



Intermountain Forensics

SOP # WGS-200

Revision # 01

Forensic DNA Technical Leader Approval

Issue Date

04/03/2023

NovaSeq 6000 Operation

1. Purpose

This document describes setup and load of the NovaSeq 6000 for whole genome sequencing.

2. Summary

Whole genome sequencing is used by Intermountain Forensics to generate data files suitable for upload to genetic genealogy databases for uses in the identification of human remains, Keepsake analysis for genealogists, and crime investigation by law enforcement.

3. Procedure

Documentation

1. The samples included in the pool are documented on the Run Pooling Template page of the Library Preparation workbook.
2. A Case Notes Document is generated at the end of the run to document the quality metrics of the run. The Quality metrics are copied and pasted from the Sequence Analysis Viewer.

Instrument Preparation

1. Prior to initiating any of the following steps, it is recommended that the instrument is checked to ensure all appropriate maintenance has been completed and no updates are pending. Either of these can delay the run and should be addressed prior to the thawing of reagents or denaturation of the library pool, to reduce the risk of reagent waste or sample loss.
 - a. A maintenance wash will be prompted by the instrument and requires **80 minutes** to complete. Refer to the NovaSeq 6000 Sequencing System guide for the procedure.

Cartridge Preparation

1. Remove the SBS and cluster cartridges from the freezer and thaw in a room temperature water bath up to 4 hours prior to the intended start of the run.
 - a. Place the cartridges in the manufacture provided wire racks to ensure they do not capsize in the water bath.
 - b. Once thawed, the cartridges can be stored at 2-8°C for up to 24 hours before they must be used.
2. After the cartridges are thawed, remove from the water bath and dry the bottoms thoroughly with paper towels and wipe the foil cover with a lint free tissue.
 - a. Ensure no water remains between the wells when drying.
 - b. Complete thawing can be confirmed by inspecting the underside of each cartridge for the visible presence of ice in the reservoirs.
3. Invert the cartridge 10 times to mix the reagents.
4. Gently tap the bottom of each cartridge on the lab bench to reduce the presence of air bubbles.



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Sample Denaturation

1. Shortly before the intended start time, prepare the 0.2 N NaOH according to the table below.
 - a. Ensure to mix the dilution thorough after the components are pipetted.
 - b. The diluted NaOH must be used within 12 hours of preparation, to ensure optimal yield.

Reagent	Volume for 1 Flow Cell	Volume for 2 Flow Cells
Laboratory-grade Water	80 μ L	160 μ L
Stock 1 N NaOH	20 μ L	40 μ L

2. Add 77 μ L of the diluted NaOH to the 310 μ L of the library pool.
3. Cap the tube and vortex it briefly.
4. Centrifuge the tube at 280 x g for up to 1 minute then allow it to incubate at room temperature for 8 minutes.
5. After 8 minutes, add 78 μ L of 400 mM Tris-HCl, pH 8.0 to the tube to stop the denaturation.
6. Cap the tube and vortex it briefly.
7. Centrifuge the tube at 280 x g for up to 1 minute.
8. Transfer the volume into the library tube provided with the NovaSeq 600 Reagent Kit.
9. Load the tube into the cluster cartridge.
 - a. Once loaded the library pool must be loaded on to the instrument within 30 minutes or stored at -25°C to -15°C for up to 3 weeks. The tube can only be thawed for use once.

NovaSeq Set Up

1. Remove the flow cell package from refrigeration and set it at room temperature for 10-15 minutes.
 - a. The flow cell must be used within 24 hours of being warmed to room temperature.
2. Empty the used reagent bottles located in the lower portion of the NovaSeq 6000.
 - a. It is essential this is performed with every run to avoid a run stoppage due to overflow.
 - b. To remove the small bottle, raise the lever and grasp the bottle by its sides.
 - c. To remove the large bottle, grasp the bottle by its handles and lift up.
 - d. Return the empty bottles to their appropriate locations in the instrument.
3. Inspect the flow cell dock and ensure there is no particulate matter on its surface.
4. Remove the flow cell from its package by grasping the side to avoid touching the glass or underside gaskets.
5. Clean any particulate matter off the flow cell with an alcohol wipe and dry it with a low-lint/lint free tissue
6. Invert the flow cell so that the top surface faces downward.
7. Gently place the flow cell into the dock.





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8. With the wells facing up, load the NovaSeq Xp manifold of the inlet end of the flow cell and ensure it fits securely.
9. Load the SBS and Cluster Cartridges
 - a. Open the liquid compartment doors and then the reagent chiller door.
 - b. Remove any used SBS and cluster cartridges
 - c. Load the SBS cartridge into the left position with the Insert label facing the back of the instrument
 - d. Load the cluster cartridge containing the uncapped library tube into the right position with the Insert label facing the back of the instrument.



- e. Slide the drawer into the chiller and close the reagent chiller door.
10. Load the buffer cartridge
 - a. Open the buffer drawer by pulling on the metal handle
 - b. Remove any used buffer cartridges
 - c. Place the new cartridge in the drawer so that the Illumina label faces the front of the drawer and the cartridge is aligned with the raised sides of the drawer.
 - d. Close the Drawer
11. Acknowledge that the reagent bottles have been emptied on the instrument screen.
12. Enter the run parameters:
 - a. NovaSeq Standard
 - b. Run Name
 - i. Enter using the following format: YYYYMMDDInitials (example 20230301SEW)
 - c. Read 1: 151
 - d. Index 1: 8
 - e. Index 2: 8
 - f. Read 2: 151
13. Confirm the run parameters were entered correctly in the Review screen.
14. Click Start Run



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Reviewing Quality Metrics

1. Access the Quality Metrics for the run in the Sequence Analysis Viewer
2. Review the following Quality Metrics to ensure that the run was successful.
 - a. %Q30 – Must be greater than 75% of bases at 2 x 150 bp
 - b. Yield – The number of bases generated in the run should be greater than 2400-3000 Gb per run.
 - c. % Pass Filter – The number should be greater than 75%.
3. Paste the values into an IMF Notes Document, or its equivalent to be included in the support documents for all of the cases associated with the run.
4. Generate a report for the following quality metrics and provide it to the DNA Technical Leader to monitor the performance of the instrument, reagents, and the process, as a continue performance check.
 - a. Cluster Count: Value should be greater than 16-20 billion ($16 - 20 \times 10^9$) per run
 - b. Phasing/Prephasing: Values should be $> 0.1/0.1$ for read 1 and Read 4 to ensure the reagents are performing well and the temperatures are not varying.
 - c. % Occupied by %Pass Filter: Indicates if the run is over or underloaded to help assess the library pools that are being created
 - d. Q Score Distribution: Graphical capture of the Q30 scores. Should be greater than 75% of bases greater than or equal to Q30.

Raw Sequencing Data Files

1. The output files are automatically generated by the NovaSeq Control Software in the course of analysis. The files can be accessed for bioinformatic analysis either through direct access to Base Space, granted only to authorized individuals, or through the release of an external hard drive.
2. If IMF is providing bioinformatic analysis to the customer via internal processes or through the use of an approved vendor, a technical review of the NovaSeq run, to ensure the quality metrics have been met, must be completed prior to release of the data files to the customer.

Release of SNP data files

3. Data files resulting from bioinformatic analysis are only provided to the customer after a technical review of the NovaSeq run, to ensure the quality metrics have been met must be completed.

4. References

NovaSeq 6000 System Denature and Dilute Libraries Guide Document 1000000106351v03 November 2020
Sequencing Analysis Viewer Software Guide 15066069 v04 March 2019

5. Definitions

N/A