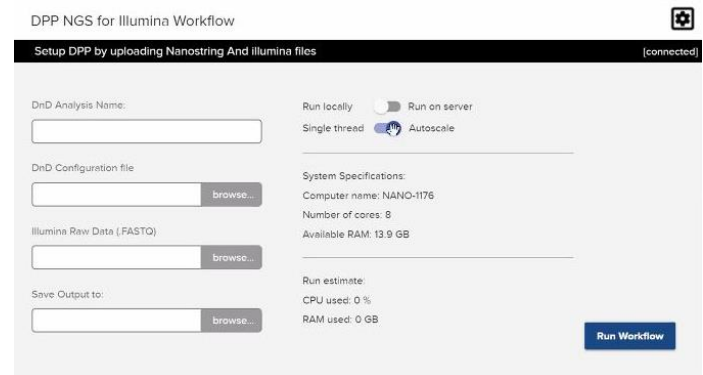


GeoMx NGS Pipeline v2.0 Software Release Notes

The GeoMx DSP technology expands its functionality through the release of **GeoMx NGS Pipeline software v2.0**, released in November 2020. The Pipeline uses a series of novel algorithms to process RNA sequencing files (FASTQ files) from Illumina sequencers and produce *.dcc* files, which can then be uploaded to the GeoMx DSP system for data analysis. The following is a summary of v2.0 features and known issues. Contact GeoMxSupport@nanoString.com for additional information and assistance.



Features

- Processes FASTQ files from Illumina sequencers according to parameters defined in the Configuration File (which is generated from the GeoMx DSP run).
- Outputs *.dcc* files, which can then be uploaded to the GeoMx DSP system for data analysis.
- A graphical user interface (GUI) which can be installed on Windows or MacOS. This GUI allows the user to browse to input files needed, designate an output location, choose server or local processing, and monitor progress.
- Users may also choose to use command line interface (CLI) to run the GeoMx NGS Pipeline on Linux systems.
- The Pipeline program itself may run locally or on a server (most common). Linux and Amazon Web Services (AWS) servers are supported.

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Feature Updates from v1.0

- Support for sample ID translator, allowing user-preferred sample name in lieu of sample name provided in the sample sheet.
- Improvements to processing including:
 - 10X faster than DNDv1.0 to support the processing of WTA data
 - Better performance allowing pipeline to run on a standard work computer/laptop for >96 segments worth data
 - No Docker requirement
 - No AWS Batch processing requirement to attain acceptable run times.
 - Submitting multiple jobs from the GUI to a server while a job is already processing will result in new run queueing functionality; you will be notified of position in the queue and as soon as resources from the server become available, the queued run will begin automatically.
 - Processing log and error log (if errors have occurred) from each run will be output within the user-specified output directory.
 - Upon installation of GeoMxNGSPipeline on a Linux Ubuntu server, user can specify a known listening port (other than the default, port 5000).
- Bug fixes and usability improvements.

Known Issues

- bcl to FASTQ file conversion software may be set to use custom headers; ensure the software you use to generate FASTQ files follows the FASTQ naming conventions set provided by Illumina, using the following link
https://support.illumina.com/help/BaseSpace_OLH_009008/Content/Source/Informatics/BS/NamingConvention_FASTQ-files-swBS.htm
- Do not modify FASTQ filenames from the [Illumina FASTQ file naming conventions](#). If possible, retain the naming from the sample sheets exported from DSP, which incorporate the plate barcode. If you must change the sample names, be advised the GeoMx NGS Pipeline software parses the Illumina-written portion of the file names to identify well/sample, lane, and read. In addition, if the sample name portions of your filenames are modified from the default pipeline workflow, you will need to update the names in your config file accordingly, or optionally use a sample ID translator, before you begin your NGS processing pipeline run.
- Processing FASTQ files for only a subset of the ROIs listed in the GeoMx NGS Pipeline (DND) configuration file will result in empty *.dcc* files for the missing ROIs. Uploading these empty *.dcc* files will overwrite any data that may exist for these segments from a previous DND run. The GeoMx NGS Pipeline configuration file can be revised to exclude any ROIs for which FASTQ files were previously processed. The resulting *.dcc* zip uploaded should then only update segments associated with blank data.

- Always review the summary.txt file to note any unexpected errors or zero reads for any intermediate steps for ROIs. Logs for any given job may be retrieved from the user specified output directory.
- If executing a run from the GUI, any errors or failures will be indicated in the notification menu and listed by segment name.