|  |
| --- |
| ***Insert Laboratory Specific Name Here*** |
| **iSeq Employee Training Form** |

|  |  |
| --- | --- |
| **Employee Name** | **Training Start Date** |
|  |  |

**Section I – Base Knowledge (Video and Reading Requirements)** *[select videos and documents relevant to your lab processes; add other videos and documents as appropriate]*

|  |  |  |
| --- | --- | --- |
| **Video Title** | **Trainee Initials** | **Date Watched** |
| [Sequencing: Illumina Technology](https://support.illumina.com/content/dam/illumina-support/courses/Sequencing_Illumina_Technology/story_html5.html) |  |  |
| [Sequencing: Fundamentals](https://support.illumina.com/content/dam/illumina-support/courses/sequencing-fundamentals/story_html5.html) |  |  |
| [iSeq 100: Introduction](https://illuminasupport.webex.com/cmp3300/webcomponents/docshow/docshow.do?siteurl=illuminasupport&setupStatus=1) |  |  |
| [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) |  |  |
| [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) |  |  |
| [AmpliSeq for Illumina: Library Prep Protocol](https://support.illumina.com/content/dam/illumina-support/courses/ampliseq-for-illumina-protocol/story_html5.html) |  |  |
| [AmpliSeq for Illumina: Overview](https://support.illumina.com/content/dam/illumina-support/courses/ampliseq-for-illumina-overview/story_html5.html) |  |  |
| [Nextera DNA Flex Library Preparation](https://support.illumina.com/content/dam/illumina-support/courses/nextera-flex/story_html5.html) |  |  |
| [BaseSpace Sequence Hub: Introduction](https://illuminasupport.webex.com/cmp3300/webcomponents/docshow/docshow.do?siteurl=illuminasupport&setupStatus=1) |  |  |
| **Document Name** | **Trainee Initials** | **Date Read** |
| [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) |  |  |
| [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) |  |  |
| [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) |  |  |
| [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) |  |  |
| [Indexed Sequencing Overview Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/indexed-sequencing-overview-guide-15057455-05.pdf) |  |  |
| [Cluster Optimization Overview Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/cluster-optimization-overview-guide-1000000071511-00.pdf) |  |  |

**Section II – Observation: Trainee observes the trainer perform all steps in the sequencing SOP**

|  |  |  |
| --- | --- | --- |
| **Discussion Points** | **Trainer Initials** | **Date** |
| Why is fragment size important for producing high quality libraries? |  |  |
| Describe Illumina’s on-board clustering. |  |  |
| What is an acceptable percentage of bases with a quality score greater than Q30 for a 2 x 150 bp run? |  |  |
| Why would you add PhiX to the library and how much is appropriate to add for an iSeq 100 run? |  |  |
| Why is the iSeq 100 cartridge inverted and then tapped on the benchtop prior to loading the library? |  |  |

**Section III – Performance under Supervision: Trainee performs all steps in the sequencing SOP under direct trainer supervision**

Previously run, well characterized sample(s) will be provided to the trainee. The trainee will:

1. Extract the DNA
2. Perform Quality Control on the extracted DNA
3. Shear the DNA (as applicable) and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Load the prepared library onto the iSeq

Successful performance criteria: All samples result in good quality sequence data.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Performance Assessment** | **Yes** | **No** | **Trainer Initials** | **Date** |
| Extracted DNA met quality requirements | 🞏 | 🞏 |  |  |
| Sheared DNA (as applicable) met quality requirements | 🞏 | 🞏 |
| Library Preparation met quality requirements | 🞏 | 🞏 |
| Sequence data metrics met quality requirements | 🞏 | 🞏 |
| **Comments:** | | | | |

**Section IV – Independent Performance: Trainee individually executes all steps in the sequencing SOP**

Sample(s) will be provided to the trainee. The trainee will:

1. Extract the DNA
2. Perform Quality Control on the extracted DNA
3. Shear the DNA (as applicable) and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Load the prepared library onto the iSeq

Successful performance criteria: All samples result in good quality sequence data.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Performance Assessment** | **Yes** | **No** | **Trainer Initials** | **Date** |
| Extracted DNA met quality requirements | 🞏 | 🞏 |  |  |
| Sheared DNA (as applicable) met quality requirements | 🞏 | 🞏 |
| Library Preparation met quality requirements | 🞏 | 🞏 |
| Sequence data metrics met quality requirements | 🞏 | 🞏 |
| **Comments:** | | | | |

**Section VI – Employee Attestation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Attestations** | **Yes** | **No** | **Trainee Initials** |
| I read and understand the procedures listed in the required reading. | **🞏** | **🞏** |  |
| I had an opportunity to discuss my questions with the trainer. | **🞏** | **🞏** |  |
| I am satisfied with the explanations provided to me; all my questions were answered. | **🞏** | **🞏** |  |
| I understand the risks and mitigation practices that eliminate/minimize these risks. | **🞏** | **🞏** |  |
| I agree to comply with risk mitigation controls to eliminate/minimize these risks. | **🞏** | **🞏** |  |

**Section VII – Review and Signatures**

|  |  |  |
| --- | --- | --- |
| **Trainee Name** | **Signature** | **Date** |
|  |  |  |
| **Trainer Name** | **Signature** | **Date** |
|  |  |  |
| **Quality Assurance** | **Signature** | **Date** |
|  |  |  |

**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.*