

DRAGEN Microbial Enrichment Plus for MiSeq i100 v1.1.7

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1 App Highlights

DRAGEN Microbial Enrichment Plus offers a dedicated informatics solution with flexible analysis options for Illumina Infectious Disease and Microbiology target-capture enrichment panel kits. The app delivers easy-to-use, powerful secondary analysis of Illumina sequencing data, with workflows for sample QC, viral WGS (whole-genome sequencing), pathogen detection and quantification, and antimicrobial resistance (AMR) marker profiling. It also supports user-defined microorganism reporting thresholds and custom reference sequence analysis.

1.1 Input files

- FASTQ files
- User-defined microorganism reporting file in TSV or XLSX format (optional)
- Custom reference FASTA file (if applicable)
- Custom reference BED file (if applicable)

1.2 Analysis Pipeline

(all panels except where noted, (*) indicates applicable to custom reference sequence analysis)

1. **Read QC*** (optional)
2. **Dehosting*** (human read removal)
3. **Sample QC** (sample composition and enrichment factor calculations. Internal control required to calculate the enrichment factor) – RPIP, UPIP, VSP V2
4. **Microorganism classification** (configurable sensitivity) - RVOP, VSP, VSP V2
5. **Microorganism detection** (alignment, consensus generation, variant calling)
6. **Microorganism quantification** (quantitative internal control required) – RPIP, UPIP, VSP V2
7. **Microorganism reporting thresholds** (proprietary algorithms or user-defined reporting logic)
8. **Bacterial AMR marker analysis** (nucleotide and protein alignment, consensus generation, variant calling and annotation) – RPIP, UPIP
9. **Viral AMR marker analysis** (variant calling and annotation) – RPIP, RVOP, VSP, VSP V2
10. **Viral clade and lineage prediction** (Pangolin, Nextclade) – RPIP, RVOP, VSP, VSP V2
11. **Result filters** (user-specified filters applied)
12. **Reporting***

1.3 Output files

- Analysis-level outputs: **XLSX, HTML, ZIP**
- Sample-level outputs: **JSON, HTML, FASTA** (consensus sequences), **VCF** (viral variants)

1.4 Important Notes

DRAGEN Microbial Enrichment Plus is a secondary analysis tool for research use only. Further interpretation, statistical analysis, and downstream analysis of results may be necessary.

1.5 DRAGEN core on Gitbook

More information about the DRAGEN Microbial Enrichment Plus (Explify) pipeline can be found in core DRAGEN documentation:

<https://help.dragen.illumina.com/product-guide/dragen-v4.4/pipeline>

1.6 Demo Data

The [DRAGEN Microbial Enrichment Plus Demo Project](#) includes external control, contrived, and environmental samples prepared using the RPIP, UPIP, RVOP, VSP, and VSP V2 target-capture enrichment kits. Example custom reference sequence FASTA and BED files are also included.

2 Pipeline Configuration

DragenExplify_Settings

Parameter	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software used to process the DRAGEN Microbial Enrichment Plus pipeline, including conversion to FASTQ, specified using all three integers included in the DRAGEN version name. For example, 4.4.6.
AppVersion	Yes	The version of the workflow-specific application (i.e., DRAGEN Enrichment), using all three integers included in the version name. For example, 1.0.0.
KeepFastQ	Yes	Select whether FASTQs are saved (true) or discarded (false).
MapAlignOutFormat	Yes	Formatting of the output files. Accepted values are bam, cram, or none. Selecting none produces no map/align output. If MapAlignOutFormat is None, VariantCallingMode cannot be None for any sample.
TestType	Yes	Allowed values: RPIP, UPIP, VSPv2, VSPv1, RVOP, Custom
ReacQcEnabled	No	Allowed values: true, false
CustomReferenceFastaFile	Conditionally required	File name with a specific header format (see below). Required when TestType is Custom.
CustomReferenceBedFile	No	File name with specific columns, paired with custom reference FASTA file. Important for segmented viruses, especially. Tab-delimited .tsv file. Only applicable when TestType is Custom.
PreDefinedMicroorganismReportingList	No	Report filter setting
UserDefinedMicroorganismReportingListFile	No	Report filter setting
BelowThresholdEnabled	No	Report filter setting
QuantitativeInternalControlName	Conditionally required	Valid selections for UPIP and RPIP: Allobacillus halotolerans, Armored RNA Quant Internal Process Control, Enterobacteria phage T7, Escherichia virus MS2, Escherichia virus Qbeta, Escherichia virus T4,

		<p>Imtechella halotolerans, Phocid alphaherpesvirus, Phocine morbillivirus, Truepera radiovictrix, NONE</p> <p>Valid selections for VSPv2: Armored RNA Quant Internal Process Control, Enterobacteria phage T7, Escherichia virus MS2, Escherichia virus T4, NONE</p> <p>Required when TestType = RPIP, UPIP, VSPv2</p>
QuantitativeInternalControlConcentration	No	<p>Integer in scientific notion format: $###x10^{\#}$</p> <p>Default value is $1.21x10^7$</p> <p>Will accept more values beyond the decimal point, but the pipeline will only use the first 3 values after the decimal.</p> <p>Cannot be set when TestType = VSP, RVOP, Custom</p>
BacterialAmrMarkerMicroorganismRequired	No	Report filter setting
NextCladeEnabled	No	Allowed values: true, false
BacterialAmrMarkersOnly	No	Allowed values: true, false. Only applicable for RPIP and UPIP
ReadClassificationSensitivity	No	1-1000 (cannot be set to 0). Only applicable for RVOP, VSPv1 and VSPv2

DragenExplify_Data

Parameter	Required	Description
Sample_ID	Yes	Alphanumeric string with _ or - with no spaces allowed
ExternalControlFlag	No	Allowed values are positive, negative, blank, or none

2.1 Custom database FASTA file format

A custom reference FASTA file containing one or more reference sequences is required to run the custom reference sequence analysis. In the FASTA file, sequence names must be unique and should not contain any spaces. If there is any space in the FASTA header, the part before the first space is assumed to be the sequence name. It is recommended to use only the following in sequence names: alphabets, numbers, underscore (_), hyphen (-), parentheses ((,)), and period (.). Otherwise, the sequence names may appear different in the output.

3 Modes of Operation

3.1 Cloud and Local Execution:

DRAGEN can be scheduled to operate in the following modes:

- Cloud:
 - Run planning:
 - Occurs in the Cloud via the Cloud User Interface (UI), or alternatively, sample sheets can be imported into the Cloud UI
 - Sequencing execution:
 - Runs are identified on the sequencer, and are executed locally on the instrument
 - DRAGEN Execution:
 - Occurs in the Cloud automatically after sequencing is complete
- Local:
 - Run planning:
 - Occurs on the instrument via the Instrument UI, or alternatively, sample sheets can be imported into the Instrument UI
 - Sequencing execution:
 - Runs are executed locally on the instrument
 - DRAGEN Execution:
 - Occurs locally on the instrument, automatically after sequencing is complete

3.2 Requeues:

After a sequencing run is complete, it may be desirable to run or re-run DRAGEN with, for example, an updated configuration. Requeues can occur on the instrument or in the Cloud. For on-instrument requeues, the following pre-requisites are necessary:

- The BCL's must be stored on the instrument
- The system must be idle

4 Output Files

4.1 Summary

- Analysis-level outputs: **XLSX, CSV, HTML, ZIP**
- Sample-level outputs: **JSON, CSV, HTML, TXT.GZ**

4.2 Toplevel DRAGEN

<run_id>/Analysis/<no>/inputs

- <SampleSheet>.csv

<run_id>/Analysis/<no>/Data

- Secondary_Analysis_Complete.txt
- summary
 - <x.y.z> (Note: DRAGEN version)
 - highlevel_summary.json
 - detailed_summary.json
- AggregateReports
 - report.html
 - report_files
 - *Links to workflow level reports*
- Demux
 - AggregateReports
 - *Links to lower-level reports*
 - Demultiplex_Stats.csv
 - *other stats*
- Dragen<Workflow>
 - Data (including FASTQ) for samples configured for the workflow (see below)
- RunInstrumentAnalyticsMetrics
- logs
 - *high-level logs*

4.3 Workflow Level Output

<run_id>/Analysis/<no>/Data/DragenMicrobialEnrichmentPlus

- Dragen<workflow>
 - AggregateReports
 - *Links to workflow specific sample level reports*

- fastq (or ora_fastq)
 - <sample_ID>.S0_Rm_001.fastq.gz or *.fastq.ora (m=1-2)
 - *Additional samples*
 - Reports
 - *Adapter_Metrics.csv*
 - *Quality_Metrics.csv*
 - *Additional metrics files*
- <sample_ID>
 - sample-specific outputs
 - logs
 - *Sample specific logs*
- logs
 - *Workflow specific logs*

5 How To Install DRAGEN and DRAGEN Applications

5.1 Install DRAGEN Versions:

- When a new DRAGEN version is available, download the DRAGEN installer (*.ires) from the MiSeq i100 Series support page. Save the installer locally or to a network drive.
- Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
- Select the instrument icon to open the global navigation menu.
- Select Settings, and then select DRAGEN.
- On the Versions tab, select Install version.
- Navigate to the installer, and then select Open.
- Select Install. A message indicates if the installation was successful or failed

5.2 Uninstall DRAGEN Versions:

- Select the instrument icon to open the global navigation menu.
- Select Settings, and then select DRAGEN.
- To uninstall a previous DRAGEN version, do as follows.
 - a. On the Versions tab, select the ellipsis icon in the Actions column.
 - b. Select Uninstall.
 - c. Select Yes, uninstall.
- To uninstall the latest DRAGEN version, do as follows.
 - a. On the Versions tab, select the ellipsis icon in the Actions column.
 - b. Select Uninstall all.
 - c. Select Yes, uninstall all

5.3 Application Installation:

- Download the application (*.iapp) from the MiSeq i100 Series support page. Save the installer to a network drive.
- Select the instrument icon to open the global navigation menu.
- Select Settings, and then select Applications.
- Select Install application.
- Navigate to the application file, and then select Open. After the file uploads, information about the application displays.
- Select Install. After the application installs, you can review the application configuration.

5.4 View Application Settings:

The DRAGEN application provides a default library prep kit, index adapter kit, read information, index information, and permissions. Some applications also provide settings and configuration for secondary analysis.

- Select the instrument icon to open the global navigation menu.
- Select Settings, and then select Applications.
- Select the application to view. After you install an application, the Configuration screen opens automatically.
- Edit any of the following information:
 - Library prep kits
 - Index adapter kits
 - Index reads
 - Read type
 - Index lengths
 - Read length
- Select Save.

5.5 Uninstall Applications

Administrators can uninstall applications.

- Select the instrument icon to open the global navigation menu.
- Select Settings, and then select Applications.
- Select the application to uninstall.
- Select Uninstall.
- Confirm to uninstall the application.

Release History

Revision	Release Reference	Originator	Description of Change
00	1130656	Mark Bilstad	Initial release