HiSeq Overview

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Learning Objectives

After completing this lesson, you will be able to:

- List the HiSeq reagents
- Identify key characteristics of the HiSeq flow cell
- Describe how the HiSeq performs dual surface imaging

Overview

HiSeq 2000

Most widely adopted and proven chemistry	• Leverages Illumina's reversible terminator-based SBS chemistry
Innovative Engineering	• Dual surface imaging • 4-camera system with TDI scanning technology • Fully-integrated paired-end fluidics
Highest Output	• v3 flow cells • 600Gb/run
Fastest Data Rate	• v3 flow cells • 66Gb/day • 11 days for 2x100 bp
Highest Number of Reads	• v3 produces 3 billion SR reads or 6 billion PE reads

HiSeq 2000/1000 System

	HiSeq 2000	HiSeq 1000
Output (2 × 100 bp)	600 Gb	300 Gb
Run Time (2 × 100 bp)	~11 days	~8.5 days
Cluster Generation	cBot	cBot
Paired-end Reads	6 Billion	3 Billion
Single Reads	3 Billion	1.5 Billion
Maximum Read Length**	2 × 100 bp	2 × 100 bp
Quality Scores***	> 85% (2 x 50 bp) > 80% (2 x 100 bp)	

***Based on an Illumina PhiX library at supported cluster densities.

HiSeq 2500/1500 System

	HiSeq 2500		HiSeq 1500	
Run Mode	High Output	Rapid Run*	High Output	Rapid Run*
Output (2 × 100 bp)	600 Gb	120 Gb	300 Gb	60 Gb
Run Time (2 × 100 bp)	~11 days	~27 hours	~8.5 days	~27 hours
Cluster Generation	cBot	On board	cBot	On board
Paired-end Reads	6 Billion	1.2 Billion	3 Billion	600 Million
Single Reads	3 Billion	600 Million	1.5 Billion	300 Million
Maximum Read Length**	2 × 100 bp	2 × 150 bp	2 × 100 bp	2 × 150 bp
Quality Scores***	> 85% (2 x 50 bp) > 80% (2 x 100 bp)			

***Based on an Illumina PhiX library at supported cluster densities.

HiSeq Reagents

HiSeq Reagent kits

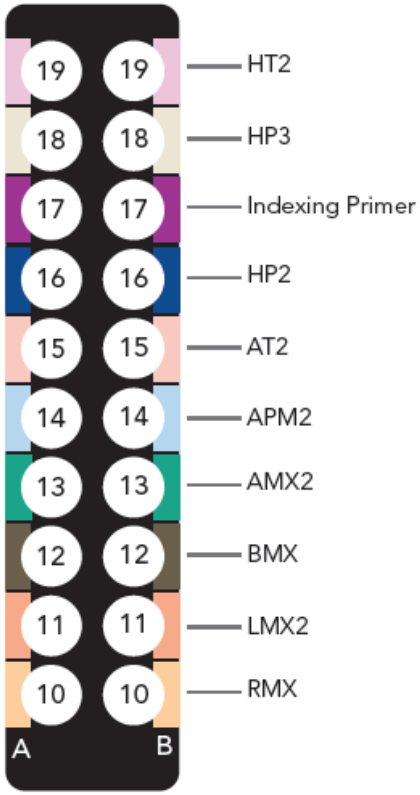
Reagent Kits	
200 cycles	209 cycles of sequencing
50 cycles	58 cycles of sequencing

TruSeq SBS Kit v3 – HS (200-cycle)

TruSeq SBS Kit v3 – HS (50-cycle)

Reagents Layout HiSeq 2000

Rear of Fridge



Paired-End Rack

Flow Cell A Rack

Flow Cell B Rack

Front of Fridge

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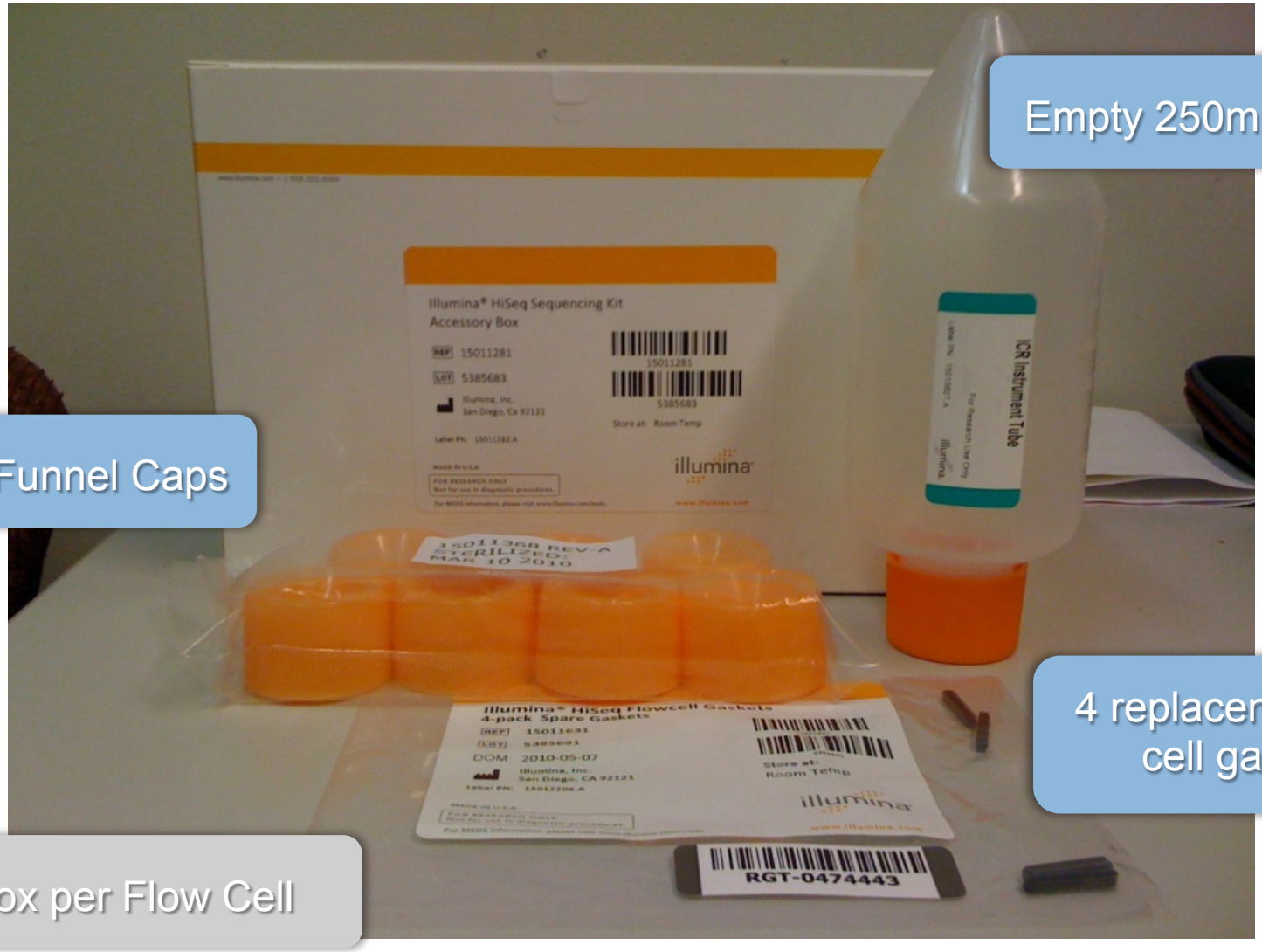
HiSeq Accessory Box (shipped with cBot kit)



Empty 250mL Conical

7-pack Funnel Caps

4 replacement flow cell gaskets

1 Box per Flow Cell

Gasket Maintenance and Best Practices

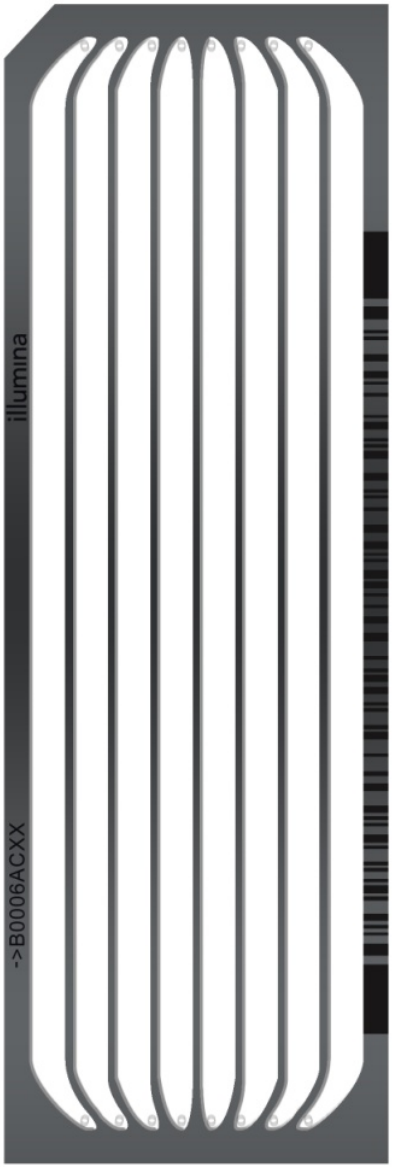
Gasket placement

Replace before every maintenance wash

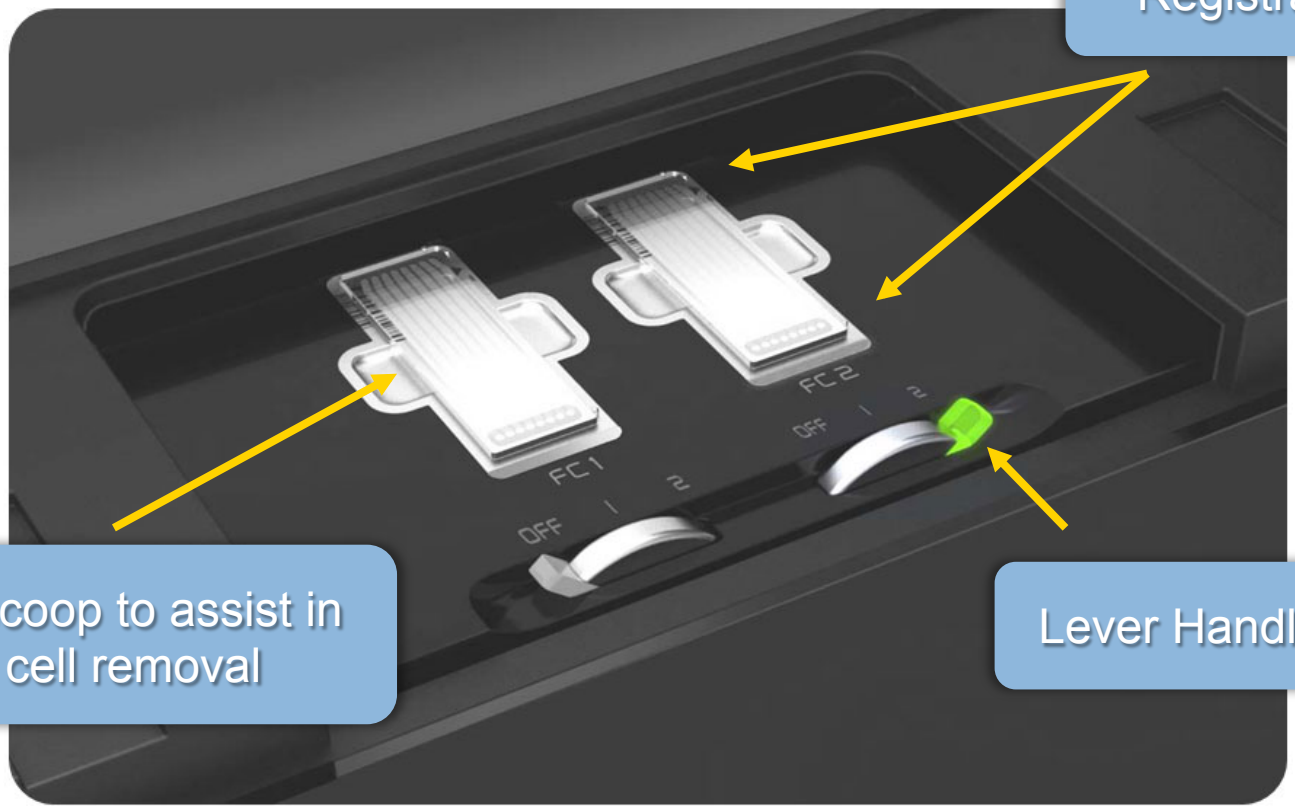
HiSeq System Hardware

Flow Cell Design

Dual-surface enabled
- 8 lane format
- 25mm wide x 75mm long
- 1.7mm-wide lanes
- Only compatible with cBot



Flow Cell Stage



Flow Cell Loading

cBot

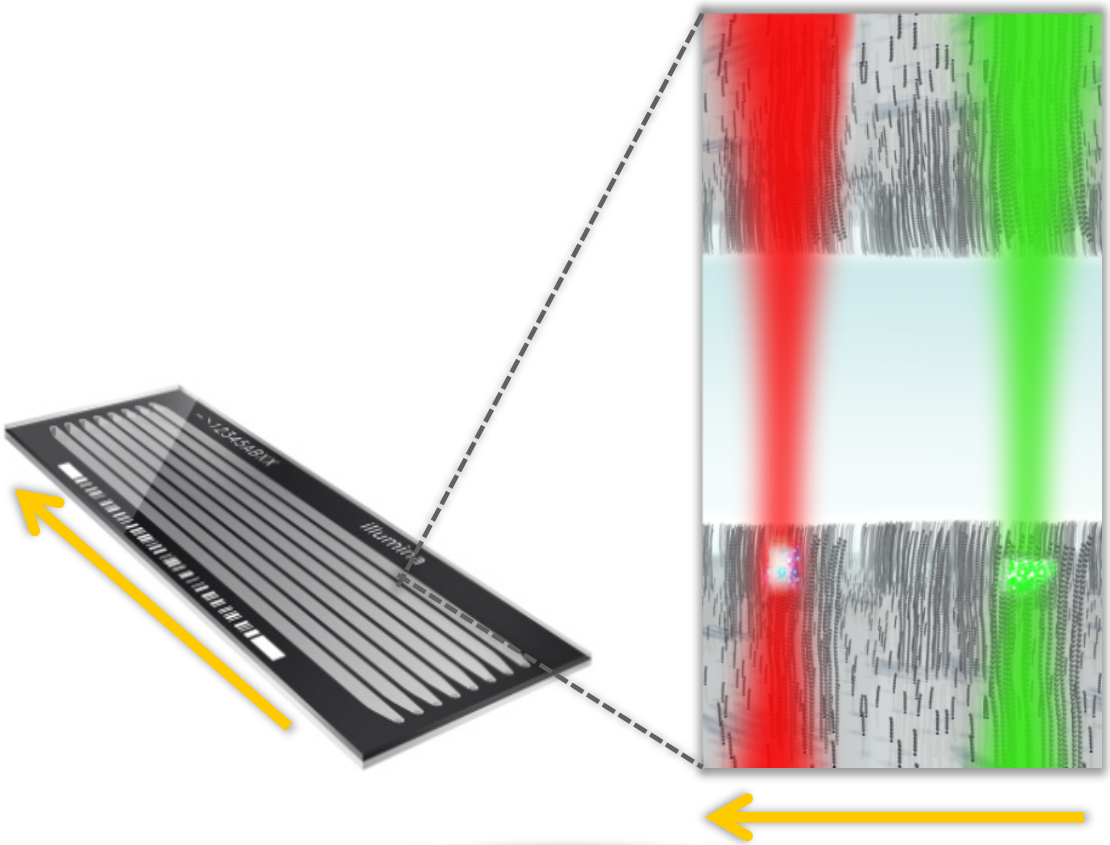
- Ensure the notched position is on the **upper-right** corner of the flow cell, relative to the instrument operator

HiSeq

- Ensure the notched position is on the **upper-left** corner of the flow cell, relative to the instrument operator

Performing this simple check will ensure correct sample orientation

HiSeq System Imaging



3 swaths/lane + 2 surfaces/lane = 6 scanning events/lane

Operates in epi-illumination mode

- Fluorescence and emission from the same side of the sample

Continuous Scanning

- Cameras operate in time delay integration (TDI)
- Fluorescence image continually read

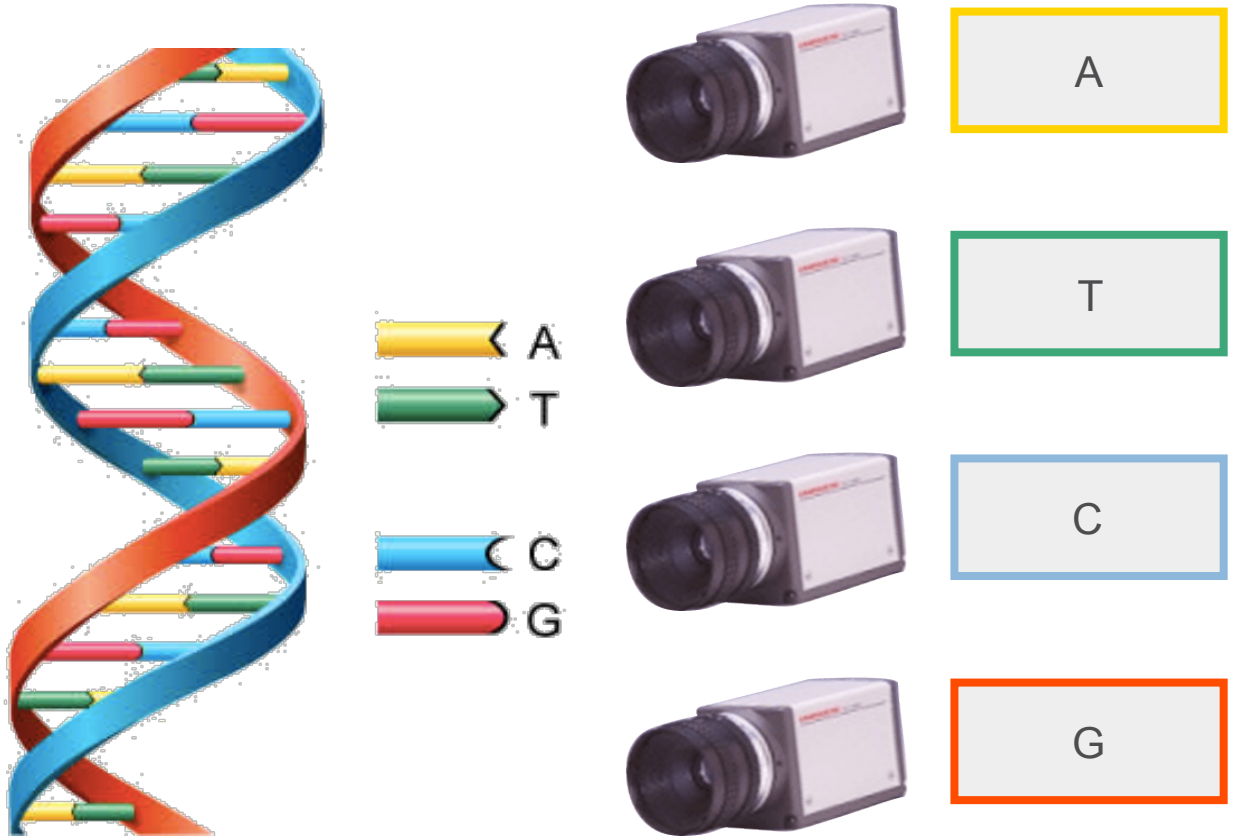
Dual Surface Scanning

- First: Top of flow cell, all lanes
- Second: Bottom of flow cell, all lanes

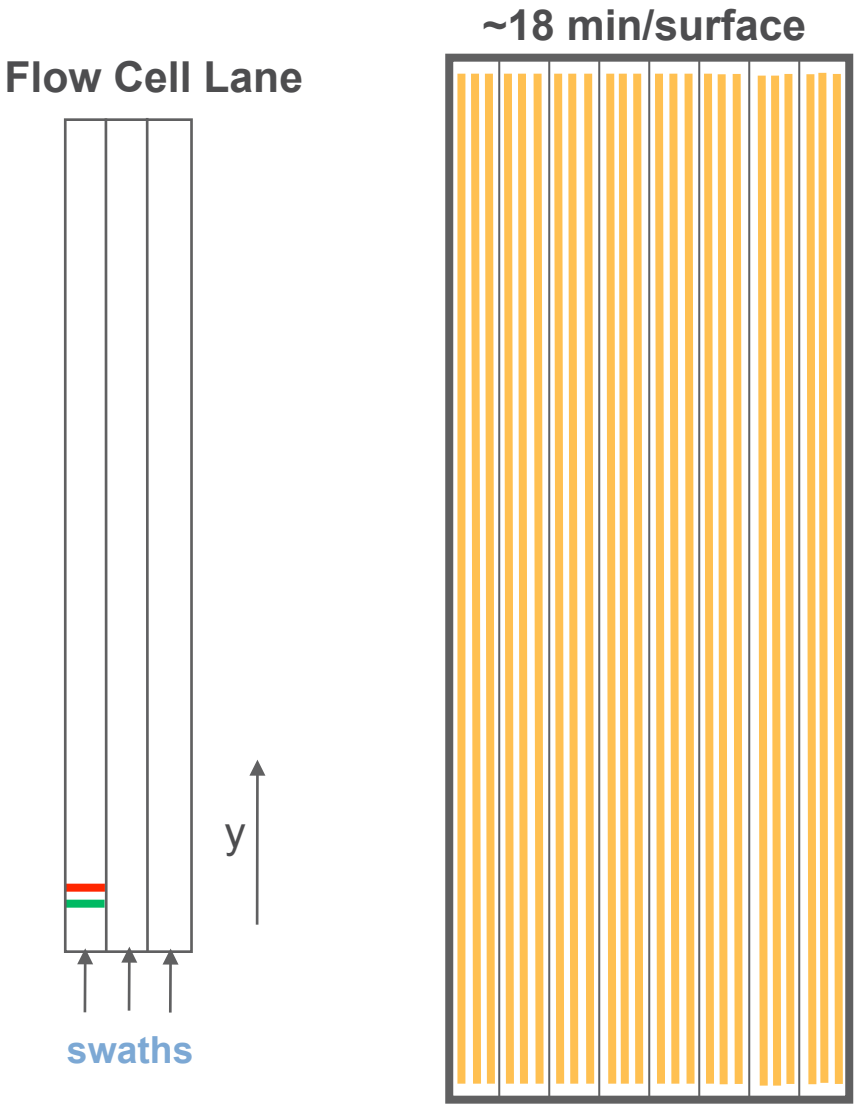
Emission Capture

Capture image for each swath simultaneously

4 cameras—1 per base

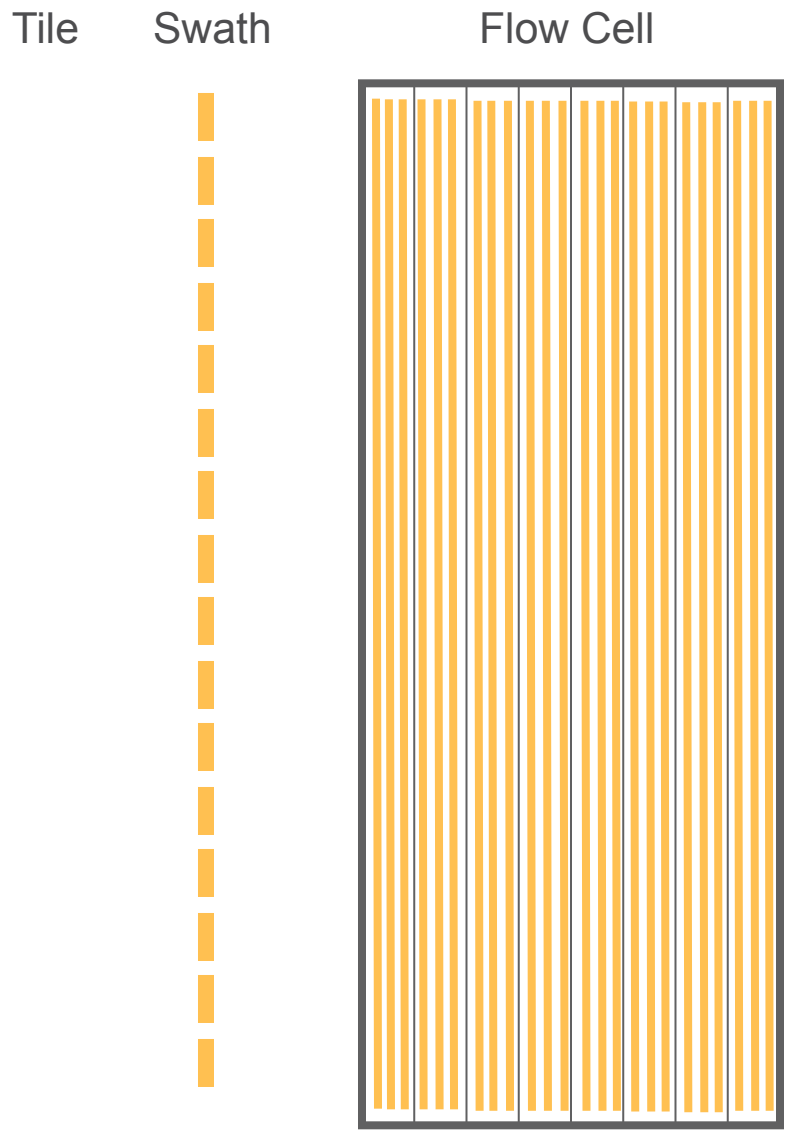


Excitation and Scanning on HiSeq System



Flow Cell Swath/Tile

HiSeq Control Software (HCS) divides 1 swath into 16 tiles for image analysis



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HiSeq Control Software

HiSeq Control Software (HCS)

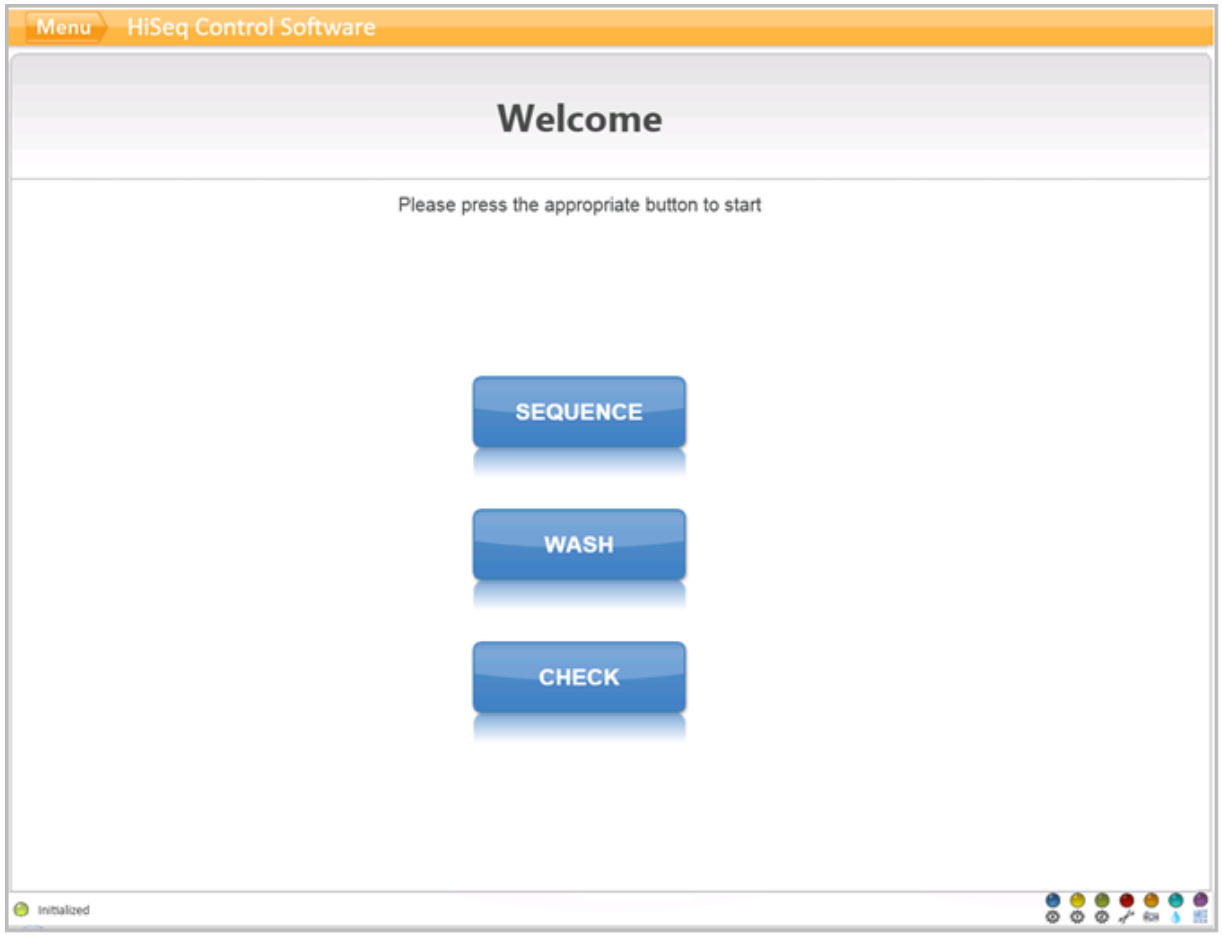
Simple Operation
- Step-by-step run setup
- Enter read length: single, paired-read, indexing

- Real-time run monitoring

- Reagents usage tracking

- Remote monitoring from Web browser

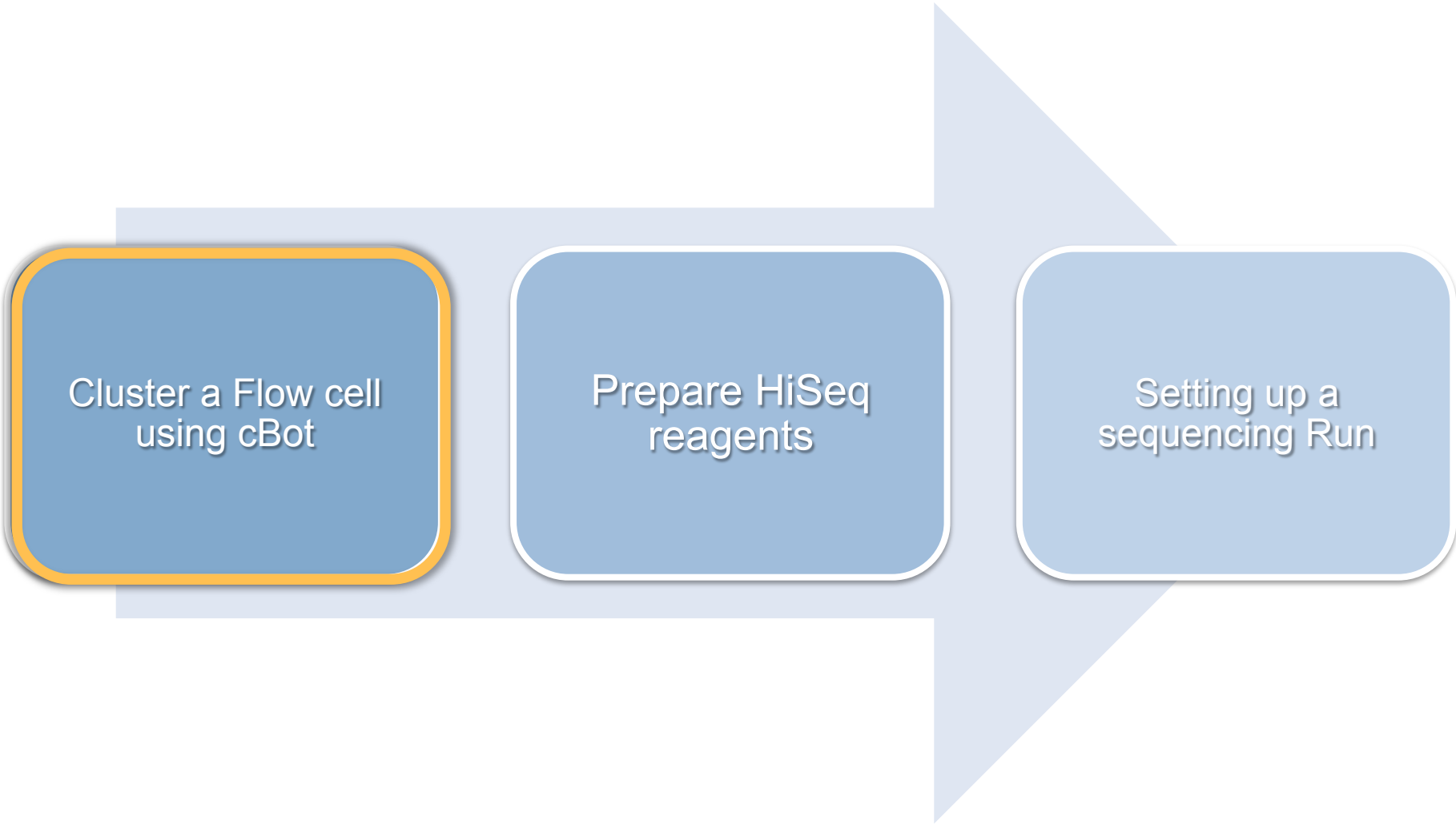
- Multimedia help menu



Starting a Run Using the HiSeq System

How to Start a Run

Workflow



Clustering a Flow cell using cBot

Automates the formation of amplified clonal clusters from the DNA single molecules

- Aspirates DNA samples into flow cell
- Delivers fluidics and controls temperature
- Clustering takes 4.5 hr
- Single manifold per run

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Cluster a Flow cell using cBot workflow

Thaw reagents

- In RT water bath, 1 hour
- Keep on ice or 4 CC after they are thawed

Prepare Samples

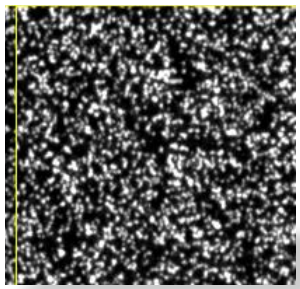
- Denature
- Dilute
- Spike in PhiX if possible

Set up clustering process

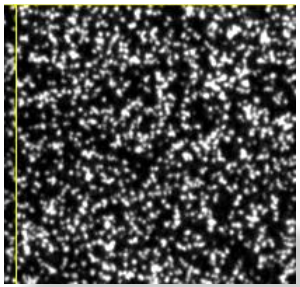
- Load FC
- Load Reagents
- Load Template/Custom Primers

Maximize data quality and quantity

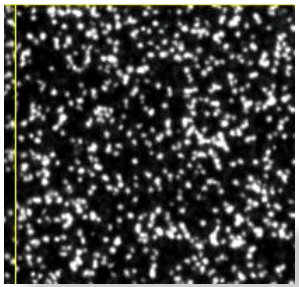
Optimized flow cell clustering determines data quality and overall data yield



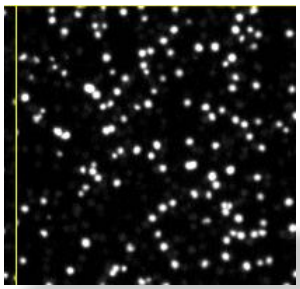
20pM



10pM



5pM



1pM

Overclustering can result in:

- Loss of data quality and data output
- Loss of focus
- Reduced base calls and Q30 scores
- Complete run failure

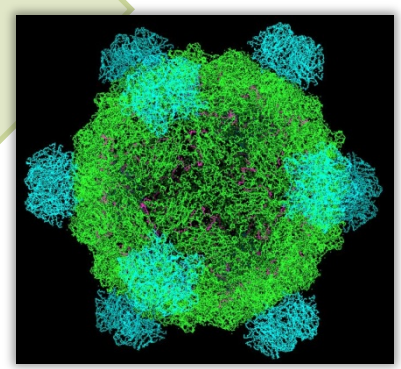
Underclustering can result in:

- Loss of time and money

Using a PhiX Control for HiSeq Sequencing Runs

What are PhiX Control Libraries?

- **Small:** PhiX is a small genome, which enables quick alignment and estimation of error rates
- **Diverse:** PhiX genome contains ~45% GC and 55% AT
- **Well-defined:** PhiX has a well-defined genome sequence



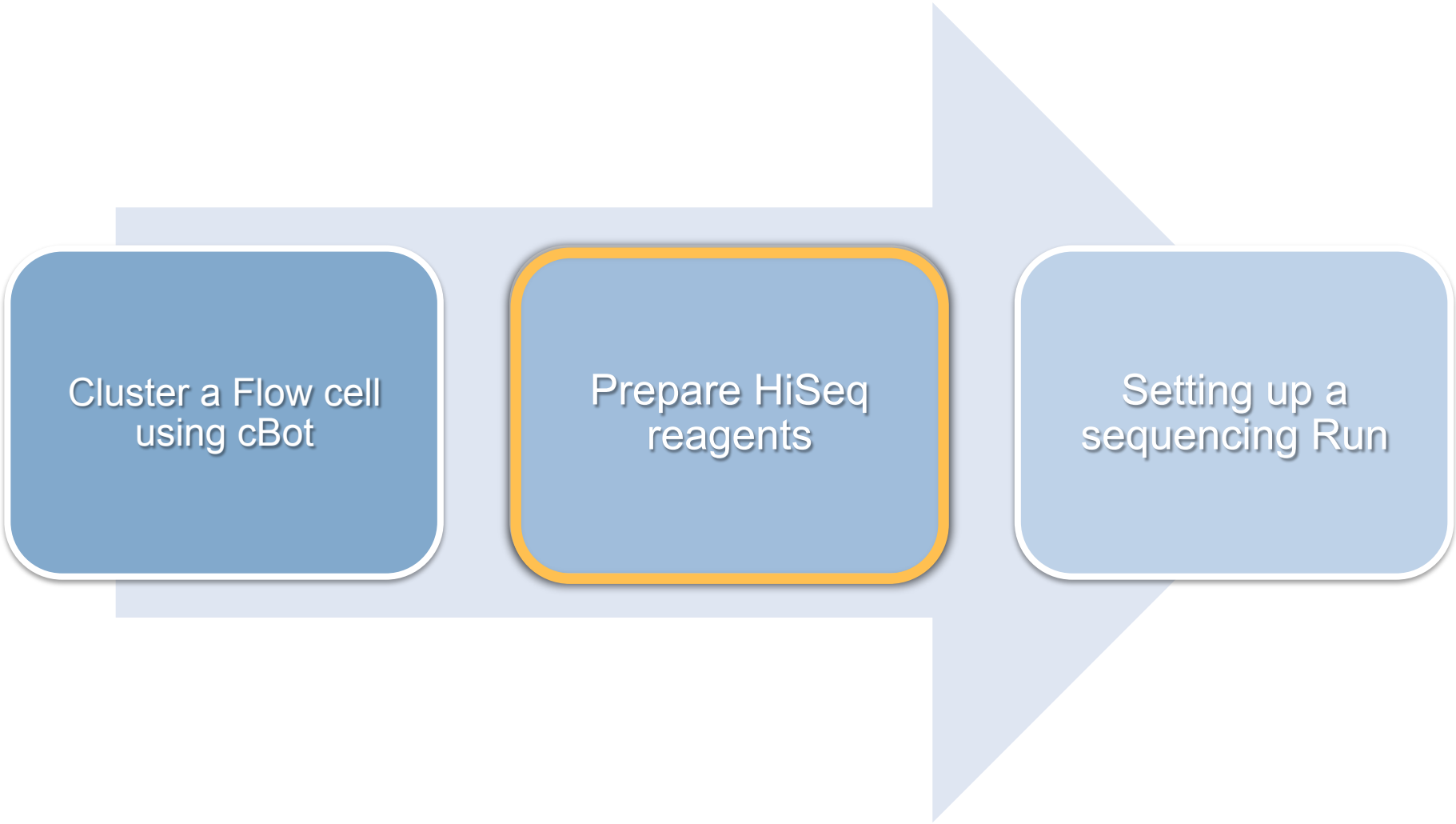
Structure of phage phiX 174 capsid

How to use a PhiX Control

- Low-concentration spike-in of 1%
- Dedicated control lane
- High-concentration spike-in of 40% or higher

How to Start a Run

Workflow



Prepare HiSeq Reagents Workflow

Thaw reagents

- Leave EDP in -15-25 CC storage until you are ready to prepare ICB
- Thaw SRE and in a RT water bath for ~90 min
- Thaw the LFN in a RT water bath for 20 min.

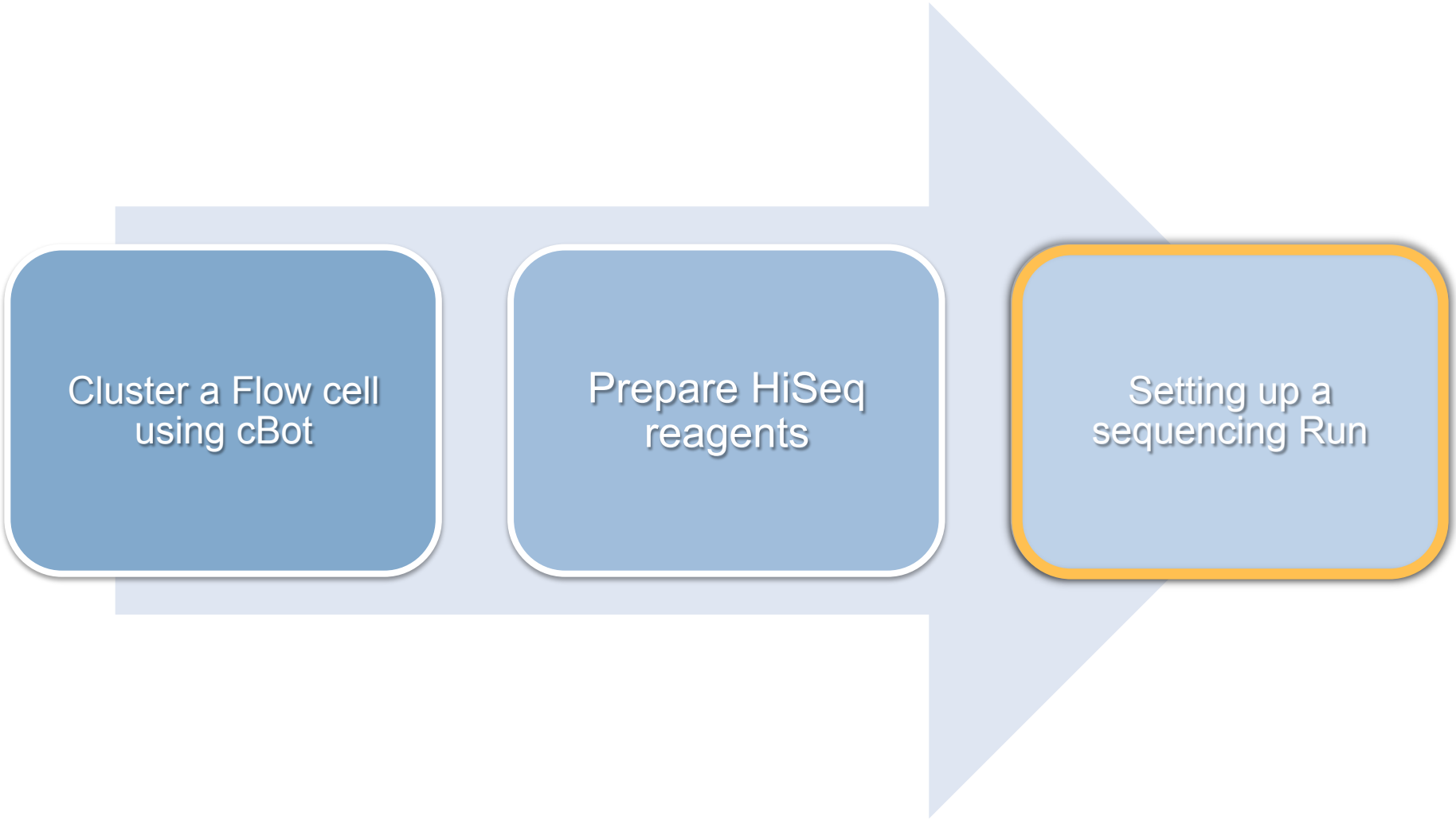
Prepare ICB

- Prepare ICB for Read 1 and Read 2 separate
- Prepare ICB for Read 1 and Read 2 (Alternative Workflow)

Keep reagents on 2-8 CC storage until you are ready to load

How to Start a Run

Workflow



Setting up a sequencing run workflow

Prerequisites

- You have recently performed an instrument wash
- You have prepared sequencing reagents
- You have a clustered HiSeq FC

Enter Run parameters

- Provide a Sample Sheet for indexed runs

Load Reagents

- Load SBS Reagents
- Load Indexing Reagents
- Prime Reagents, collect and measure priming waste

Load the clustered FC, start the run

- Confirm Proper Flow

Questions?