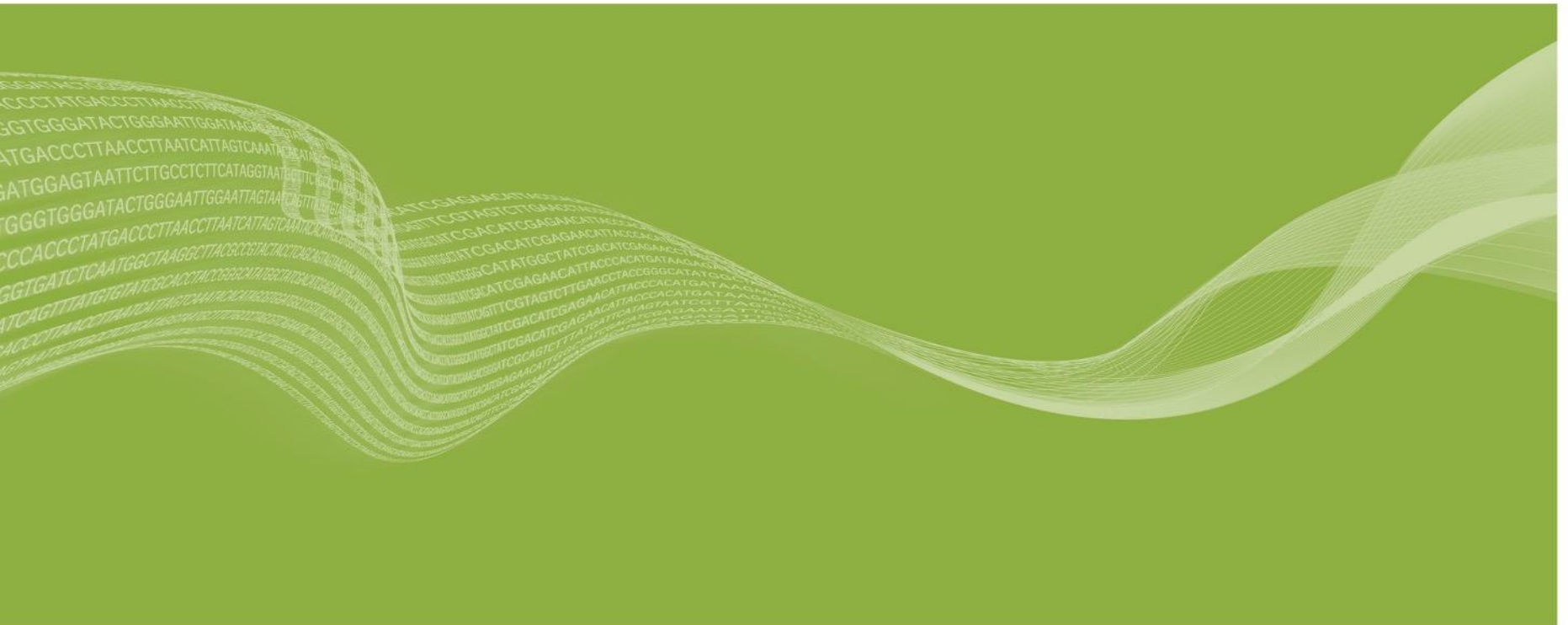
# Introduction to loading the MiSeq™ and the NextSeq™

Rayne Lawrence, FAS



For Research Use Only. Not for use in diagnostic procedures.

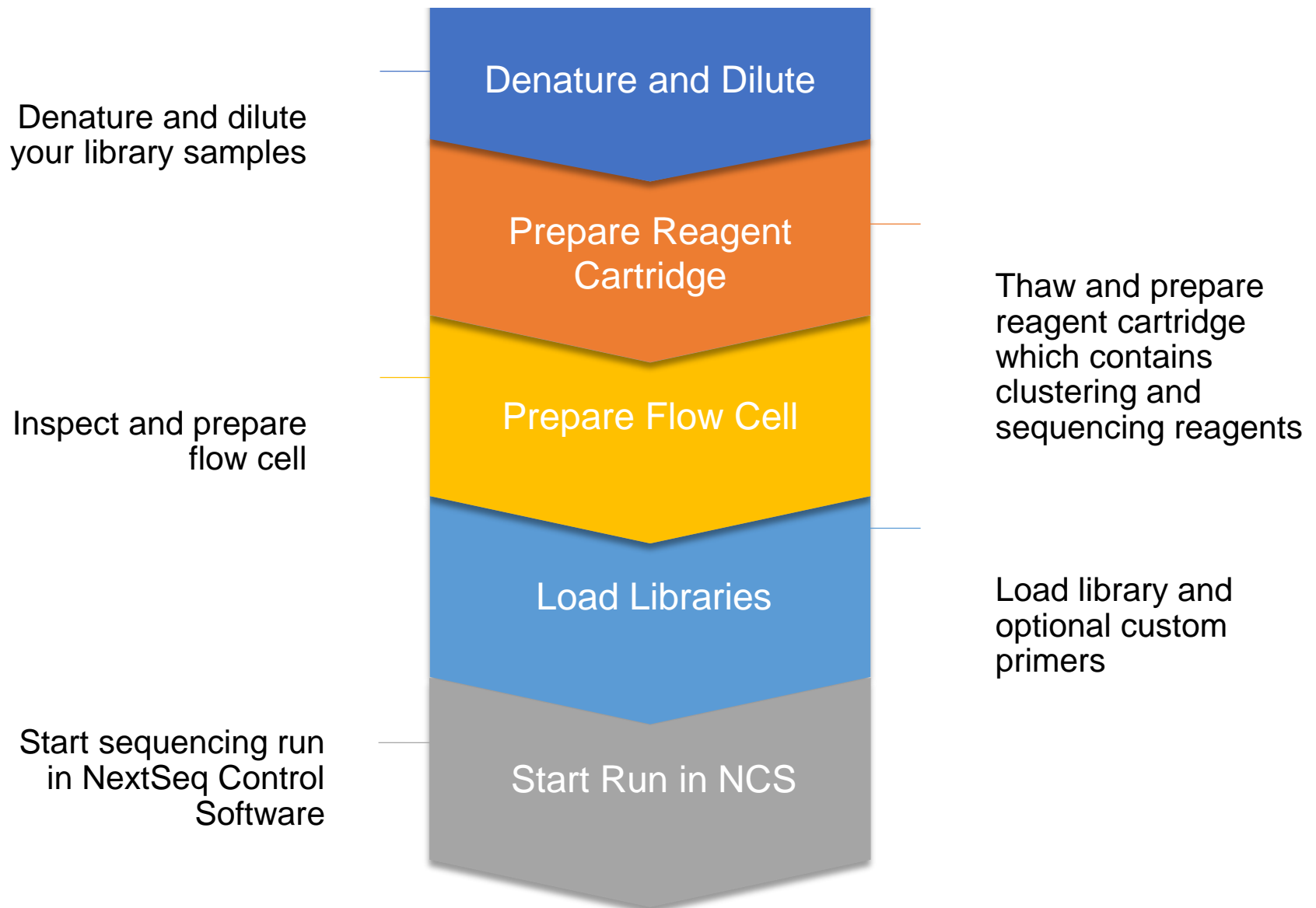
# Starting a Run Using the NextSeq System



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# How to Start a Run

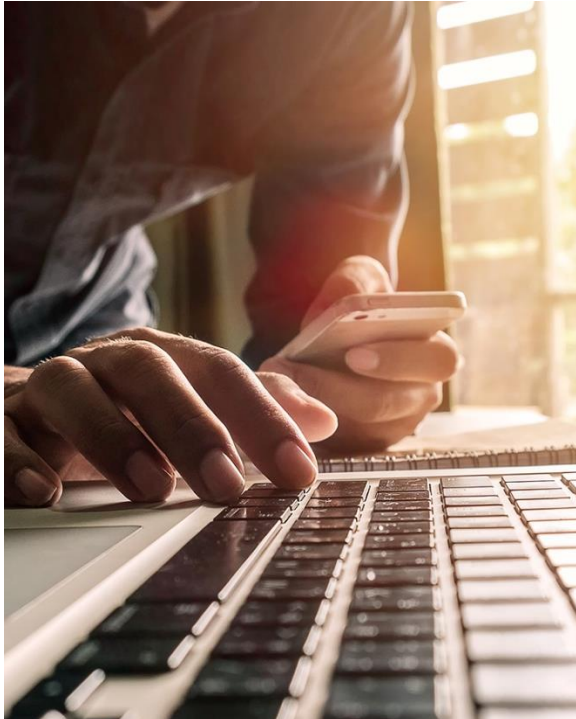
*NextSeq workflow*



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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



### Manual\*

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



### BaseSpace™ (optional)

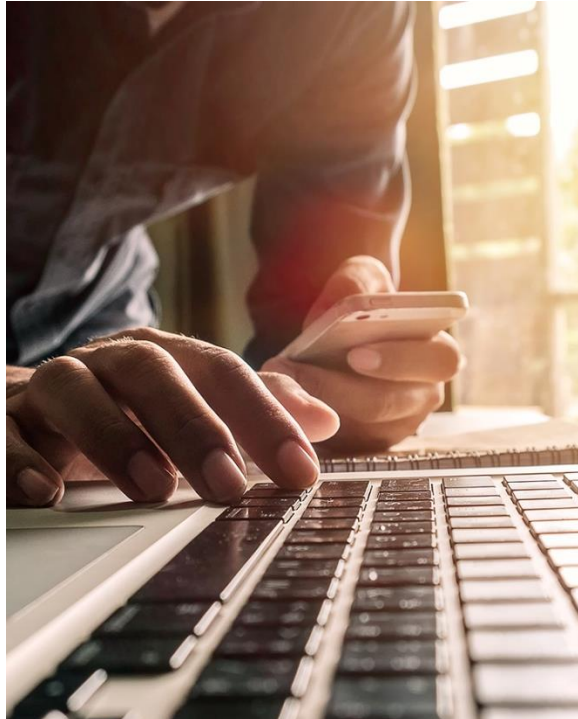
Requires BaseSpace Sequence Hub account, internet connection, sample sheet for integrated analysis

\*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



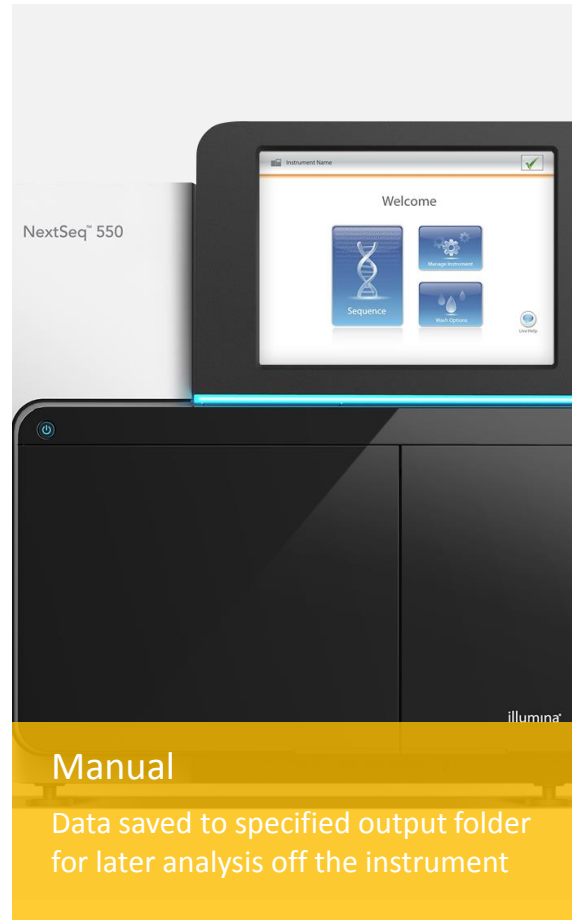
### Local Run Manager

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



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



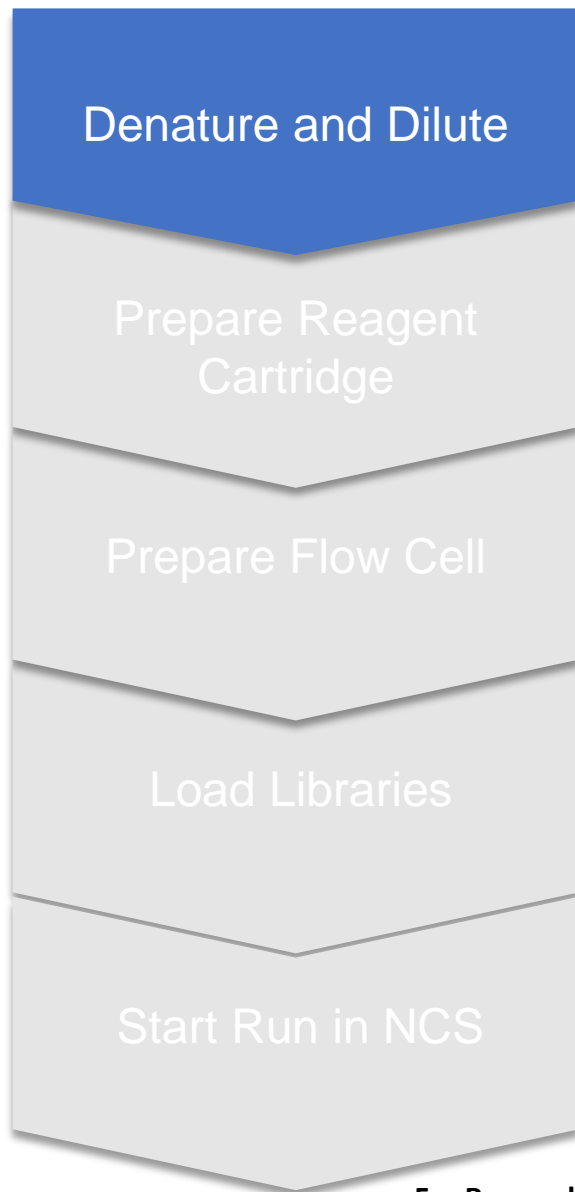
**BaseSpace (optional)**

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



# How to Start a Run

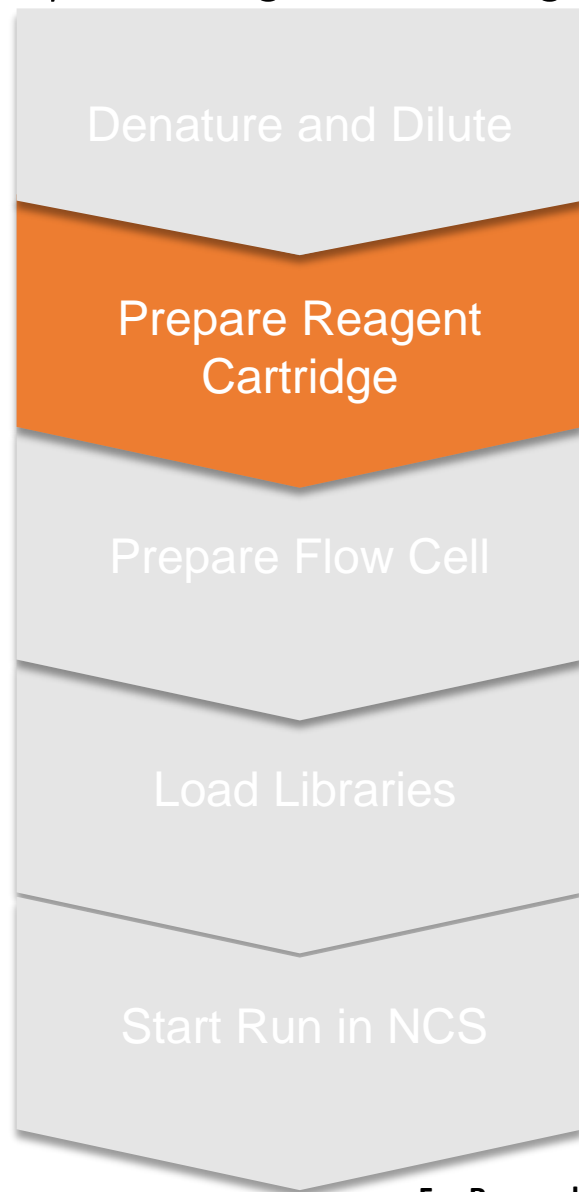
## *Denature and Dilute*



- Denature and dilute your sample
  - Target cluster densities (170 – 220 K clusters/mm<sup>2</sup>)
  - 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*



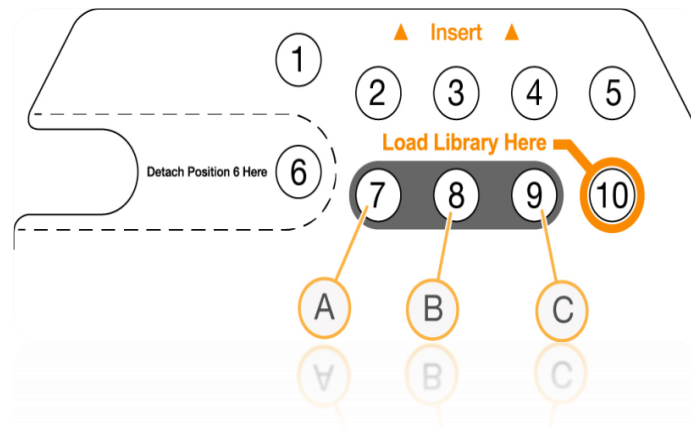
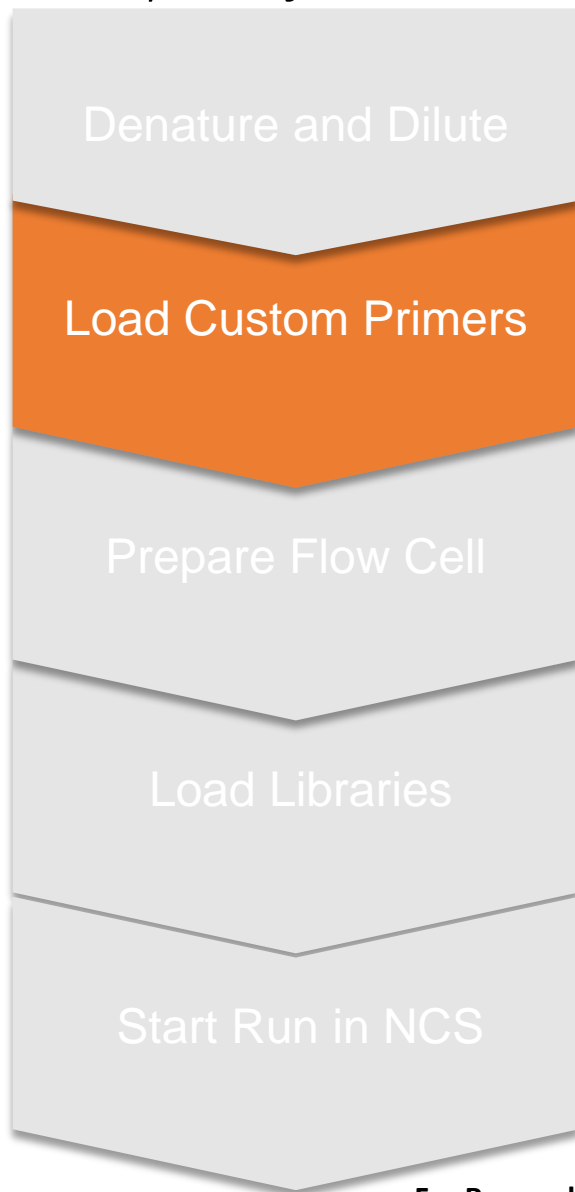
- Thaw cartridge in room temperature water bath for approximately 60 minutes
  - Do not submerge above lower lid edge
  - Ensure all reagents are fully thawed
- Gently dry the base of the cartridge
- Manually invert the cartridge 5 times to mix the thawed reagents
- Gently tap the cartridge on the bench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



**Water Line**

# How to Start a Run

*NextSeq workflow*



- 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

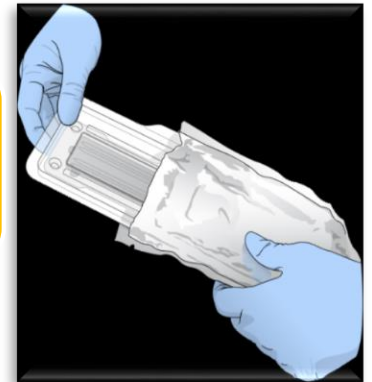
Prepare Flow Cell

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

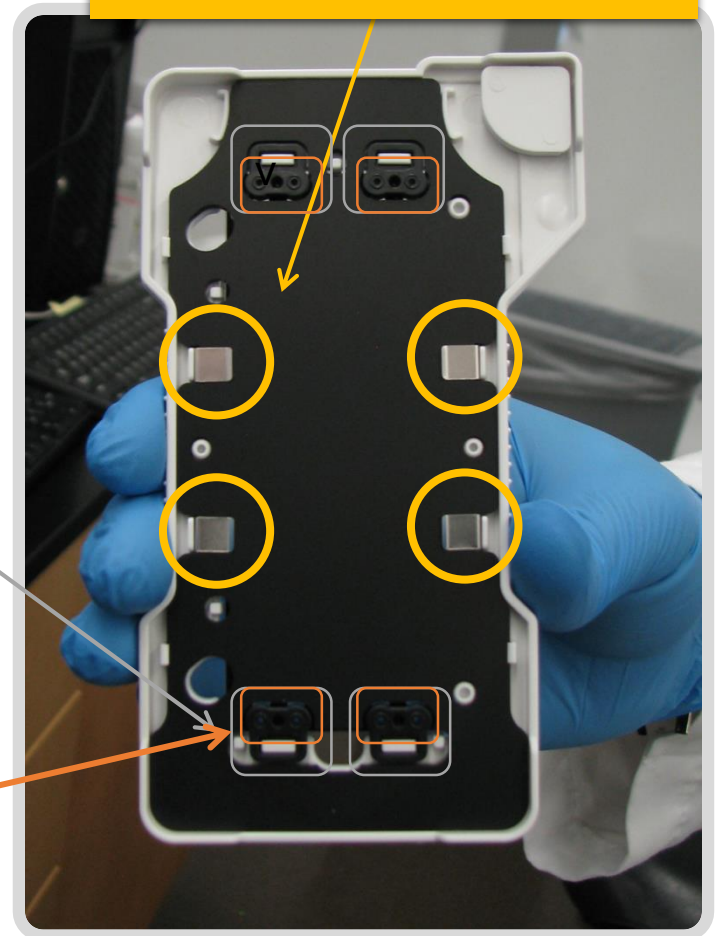
## *Inspect the flow cell*

- 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

Make sure the port gaskets are seated flat against the surface.

Make sure that the ports are free of obstructions.

Make sure that the black carrier plate is flat and secure under the four spring clips



# How to Start a Run

## *Load Libraries*

Denature and Dilute

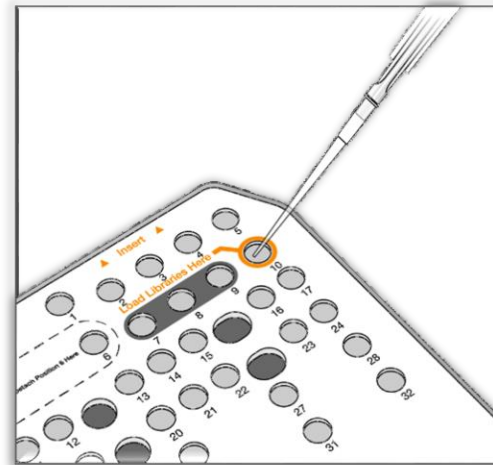
Load Custom Primers

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

The screenshot shows the 'Run Setup' window in the BaseSpace Sequencing Hub. The window has a title bar with a 'None' icon and a green checkmark icon. Below the title bar is a progress bar with five steps: 1. Run Setup (active), 2. Load, 3. Review, 4. Check, and 5. Sequence. The main content area is titled 'Select run, monitoring and storage mode.' and contains two sections. The first section, 'Setup Runs Using', has two radio buttons: 'Manual' (unselected) and 'Local Run Manager' (selected). The second section, 'BaseSpace Sequence Hub Settings', has three options: 'Use BaseSpace Sequencing Hub' (checked, with a 'View Terms' link), 'Run Monitoring and Storage' (unselected), and 'Run Monitoring Only' (selected). At the bottom of the window are three buttons: 'Back' (with a left arrow), 'Exit' (with an 'X' icon), and 'Next' (with a right arrow).

# How to Start a Run

## *Setting up a Sequencing Run*

Denature and Dilute

Load Custom Primers

Prepare Flow Cell

Load Libraries

BaseSpace Settings

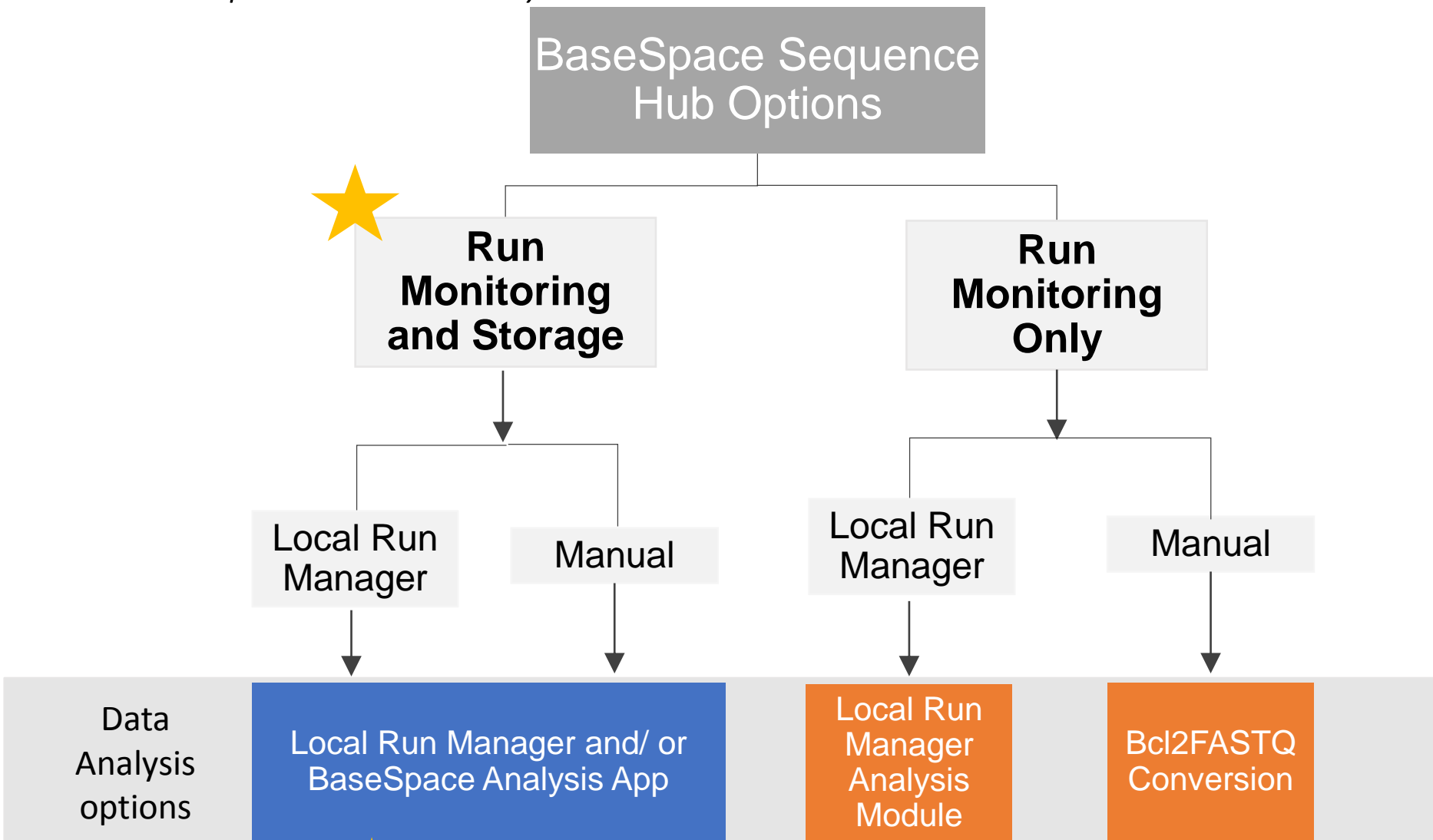
### ► BaseSpace Connectivity

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

The screenshot shows a software window titled "None" with a progress bar at the top indicating five steps: 1. Run Setup, 2. Load, 3. Review, 4. Check, and 5. Sequence. The "Run Setup" step is currently active. Below the progress bar, a message states: "In order to enable run monitoring, you must log in to BaseSpace." The main area of the window displays the "BaseSpace SEQUENCE HUB" logo and a login form with fields for "Email Address" and "Password", followed by a "Sign In" button. At the bottom of the window, there are three buttons: "Back" (with a left arrow), "Exit" (with an 'X'), and "Next" (with a right arrow).

# Run Mode Selection

*with BaseSpace Connectivity*



★ Sample Sheet required at run set up screen

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# How to Start a Run

## *Setting up a Sequencing Run*

Denature and Dilute

Load Custom Primers

Prepare Flow Cell

Load Libraries

Local Run Manager

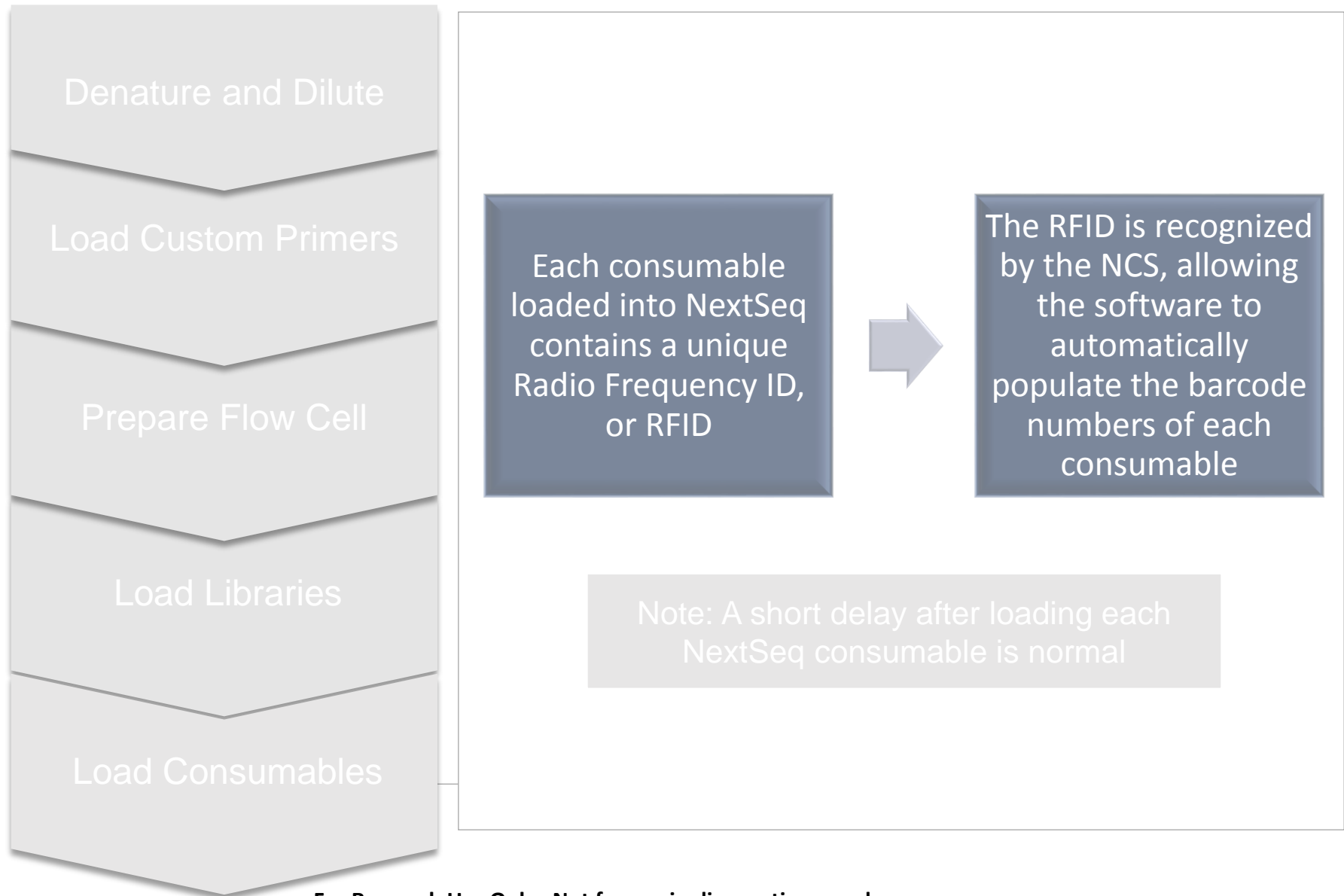
- ▶ Select a run name from the list of available runs
  - Confirm run parameters
  - Option available to edit and preview sample list

The screenshot displays the 'Local Run Manager' software interface, specifically the 'Run Setup' step (indicated by a blue circle with the number 1). The interface features a progress bar at the top with five steps: Run Setup, Load, Review, Check, and Sequence. Below the progress bar, a message box states: 'Select a run from the list of runs from Local Run Manager. Select Next to Load Consumables.' A search bar labeled 'Search for Run Name' is present. Below it, a list of runs is shown, with 'demo training run' selected and highlighted by a blue arrow. To the right of the list, details for the selected run are displayed: Created by System User, Run ID 1001, Date 2018-10-04, and Module GenerateFastQWorkflow. Below these details, a table shows the sequencing parameters: Read 1 (151), Index 1 (8), Index 2 (8), and Read 2 (151). The Output Folder is set to D:\Output. At the bottom, there are buttons for 'Back', 'Exit', 'Edit', 'Preview Samples', and 'Next'.

Read 1	Index 1	Index 2	Read 2
151	8	8	151

# How to Start a Run

## *Setting up a Sequencing Run*



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# 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

The screenshot shows the 'Run Setup' dialog box in the NCS software. The dialog has a title bar with a 'None' icon and a green checkmark icon. Below the title bar is a progress bar with five steps: 1. Run Setup (active), 2. Load, 3. Review, 4. Check, and 5. Sequence. The main content area contains the following fields and controls:

- Run Name \***: A text input field.
- Library ID**: A text input field.
- Read Type**: Two radio buttons, 'Single Read' (selected) and 'Paired End'.
- Read Length \***: Four input fields for 'Read 1', 'Index 1', 'Index 2', and 'Read 2', all containing the value '0'.
- Custom Primers**: Four checkboxes for 'Read 1', 'Index 1', 'Index 2', and 'Read 2', all of which are unchecked.
- Output Folder \***: A text input field containing 'D:\Output' and a 'Browse' button.
- Sample Sheet**: A text input field and a 'Browse' button.
- Purge consumables for this run**: A checked checkbox.

At the bottom of the dialog are three buttons: 'Back' (with a left arrow), 'Exit' (with an 'X' icon), and 'Next' (with a right arrow).

Save run to a network drive



# How to Start a Run

## *Start Run in NextSeq Control Software*

Denature and Dilute

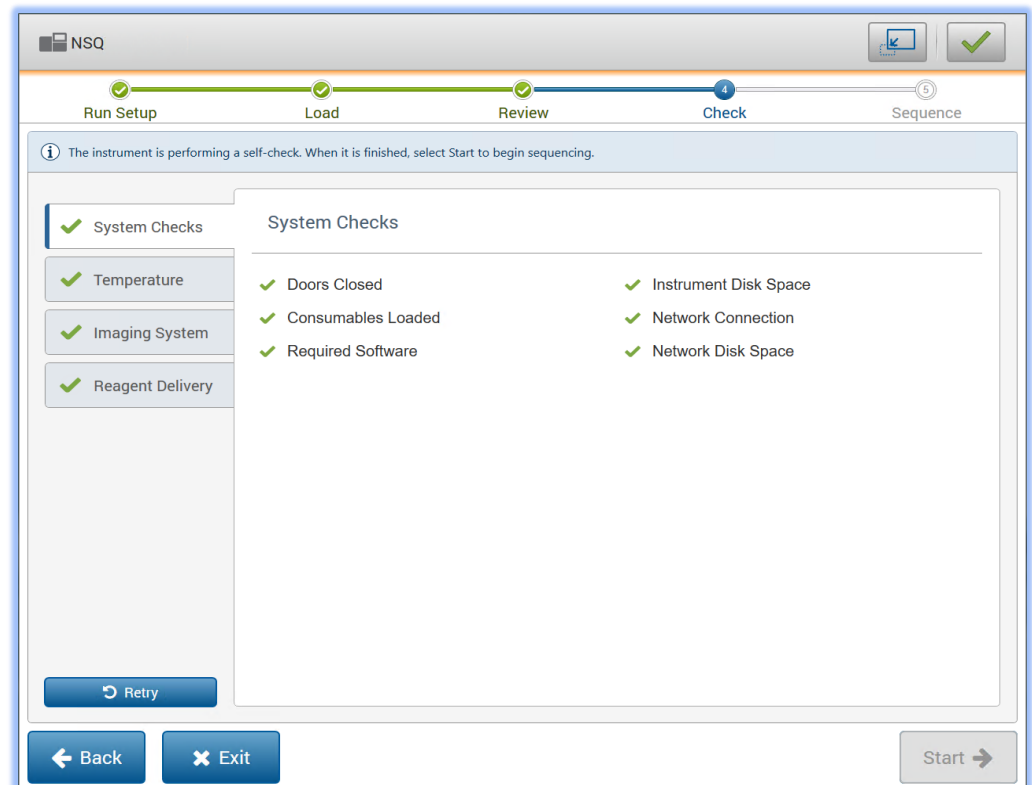
Load Custom Primers

Prepare Flow Cell

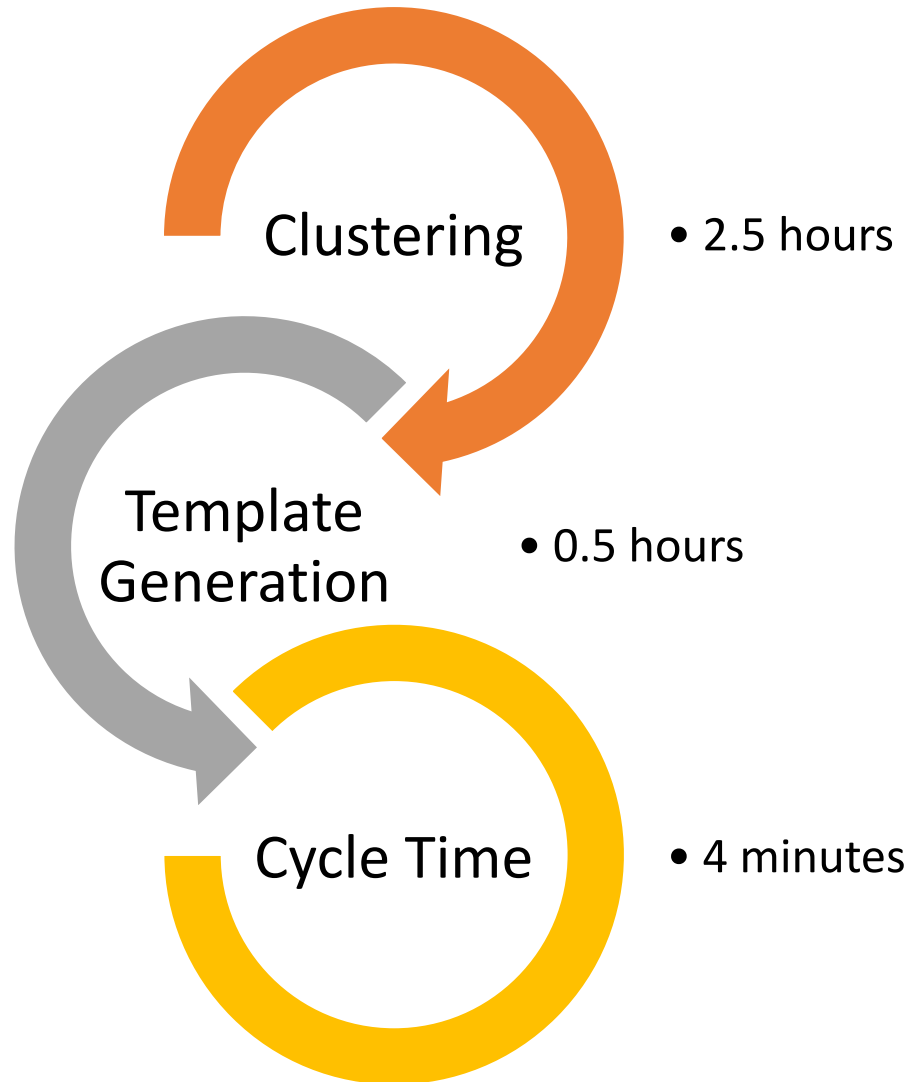
Load Libraries

Automated Pre Run  
Check

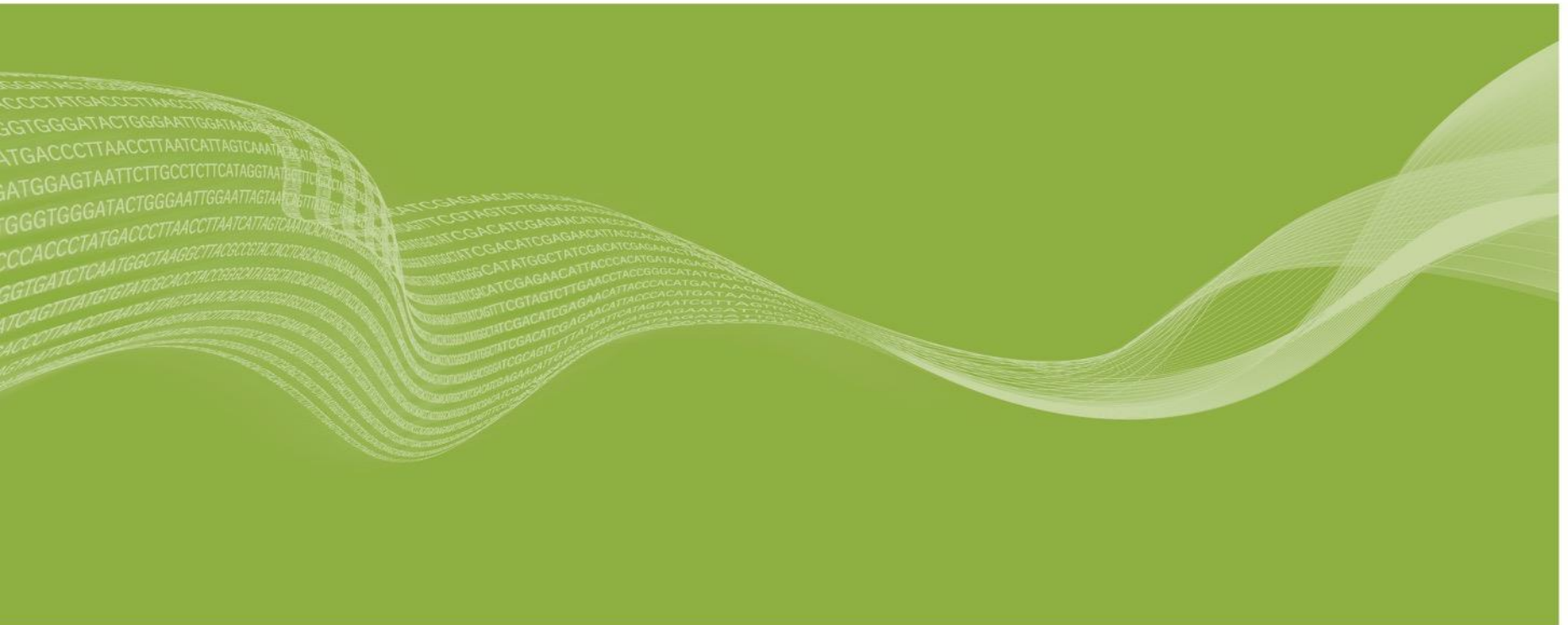
- ▶ The software performs an automated check of the system.
- ▶ When the automated check is complete, select Start. The sequencing run begins



# Timing on NextSeq System



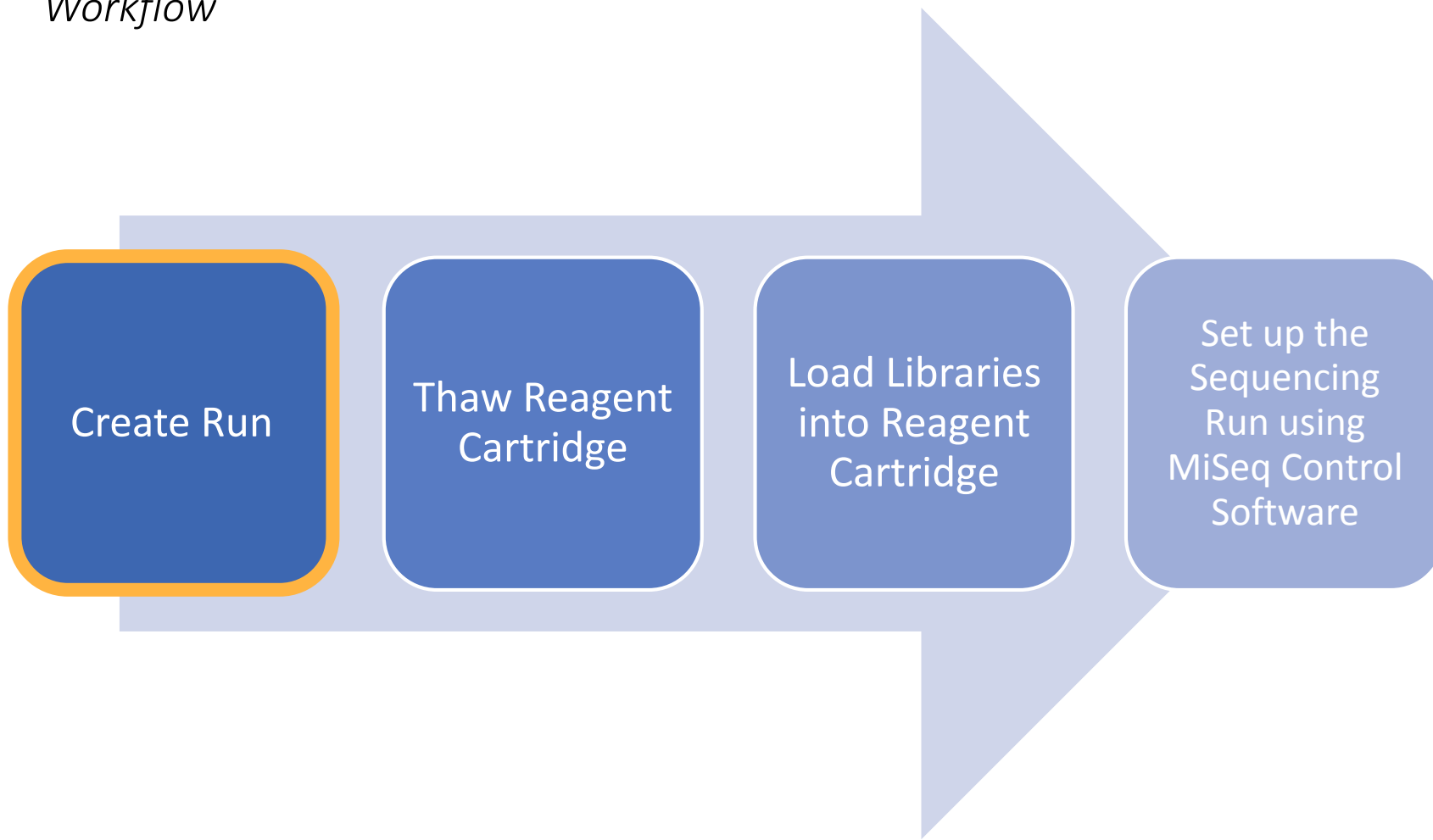
# Starting a Run Using the MiSeq System



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# How to Start a Run

## *Workflow*



# Create Run

- There are multiple options for completing the Create Run step

MiSeq Control Software

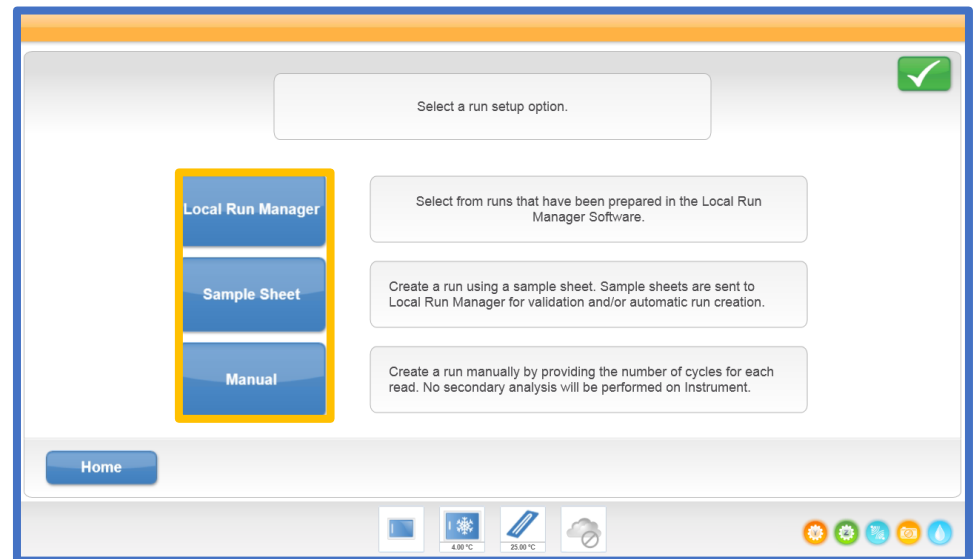
Create Run

Illumina  
Experiment  
Manager

Local Run  
Manager

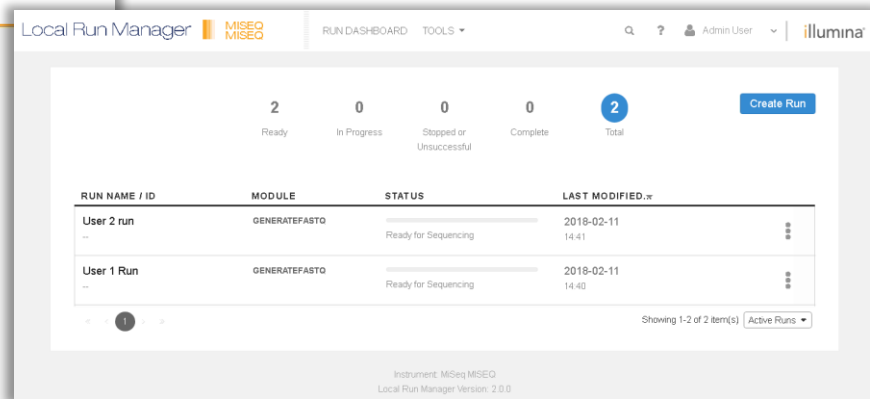
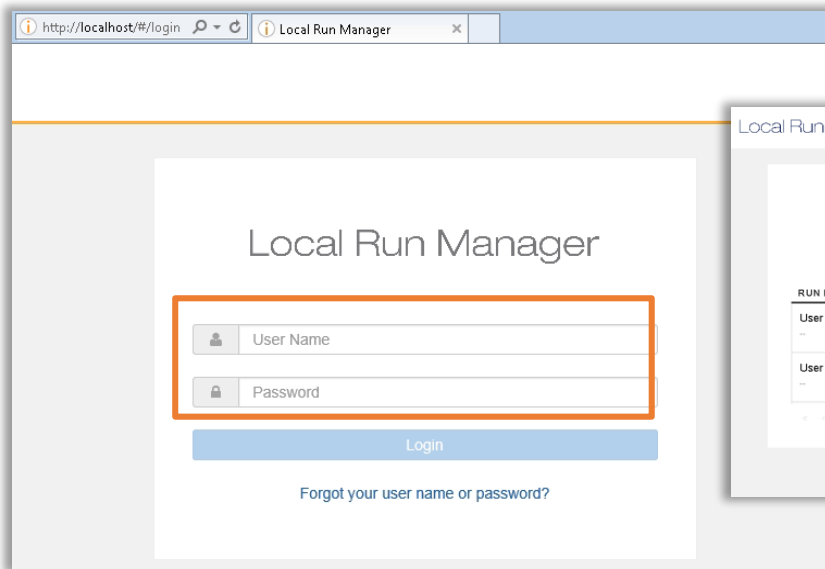
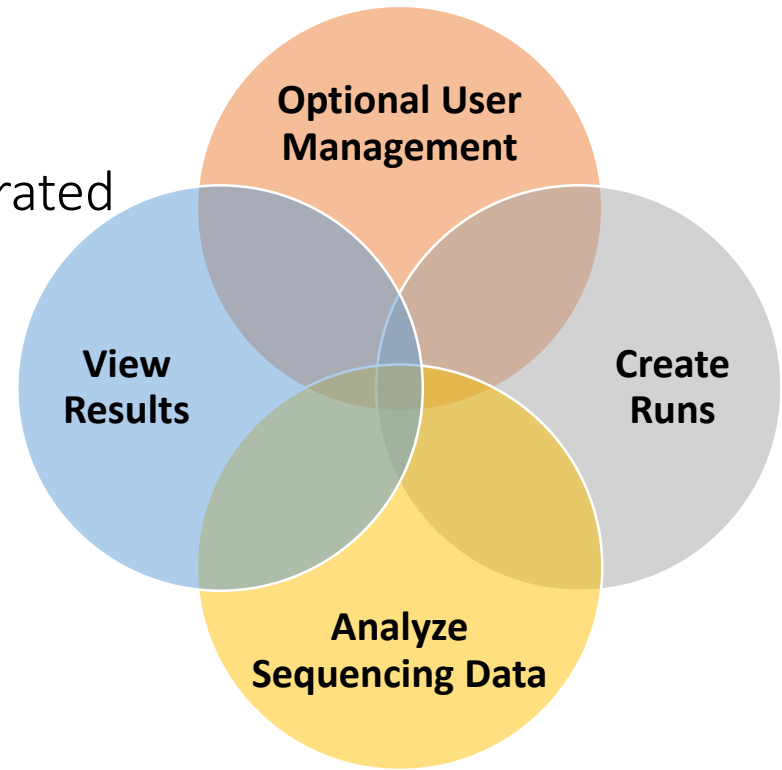
Manual

Recommended  
Method



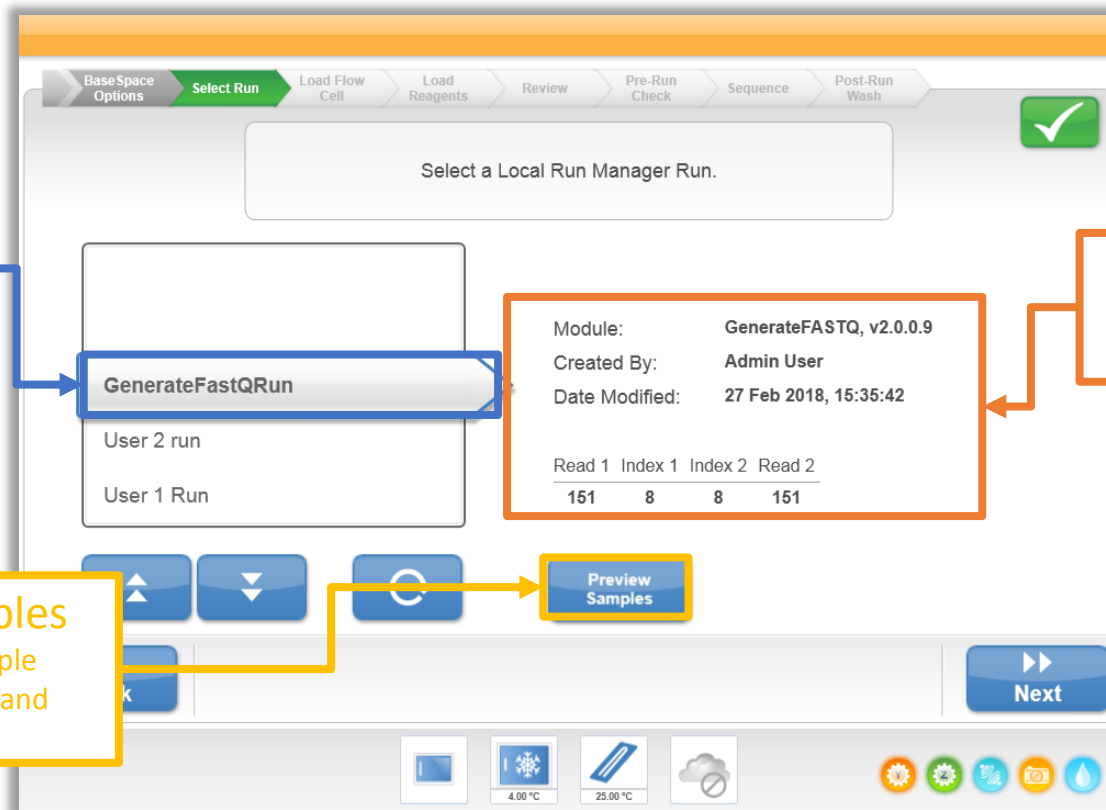
# Create Run: Local Run Manager

- 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

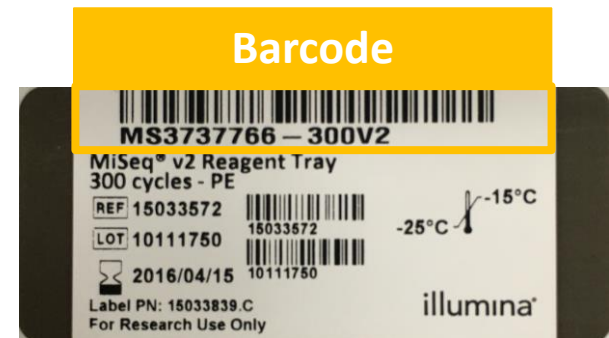
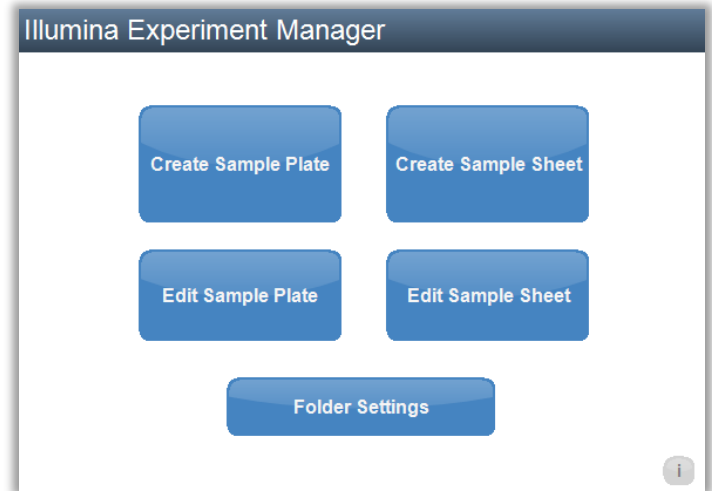
- Select Run
  - Preview samples
  - Edit the run in Local Run Manager





# 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

BaseSpace Options **Run Settings** Load Flow Cell Load Reagents Review Pre-Run Check Sequence Post-Run Wash

Setup the run

Read Type: ☒ Single Read ☐ Paired End

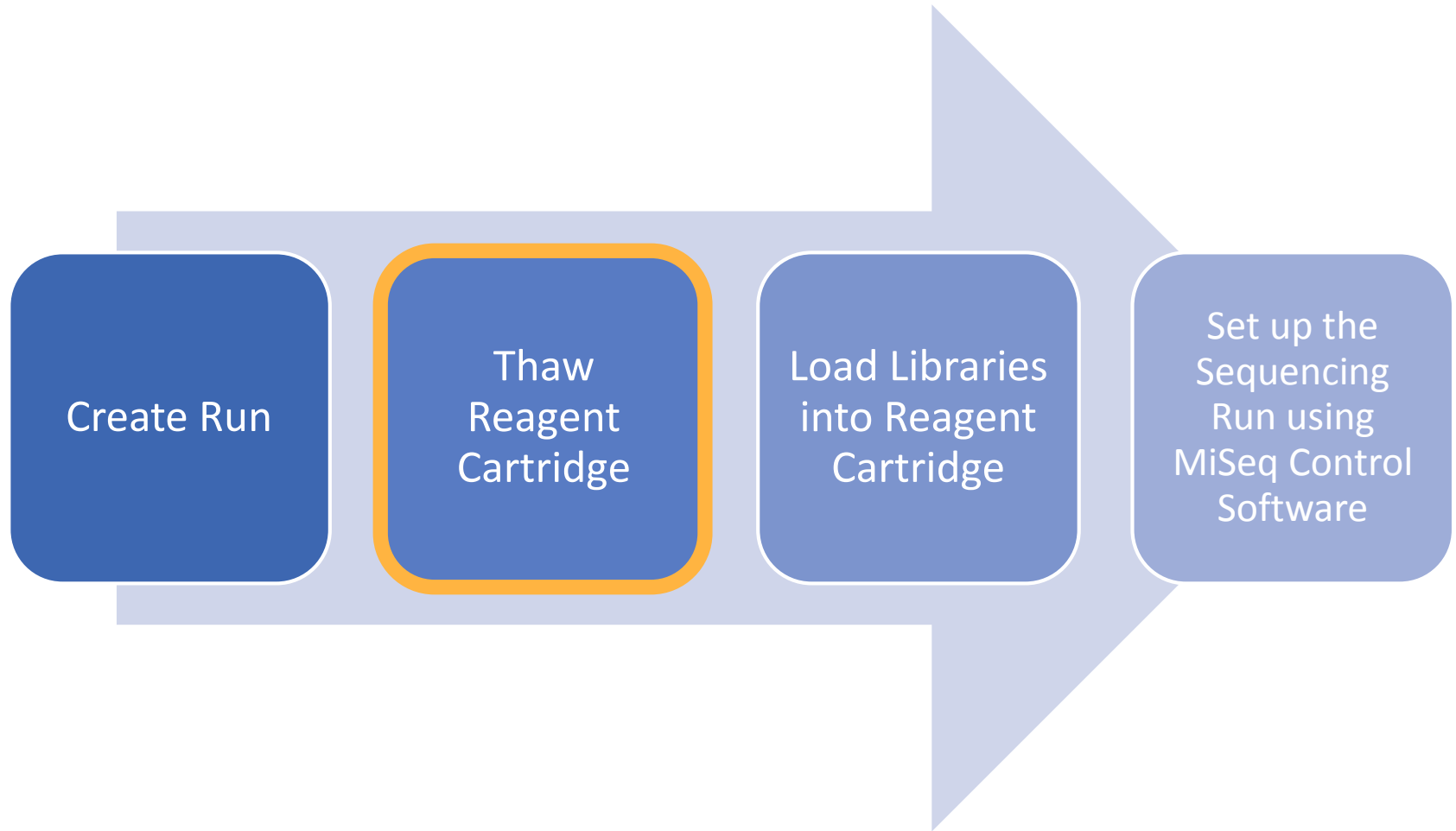
	READ 1	INDEX 1	INDEX 2	READ 2
Read Length:	0	0	0	0
Custom Primers:	<input type="checkbox"/> Read 1	<input type="checkbox"/> Index	<input type="checkbox"/> Read 2	

Back Exit Change Folder Next

4.00 °C 25.00 °C

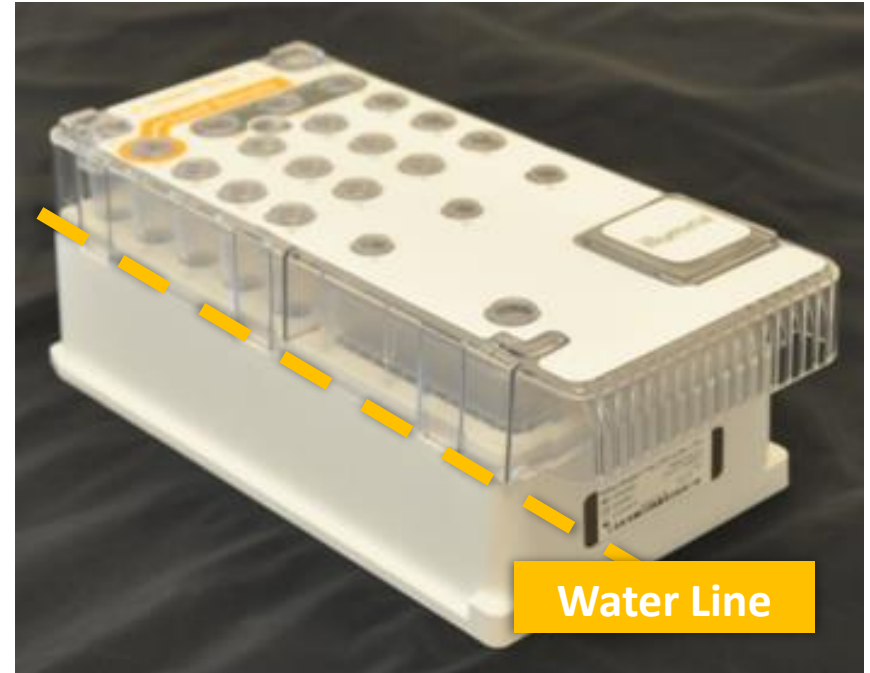
# How to Start a Run

## *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	MS-102-3003
		150	MS-102-3001
MiSeq Reagent Kit v2	14 tiles, dual surface (28 tiles total )	500	MS-102-2003
		300	MS-102-2002
		50	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	MS-103-1003
		300	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

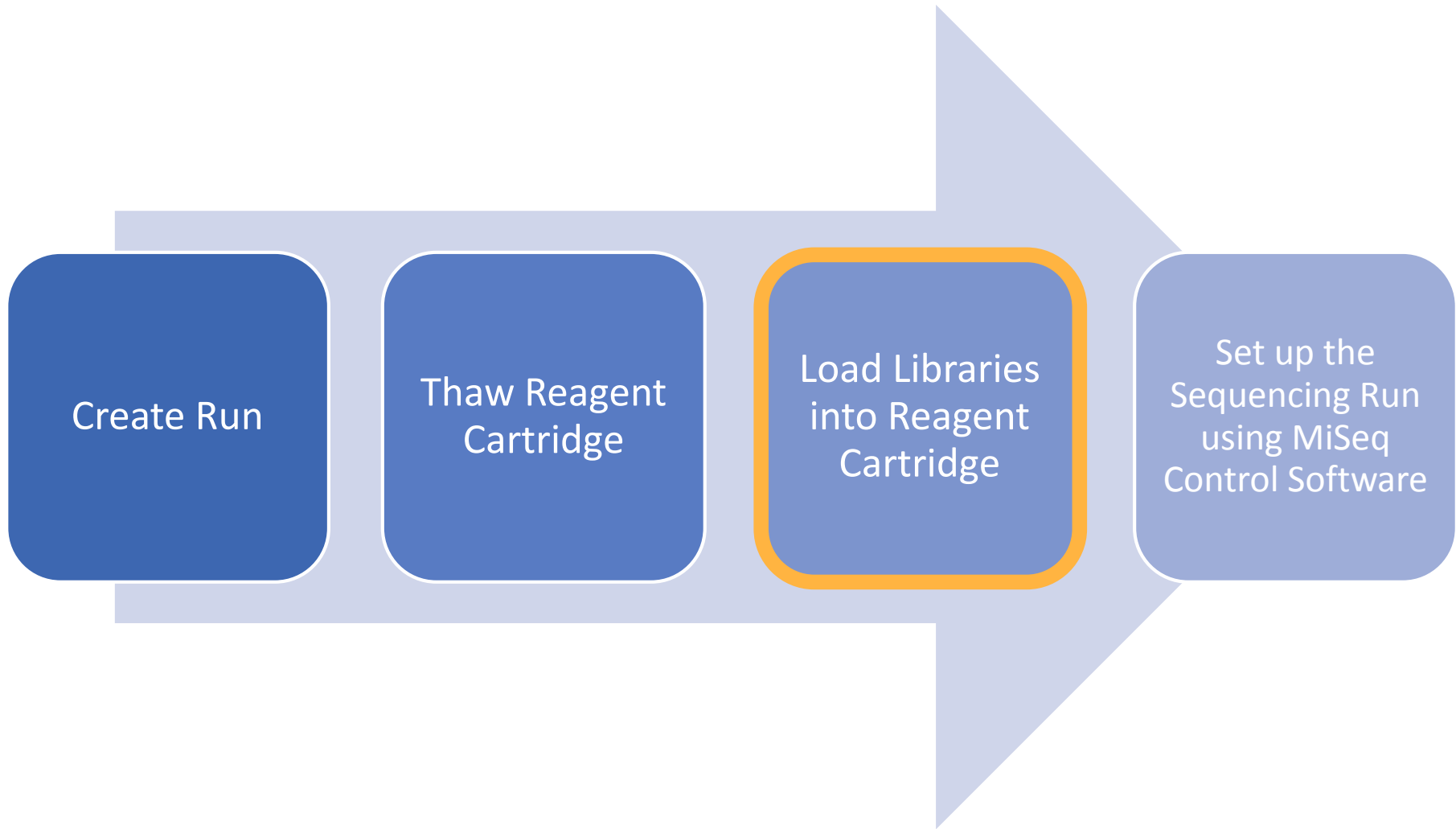
## Low Output Applications

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

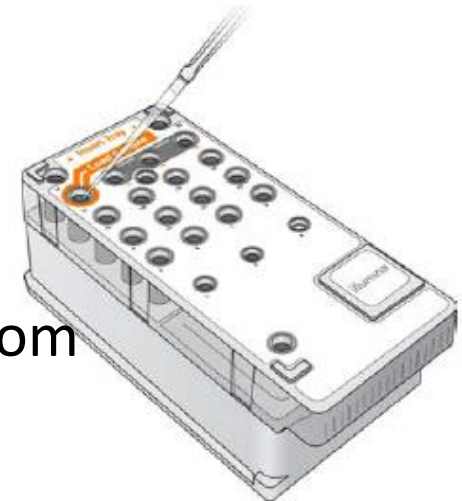
## *Workflow*





# 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

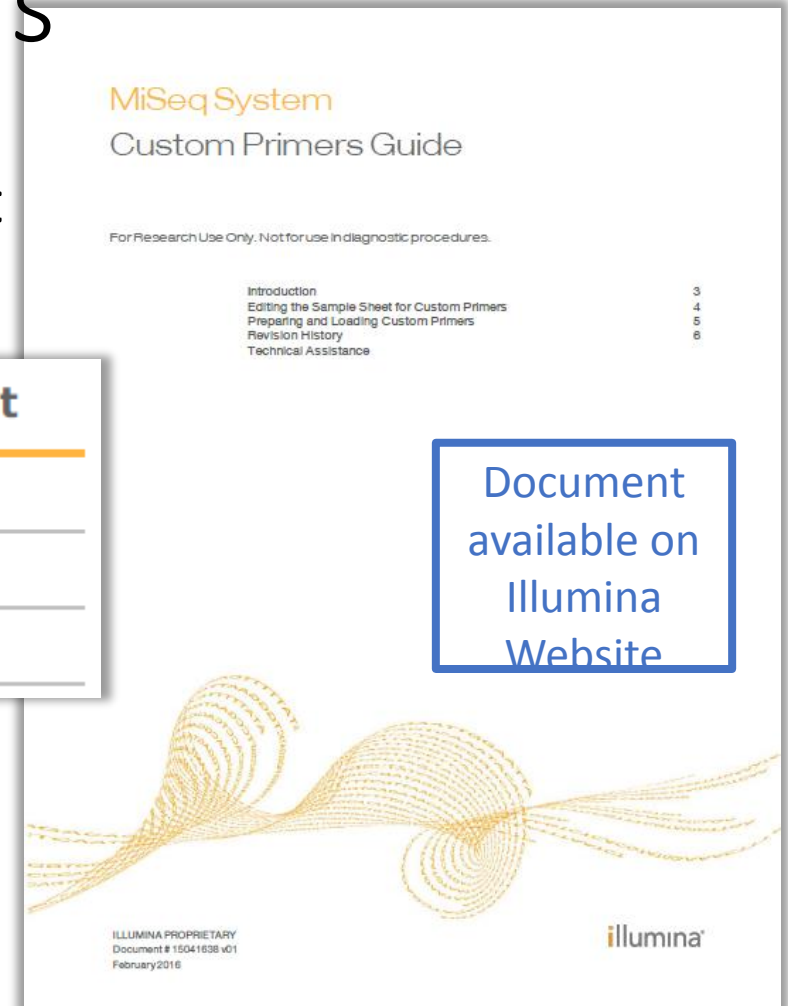


# Using Custom Primers

- Use Ports 18 -20 on MiSeq reagent cartridge

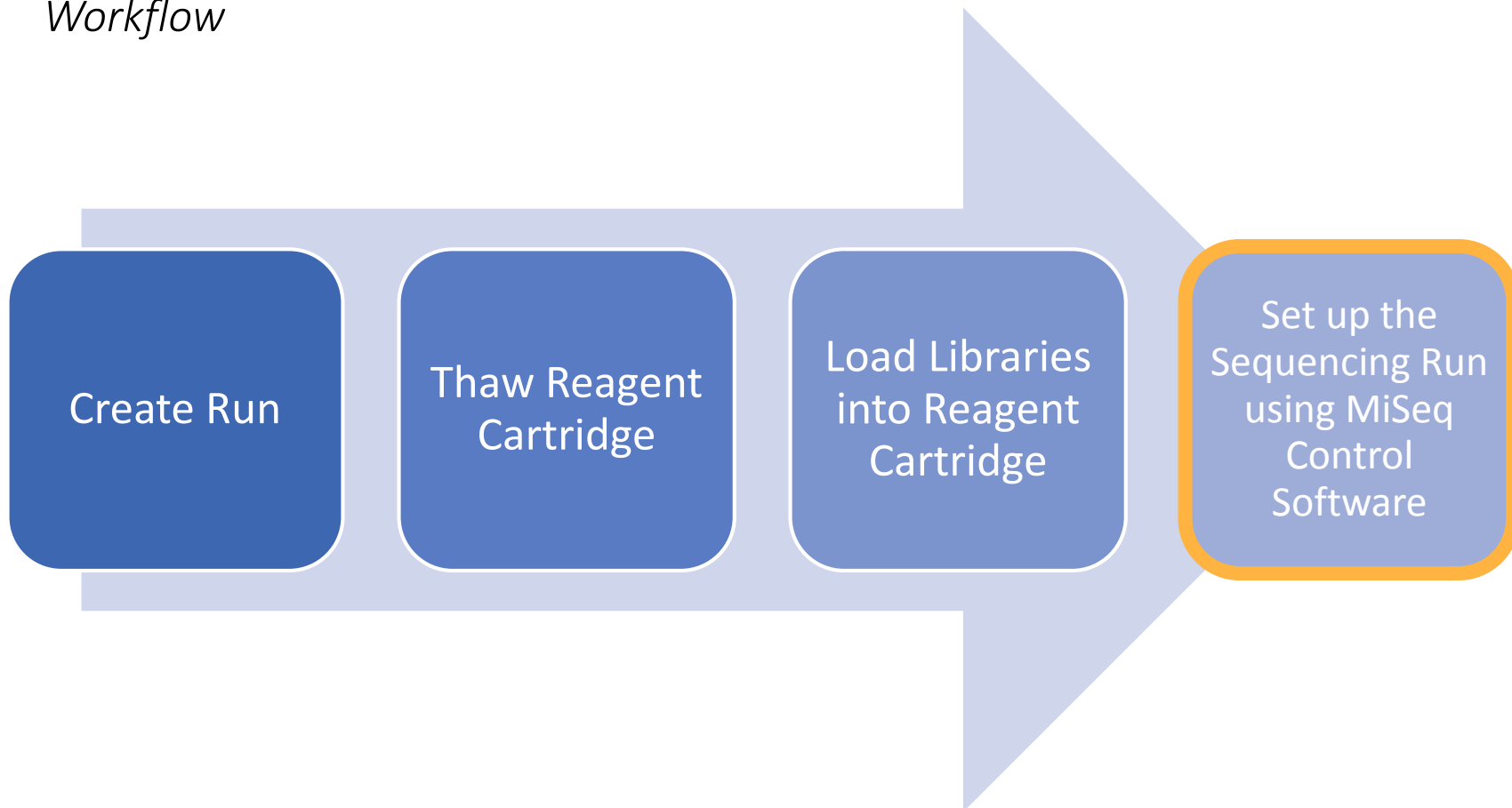
Read	Port
Custom Primer for Read 1	18
Custom Primer for the Index Read	19
Custom Primer for Read 2	20

- 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



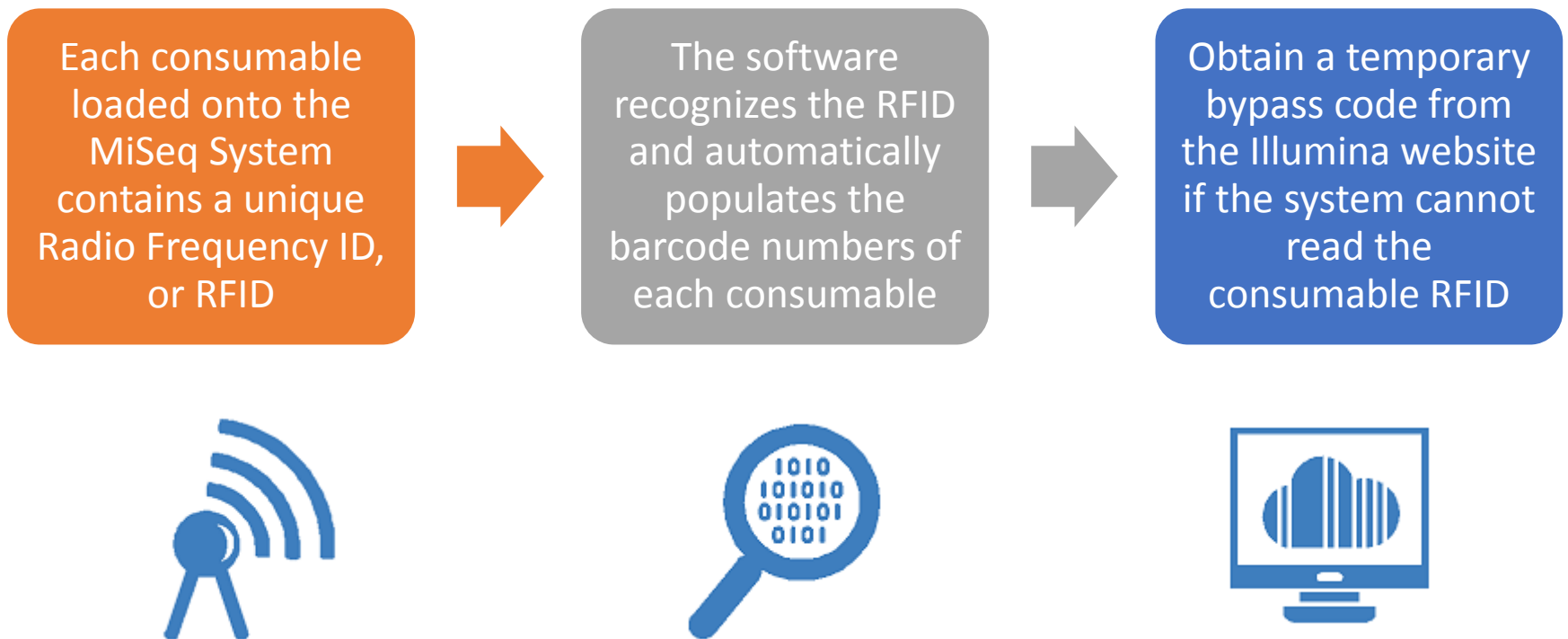
# How to Start a Run

## *Workflow*



# MiSeq Control Software and Radio Frequency

*Identification (RFID)*



# MyIllumina

## *How to obtain a temporary bypass code*

**1** Click the menu icon.

**2** Click MY TOOLS.

**3** Click MiSeq Self-Service.

**4** Click Get Code.

**Self Service for MiSeq**

Serial Number

Description of the Issue

Type of Override Code  
Please select...

**Note:** The MiSeq serial number (M####) can be found under the title "Instrument Name" in the About menu. This Self Service tool is not available for MiSeqDx instruments. If an RFID override code is needed for a MiSeqDx, please contact [Illumina Technical Support](#) with the instrument serial number, the part number and the lot number of the component with the failed RFID.)

**MiSeq™ Control Software**

Version:	1.2.3
Recipe Fragment Version:	1.2.0.0
Build Date:	5/3/2012 10:18:41 AM
Computer:	PC
Instrument ID:	M00240
Uptime:	0d 1h 33m 15s
Runs:	2
FPGA Version:	9.3.0
MiSeq Reporter Version:	1.3.17.0

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# MyIllumina

## *Input Bypass Code*

Illumina MiSeq

BaseSpace Options Load Flow Cell **Load Reagents** Review Pre-Run Check Sequence Post-Run Wash

Valid bypass code has been entered.  
Please enter the reagent kit barcode and version.  
Allowed format for barcode is 'AA1234567-12345'

Reagent Kit Barcode: MS1234567-600V3

Reagent Kit Version: Select Version

- Select Version
- Version 1
- Version 2
- Version 3

Back Next

19.25 °C 27.78 °C

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# How to Calculate the Estimated Run Duration on a MiSeq

