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Local Run Manager
VeriSeq PGS Analysis Module

Workflow Guide

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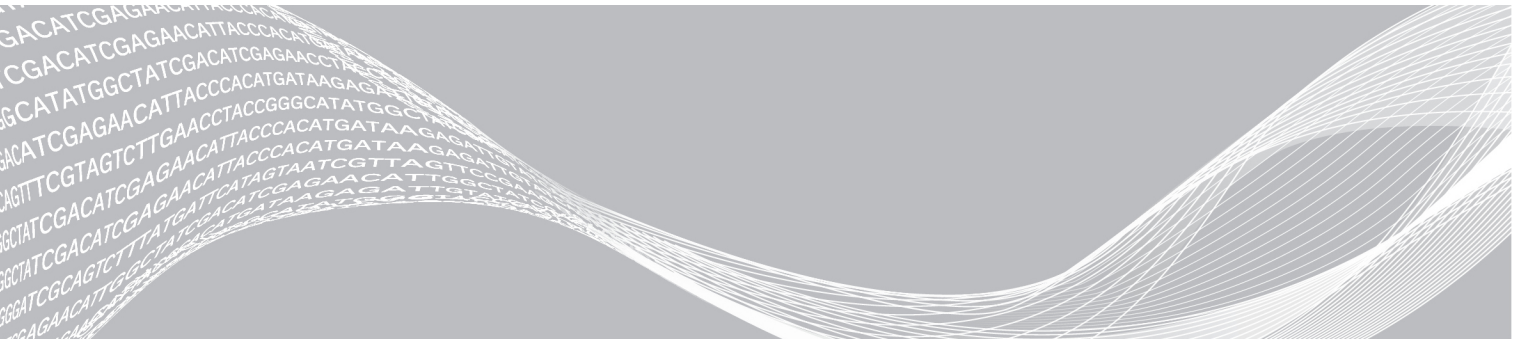

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Overview

The Local Run Manager VeriSeq™ PGS analysis module creates runs for sequencing and performs secondary analysis of the sequencing data by demultiplexing and aligning the reads to the human reference genome. BAM files from the sequencing system are imported directly into the BlueFuse™ Multi Analysis Software using the prepared sample sheet. This workflow is designed specifically for VeriSeq PGS libraries.

Input Requirements

The VeriSeq PGS analysis module requires the base call files (*.bcl) and the run summary files generated during the sequencing run. In addition to sequencing data files generated during the sequencing run, such as base call files, the VeriSeq PGS analysis module requires the following files.

- **Human reference genome**—The VeriSeq PGS analysis module requires the human reference genome for alignment.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis parameters for the VeriSeq PGS analysis module. For information about the Local Run Manager dashboard and system settings, see the *Local Run Manager Software Guide (document # 1000000002702)*.

NOTE

To import a run and sample sheet into the Local Run Manager dashboard, see the *MiSeq™ System Guide (document # 15027617)* and the *BlueFuse Workflow Manager for VeriSeq PGS User Guide (document #1000000028842)* on the Illumina® support site.

Set Parameters

1. If needed, log in to Local Run Manager.
2. Select **Create Run**, and select **VeriSeq PGS**.
3. Enter a run name that identifies the run from sequencing through analysis.
   The run name can contain alphanumeric characters and dashes.
4. Enter a run description to identify the run.
   The run description can contain alphanumeric characters, spaces, and the following special characters: `~!@#$%-_{}.

Specify Run Settings

1. Select the number of samples from the Number of Samples drop-down list.
   - **Up to 12 (Single Index)**
   - **Up to 24 (Dual Index)**
2. Select an index plate layout.
   - **Standard (recommended)**—Indexes are preassigned to samples.
   - **Custom**—Indexes can be selected for each sample. When the Number of Samples is set to Up to 12 (Single Index), you can select from the following I7 indexes: N701- N712. When the Number of Samples is set to Up to 24 (Dual Index), you can also select from the following I5 indexes: S503 and S504.
3. Select **Next**.

Specify Sample Defaults

1 Select a cell type from the Default Cell Type drop-down list.
- PB1 (Polar Body 1)
- PB2 (Polar Body 2)
- Blastomere
- Trophectoderm
- Genomic DNA
- Other

2 Select Apply Changes.

Specify Samples for the Run

Specify samples for the run using the following option:

- Enter samples manually—Use the blank table at the bottom of the Create Run screen.

Enter Samples Manually

1 Enter a cycle ID in the Cycle ID field.
The cycle ID can be pasted directly from a spreadsheet table or entered manually. Multiple cycle IDs can be pasted simultaneously. The cycle ID can contain alphanumeric characters and dashes.

2 Enter an embryo ID in the Embryo ID field.
The embryo ID can be pasted directly from a spreadsheet table or entered manually. Multiple embryo IDs can be pasted simultaneously. The embryo ID can contain alphanumeric characters and dashes.

3 [Optional] If you have multiple cell types, select the cell type for each sample from the Cell Type drop-down list.
The cell type is automatically populated with the Default Cell Type.

4 Calculate the dilution:

   a Enter the DNA concentration of the 1/10 diluted SurePlex reaction obtained from dsDNA quantification in the 1/10 dsDNA field.
   b Enter the volume of the 1/10 dilution that is transferred to the clean plate for library preparation in the Volume 1/10 dsDNA field.
   c The volume of MBG water required to prepare dsDNA at 0.2 ng/µl is populated in the Added Water field.

5 [Optional] If you chose a custom index plate layout, you can select an Index 1 (i7) in the Index 1 drop-down list.
By default, an optimal Index 1 layout is selected.

6 [Optional] If you chose a custom index plate layout for dual indexes, you can select an Index 2 (i5) in the Index 2 drop-down list.
By default, an optimal Index 2 layout is selected.

7 [Optional] Enter a sample description in the Description field.

8 [Optional] Select Plate Graphic to view the sample plate.
Wells with samples display the Cycle ID, Embryo ID, Index 1 (i7), and Index 2 (i5).

   a [Optional] Select Print to print the Plate Graphic, and then select Close.

9 Select **Save Run**.

Analysis Methods

The VeriSeq PGS analysis module performs the following analysis steps and then writes analysis output files to the Analysis folder.

- Generates a BAM file for each sample
- Generates run and sample-level statistics

BAM File Generation

The software generates BAM files to import into the BlueFuse Multi Analysis Software.

Statistics Evaluation

After a successful VeriSeq PGS sequencing run, the run and sample-level statistics are accessible.

View Analysis Results

1 From the Local Run Manager dashboard, select the run name.

2 From the Run Overview tab, review the sequencing run metrics.

3 To change the analysis data file location for future requeues of the selected run, select the **Edit** icon, and edit the output run folder file path.
The file path leading up to the output run folder is editable. The output run folder name cannot be changed.

4 **[Optional]** Select the **Copy to Clipboard** icon to copy the output run folder file path.

5 Select the Sequencing Information tab to review run parameters and consumables information.

6 Select the Samples & Results tab to view the analysis report.
- If analysis was requeued, select the appropriate analysis from the Select Analysis drop-down list.

7 **[Optional]** Select the **Copy to Clipboard** icon to copy the Analysis Folder file path.

Analysis Report

Analysis results are provided on the Samples & Results tab.

Run Metrics

Field Heading	Description
Total Clusters	The number of clusters in a run.
Cluster PF	The percentage of clusters passing filter.
Yield (kb)	The number of sequenced bases passing filter.
Aligned Reads PF (%)	The percentage of aligned reads passing filter.

Sample Metrics

Column Heading	Description
Sample No.	The number of each sample in a run.
Cycle ID	The Cycle ID of each sample entered when the run is created.
Embryo ID	The Embryo ID of each sample entered when the run is created.
Raw Reads	The number of sequencing reads in a run.
Aligned Reads (%)	The percentage of sequencing reads aligned to the reference genome.

Additional Analysis [Optional]

Additional analysis information is available from the following sources.

- Run-level and sample-level QC metrics for the sequencing run are available after the Local Run Manager VeriSeq PGS Analysis Module completes analysis. For more information, see the *VeriSeq PGS-MiSeq QC Assessment Guide* on the Illumina support site.
- For a visualization of the results, sample and sequencing run information can be imported into BlueFuse Multi Analysis Software. For more information, see the *BlueFuse Multi Software Guide (document # 15053620)* on the Illumina support site.

Analysis Output Files

The following analysis output files are generated for the VeriSeq PGS analysis module.

File Name	Description
BAM (*.bam)	Binary files used to represent aligned sequences.

BAM File Format

BAM files are binary files used to represent aligned sequences. A BAM file is the output file resulting from the sequencing data analysis. BAM files are required to import the data into the BlueFuse Multi Software Guide for visualization purposes.

Supplementary Output Files

The following output files provide supplementary information, or summarize run results and analysis errors. Although these files are not required for assessing analysis results, they can be used for troubleshooting purposes.

File Name	Description
AnalysisLog.txt	Processing log that describes every step that occurred during analysis of the current run folder. This file does not contain error messages.
AnalysisError.txt	Processing log that lists any errors that occurred during analysis. This file will be empty if no errors occurred.
LibraryQCRunStatistics.xml	Contains summary statistics specific to the run.

Analysis Folder

The analysis folder holds the files generated by the Local Run Manager software.

The relationship between the output folder and analysis folder is summarized as follows:

- During sequencing, Real-Time Analysis (RTA) populates the output folder with files generated during image analysis, base calling, and quality scoring.
- RTA copies files to the analysis folder in real time. After RTA assigns a quality score to each base for each cycle, the software writes the file to both folders.
- When the file is present, analysis begins.
- As analysis continues, Local Run Manager writes output files to the analysis folder, and then copies the files back to the output folder.

Folder Structure

- Thumbnail_Images
- Recipe
- Logs
- InterOp
- Data
- CopyLog_[timestamp]
- Config
- SoftwareVersionsFile.csv
- SampleSheet.csv
- runParameters.xml
- RunInfo.xml
- RTAComplete.txt
- ReportInfo.dat
- ImageAnalysis_Netcopy_complete.txt
- ImageAnalysis_Netcopy_complete_Read3.txt
- ImageAnalysis_Netcopy_complete_Read2.txt
- ImageAnalysis_Netcopy_complete_Read1.txt
- copy_on_hold.txt
- CompletedJobInfo.xml
- Basecalling_Netcopy_complete.txt
- Basecalling_Netcopy_complete_Read3.txt
- Basecalling_Netcopy_complete_Read2.txt
- Basecalling_Netcopy_complete_Read1.txt
- AnalysisError.txt
- AnalysisLog.txt
- Alignment_## or Alignment_Imported_##
  - [Timestamp of Run]
    - DataAccessFiles
    - Logging
    - Plots
    - [last 5 characters of flow cell barcode]-[Cycle ID]-[Embryo ID]_S[Sample #].CoverageHistogram.txt
    - [last 5 characters of flow cell barcode]-[Cycle ID]-[Embryo ID]_S[Sample #].bam.bai
    - [last 5 characters of flow cell barcode]-[Cycle ID]-[Embryo ID]_S[Sample #].bam

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

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South Korea	+82 80 234 5300	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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