# Table of Contents

## ILLUMINA MISEQ SEQUENCER TRAINER DESIGNATION FORM

The individual identified below is designated as a trainer for the Illumina MiSeq Sequencer. Once completed, retain this form in the trainer's employee file.

Trainer Name:

_____

Trainer Signature:

_____ Date: _____

Team Lead Name:

_____

Team Lead Signature:

_____ Date: _____

# CONDUCTING TRAINING FOR THE ILLUMINA MISEQ SEQUENCER

## 1.0 Purpose

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

## 2.0 Scope

This document applies to all staff that operate the Illumina MiSeq next generation sequencer and supervisors that oversee these operations. Training on the Illumina MiSeq 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.

## 3.0 Related Documents

| Title | Document Control Number |
|---|---|
| MiSeq Employee Training Form | |
| MiSeq Trainer Designation Form | |
| *"Lab-developed Risk Assessment/Mitigation document"* | |

## 4.0 Responsibilities

| Position | Responsibility |
|---|---|
| All laboratory staff | • Complete all necessary training requirements |
| Team Lead | • Determine the training needs for the laboratory team<br>• Ensure all staff are trained and evaluated according to this procedure<br>• Designate the trainer by completing the MiSeq Trainer Designation Form<br>• Create training plans, review training materials, and assign trainers as needed |
| Trainers | • Develop training materials<br>• Train staff as directed by the Team Lead<br>• Document training activities |
| Branch Chief | • Ensure applicable laboratory staff are accountable for completing all training and evaluation requirements described in this procedure<br>• Review and approve this procedure |
| Quality Manager | • Review training documentation |

## 5.0 Training Information Resources

**5.1** *Reference your laboratory SOP or the Illumina SOP your laboratory uses here.*

**5.2** *Reference your laboratory-developed risk assessment/mitigation document here; this may be specific to the MiSeq or to the specific nucleic acid source.*

**5.3** Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication Number (CDC) 21-1112.

**5.4** Illumina Support Training Videos *(select the videos relevant to your lab processes; add other videos as appropriate)*
**https://support.illumina.com/sequencing/sequencing_instruments/miseq/training.html**
- **a. MiSeq: Introduction to the MiSeq System**
- **b. MiSeq: How to Start a Run**
- **c. MiSeq: Instrument Washes**
- **d. TruSeq: Best Practices**
- **e. TruSeq: Controls**
- **f. TruSeq: Sample Purification Bead Size Selection and Best Practices**
- **g. Nextera DNA Sample Prep**
- **h. Nextera Sample Prep: Best Practices**
- **i. Illumina Experiment Manager**
- **j. MiSeq: Does My Run Look Good?**

**5.5** Required Reading *(select the documents relevant to your lab processes; add other documents as appropriate)*
- **a.** Illumina MiSeq System User Guide
- **b.** TruSeq DNA Sample Preparation Guide
- **c.** Nextera DNA Sample Preparation Guide
- **d.** Preparing Libraries for Sequencing on the MiSeq

## 6.0 Equipment/Materials

**6.1** Illumina MiSeq Sequencer

**6.2** Library preparation and sequencing reagents

## 7.0 Safety Precautions

**7.1** 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.

**7.2** Appropriate PPE must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses (if splashes are anticipated).

## 8.0 Procedure

**8.1** The trainee will build a basic understanding of MiSeq next generation sequencing (NGS) technology by:
- **a.** Reviewing the Illumina support training videos (5.4), and
- **b.** Completing the required reading (5.5).

**8.2** The trainer will perform all steps within the sequencing SOP in the laboratory while the trainee observes.
- **a.** 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**).

**b.** 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.

**8.3** The trainee will perform all steps within the sequencing SOP under direct and full observation of the trainer.

**a.** The trainer will quiz the trainee on multiple aspects of the protocol, including the questions below.

**i.** What should be done when there is not enough DNA in the library prep?

**ii.** *Add additional questions the trainer should ask the trainee to determine level of understanding specific to your protocol.*

**b.** 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.

**8.4** Once the trainee successfully performs a sequencing run under the observation of the trainer, the trainee will perform an unaccompanied sequencing run.

**a.** 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.

**8.5** It is the responsibility of the primary user to ensure that preventative maintenance is scheduled and executed.

**a.** The trainee will observe proper user performed preventive maintenance.

**b.** The trainee will perform user performed preventive maintenance.

**c.** The trainer will assess the trainee's ability to properly maintain the instrument according to established maintenance procedures.

## 9.0 Continued Learning

**9.1** Trainers and primary users should regularly attend Illumina MiSeq webinars, read primary literature, and review new product releases.

**9.2** It is expected that trainers will try new protocols in the laboratory and teach new skills to primary users on a semiannual basis.

## 10.0 Revision History

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

## 11.0 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

# MiSeq 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 |
|---|---|---|
| MiSeq: Introduction to the MiSeq System | | |
| MiSeq: How to Start a Run | | |
| MiSeq: Instrument Washes | | |
| TruSeq: Best Practices | | |
| TruSeq: Controls | | |
| TruSeq: Sample Purification Bead Size Selection and Best Practices | | |
| Nextera DNA Sample Prep | | |
| Nextera Sample Prep: Best Practices | | |
| Illumina Experiment Manager | | |
| MiSeq: Does My Run Look Good? | | |
| **Document Name** | **Trainee Initials** | **Date Read** |
| Illumina MiSeq System User Guide | | |
| TruSeq DNA Sample Preparation Guide | | |
| Nextera DNA Sample Preparation Guide | | |
| Preparing Libraries for Sequencing on the MiSeq | | |

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

| Discussion Points | Trainer Initials | Date |
|---|---|---|
| During a library preparation it is common to have a size selection step. Why is size selection important for producing high quality libraries? | | |
| Describe Illumina's on-board clustering technique to produce a polymerase colony. What are acceptable cluster densities for a sequencing run? | | |
| Describe how Illumina chemistry utilizes color-balanced barcodes and de-multiplexes sequence data. | | |

**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 and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Load the prepared library onto the MiSeq

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 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 and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Load the prepared library onto the MiSeq

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 met quality requirements | ☐ | ☐ | | |

| | | | | |
|---|---|---|---|---|
| Library Preparation met quality requirements | ☐ | ☐ | | |
| Sequence data metrics met quality requirements | ☐ | ☐ | | |
| **Comments:** | | | | |

**Section V – Instrument Preventive Maintenance: Trainee individually executes all steps in the preventive maintenance SOP**

| Performance Assessment | Yes | No | Trainer Initials | Date |
|---|---|---|---|---|
| Performed Post Run Wash | ☐ | ☐ | | |
| Performed Maintenance Wash | ☐ | ☐ | | |
| Understands when to perform Standby Wash | ☐ | ☐ | | |
| **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** |
| | | |

## ION PGM SEQUENCER TRAINER DESIGNATION FORM

The individual identified below is designated as a trainer for the Ion PGM Sequencer. Once completed, retain this form in the trainer's employee file.

Trainer Name:

_____

Trainer Signature:

_____ Date: _____

Team Lead Name:

_____

Team Lead Signature:

_____ Date: _____

## 1.0 Purpose

This procedure outlines the steps for training personnel to acquire the skills and knowledge necessary for sample preparation and sequencing on the Ion Personal Genome Machine (PGM) system.

## 2.0 Scope

This document applies to all staff that use the Ion PGM system and supervisors that oversee these operations. Training on the Ion PGM system is a four-step process that includes 1) building a knowledge base of Ion PGM technology, 2) observing the trainer perform the procedures, 3) performing procedures under direct trainer supervision, and 4) independently executing the procedures as instructed.

## 3.0 Related Documents

| Title | Document Control Number |
|---|---|
| Ion PGM Sequencer Employee Training Form | |
| Ion PGM Sequencer Trainer Designation Form | |
| *"Lab-developed Risk Assessment/Mitigation document"* | |

## 4.0 Responsibilities

| Position | Responsibility |
|---|---|
| All laboratory staff | • Complete all necessary training requirements |
| Team Lead | • Determine the training needs for the laboratory team<br>• Ensure all staff are trained and evaluated according to this procedure<br>• Designate the trainer by completing the Ion PGM Sequencer Trainer Designation Form<br>• Create training plans, review training materials, and assign trainers as needed |
| Trainers | • Develop training materials<br>• Train staff as directed by the Team Lead<br>• Document training activities |
| Branch Chief | • Ensure applicable laboratory staff are accountable for completing all training and evaluation requirements described in this procedure<br>• Review and approve this procedure |
| Quality Manager | • Review training documentation |

## 5.0 Training Information Resources

**5.1** *Reference your laboratory SOP or the Ion PGM SOP your laboratory uses.*

**5.2** *Reference your laboratory-developed risk assessment/mitigation document here; this may be specific to the PGM or to the specific nucleic acid source.*

**5.3** Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication Number (CDC) 21-1112.

**5.4** ThermoFischer ION and NGS videos *(select the videos relevant to your lab processes; add other videos as appropriate)*

- **a.** **Ion Torrent Technology**
- **b.** **NGS Library Preparation**
- **c.** **Ion Chef: Template Preparation**
- **d.** **Ion Chef: Workflow**
- **e.** **NGS Acronyms and Terms**

**5.5** Required Reading *(select the documents relevant to your lab processes; attach other documents as appropriate)*

- **a.** Ion Torrent Amplicon Sequencing
- ***b.*** *Insert applicable Library Preparation User Guide*
- **c.** Ion PGM Template OT 200 Kit Appendix A: Quality Control of Ion PGM Template OT2 Ion Sphere Particles
- **d.** Ion PGM sequencing protocols such as Ion PGM Sequencing 200 or 400 Kit User Guide
- ***e.*** *Insert applicable Ion Chef kit User Guide if applicable in the laboratory*

**5.6** Manuals & Protocols

- **a.** **Quick Reference: Ion PGM Hi-Q View Sequencing Kit**
- **b.** **Quick Reference: Ion PGM IC 200 Kit**
- **c.** **Reference Guide: Ion Personal Genome Machine (PGM) System**
- **d.** **Torrent Suite Software Help Document**
- **e.** **User Guide: Ion PGM Hi-Q Chef Kit**
- **f.** **User Guide: Ion PGM Hi-Q View Sequencing Kit**
- **g.** **User Guide: Ion PGM IC 200 Kit**

## 6.0 Equipment/Materials

**6.1** Ion PGM Sequencer

**6.2** Refer to Ion Torrent User Guides or laboratory documents and procedures for a complete description of laboratory equipment, material, and reagent requirements for library construction and template preparation.

**6.3** Qubit fluorimeter and reagents

**6.4** Library preparation reagents

**6.5** Template and enrichment reagents

**6.6** Sequencing reagents

**6.7** Ion Quality Control kit

## 7.0 Safety Precautions

**7.1** Reference the current version of Biosafety in Microbiological and Biomedical Laboratories (BMBL) for guidance on safe laboratory practices when conducting research in biomedical and clinical laboratories.

**7.2** Review and follow all safety regulations and requirements specific to the laboratory in which this training will be conducted.

**7.3** Appropriate Personal Protective Equipment (PPE) must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses (if splashes are anticipated).

## 8.0 Procedure

**8.1** The trainee will build a basic understanding of Ion PGM next generation sequencing (NGS) technology by:
- **a.** Reviewing the relevant Ion support videos (5.4), and
- **b.** Completing the required readings (5.5).
- **c.** Reviewing the Manuals & Protocols (5.6).

**8.2** The trainer will guide the trainee through the entire sequencing process while referencing the applicable documents or procedures (5.1). This 1:1 review will cover safety requirements, initial sample quality control, library and template preparations, instrument set-up, sequencing, post-sequencing clean-up, review of sequencing run quality control metrics, and other information as warranted.

**8.3** The trainee will carefully observe the procedure as it is performed by the trainer in the laboratory.

**8.4** The trainee will perform all steps within the sequencing SOP under direct and full observation of the trainer.
- **a.** The trainer will quiz the trainee on multiple aspects of the protocol, including the questions below.
  - *i. Add additional questions the trainer should ask the trainee to determine level of understanding specific to your protocol.*
  - *ii.* What is the minimum concentration of gDNA required to begin library construction?
  - *iii.* What is the minimum concentration of gDNA library necessary for template preparation?
- **b.** The trainer will review the trainee's results to assess the trainee's competency in performing the procedure.

**8.5** Once the trainee competently performs the procedure under direct observation, the trainee will perform the procedure unaccompanied.
- **a.** The trainer will review the trainee's results to assess the competency of the trainee.

**8.6** It is the responsibility of the primary user to ensure that preventative maintenance is scheduled and executed.
- **a.** The trainee will observe proper user performed preventive maintenance.
- **b.** The trainee will perform user performed preventive maintenance.
- **c.** The trainer will assess the trainee's ability to properly maintain the instrument according to established maintenance procedures.

## 9.0 Continued Learning

**9.1** Trainers and primary users should regularly check the Ion Community website for updated tutorials. They should attend Ion PGM webinars, read primary literature, and review new product releases.

**9.2** It is expected that trainers will try new protocols in the laboratory and teach new skills to primary users.

## 10.0 Revision History

| Rev # | DCR # | Change Summary | Date |
|---|---|---|---|
| | | | |

## 11.0 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

# Ion PGM Sequencer Training Form



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

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

| Video Title | Trainee Initials | Date Watched |
|---|---|---|
| Next Generation Sequencing (NGS) - An Introduction | | |
| NGS Library Preparation | | |
| Ion Chef: Template Preparation | | |
| Ion Torrent next-gen sequencing technology | | |
| **Document Name** | **Trainee Initials** | **Date Read** |
| Ion Torrent Amplicon Sequencing | | |
| *Attach laboratory-specific library preparation kit's user guide*<br>**Kit: _____** | | |
| *Attach laboratory-specific Ion Chef kit's user guide, if applicable (e.g. Ion PGM IC 200)*<br>**Kit: _____** | | |
| Ion PGM Template OT2 200 Kit Appendix A: Quality control of Ion PGM™ Template OT2 200 Ion Sphere™ Particles | | |
| Ion PGM Template OT2 200 Kit User Guide Chapter 4: Enrich the template-positive Ion PGM Template OT2 200 ISPs | | |
| Ion PGM™ Sequencing 200 or 400 Kit v2 User Guide | | |
| How to Assess a PGM Sequencing Run Report | | |

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

| Discussion Points | Trainer Initials | Date |
|---|---|---|
| During a library preparation, it is common to have a size selection step. Why is size selection important for producing high quality libraries? | | |
| During template preparation, it is important to have an optimized ratio of ISPs and DNA. Why is this important and what quality control measure is taken after template preparation? | | |
| Describe how the Ion Torrent chemistry identifies signals from the library fragment during sequencing. | | |

**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 conduct the following steps that are applicable to the laboratory's specific procedures for the sample type:

1. Extract the DNA
2. Perform Quality Control on the extracted DNA
3. Shear the DNA and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Perform Template Preparation
6. Perform Quality Control for Template Preparation
7. Load the prepared library onto appropriate chip
8. Sequence

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

| Performance Assessment | Yes | No | N/A | Trainer Initials | Date |
|---|---|---|---|---|---|
| Extracted DNA met quality requirements | ☐ | ☐ | ☐ | | |
| Sheared DNA met quality requirements | ☐ | ☐ | ☐ | | |
| Library Preparation met quality requirements | ☐ | ☐ | ☐ | | |
| Template 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 conduct the following steps that are applicable to their laboratory's specific procedures for that sample type:

1. Extract the DNA
2. Perform Quality Control on the extracted DNA
3. Shear the DNA and perform the Library Preparation
4. Perform Quality Control on the sheared DNA and the Library Preparation
5. Perform Template Preparation on Library

6. Perform Quality Control on Template
7. Load the prepared template into appropriate chip
8. Sequence

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

| Performance Assessment | Yes | No | N/A | Trainer Initials | Date |
|---|---|---|---|---|---|
| Extracted DNA met quality requirements | ☐ | ☐ | ☐ | | |
| Sheared DNA met quality requirements | ☐ | ☐ | ☐ | | |
| Library Preparation met quality requirements | ☐ | ☐ | ☐ | | |
| Template Preparation met quality requirements | ☐ | ☐ | ☐ | | |
| Sequence data metrics met quality requirements | ☐ | ☐ | ☐ | | |
| **Comments:** | | | | | |

**Section V – Instrument Preventive Maintenance: Trainee individually executes all steps in the preventive maintenance SOP**

| Performance Assessment | Yes | No | Trainer Initials | Date |
|---|---|---|---|---|
| Performed Post Run Wash | ☐ | ☐ | | |
| Performed Weekly Chlorite Maintenance Wash | ☐ | ☐ | | |
| Understands when to perform Standby Wash | ☐ | ☐ | | |
| **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** |
| | | |

# CONDUCTING PERSONNEL COMPETENCY ASSESSMENT FOR THE ILLUMINA MISEQ SEQUENCER

## 1.0 Purpose

This procedure outlines the steps for assessing the competency of personnel to run the Illumina MiSeq next generation sequencer from initial sample quality control to the review of sequencing run quality metrics. Competency should be assessed after successful completion of training. Competency assessment is required every six months during the first year the individual tests patient specimens. Thereafter, competency assessment must be performed at least annually. Competency assessment can be done throughout the year by coordinating it with routine practices and procedures to minimize impact on workload.

## 2.0 Scope

This document applies to all staff that operate the Illumina MiSeq next generation sequencer and supervisors that oversee these operations. CLIA competency assessment consists of six criteria: 1) Direct observation of testing; 2) Monitor recording and reporting results; 3) Review intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records; 4) Direct observation of instrument maintenance and function checks; 5) Assess test performance; and 6) Assess problem solving skills.

*Note that this document is written to meet CLIA requirements; if your laboratory is not subject to the requirements of CLIA, please consult your Quality Manager to tailor the content of this document to meet your laboratory's specific needs.*

## 3.0 Related Documents

| Title | Document Control Number |
|---|---|
| MiSeq Competency Assessment Form | |
| *List your laboratory's SOPs as appropriate* | |

## 4.0 Responsibility

| Position | Responsibility |
|---|---|
| All laboratory staff | • Complete all necessary competency assessment requirements |
| Team Lead | • Determine the competency assessment needs for the laboratory team<br>• Designate the assessor |
| Assessor | • Assess staff as directed by the Team Lead<br>• Document competency assessment activities |
| Technical Supervisor | • Document final assessment results and remediation activities, if required |

| Position | Responsibility |
|---|---|
| Branch Chief | • Ensure applicable laboratory staff are accountable for completing all competency assessment requirements described in this procedure<br>• Review and approve this procedure |
| Quality Manager | • Review competency assessment documentation |

## 5.0 Definitions

| Term | Definition |
|---|---|
| Competency | The ability of laboratory personnel to apply their skill, knowledge, and experience to perform their laboratory duties correctly. |
| Competency assessment | Ensures that laboratory personnel are fulfilling their duties as required by federal regulation. |

## 6.0 Equipment/Materials

**6.1** Illumina MiSeq Sequencer

**6.2** Library preparation and sequencing reagents

## 7.0 Safety Precautions

**7.1** 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.

**7.2** Appropriate PPE must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses (if splashes are anticipated).

## 8.0 Procedure

**8.1** Perform direct observation of routine testing, including sample preparation (if applicable), specimen handling, specimen processing, and test performance.

- **a.** Observe the performance of the skill/knowledge areas listed in the MiSeq Competency Assessment Form section 1) Direct Observation of Testing.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.2** Monitor recording and reporting test results; assess during direct observation of testing.

- **a.** Observe the performance of all skill/knowledge areas listed in the MiSeq Competency Assessment Form section 2) Monitor Recording and Reporting Results.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.3** Review intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records.

- **a.** Observe the performance of all skill/knowledge areas listed in the MiSeq Competency Assessment Form section 3) Review Intermediate Test Results / Worksheets, QC Records, PT Results, PM Records.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.4** Perform direct observation of instrument maintenance and function checks.
- **a.** Observe the performance of all skill/knowledge areas listed in the MiSeq Competency Assessment Form section 4) Direct Observation of Instrument.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.5** Assess test performance by comparing results with previously analyzed specimens, internal blind testing samples, or external proficiency testing samples.
- **a.** Observe the performance of all skill/knowledge areas listed in the MiSeq Competency Assessment Form section 5) Assess Test Performance.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.6** Assess problem solving skills.
- **a.** Observe the performance of all skill/knowledge areas listed in the MiSeq Competency Assessment Form section 6) Assess Problem Solving Skills.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.7** Document completion of competency assessment.
- **a.** Document employee acknowledgement through signature and date.
- **b.** Indicate assessment result and if required, document remediation actions taken (to be completed by the Technical Supervisor).
- **c.** Obtain final review and signatures by the Technical Supervisor, Team Lead, and Quality Manger.
- **d.** List all assessors who participated in the assessment with signature, initials, and date. *(Add lines to the form as needed.)*

## 9.0 Revision History

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

## 1.0 Approval

Approval Signature: _____ Date:_____

# MiSeq Competency Assessment Form



| **Employee Name** (Print) | | **Assessment Start Date** | | **Test/Assay Name** | |
|---|---|---|---|---|---|

| **Assessment Type:** (check one) | **Initial Assessment** | ❑ | **6-Month Assessment** | ❑ | **Annual Assessment** | ❑ |
|---|---|---|---|---|---|---|

| **1) Direct Observation of Testing** | | | | | |
|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Specimen Processing: DNA Extraction | Performs specimen preparation in accordance with approved procedure *(DNA extraction)* | ☐ | ☐ | ☐ | |
| | Ensures positive specimen identification is maintained | ☐ | ☐ | ☐ | |
| | Identifies acceptable specimen types and storage requirements | ☐ | ☐ | ☐ | |
| Specimen Processing: DNA Quality Control | Performs quality control of DNA in accordance with approved procedure *(Nanodrop, Qubit, Bioanalyzer, or Fragment Analyzer)* | ☐ | ☐ | ☐ | |
| | Ensures positive specimen identification is maintained | ☐ | ☐ | ☐ | |
| Specimen Processing: DNA Shearing and Library Preparation | Performs DNA Shearing in accordance with approved procedure *(Covaris)* | ☐ | ☐ | ☐ | |
| | Performs Sheared DNA Quality Control testing in accordance with approved procedure *(Bioanalyzer, TapeStation, Fragment Analyzer)* | ☐ | ☐ | ☐ | |
| | Performs Library Preparation in accordance with approved procedure *(TruSeq)* | ☐ | ☐ | ☐ | |
| | Performs Library Preparation Quality Control Testing in accordance with approved procedure *(Bioanalyzer, TapeStation, Fragment Analyzer)* | ☐ | ☐ | ☐ | |
| | Performs Library Preparation Quantitation in accordance with approved procedure *(Qubit, qPCR)* | ☐ | ☐ | ☐ | |
| Loading the MiSeq Cartridge | Performs Loading of the MiSeq Cartridge in accordance with approved procedure | ☐ | ☐ | ☐ | |
| **Comments** | | | | | |

| **Criterion 1) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | |
|---|---|---|---|---|---|

| 2) Monitor Recording and Reporting Results | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Record NGS process data | Complies with applicable documentation requirements *(e.g. completes NGS Run Data Capture Tool)* | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 2) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| 3) Review Intermediate Test Results / Worksheets, QC Records, PT Results, PM Records | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Updates equipment records (calibration, maintenance, etc.) | Updates the preventive maintenance equipment log after each sequencing run | | | ☐ | ☐ | ☐ | |
| Reviews NGS process quality control checkpoint data | Records quality control data in the NGS Data Capture Tool and shows ability to make correct decisions on whether to proceed with the NGS process according to the approved procedure | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 3) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| 4) Direct Observation of Instrument Maintenance and Function Checks | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Performs the Post-Run Wash | Performs Post-Run Wash in accordance with approved procedure | | | ☐ | ☐ | ☐ | |
| Perform Maintenance Wash | Performs Maintenance Wash in accordance with approved procedure | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 4) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| 5) Assess Test Performance | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Review of quality control checkpoint data and sequence data demonstrate acceptable performance | The results of testing one of the below are as expected:<br>(select one; attach record)<br><br>☐ Previously tested specimens<br>☐ Internal blind specimens<br>☐ Proficiency test specimens result | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 5) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| 6) Assess Problem Solving Skills | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Troubleshooting | Able to describe potential sources of error and preventive actions | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 6) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | |

| Employee Acknowledgement | | | |
|---|---|---|---|
| I certify that I completed the assessment outlined above. | | | |
| | **Name** (Print) | **Signature** | **Date** |
| **Employee** | | | |

| Assessment Result (To be completed by the Technical Supervisor) | | |
|---|---|---|
| **Criteria for Success: 100% Compliance to Procedures** | ☐ Successful | ☐ Remediation Required |
| **Remediation:** (If required) | | |
| | | |
| | **Name** (Print) | **Signature** | **Date** |

| | | | |
|---|---|---|---|
| **Technical Supervisor** | | | |

| **Reviews** | | | |
|---|---|---|---|
| | **Name** (Print) | **Signature** | **Date** |
| **Team Lead** | | | |
| **Quality Manager** | | | |

| **Assessor Reference** | | | |
|---|---|---|---|
| I certify that I conducted the assessment outlined above and the employee completed activities as identified. | | | |
| | **Name** (Print) | **Signature** | **Initials / Date** |
| **Assessor** | | | |
| **Assessor** | | | |

## CONDUCTING PERSONNEL COMPETENCY ASSESSMENT FOR THE ION PGM SEQUENCER

### 1.0 Purpose

This procedure outlines the steps for assessing the competency of personnel to run the Ion PGM next generation sequencer from initial sample quality control to the review of sequencing run quality metrics. Competency should be assessed after successful completion of training. Competency assessment is required every six months during the first year the individual tests patient specimens. Thereafter, competency assessment must be performed at least annually. Competency assessment can be done throughout the year by coordinating it with routine practices and procedures to minimize impact on workload.

### 2.0 Scope

This document applies to all staff that operate the Ion PGM next generation sequencer and supervisors that oversee these operations. CLIA competency assessment consists of six criteria: 1) Direct observation of testing; 2) Monitor recording and reporting results; 3) Review intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records; 4) Direct observation of instrument maintenance and function checks; 5) Assess test performance; and 6) Assess problem solving skills.

*Note that this document is written to meet CLIA requirements; if your laboratory is not subject to the requirements of CLIA, please consult your Quality Manager to tailor the content of this document to meet your laboratory's specific needs.*

### 3.0 Related Documents

| Title | Document Control Number |
|---|---|
| Ion PGM Sequencer Competency Assessment Form | |
| *Insert laboratory specific protocol for sample processing* | |
| *Insert laboratory specific protocols for associated processes (e.g. nucleic acid extraction)* | |

### 4.0 Responsibility

| Position | Responsibility |
|---|---|
| All laboratory staff | • Complete all necessary competency assessment requirements |
| Team Lead | • Determine the competency assessment needs for the laboratory team<br>• Designate the assessor |
| Trainers | • Assess staff as directed by the Team Lead<br>• Document competency assessment activities |
| Branch Chief | • Ensure applicable laboratory staff are accountable for completing all competency assessment requirements described in this procedure<br>• Review and approve this procedure |
| Quality Manager | • Review competency assessment documentation |

## 5.0 Definitions

| Term | Definition |
|---|---|
| Competency | The ability of laboratory personnel to apply their skill, knowledge, and experience to perform their laboratory duties correctly. |
| Competency assessment | Ensures that laboratory personnel are fulfilling their duties as required by federal regulation. |

## 6.0 Equipment/Materials

**6.1** Ion PGM Sequencer

**6.2** Ion Torrent Library and/or Template Preparation instruments (such as designated PCR machine, Ion Chef, or Ion OneTouch 2 and Ion OneTouch ES instruments)

**6.3** Library preparation and sequencing reagents

## 7.0 Safety Precautions

**7.1** All BSL practices, safety equipment, and facility design must comply with the requirements listed in the most current version of Biosafety in Microbiology and Biomedical Laboratories for the applicable biosafety level for your laboratory.

**7.2** Appropriate PPE must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses (if splashes are anticipated).

## 8.0 Procedure

**8.1** Perform direct observation of routine testing, including sample preparation (if applicable), specimen handling, specimen processing, and test performance.

- **a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Sequencer Competency Assessment Form section 1) Direct Observation of Testing.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.2** Monitor recording and reporting test results; assess during direct observation of testing.

- **a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Sequencer Competency Assessment Form section 2) Monitor Recording and Reporting Results.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.
- **c.** Record the outcome (Pass or Fail), initial and date.

**8.3** Review intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records.

- **a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Sequencer Competency Assessment Form section 3) Review Intermediate Test Results / Worksheets, QC Records, PT Results, PM Records.
- **b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.

**c.** Record the outcome (Pass or Fail), initial and date.

**8.4** Perform direct observation of instrument maintenance and function checks.

**a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Competency Assessment Form section 4) Direct Observation of Instrument.

**b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.

**c.** Record the outcome (Pass or Fail), initial and date.

**8.5** Assess test performance by comparing results with previously analyzed specimens, internal blind testing samples, or external proficiency testing samples.

**a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Sequencer Competency Assessment Form section 5) Assess Test Performance.

**b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.

**c.** Record the outcome (Pass or Fail), initial and date.

**8.6** Assess problem solving skills.

**a.** Observe the performance of the skill/knowledge areas listed in the Ion PGM Sequencer Competency Assessment Form section 6) Assess Problem Solving Skills.

**b.** Indicate Yes, No, or N/A for each Task Observed, initial and date; include Comments as needed for tasks marked No or N/A.

**c.** Record the outcome (Pass or Fail), initial and date.

**8.7** Document completion of competency assessment.

**a.** Document employee acknowledgement through signature and date.

**b.** Indicate assessment result and if required, document remediation actions taken (to be completed by the Technical Supervisor).

**c.** Obtain final review and signatures by the Technical Supervisor, Team Lead, and Quality Manger.

**d.** List all assessors who participated in the assessment with signature, initials, and date. *(Add lines to the form as needed.)*

## 9.0 Revision History

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

## 10.0 Approval

Approval Signature: _____ Date: _____

# Ion PGM Sequencer Competency Assessment Form



| **Employee Name** (Print) | | **Assessment Start Date** | |
|---|---|---|---|

| **Assessment Type:** (check one) | **Initial Assessment** | ❑ | **6-Month Assessment** | ❑ | **Annual Assessment** | ❑ |
|---|---|---|---|---|---|---|

| **1) Direct Observation of Testing** | | | | | |
|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Specimen Processing: DNA Extraction | Performs specimen preparation in accordance with approved procedure *(DNA extraction)* | ☐ | ☐ | ☐ | |
| | Ensures positive specimen identification is maintained | ☐ | ☐ | ☐ | |
| | Identifies acceptable specimen types and storage requirements | ☐ | ☐ | ☐ | |
| Specimen Processing: DNA Quality Control | Performs quality control of DNA in accordance with approved procedure *(Nanodrop and Qubit, Bioanalyzer, or Fragment Analyzer)* | ☐ | ☐ | ☐ | |
| | Ensures positive specimen identification is maintained | ☐ | ☐ | ☐ | |
| Specimen Processing: DNA Shearing and Library Preparation | Performs DNA Shearing and Library Preparation in accordance with approved procedures *(example: Covaris or enzymatic)* | ☐ | ☐ | ☐ | |
| | Performs Sheared DNA Quality Control testing in accordance with approved procedure *(Bioanalyzer, TapeStation, Fragment Analyzer)* | ☐ | ☐ | ☐ | |
| | Performs Library Preparation Quality Control Testing in accordance with approved procedure *(gel electrophoresis, Bioanalyzer)* | ☐ | ☐ | ☐ | |
| | Performs Library Preparation Quantitation in accordance with approved procedure *(bioanalyzer, qPCR, Qubit)* | ☐ | ☐ | ☐ | |
| Automated System: Loading the Ion Chef | Prepares reagents and loads libraries into the Ion Chef in accordance with approved procedure (check N/A if the Ion Chef is not used) | ☐ | ☐ | ☐ | |
| Template Preparation | Performs Template Preparation and enrichment in accordance with approved procedure (check N/A if the Ion Chef was used) | ☐ | ☐ | ☐ | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Loading the Ion chip | Performs loading of the Ion chip in accordance with approved procedure (check N/A if the Ion Chef was used) | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 1) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| **2) Monitor Recording and Reporting Results** | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Record NGS process data | Complies with applicable documentation requirements *(e.g. completes NGS Run Data Capture Tool, records in lab notebook)* | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 2) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | | |

| **3) Review Intermediate Test Results / Worksheets, QC Records, PT Results, PM Records** | | | | | |
|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Updates equipment records (calibration, maintenance, etc.) | Updates the preventive maintenance equipment log after each sequencing run | ☐ | ☐ | ☐ | |
| Reviews NGS process quality control checkpoint data | Records quality control data in the NGS Data Capture Tool or other record and shows ability to make correct decisions on whether to proceed with the NGS process according to the approved procedure | ☐ | ☐ | ☐ | |

| | | | | | |
|---|---|---|---|---|---|
| **Comments** | | | | | |
| **Criterion 3) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | |

| **4) Direct Observation of Instrument Maintenance and Function Checks** | | | | | |
|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Performs the Post-Run Wash | Performs Post-Run Wash on all required instruments in accordance with approved procedure | ☐ | ☐ | ☐ | |
| Perform Maintenance Wash | Performs other maintenance washes on all required instruments in accordance with approved procedure | ☐ | ☐ | ☐ | |
| **Comments** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Criterion 4) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | |

| **5) Assess Test Performance** | | | | | |
|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Review of quality control checkpoint data and sequence data demonstrate acceptable performance | The results of testing one of the below are as expected:<br>(select one; attach record)<br><br>☐ Previously tested specimens<br>☐ Internal blind specimens<br>☐ Proficiency test specimens result | ☐ | ☐ | ☐ | |
| **Comments** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| **Criterion 5) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | |

| 6) Assess Problem Solving Skills | | | | | | |
|---|---|---|---|---|---|---|
| **Skill / Knowledge Assessed** | **Task Observed** | | | **Yes** | **No** | **N/A** | **Assessor Initial / Date** |
| Troubleshooting | Able to describe potential sources of error and preventive actions | | | ☐ | ☐ | ☐ | |
| **Comments** | | | | | | | |
| **Criterion 6) Outcome:** | **Pass** ☐ | **Fail** ☐ | **N/A** ☐ | **Assessor Initial / Date** | | |

| Employee Acknowledgement | | | |
|---|---|---|---|
| I certify that I completed the assessment outlined above. | | | |
| | **Name** (Print) | **Signature** | **Date** |
| **Employee** | | | |

| Assessment Result (To be completed by the Technical Supervisor) | | | |
|---|---|---|---|
| **Criteria for Success: 100% Compliance to Procedures** | ☐ Successful | ☐ Remediation Required | |
| **Remediation:** (If required) | | | |
| | | | |
| | **Name** (Print) | **Signature** | **Date** |

| | | | |
|---|---|---|---|
| **Technical Supervisor** | | | |

| **Reviews** | | | |
|---|---|---|---|
| | **Name** (Print) | **Signature** | **Date** |
| **Team Lead** | | | |
| **Quality Manager** | | | |

| **Assessor Reference** | | | |
|---|---|---|---|
| I certify that I conducted the assessment outlined above and the employee completed activities as identified. | | | |
| | **Name** (Print) | **Signature** | **Initials / Date** |
| **Assessor** | | | |
| **Assessor** | | | |

## Oxford Nanopore MinION Sequencer Trainer Designation Form

The individual indentified below is designated as a trainer for the Oxford Nanopore MinION Sequencer. Once completed, retain this form in the trainer's employee file.

Trainer Name: _____

Trainer Signature: _____ Date: _____

Team Lead Name: _____

Team Lead Signature: _____ Date: _____

## 1.0 Purpose

This procedure outlines the steps for training personnel to acquire the skills and knowledge necessary to run the Oxford Nanopore MinION sequencer from initial sample quality control to the review of sequencing run quality metrics.

## 2.0 Scope

This document applies to all staff that operate the Oxford Nanopore MinION next generation sequencer and supervisors that oversee these operations. Training on the Oxford Nanopore MinION 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.

## 3.0 Related Documents

| Title | Document Control Number |
|---|---|
| MinION Employee Training Form | |
| MinION Trainer Designation Form | |
| *"Lab-developed Risk Assessment/Mitigation Steps"* | |

## 4.0 Responsibilities

| Position | Responsibility |
|---|---|
| All laboratory staff | • Complete all necessary training requirements |
| Team Lead | • Determine the training needs for the laboratory team<br>• Ensure all staff are trained and evaluated according to this procedure<br>• Designate the trainer by completing the MinION Trainer Designation Form<br>• Create training plans, review training materials, and assign trainers as needed |
| Trainers | • Develop training materials<br>• Train staff as directed by the Team Lead<br>• Document training activities |
| Branch Chief | • Ensure applicable laboratory staff are accountable for completing all training and evaluation requirements described in this procedure<br>• Review and approve this procedure |
| Quality Manager | • Review training documentation |

## 5.0 Training Information Resources

**5.1** *Reference your laboratory SOP or the MinION SOP your laboratory uses here.*

**5.2** *Reference your laboratory-developed risk assessment/mitigation document here; this may be specific to the MinION or to the specific nucleic acid source.*

**5.3** Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication Number (CDC) 21-1112

**5.4** MinION Support Training Videos (select the videos relevant to your lab processes; add other videos as appropriate)

- **a.** **MinION: A Portable, Real-Time DNA/RNA Sequencing Device**
- **b.** **Flongle: For Rapid Nanopore Sequencing of Smaller Samples**
- **c.** **Loading an Oxford Nanopore Flow Cell**
- **d.** **RNA Sequencing with Nanopore Technology**

**5.5** Required Reading *(select documents relevant to your lab processes; add other documents as appropriate)*

- **a.** MinION Flow Cell Check Protocol
- **b.** MinION Rapid Sequencing Protocol
- **c.** MinION 1D Genomic DNA by Ligation Protocol
- **d.** Oxford Nanopore Community Discussion Board

## 6.0 Equipment/Materials

**6.1** Oxford Nanopore MinION Sequencer

**6.2** Library preparation and sequencing reagents

## 7.0 Safety Precautions

**7.1** 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.

**7.2** Appropriate PPE must be worn at all times when working in the laboratory, including laboratory coat, gloves, and safety glasses (if splashes are anticipated

## 8.0 Procedure

**8.1** The trainee will build a basic understanding of MinION next generation sequencing (NGS) technology by:

- **a.** Reviewing the MinION support training videos (Section 5.4)
- **b.** Complete the required readings (Section 5.5)

**8.2** The trainer will perform all steps within the sequencing SOP in the laboratory while the trainee observes.

- **a.** The trainer will verbally walk the trainee through the entire sequencing process from the beginning to end using the operational protocol as a training guide (Section 5.5)

**b.** 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.

**8.3** The trainee will perform all steps within the sequencing SOP under direct and full observation of the trainer.

**a.** The trainer will quiz the trainee on multiple aspects of the protocol, including the questions below, see Appendix A for answer sheet. *(the laboratory should populate this section with additional questions relevant to their procedure)*:

***i.*** *Describe how the MinION generates raw signals during sequencing.*

***ii.*** *What can be done if the library does not absorb by capillary action into the SpotON priming port?*

***iii.*** *Why do you open the flow cell priming port and then add 200 µl?*

***iv.*** *Why is it important to have greater than 800 active pores?*

***v.*** *What could cause a large number of unavailable/inactive pores after loading the flow cell?*

**b.** The trainer will review the trainee's quality control data as described in the sequencing protocol to assess the competency of the trainee.

**8.4** Once the trainee successfully performs a sequencing run under the observation of the trainer, the trainee will perform an unaccompanied sequencing run.

**a.** The trainer will review the trainee's quality control data and run data to assess the competency of the trainee.

**8.5** It is the responsibility of the primary user to ensure that preventative maintenance is scheduled and executed.

**a.** The trainee will observe proper user performed preventive maintenance.

**b.** The trainee will perform user performed preventive maintenance.

**c.** The trainer will assess the trainee's ability to properly maintain the instrument according to established maintenance procedures.

## 9.0 Continued Learning

**9.1** Trainers and primary users should regularly attend Oxford Nanopore MinION webinars, read primary literature, and review new product releases.

**9.2** It is expected that trainers will try new protocols in the laboratory and teach new skills to primary users on a semiannual basis.

## 10.0 References

## 11.0 Appendices

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

## 12.0 Revision History

| Rev # | DCR # | Change Summary | Date |
|---|---|---|---|
| | | | |

## 13.0 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

i. ***Describe how the MinION generates raw signals during sequencing.***
*An ionic current passes through the nanopores. As biological molecules pass through the nanopore the disruption/change in current is measured. The information about the change in current is used to identify the biological molecule.*

ii. ***What can be done if the library does not absorb by capillary action into the SpotON priming port?***
*Remove a small amount of priming solution and then re-add the priming solution to re-activate the capillary action.*

iii. ***Why do you open the flow cell priming port and then add 200 µl?***
*To remove any bubbles from the flow cell's array. This process also creates capillary action in SpotON port, so when the library is loaded it is slowly absorbed into the port.*

iv. ***Why is it important to have greater than 800 active pores?***
*A low number of available sequencing channels will reduce the throughput of the run.*

v. ***What could cause a large number of unavailable/inactive pores after loading the flow cell?***
*Loading the priming solution too quickly can cause the membrane to burst, damaging the pores and resulting in unavailable/inactive pores. Over loading the flow cell with too much DNA, and contaminants in the sample can also cause a large number of pores to become unavailable, or inactive.*

# MinION Employee Training Form



I am hoping that there will be a discussing around vehicles and knowing how to direct clients to the best one. Is there a list somewhere and who maintains it?

| 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 |
|---|---|---|
| MinION: A Portable, Real-Time DNA/RNA Sequencing Device | | |
| Flongle: For Rapid Nanopore Sequencing of Smaller Samples | | |
| Loading an Oxford Nanopore Flow Cell | | |
| RNA Sequencing with Nanopore Technology | | |
| **Document Name** | **Trainee Initials** | **Date Read** |
| MinION Flow Cell Check Protocol | | |
| MinION Rapid Sequencing Protocol | | |
| MinION 1D Genomic DNA by Ligation Protocol | | |
| Oxford Nanopore Community Discussion Board | | |

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

| Discussion Points | Trainer Initials | Date |
|---|---|---|
| Describe how the MinION generates raw signals during sequencing. | | |
| What can be done if the library does not absorb by capillary action in the SpotON priming port? | | |
| Why do you open the flow cell priming port and then add 200µL? | | |
| Why is it important to have greater than 800 active pores? | | |
| What could cause a large number of unavailable/inactive pores after loading the flow cell? | | |

**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 optional DNA Fragmentation/DNA repair **(1D)**
3. Perform Library Preparation
4. Load the prepared library onto the MinION

I am hoping that there will be a discussing around vehicles and knowing how to direct clients to the best one. Is there a list somewhere and who maintains it?

5. Perform all necessary Quality Control Checkpoints throughout the process

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

| Performance Assessment | Yes | No | Trainer Initials | Date |
|---|---|---|---|---|
| Extracted DNA met quality requirements | ☐ | ☐ | | |
| Fragmented/Repaired DNA met quality requirements (1D) | ☐ | ☐ | | |
| 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 optional DNA Fragmentation/DNA repair **(1D)**
3. Perform Library Preparation
4. Load the prepared library onto the MinION
5. Perform all necessary Quality Control Checkpoints throughout the process

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

| Performance Assessment | Yes | No | Trainer Initials | Date |
|---|---|---|---|---|
| Extracted DNA met quality requirements | ☐ | ☐ | | |
| Fragmented/Repaired DNA met quality requirements (1D) | ☐ | ☐ | | |
| Sequence data metrics met quality requirements | ☐ | ☐ | | |
| **Comments:** | | | | |

**Section V – Instrument Preventive Maintenance: Trainee individually executes all steps in the preventive maintenance SOP**

| Performance Assessment | Yes | No | Trainer Initials | Date |
|---|---|---|---|---|
| Performed Post Run Wash | ☐ | ☐ | | |

I am hoping that there will be a discussing around vehicles and knowing how to direct clients to the best one. Is there a list somewhere and who maintains it?

| 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** |
| | | |