**NextSeq Training SOP**

**Conducting TrainIng for the Illumina NextSeq Sequencer**

1. **Purpose**

This procedure outlines the steps for training personnel to acquire the skills and knowledge necessary to run the Illumina NextSeq next generation sequencer from initial sample quality control to the review of sequencing run quality metrics.

1. **Scope**

This document applies to all staff that operate the Illumina NextSeq next generation sequencer and supervisors that oversee these operations. Training on the Illumina NextSeq sequencer is a process that includes building a base of sequencing knowledge, observing the trainer perform the sequencing procedures, performing sequencing procedures under direct trainer supervision, and individually executing the sequencing procedures.

1. **Related Documents**

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| --- | --- |
| **Title** | **Document Control Number** |
| NextSeq Training Form |  |
| NextSeq Trainer Designation Form |  |
| *“Lab-developed Risk Assessment/Mitigation document”* |  |

1. **Responsibilities**

| **Position** | **Responsibility** |
| --- | --- |
| All laboratory staff | * Complete all necessary training requirements |
| Team Lead | * Determine the training needs for the laboratory team * Ensure all staff are trained and evaluated according to this procedure * Designate the trainer by completing the NextSeq Trainer Designation Form, as needed * Create training plans, review training materials, and assign trainers as needed |
| Trainers | * Develop training materials * Train staff as directed by the Team Lead * Document training activities |
| Quality Manager | * Review training documentation |

1. **Training Information Resources**
   1. *Reference your laboratory SOP, the Illumina NextSeq System User Guide.*
   2. *Reference your laboratory-developed risk assessment/mitigation document here; this may be specific to the NextSeq or to the specific nucleic acid source.*
   3. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication Number (CDC) 21-1112.
   4. Illumina Support Training Videos (*select the videos relevant to your lab processes; add other videos as appropriate)*
      1. **[NextSeq: System Overview](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)**
      2. **[Sequencing: Introduction to Sequencing by Synthesis](https://illuminasupport.webex.com/cmp3300/webcomponents/docshow/docshow.do?javaEnable=false&setupStatus=0&siteurl=illuminasupport&jvm=false&rnd=0.8037636928420097)**
      3. [**AmpliSeq for Illumina: Library Prep Protocol**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)
      4. **[AmpliSeq for Illumina: Overview](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)**
      5. [**Nextera DNA Flex Library Preparation**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)
      6. [**NextSeq: How to Start a Run**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)
      7. [**NextSeq: Does My Run Look Good?**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/training.html)
   5. Required Reading *(select the documents relevant to your lab processes; add other documents as appropriate and insert below)*
      1. **[NextSeq System Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/nextseq-500-system-guide-15046563-06.pdf)**
      2. **[NextSeq System Denature and Dilute Libraries Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/nextseq-denature-dilute-libraries-guide-15048776-11.pdf)**
      3. [**NextSeq System Custom Primers Guide**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/documentation.html)
      4. [**NextSeq System Site Prep Guide**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/documentation.html)
      5. [**NextSeq System Safety and Compliance Guides**](https://support.illumina.com/sequencing/sequencing_instruments/nextseq-500/documentation.html)
2. **Equipment/Materials**
   1. Illumina NextSeq Sequencer
   2. Library preparation and sequencing reagents
3. **Safety Precautions**
   1. All BSL-2 practices, safety equipment, and facility design must comply with the requirements listed in the most current version of Biosafety in Microbiology and Biomedical Laboratories.
   2. Appropriate PPE must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses.
4. **Procedure**
   1. The trainee will build a basic understanding of NextSeq next generation sequencing (NGS) technology by:
      1. Reviewing the Illumina support training videos (5.4), and
      2. Completing the required reading (5.5).
   2. The trainer will perform all steps within the sequencing SOP in the laboratory while the trainee observes.
      1. The trainer will verbally walk the trainee through the entire sequencing process from beginning to end using the operational SOP as a training guide (**5.5, a**).
      2. This 1:1 review will cover initial sample quality control, preparing sample libraries, preparing the sequencing instrument, running the sequencing instrument, clean-up, and review of sequencing run quality control metrics.
   3. The trainee will perform all steps within the sequencing SOP under direct and full observation of the trainer.
      1. The trainer will quiz the trainee on multiple aspects of the protocol, including the questions below.
         1. When is a manual post-run wash necessary?
         2. Briefly explain how clusters are imaged using 2-channel sequencing chemistry.
         3. Briefly explain the configuration of the NextSeq flow cell.
         4. Why is fragment size important for producing high quality libraries?
         5. Why is it essential to prepare freshly diluted NaOH for denaturing libraries for cluster generation?
         6. *Add additional questions the trainer should ask the trainee to determine level of understanding specific to your protocol.*
      2. The trainer will review the trainee’s quality control data as described in the sequencing SOP (**5.5, a**) to assess the competency of the trainee.
   4. Once the trainee successfully performs a sequencing run under the observation of the trainer, the trainee will perform an unaccompanied sequencing run.
      1. The trainer will review the trainee’s quality control data as described in the sequencing SOP (**5.5, a**) to assess the competency of the trainee.
   5. It is the responsibility of the primary user to ensure that preventative maintenance is scheduled and executed.
      1. The trainee will observe proper user performed preventive maintenance.
      2. The trainee will perform user performed preventive maintenance.
      3. The trainer will assess the trainee’s ability to properly maintain the instrument according to established maintenance procedures.
5. **Appendices**

Appendix A – Trainer Question and Answer Sheet

1. **Revision History**

|  |  |  |  |
| --- | --- | --- | --- |
| **Rev #** | **DCR #** | **Changes Made to Document** | **Date** |
|  |  |  |  |

1. **Approval**

Approved By: Date:

Author

Print Name and Title

Approved By: Date:

Technical Reviewer

Print Name and Title

Approved By: Date:

Quality Manager / Designee

Print Name and Title

**Appendix A – Trainer Question and Answer Sheet**

**When is a manual post-run wash necessary?**

*A manual post-run wash is required when the automatic post-run wash is not performed, such as when a run is ended early and the flow cell is saved for later rehybridization.*

**Briefly explain how clusters are imaged using 2-channel sequencing chemistry**

*2-channel sequence by synthesis uses a mix of dyes. Images are taken of each DNA cluster using red and green wavelength filter bands. Clusters seen in red or green images are interpreted as C and T bases, respectively. Cluster observed in both red and green images are flagged as A bases (appearing as yellow clusters), while unlabeled clusters are identified as G bases.*

**Why is fragment size important for producing high quality libraries?**

*High-quality size selection can boost sequencing efficiency, improve assemblies, and allow sequencing of low-input samples. MiniSeq operates best when fed DNA libraries that contain fragments of similar sizes, when fragments have been improperly size selected efficiency is compromised. For example, it might take two lanes of sequencing to accomplish what could have been done in a single lane when a well sized library is selected.*

**Why is it essential to prepare freshly diluted NaOH for denaturing libraries for cluster generation?**

*Using a fresh dilution of NaOH is essential in order to completely denature samples for cluster generation. Diluted NaOH should be prepared within 12 hours of the run.*

**Briefly explain the configuration of the NextSeq flow cell.**

*The NextSeq flow cell contains four physical lanes. However, libraries are loaded onto the flow cell from a single reservoir. You can sequence a single library or multiple pooled libraries on the flow cell. There are two types of flow cells available for the NextSeq system: the high-output flow cell and the mid-output flow cell. Both flow cells contain four lanes of different width, resulting in a different number of tiles. Lanes on the high-output flow cell are imaged top and bottom in three swaths, or columns, for a total of 864 tiles. Lanes on the mid-output flow cell are imaged top and bottom in one swath for a total of 288 tiles.*