NextSeq 500 System Overview

Page	Audio
Welcome Page	Welcome to the NextSeq 500 System Overview. This course takes 20 minutes to complete and contain audio. Click Next to continue.
Navigation	Take a moment to familiarize yourself with the navigation of the course. If you need help while working in the course, click the help link in the top right corner of the page.
Course Objectives	By the end of this course, you will be able to: -Identify features of the NextSeq 500 system -Describe NextSeq 500 sequencing technology, and -Describe the simplified sequencing workflow of the NextSeq 500 System
What is NextSeq 500?	The Illumina NextSeq 500 system is a flexible, scalable, high-throughput desktop sequencer enabling a wide range of applications with the accuracy of Illumina SBS technology.
NextSeq 500 Sample-to-Answer Workflow	The Illumina NextSeq 500 system combines proven sequencing by synthesis (SBS) technology with a revolutionary workflow that enables you to go from DNA to analyzed data in as little as 30 hours.
NextSeq 500 Reagent Cartridge	The NextSeq 500 system reagent cartridge includes all the reagents required for cluster generation, sequencing, and paired-end chemistry.
NextSeq 500 Buffer Cartridge	The NextSeq 500 system buffer cartridge is a single-use consumable containing three reservoirs filled with buffers and wash solution. Contents of the buffer cartridge are sufficient for one sequencing run.
NextSeq 500 Flow Cell	NextSeq 500 system uses a four-lane flow cell. The NextSeq 500 system applies the pooled libraries from a loaded reagent cartridge onto the loaded flow cell, without the need for user intervention. The same library or library pool is distributed across all lanes: lanes are not independent with respect to library.
NextSeq 500 Control Software (NCS)	NextSeq Control Software, or NCS, guides the NextSeq 500 system user through all the steps to load the flow cell, load the reagents, and start a run. See the NextSeq 500: How to Start a Run online training for more information.

	Click the magnifying glass for a closer look.
BaseSpace Integration	The NextSeq 500 sequencing workflow is integrated with BaseSpace, the Illumina genomics computing environment for data analysis, storage, and collaboration. Before beginning a sequencing run, you can enter library information and run parameter in BaseSpace. Runs that were set up in BaseSpace appear on the software interface for selection during run setup. During the sequencing run, data can be streamed in real time to BaseSpace. BaseSpace is available on the cloud or on BaseSpace Onsite, an on-premises computing platform.
Reagent and Flow Cell Loading	NextSeq 500 system guides the user through loading the flow cell, loading reagents, and starting a run. Click Show Me the Steps to view the loading process.
Cluster Generation	At the start of NextSeq 500 system sequencing, pooled libraries loaded into the reagent cartridge flow through all 4 lanes of the flow cell in order for hybridization and cluster generation to occur. This process is automated on the NextSeq 500 system. First, single DNA molecules of the prepared libraries bind to the oligos on the flow cell surface. DNA molecules are then amplified to form clusters.
Sequencing or Imaging Cycles	Using two-channel sequencing only requires two images and hence all the data from the four nucleotides or bases is enclosed in these two images. The two-channel sequencing is much like the four channel sequencing and uses the same sequencing by synthesis (SBS) method but allows more efficient acquisition of the data. Click each button for more information. *{Four Channel button audio}* Four-channel sequencing works by using four images to determine which base occurs in each cluster. Each of the four DNA bases will emit an intensity of a unique wavelength. Therefore, during a cycle, each cluster appears in only one of the four images. *{start animation here}* For example, when a strong intensity signal is detected in the wavelength related to the G base, a G is called. When a strong intensity signal is detected in the wavelength related to the T base, a T is called, and so forth. Four-Channel Sequencing requires all four images to build up the DNA sequence. {Two Channel button audio} Rather than using four-images from four channels, two-channel sequencing uses only two images: an image from a red channel and an image from a green channel. In Two Channel Sequencing the intensity

	emitted by each base is as follows. A emits 50% green and 50% red intensities, C emits 100% red intensity, G is dark and does not emit any intensity, and T emits 100% green intensity. Therefore if we take a green image and a red image, A is presented in both images represented here as a blend of the red and green image, C is only presented in red, T is only presented in the green, and G is represented here with a blank image. The template is still built up over multiple cycles, so although a cluster starting on a G base would not be detected in the early cycles, it would be detected as it moves though the cycles and changes to A, C or T. Let us look at how each base call is made in two-channel sequencing. Intensity from each cluster is plotted onto a scatter plot of red intensity versus green intensity. Each base is called according to the region of the scatter plot, or base population, that the cluster falls into.
Flow Cell Tiles	Tiles are small imaging areas on the flow cell defined as one field of view by the camera. The total number of tiles depends on the number of lanes, swaths, and surfaces that are imaged on the flow cell, and how the camera works together to collect the images. Click the links for more information. *{Lane Numbering audio}* As mentioned previously there are 4 lanes in the NextSeq 500 flow cell that are grouped into lane pairs: lane 1 and 3 make up lane pair A and lane 2 and 4 make up lane pair B. Roll over the highlighted area to view lane numbering. *{Swath Numbering audio}* Each lane is imaged in three swaths. Swaths are numbered 1–3 for a high output flow cell. Roll over the highlighted area to view swath numbering. *{Camera Numbering audio}* The NextSeq 500 uses six cameras to image the flow cell. Cameras numbered 1, 2, and 3 image lane 1. Cameras numbered 4, 5, and 6 image lane 3. After lanes 1 and 3 are imaged, the imaging module moves on the X-axis to image lanes 2 and 4.

	Roll over the highlighted area to view camera numbering. *{Tile Numbering audio}* There are 12 tiles in each swath of each camera segment. Tiles are numbered 1–12, regardless of swath number or camera segment. The total number of tiles in a high-output flow cell is 864. Mid-output flow cells have a total of 288 tiles. Roll over the highlighted area to view tile numbering.
Primary Data Analysis	Primary data analysis, including base calling and quality scoring is, performed by real-time analysis or RTA on the instrument. RTA performs image analysis and base calling during the sequencing run. The NextSeq 500 uses a new implementation of RTA called RTA 2.0. The primary analysis workflow includes the following steps: Template generation, Imaging, and base calling. After a base is called, RTA 2.0 assigns a quality score. Sequencing quality metrics can be viewed in NCS during runs or remotely through BaseSpace or Sequencing Analysis Viewer (SAV). Click the links for more information.
NextSeq 500 Simplified Workflow	There are 10 items that make up the NextSeq 500 workflow. Roll over the workflow icons to learn more about the NextSeq 500 workflow.
Summary	You should now be familiar with the NextSeq 500 system. Take a moment to review the key points summarized here.
Lesson Completion	Congratulations! You have completed this course. For more information on NextSeq 500 system visit the NextSeq 500 System support page.