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| ***Insert Laboratory Specific Name Here*** |
| **iSeq Employee Training SOP** |

1. **Purpose**

This procedure outlines the steps for training personnel to acquire the skills and knowledge necessary to run the Illumina iSeq 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 iSeq next generation sequencer and supervisors that oversee these operations. Training on the Illumina iSeq sequencer is a four-step 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** |
| iSeq Employee Training Form |  |
| iSeq 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 iSeq Trainer Designation Form * 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 |
| Branch Chief | * Ensure applicable laboratory staff are accountable for completing all training and evaluation requirements described in this procedure * Review and approve this procedure |
| Quality Manager | * Review training documentation |

1. **Training Information Resources**
   1. *Reference your laboratory SOP, the Illumina iSeq System User Guide.*
   2. *Reference your laboratory-developed risk assessment/mitigation document here; this may be specific to the iSeq 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. [**Sequencing: Illumina Technology**](https://support.illumina.com/content/dam/illumina-support/courses/Sequencing_Illumina_Technology/story_html5.html)
      2. [**Sequencing: Fundamentals**](https://support.illumina.com/content/dam/illumina-support/courses/sequencing-fundamentals/story_html5.html)
      3. [**iSeq 100: Introduction**](https://illuminasupport.webex.com/cmp3300/webcomponents/docshow/docshow.do?siteurl=illuminasupport&setupStatus=1)
      4. [**iSeq 100: How to Start a Run**](https://support.illumina.com/content/dam/illumina-support/courses/iseq-100-how-to-start-a-run/story_html5.html)
      5. [**iSeq 100: Does My Run Look Good?**](https://support.illumina.com/content/dam/illumina-support/courses/iseq-100-how-to-start-a-run/story_html5.html)
      6. [**AmpliSeq for Illumina: Library Prep Protocol**](https://support.illumina.com/content/dam/illumina-support/courses/ampliseq-for-illumina-protocol/story_html5.html)
      7. [**AmpliSeq for Illumina: Overview**](https://support.illumina.com/content/dam/illumina-support/courses/ampliseq-for-illumina-overview/story_html5.html)
      8. [**Nextera DNA Flex Library Preparation**](https://support.illumina.com/content/dam/illumina-support/courses/nextera-flex/story_html5.html)
      9. [**BaseSpace Sequence Hub: Introduction**](https://illuminasupport.webex.com/cmp3300/webcomponents/docshow/docshow.do?siteurl=illuminasupport&setupStatus=1)
      10. [**Illumina Experiment Manager**](https://support.illumina.com/content/dam/illumina-support/courses/illumina-experiment-manager/story.html?iframe)
   5. Required Reading *(select the documents relevant to your lab processes; add other documents as appropriate)*
      1. [**iSeq 100: Sequencing System Guide**](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/iseq100/iseq-100-sequencing-system-guide-1000000036024-05.pdf)
      2. [**Sequencing Library QC with the iSeq System**](https://support.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/novaseq-qc-iseq-app-note-770-2018-019.pdf)
      3. [**iSeq 100 Sequencing System Safety and Compliance Guide**](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/iseq100/iseq-100-safety-compliance-guide-1000000035336-00.pdf)
      4. [**iSeq 100 Sequencing System Site Prep Guide**](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/iseq100/iseq-100-site-prep-guide-1000000035337-05.pdf)
      5. [**Indexed Sequencing Overview Guide**](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/indexed-sequencing-overview-guide-15057455-05.pdf)
      6. [**Cluster Optimization Overview Guide**](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/cluster-optimization-overview-guide-1000000071511-00.pdf)
2. **Equipment/Materials**
   1. Illumina iSeq 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 (if splashes are anticipated).
4. **Procedure**
   1. The trainee will build a basic understanding of iSeq 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 (**5.1**).
      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. Why is fragment size important for producing high quality libraries?
         2. Describe Illumina’s on-board clustering technique.
         3. What is an acceptable percentage of bases with a quality score greater than Q30 for a 2 x 150 bp run?
5. Why would you add PhiX to the library and how much is appropriate to add for an iSeq 100 run?
6. Why is the iSeq 100 cartridge inverted and then tapped on the benchtop prior to loading the library?
7. *Add additional questions the trainer should ask the trainee to determine level of understanding specific to your protocol.*
   1. The trainer will review the trainee’s quality control data as described in the sequencing SOP (5.1) to assess the competency of the trainee.
   2. 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 (5.1) to assess the competency of the trainee.
   3. 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.
8. **Continued Learning**
   1. Trainers and primary users should regularly attend Illumina iSeq webinars, read primary literature, and review new product releases.
   2. It is expected that trainers will try new protocols in the laboratory and teach new skills to primary users on a semiannual basis.
9. **Appendices**

Appendix A – Trainer Question and Answer Sheet

1. **Revision History**

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| --- | --- | --- | --- | --- |
| **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 – Trainee Answer Sheet**

**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. iSeq 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.*

**Describe Illumina’s on-board clustering technique.**

*A cluster is a clonal group of DNA strands generated from the library fragments that attach to a flow cell. Each cluster will produce one single read or one paired-end read.*

*During clustering, each strand of the library will bind to the flow cell. This template is amplified until the cluster consists of many copies. During a run the location and number of clusters is fixed. Each fragment is tagged with a fluorescent-labeled nucleotide by an incorporation mix that flows through the cell. Light is emitted which is detected by sensors in the instrument which results in base calls from each cluster.*

**What is an acceptable percentage of bases with a quality score greater than Q30 for a 2 x 150 bp run?**

*Illumina considers a run successful if > 80% of bases are higher than Q30.*

**Why would you add PhiX to the library and how much is appropriate to add for an iSeq 100 run?**

*PhiX Control v3 is a ready-to-use library that serves as a defined control genome. The iSeq 100 software is designed to look for it during a run to provide quality control for cluster generation, sequencing, and alignment, and a calibration control for cross-talk matrix generation, phasing, and prephasing. Illumina recommends users start with 5% PhiX in their library, at least initially, to ensure the instrument is performing adequately.*

**Why is the iSeq 100 cartridge inverted and then tapped on the benchtop prior to loading the library?**

*The iSeq 100 cartridge is inverted to mix reagents within the cartridge. Tapping the cartridge afterward on the benchtop dislodges air bubbles that may have formed during the inversion step.*