**MiSeqDx Instrument Procedure**

1. **PRINCIPLE:**
	1. The MiSeqDx Platform is a Next Generation Sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of instrument specific reagents and flow cells, imaging hardware, and data analysis software. Illumina MiSeq® systems utilize sequencing by synthesis (SBS) technology, integrating cluster generation, sequencing, and data analysis on a single instrument.
	2. In contrast to standard MiSeq instruments, the MiSeqDx Platform has two modes of operation: Diagnostic Mode, which can be used with FDA-approved/cleared assays; and RUO/Research Mode, which can be used with laboratory-developed tests and research assays.
	3. The MiSeqDx (MiSeq) has an on-board Windows operating system. In addition, a networked computer may be utilized to aid in upstream and downstream functions through the use of software tools.
	4. Software: Illumina provides software for various functions (which may be utilized or, in some cases, replaced with similar software).
		1. Illumina Experiment Manager: helps in creating sample plates and sample sheets for different workflows and library types.
			1. See individual assay procedures for utilization steps.
		2. MiSeq Control Software (MCS): provides tools for set-up, maintenance, and sequencing runs.
		3. Real-time analysis (RTA): Integrated analysis software that performs real-time on-instrument data analysis during the sequencing run, which includes image analysis and base calling, and saves valuable downstream analysis time.
		4. MiSeq Reporter: Integrated secondary analysis software that processes data from analysis by RTA to perform alignment and provide information about each sample analyzed.
		5. Sequencing Analysis Viewer (SAV): allows the review of metrics during a run as metrics are generated and later after a run has completed.
		6. Variant Studio: software which aids in data analysis and review.
2. **PROCEDURE FOR OPERATION:**
	1. Starting the MiSeqDx:
		1. For the best performance, leave the instrument on continuously (except for scheduled power-downs). If the instrument has been turned off, wait at least 60 seconds to turn the power switch back to the ON position.
		2. Locate the power switch on the back panel on the right side of the instrument.
		3. Turn the power switch to the ON position. The integrated instrument computer then starts. Allow the instrument time to fully power up.
		4. Once the computer has fully powered up, it will automatically start initializing the instrument.
		5. Once initialization is complete, click anywhere on the screen.
		6. Log into the operating system.
			1. A window will pop up prompting you to enter the MiSeqDx username and password.
			2. Select **Next**.
		7. Wait until the operating system has finished loading.
			1. When the system is ready, the MiSeqDx Control Software (MCS) launches and initializes the system automatically.
			2. After the initialization step is complete, the Home screen appears.
		8. The system will initially be in Diagnostic Mode. To switch to RUO/Research Mode, perform the following steps:
			1. Select **Manage Instrument**.
			2. Select **Reboot To Research Mode**. The system will automatically restart and initialize the instrument in Research Mode.
	2. MiSeqDx Workflow:
		1. Prepare the prefilled reagent cartridge for use.
		2. Denature and dilute libraries.
		3. Load the library mix onto the reagent cartridge.
		4. From the software interface, select **Sequence** to begin the run setup steps.
		5. Wash the flow cell with deionized water. Thoroughly dry using a Kimwipe, followed by lint-free lens paper to remove residual lint/fibers. Load the flow cell into the MiSeqDx.
		6. Load the PR2 bottle and make sure the waste bottle is empty. Load the reagent cartridge.
		7. Review run parameters and pre-run check results.
		8. Select **Start Run**.
		9. Monitor the run from the MCS interface or using the SAV software.
		10. Perform a post-run wash and power-cycle (see below).
	3. Loading the Instrument:
		1. Thaw the reagent cartridge:
			1. Option 1 (preferred): Thaw overnight at 2° to 8°C.
				1. **NOTE**: store at 2° to 8°C for up to 1 week.
			2. Option 2: Thaw the reagent cartridge for 1 hour in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge. Do not allow the water to exceed the maximum water line printed on the reagent cartridge. Once the cartridge is completely thawed, remove it from the water bath.
		2. After thawing the cartridge, gently invert 10 times and then gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge.
		3. Denature and dilute the libraries according to the assay procedure.
		4. Loading sample libraries:
			1. Once the reagent cartridge is fully thawed, it is ready for loading of the sample libraries.
			2. Pierce the foil seal with a clean 1ml pipette tip.
			3. Pipette 600ul of prepared libraries into the reservoir labeled “**Load Samples**”. Avoid touching the foil seal.
			4. Depending on the assay being run, additional reagents may be loaded into the appropriately labelled wells of the cartridge. Check the assay protocol.
			5. Proceed directly to the run setup steps using the MCS interface.
		5. Cleaning the flow cell:
			1. Lightly rinse with laboratory-grade water until both the glass and plastic cartridge are thoroughly rinsed of excess salts.
			2. Thoroughly dry the flow cell and cartridge with a Kimwipe, followed by lint-free lens paper to remove residual lint/fibers.
			3. Make sure that the glass is free of streaks, fingerprints, and lint or tissue fibers.
		6. Loading the flow cell:
			1. Raise the flow cell compartment door, and then press the release button to the right of the flow cell latch.
				1. The flow cell latch will then open.
				2. Remove the flow cell from the previous run and discard, as appropriate.
				3. **NOTE**: Always ensure that at least one spare (previously used) flow cell is by the instrument. If there are any run errors, this flow cell can be used during repair instead of opening a new one.
			2. Make sure the flow cell stage is free of lint. Holding the flow cell by the edges, place it on the flow cell stage.
			3. Gently press down the flow cell latch to close it over the flow cell.
				1. As the flow cell latch closes, the alignment pins will properly position the flow cell.
				2. An audible click indicates that the flow cell latch is secure.
			4. If the software does not identify the flow cell RFID, see the troubleshooting section for instructions.
			5. Close the flow cell compartment door.
			6. Select **Next**.
		7. Load the reagents:
			1. PR2 and waste bottles:
				1. Remove the bottle of PR2 from the 4°C storage. Invert to mix and then remove the lid.
				2. Open the reagent compartment door.
				3. Raise the sipper handle until it locks into place.
				4. Remove the wash bottle and load the PR2 bottle.
				5. Empty the contents of the waste bottle and the wash bottle into the appropriate waste containers.
				6. Slowly lower the sipper handle. Make sure the sippers lower into the PR2 and waste bottles.
				7. If the software does not identify the RFID of the PR2 bottle, see the troubleshooting section for instructions.
				8. Select **Next**.
			2. Reagent cartridge:
				1. Open the reagent chiller door.
				2. Hold the reagent cartridge on the end with the Illumina label and slide it into the reagent chiller until the cartridge stops.
				3. Close the reagent chiller door.
				4. If the software does not identify the RFID of the reagent cartridge, see the troubleshooting section for instructions.
				5. Select **Next**.
	4. Starting the run:
		1. Review run parameters:
			1. Review Worklist Name, Analysis Workflow, and Read Length. These are specified on the sample sheet (i.e., generated using the Pillar-ILMN Sample Sheet Generator).
			2. Review the folder locations in the lower left corner.
			3. Select **Next**. The Pre-Run Check screen opens and starts. This step may take a few minutes.
		2. Review the pre-run check.
			1. The system performs a check of all run components, disk space, and network connections prior to starting a run.
			2. If any items do not pass the pre-run check, a message appears on the screen with instructions on how to correct the error.
			3. When all items successfully pass the pre-run check, select **Start Run**.
	5. Monitoring the Run
		1. During the run, monitor run progress, intensities, and quality scores that appear on the Sequencing screen (which is view-only).
	6. Completing the Run
		1. When the run is complete, the “Next” button appears. Review the results on the Sequencing screen before proceeding.
		2. **NOTE**: The Sequencing screen remains viewable until **Next** is selected. After you select **Next**, it is not possible to return to the Sequencing screen.
		3. Select **Next** to exit the Sequencing screen and proceed to a post-run wash.
3. **MAINTENANCE:**
	1. After each run or once a week
		1. Post-Run Wash:
			1. Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
				1. Add 5ml 100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.
				2. Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
				3. Invert 5 times to mix.
			2. Prepare the wash components with fresh wash solution as follows:
				1. Add 6ml wash solution to each reservoir of the wash tray.
				2. Add 350ml wash solution to the 500ml wash bottle.
			3. When the run is complete, select **Start Wash**. The software automatically raises the sippers in the reagent chiller.

**Note: Do not select Perform optional template line wash on the Post-Run wash screen. The template line wash requires a different procedure.**

* + - 1. Open the reagent compartment door and reagent chiller door. Slide the used reagent cartridge from the chiller.
			2. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
			3. Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place.
			4. Remove the PR2 bottle and replace it with the wash bottle. Discard of the PR2 bottle and contents appropriately.
			5. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
			6. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
			7. Close the reagent compartment door.
			8. Select **Next**. The post-run wash begins.
			9. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.
		1. Power Cycle:
			1. This step will help ensure the data integrity of the instrument and on-board computer.
			2. **Note: Before doing a power cycle make sure that any data from the previous run has been properly transferred.**
			3. Select **Manage Instrument** from the Illumina MiSeq Home page.
				1. Select **Shutdown**.
				2. Allow the computer time to fully shut down.
			4. Switch the power button, located on the back right of the instrument above the power cord, to the OFF position.
			5. Allow the instrument to sit with everything powered down for 5 minutes.
			6. Switch the power switch back to the ON position. Allow the instrument time to fully power up.
			7. Once the computer has fully powered up, it will automatically start initializing the instrument.
			8. Once initialization is complete, click anywhere on the screen.
			9. A window will pop up prompting you to enter the MiSeqDx username and password. Select **Next**.
			10. Wait until the operating system has finished loading.
				1. When the system is ready, the MiSeqDx Control Software (MCS) launches and initializes the system automatically.
				2. After the initialization step is complete, the Home screen appears.
			11. The system will initially be in Diagnostic Mode. To switch to RUO/Research Mode, perform the following steps:
				1. Select **Manage Instrument**.
				2. Select **Reboot To Research Mode**. The system will automatically restart and initialize the instrument in Research mode.
		2. Transferring MiSeqAnalysis folders to MGPGenomicData$ shared drive:
			1. On the MiSeqDx Instrument home screen, open the Shared Data Shortcut folder.
			2. Click the **Data (D:)** folder.
			3. Click the **Illumina** folder.
			4. Open the **MiSeqAnalysis** folder.
			5. Highlight the run folders in the MiSeqAnalysis folder.
			6. Right click and in the dropdown that appears, select **Cut**.
			7. Navigate to the SharedData folder, right click and in the dropdown that appears select **Paste**.
			8. Return to the Lifespan networked computer and if necessary, follow the steps below to map the SharedData drive.
				1. Click the **Windows Start button** on the bottom left of the screen.
				2. On the far-left side of the menu that appears, select **Documents** to open the File Finder.
				3. In the File Finder, click **This PC**, then select the **Computer** tab at the top of the window.
				4. Click **Map Network Drive**.
				5. In the Map Network Drive window, change the Drive to Z:, then in the folder field enter [\\M-M70291R\SharedData](file:///%5C%5CM-M70291R%5CSharedData). If the folder has been previously mapped, it can be selected from the dropdown menu in the folder field.
				6. Mark the **Connect using different credentials** checkbox.
				7. Enter the MiSeqDx username and password in the window that appears. Then, click **Finish**.
			9. On the Lifespan networked computer, navigate to the **SharedData** folder.
			10. Highlight the MiSeqAnalysis folders. Right click and, in the dropdown that appears, click **Cut**.
			11. Open the **MGPGenomicData$** shared drive.
			12. Click the **MiSeq\_Files** folder.
			13. In the MiSeq\_Files folder, right click and select **Paste**.
			14. Verify the files have been removed from the **SharedData** folder.
			15. Once the transfer is complete, click on the SharedData drive and select **Disconnect**.
	1. Local Run Manager (LRM): Changing the Password
		1. Entering the admin password in LRM is required in order to switch the instrument from Dx mode to RUO mode.
		2. The instrument password is set to expire 90 days after creation of a new password.
		3. The instrument will remind users 15 days prior to expiration.
		4. There are two admin accounts. In the event that one becomes locked out, the other can be used.
		5. LRM instructions:
			1. While logged into the Dx mode, open LRM by selecting the Chromium icon located on the taskbar.
			2. Log in using the admin account.
			3. Select user settings (gear icon, top right), followed by user management.
			4. Accounts can be edited by selecting the pencil icon next to the account name.
			5. Create and save a new password.
	2. Monthly – Maintenance Wash:
		1. Make sure that a flow cell is loaded on the instrument.
		2. From the home screen, select **Perform Wash**.
		3. From the Perform Wash screen, select **Maintenance Wash**.
		4. Perform the first wash:
			1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
				1. Add 5ml 100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.
				2. Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
				3. Invert 5 times to mix.
			2. Prepare the wash components with fresh wash solution as follows:
				1. Add 6ml wash solution to each reservoir of the wash tray.
				2. Add 350ml wash solution to the 500ml wash bottle.
			3. Load the wash tray and wash bottle onto the instrument:
				1. Open the reagent compartment door and reagent chiller door and slide the used reagent cartridge or wash tray from the chiller.
				2. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
				3. Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place and replace the PR2 bottle with the wash bottle. Dispose of the PR2 bottle and its contents appropriately.
				4. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
				5. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
				6. Close the reagent compartment door.
			4. Select **Next**. The first wash begins.
		5. Perform the second wash:
			1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
				1. Add 5ml 100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.
				2. Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
				3. Invert 5 times to mix.
			2. When the first wash is complete, remove the wash tray and wash bottle and appropriately discard the remaining wash solution from both.
			3. Refill the wash components with fresh wash solution as follows:
				1. Add 6ml wash solution to each reservoir of the wash tray.
				2. Add 350ml wash solution to the 500ml wash bottle.
			4. Load the wash tray and wash bottle as follows:
				1. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
				2. Load the wash bottle and slowly lower the sipper handle, making sure the sippers lower into the wash bottle and waste bottle.
				3. Close the reagent compartment door.
			5. Select **Next.** The second wash begins.
		6. Perform the final wash:
			1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
				1. Add 5ml 100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.
				2. Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
				3. Invert 5 times to mix.
			2. When the second wash is complete, remove the wash tray and wash bottle and appropriately discard the remaining wash solution from both.
			3. Refill the wash components with fresh wash solution as follows:
				1. Add 6ml wash solution to each reservoir of the wash tray.
				2. Add 350ml wash solution to the 500ml wash bottle.
			4. Load the wash tray and wash bottle as follows:
				1. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
				2. Load the wash bottle and slowly lower the sipper handle, making sure the sippers lower into the wash bottle and waste bottle.
				3. Close the reagent compartment door.
			5. Select **Next**. The final wash begins.
		7. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.
	3. As needed:
		1. Instrument Shutdown:
			1. It is best to leave the instrument on at all times. However, if the instrument must be turned off, follow these instructions to prepare the fluidics lines and shut down the instrument properly.
			2. Perform a **Maintenance Wash**.
			3. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
			4. Close the reagent compartment door.
			5. From the Home Screen of MCS, select **Manage Instrument**.
			6. Select **Shut Down**.
			7. Toggle the power switch to the **OFF** position. Note: Anytime you turn off the instrument, wait a minimum of 60 seconds before turning the power switch back to the **ON** position.
		2. Standby Wash:
			1. Make sure that a flow cell is loaded on the instrument.
			2. From the home screen, select **Perform Wash**.
			3. From the Perform Wash screen, select **Standby Wash**.
			4. Perform the first wash:
				1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:

Add 5 ml100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.

Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.

Invert 5 times to mix.

* + - * 1. Prepare the wash components with fresh wash solution as follows:

Add 6ml wash solution to each reservoir of the wash tray.

Add 350ml wash solution to the 500ml wash bottle.

* + - * 1. Load the wash tray and wash bottle onto the instrument:

Open the reagent compartment door and reagent chiller door. Slide the used reagent cartridge or wash tray from the chiller.

Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.

Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place. Replace the PR2 bottle with the wash bottle.

Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.

Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.

Close the reagent compartment door.

* + - * 1. Select **Next**. The first wash begins.
			1. Perform the second wash:
				1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:

Add 5ml 100% Tween 20 to 45ml laboratory-grade water. These volumes result in 10% Tween 20.

Add 25ml 10% Tween 20 to 475ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.

Invert 5 times to mix.

* + - * 1. When the first wash is complete, remove the wash tray and wash bottle and discard the remaining wash solution.
				2. Refill the wash components with fresh wash solution as follows:

Add 6ml wash solution to each reservoir of the wash tray.

Add 350ml wash solution to the 500ml wash bottle.

* + - * 1. Load the wash tray and wash bottle as follows:

Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.

Load the wash bottle and slowly lower the sipper handle, making sure the sippers lower into the wash bottle and waste bottle.

Close the reagent compartment door.

* + - * 1. Select **Next**. The second wash begins.
			1. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.
		1. Run Data
			1. 3 copies of data are generated and stored in separate subfolders within D:\Illumina
				1. MiSeqAnalysis
				2. MiSeqOutput (= Analysis files + additional archive data)
				3. MiSeqTemp = contents are auto-deleted after 1 week
			2. To save hard disk space, the contents of the MiSeqOutput folder can safely be deleted since it is mostly a duplicate of the Analysis folder.
			3. Weekly, move the run folders within MiSeqAnalysis to the [\\lsfile14\MGPGenomicData$](file:///%5C%5Clsfile14%5CMGPGenomicData%24) shared drive for long-term storage. Moving these files will also save hard drive space on the MiSeq.
			4. The following files and folders can be found within the Run Folders
				1. Data folder --> contains FASTQ files
				2. InterOp folder --> contains files for SAV
				3. RunInfo.xml --> for SAV
				4. RunParamters.xml --> for SAV
1. **TROUBLESHOOTING:**
	1. Performing a System Check:
		1. From the home screen, select **Manage Instrument**.
		2. Select **System Check**.
		3. Select the tests you want to perform. This is usually all tests on the left and right side of the screen. The center column can only be done by a field service engineer.
		4. Select **Next**. When complete, the test results will appear on the screen.
		5. Select **Show Details** to see a summary of the results on the software interface.
		6. There are two ways to export the results:
			1. Select **Export Results** to export the results to a .csv file format.
				1. Save the file on the **SharedData** drive to transfer from the MiSeqDx computer to the lifespan computer.
			2. From the desktop, select **Computer**.
				1. Select the **Data (D:)** drive.
				2. Open the **DiagResults** folder.
				3. The System Check data will be saved in a folder with the date of the system check as the name.
				4. Compress this file and save it to the **SharedData** drive to transfer from the MiSeqDx computer to the lifespan computer.
		7. Select **Done**.
	2. Pausing a run:
		1. You can temporarily pause a run before it is completed. An example of a reason you would pause a run is if the waste bottle is full. You can resume a paused run.
		2. **DO NOT** pause a run during cluster generation or within the first 8 cycles of sequencing. It is not possible to resume a run that is paused during this time.
		3. Select **Pause** from the Sequencing screen. The current command is completed, after which the run is paused, and the flow cell is placed in a safe state.
		4. The button changes to Resume. When you are ready to resume the run, select **Resume**.
	3. Stopping a run:

**NOTE: DISCUSS WITH DIRECTOR BEFORE DOING THIS**

* + 1. You can stop a run during sequencing before the run has completed using the **Stop** button on the sequencing screen. An example of a reason to stop the run is that the setup was done incorrectly, data quality is poor, or if there is a hardware error.
		2. When a run is stopped, the current command is not completed, and the flow cell stage moves to the forward position.
		3. Stopping a run is final. The run cannot be resumed. The only option is to proceed to an instrument wash.
	1. Resolving RFID Read failures:
		1. Always select **Retry** before proceeding. If the RFID fails a second time, select **Get Code**.
		2. From a computer with internet access, go to myillumina.com and log in to your MyIllumina account.
		3. From the MyIllumina page, click **Account**. In the Resources column, click **MiSeqDx Self-Service**.
		4. On the MiSeqDx Self-Service page, enter the **MiSeqDx serial number**.
		5. From the Type of Override Code drop-down list, select **RFID Override**.
		6. To generate the code, select **Get Code**.
		7. Return to the MCS interface and select **Enter Code**.
		8. Enter the temporary bypass code and then select **Next**. The code is only good for 7 days.
		9. Enter the barcode number of the flow cell, PR2 bottle, or reagent cartridge.

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| **Consumable** | **Barcode Number Location** |
| Flow Cell | Above the barcode on the flow cell container label.Flow cell barcode numbers begin with an A, G, or D.Example: A0E61. |
| PR2 Bottle | Below the barcode on the PR2 bottle label.Example: MS0011881-PR2. |
| Reagent Cartridge | Below the barcode on the reagent cartridge label.Example: MS0010744-300. |

* + 1. If you are entering a bypass code for the reagent cartridge, enter the version number of the kit. Select **Enter Reagent Kit Barcode** to enter the reagent cartridge barcode number and kit version number manually.
		2. Select **Enter**.
	1. If a bubble is identified in a flow cell, report to Illumina Technical support.
		1. If approved, the library pool can be loaded and sequenced, and the instrument should perform system checks to ensure integrity of the flow cell.
		2. Pay attention to the run metrics to determine if sequencing should be repeated.
	2. Regenerating FASTQ files:
		1. If appropriate, edit the Sample Sheet in Illumina Experiment Manager to reflect the correct index codes.
		2. Save the corrected Sample Sheet to the correct run folder in MiSeq Analysis.
		3. Delete the original Sample Sheet.
		4. The revised Sample Sheet must be named “SampleSheet.csv”.
		5. In Windows Explorer, go to D:\Illumina\MiSeqAnalysis\
			1. Open the appropriate run folder.
			2. Delete the file named “QueuedFforAnalysis.txt”.
		6. A new “QueuedFforAnalysis.txt” file will generate; this signifies the new analysis has started.
		7. To check the progress of regenerating the new FASTQ files:
			1. Open Internet Explorer and go to http://localhost:8042 OR use the MiSeq Reporter shortcut on desktop.
			2. Click the blue “Analyses” tab on the left.
			3. Complete analyses will be at the top with a green checkmark and incomplete/in-progress analyses will be at the bottom with an arrow.
			4. When the new FASTQ files are complete, the run will move to the top and have a green checkmark.
	3. For other troubleshooting concerns, refer to Appendix A of the MiSeqDx System Guide or contact technical support.
1. **CONTACT INFORMATION:**
	1. Illumina Technical Support
		1. Phone: 800-809-4566
		2. Fax: 858-202-4766
		3. Email: techsupport@illumina.com
2. **ATTACHMENTS:**
	1. MiSeqDx Instrument Maintenance Form
3. **REFERENCES:**
	1. MiSeqDx System Guide Document # 15027617 v01 (September 2015).
	2. MiSeqDx Instrument Reference Guide Part # 15070067 Rev. 02 (March 2017).
	3. MiSeqDx Denature and Dilute Libraries Guide Document # 15039740 v01 (January 2016).
4. **REVISIONS:**
	1. 3/2/2018: Additional information on data storage and transfer to save hard disk space.
	2. 1/3/2019: Instruction on how to change the password on the Local Run Manager was added.
	3. 1/15/2020: Updated footer to reflect the new laboratory name.
	4. 5/1/2020: Added new way to thaw cartridge and generating FASTQ files.
	5. 4/28/2021: Added instructions for transferring MiSeqAnalysis folders to the MGPGenomicData$ shared drive.
	6. 10/24/2023: Updated information about Sample Sheet generation to reflect the new AMP2 assay.