Hiseq 4000 Sequencing protocol

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Abstract

This is a protocol for running an illumina Hiseq4000 sequencer, following the process from the loaded flow cells through the sequencing run. The HiSeq 4000 uses a dual flow cell capable of processing over 400 Gb per day or 1.5 Tb per run, with up to 5 billion single reads. See other protocols for DNA extraction or library construction and loading of flow cells.

Citation: Scott Edmunds Hiseq 4000 Sequencing protocol. **protocols.io**
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Guidelines

See the illumina SOP here:
https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/hiseq4000/hiseq-4000-system-guide-15066496-05.pdf

Protocol

Step 1.

After priming, once the clustered flowcells are loaded the sequencing run can start. When you are ready, select "Start" to start the sequencing run. Throughout the run the operator needs to keep checking the metrics on the run overview screen, fluidics, and imaging.

Step 2.

Following completion of the 3rd cycle of sequencing, the first base confirmation dialog box opens automatically and generates a “First Base Report”. The run pauses at this step.

Step 3.

Review the First Base Report from the confirmation dialog box (the Cluster Density should be 2353 and intensity of each base is should be higher than 5000). If the results are satisfactory, select 'Continue'. Even if you don't pause the run, throughout the sequencing process you can find the “First Base Report” in the corresponding folder, and if the First Base Report result looks good the process can be left alone.

Step 4.

Keep inspecting the Sequencing Analysis Viewer software to evaluate the metrics. Select Refresh at any time during the run to view updated metrics, and select the corresponding storage address of where to store the data. Select “Imaging” and also inspect the tile images to ensure the run is in normal progress.

Step 5.

At cycle 30, select “Refresh” again in Sequencing Analysis Viewer to check for pF. The pF value should be

more than 50%, or rehybridisation is required. If problems such as abnormal focusing, decline of Q30, and low pF value appear in Read 2, rehybridisation cannot be performed and the run has to be aborted.If the results are satisfactory, select Continue and the run will continue until completion.

Step 6.

When the run is complete, unload and weigh reagents, and then perform an instrument wash.

Warnings

Safety guide is here:
https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/hiseqsiteprep/hiseq-4000-3000-safety-compliance-guide-15066491-02.pdf