# DRAGEN 16S Plus for MiSeq i100 v1.1.7

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# 1 App Highlights

The DRAGEN 16S Plus application is a rapid, kmer-based informatics solution designed for microbial classification and community profiling from mixed flora and metagenomic sample types. The app delivers easy-to-use, powerful secondary analysis of Illumina 16S sequencing data, with workflows for read QC (optional), taxonomic classification, result filtering (optional), and reporting. It also supports custom database analysis.

## 1.1 Input files

- FASTQ files
- Custom database FASTA file (if applicable)

## 1.2 Analysis Pipeline

1. **Read QC** (optional)
2. **Taxonomic classification**
3. **Result filters** (optional read count threshold)
4. **Reporting**

## 1.3 Output files

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

## 1.4 Important Notes

DRAGEN 16S Plus is a secondary analysis tool for research use only. Further interpretation, statistical analysis, and downstream analysis of results may be necessary.

For questions on this application please contact Illumina Technical Support at techsupport@illumina.com.

## 1.5 DRAGEN core on Gitbook

More information about the amplicon pipeline can be found in core DRAGEN documentation:

https://help.dragen.illumina.com/product-guide/dragen-v4.4/dragen-16s-pipeline

## 1.6 Demo Data

The DRAGEN 16S Plus Demo Project includes 21 samples prepared using the Illumina 16S metagenomic sequencing library preparation protocol. An example custom reference sequence FASTA file is also included.

# 2 Pipeline Configuration

**Dragen16sPlus_Settings**

| Parameter | Required | Description |
|---|---|---|
| SoftwareVersion | Yes | The version of the DRAGEN software used to process the DRAGEN 16s 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. |
| Database | Yes | Select input database: Refseq-RDP-v1] or Custom |
| CustomDatabaseFastaFile | Conditionally required | Alphanumeric string with "_", "-", or "." and no spaces. File name with a specific header format.<br>Required when Database = Custom. Cannot be provided when Database != Custom |
| ReadQcEnabled | No | true, false |
| ReadCountThreshold | No | 0 to 1000, inclusive |
| PrimerTrimming | No | **(CASE INSENSITIVE)** none, length, ilmn_v3-v4_length, ilmn_v1-v6_length<br><br>Notes:<br>• ilmn_v3-v4-length signifies an application hardcoded ForwardPrimerLength=17, ReversePrimerLength=21.<br>• ilmn_v1-v6-length signifies an application hardcoded ForwardPrimerLength=20, ReversePrimerLength=19<br>• ForwardPrimerLength and ReversePrimerLength are only applicable when 'length' is chosen as the PrimerTrimming option. |
| ForwardPrimerLength | No | 1-100, inclusive<br>only applicable when PrimerTrimming is 'length' |
| ReversePrimerLength | No | 1-100, inclusive<br>only applicable when PrimerTrimming is 'length' |

**Dragen16sPlus_Data**

| Parameter | Required | Description |
|---|---|---|
| Sample_ID | Required | Alphanumeric string with _ or - with no spaces allowed |

## 2.1 Custom database FASTA file format

Custom database FASTA files:

A custom database FASTA file containing up to 500 million basepairs of reference sequence may be specified using the exact FASTA header format defined below. In the FASTA file, the SequenceID should not contain any spaces. All sequences must have seven canonical taxonomic rank prefixes specified: k__;p__;c__;o__;f__;g__;s__. However, these can all be left blank except for (k)ingdom and (s)pecies designations, which are required.

Example custom database FASTA header format:

```
>SequenceID_001:k__Fungi;p__Glomeromycota;c__Glomeromycetes;o__Glomera
les;f__Glomeraceae;g__;s__uncultured_Glomus
```

When uploading the file to the instrument, select the file type as "CustomFastaFile"

# 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/Dragen16sPlus

- 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 |