

DRAGEN BCL Convert for MiSeq i100 v1.1.8

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1 App Highlights

The DRAGEN BCL Convert Pipeline includes the following functional components:

- Decompression of input BCL data
- Sample demultiplexing
- Generation of FASTQ files
- Compression of FASTQ files to DRAGEN ORA or gzip formats
- Generation of FASTQ QC Metrics
- Additional Sample Sheet parameters

The following inputs are required:

- BCL Data generated from the instrument
- RunInfo.xml
- Sample Sheet

See Pipeline Configuration for Sample Sheet parameters

1.1 Core BCL Convert information on Gitbook

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

2 Pipeline Configuration

BCLConvert_Settings

Parameter	Required	Description
SoftwareVersion	Yes	The version of the software to be used to perform BCL conversion on the Sample_ID's that only exist in the BCL Convert Data section of the sample sheet., The version is specified using all three integers included in the DRAGEN version name. For example, 1.0.0.
FastqCompressionFormat	Yes	The compression format for the FASTQ output files. Allowed values are gzip or dragen.
GenerateFastqcMetrics	No	Enable/disable generation of FAST QC Metrics. Default = False. Not applicable for the Cloud pipeline mode.
CreateFastqForIndex Reads	No	See https://help.dragen.illumina.com/product-guide/dragen-v4.4/bcl-conversion
TrimUMI	No	See https://help.dragen.illumina.com/product-guide/dragen-v4.4/bcl-conversion
NoLaneSplitting	No	Import option only. See https://help.dragen.illumina.com/product-guide/dragen-v4.4/bcl-conversion
LibraryInputVolume	No	Input volume for Library QC calculations, float > 0

BCLConvert_Data

Parameter	Required	Description
AdapterRead1	No	The sequence of the Read 1 adapter to be masked or trimmed. To trim multiple adapters, separate the sequences with a plus sign (+) indicating independent adapters that must be independently assessed for masking or trimming for each read. Characters must be A, C, G, or T. A value of na (case insensitive) must be used if: <ul style="list-style-type: none"> the AdapterRead1 field is placed in the Data section of the Sample Sheet, and no adapter trimming is desired for the sample
AdapterRead2	No	See description of AdapterRead1, applied to AdapterRead2.
BarcodeMismatchesIndex1	No	Specifies barcode mismatch tolerance for Index 1. Possible values are 0, 1, or 2, or na. The default value is 1. Only allowed if Index is specified in RunInfo.xml file and in the Reads section of the Sample Sheet. A value of na must be used if: <ul style="list-style-type: none"> BarcodeMismatchesIndex1 exists in the Data section of the Sample Sheet, and

		<ul style="list-style-type: none"> • <code>OverrideCycles</code> exist in the data section, and • Index 1 is masked out for the sample in the <code>OverrideCycles</code>
<code>BarcodeMismatchesIndex2</code>	No	See description of <code>BarcodeMismatchesIndex1</code> , applied to <code>BarcodeMismatchesIndex2</code> .
<code>OverrideCycles</code>	No	<p>Specifies the sequencing and indexing cycles to be used when processing the sequencing data.</p> <p>The <code>OverrideCycles</code> mask elements are semicolon separated. The <code>OverrideCycles</code> setting can be specified in one of the following formats, where the two formats cannot be mixed:</p> <p>Order Dependent: <code>OverrideCycles,U7N1Y143;I8;I8;U7N1Y143</code></p> <p>Order Independent (examples): <code>OverrideCycles,R1:U7N1Y143;I1:I8;I2:I8;R2:U7N1Y143</code> <code>OverrideCycles,R1:U7N1Y143;R2:U7N1Y143;I1:I8;I2:I8</code></p> <p>Must adhere to the following requirements:</p> <ul style="list-style-type: none"> • Must be same number of fields (delimited by semicolon) as sequencing and indexing reads specified in <code>RunInfo.xml</code> and in the Reads section of the Sample Sheet. • Indexing reads are specified with I, sequencing reads are specified with Y, UMI cycles are specified with U, and trimmed reads are specified with N. • The number of cycles specified for each read must equal the number of cycles specified for that read in the <code>RunInfo.xml</code> file and in the Reads section of the Sample Sheet. • Only one Y or I sequence can be specified per read. <ul style="list-style-type: none"> • 'I' cycles can only be specified for index reads • 'Y' cycles can only be specified for genomic reads • The total number of cycles used for demultiplexing each index cannot exceed 27. Note that this includes all cycles between the first "I" cycle used by any sample and the last "I" cycle used by any sample within each index. <ul style="list-style-type: none"> ○ "I5N3;I5N3" : counts as 5 bases toward the limit of 27 for Index1, and as 5 bases toward the limit of 27 for Index2 ○ "I4N1I3;I5N3": counts as 8 bases toward the limit of 27 for Index1, and as 5 bases toward the limit of 27 for Index2 <p>The following are (order-dependent) examples of <code>OverrideCycles</code> input: <code>U8Y143;I8;I8;U8Y143</code> <code>N10Y66;I6;N10Y66</code></p> <p>For a sample sheet containing two samples having the following <code>OverrideCycles</code></p>

		<ul style="list-style-type: none"> • Y151; I8N2; N10; Y151 • Y151; N2I8; I8N2; Y151 <p>the number of cycles used for demultiplexing sums to 18.</p>
Sample_ID	Yes	<p>ID for the sample with the following requirements:</p> <ul style="list-style-type: none"> • Must be alphanumeric string with _ or - and no spaces. • Case sensitive (i.e., a Sample Sheet with the samples MySample and mysample is not allowed) • The same Sample_ID may exist on more than one row of the Sample Sheet (e.g., one sample spanning more than one lane) • Undetermined is not allowed as a Sample_ID
Lane	Yes	<p>Specifies FASTQ files only for the samples with the specified lane number.</p> <p>Must adhere to the following requirements:</p> <ul style="list-style-type: none"> • Must be an integer • Value must be in the range of lanes specified in RunInfo.xml • Ranges are not supported with '-' or '+' • If not supplied, it is assumed that all samples are present in all lanes specified in the RunInfo.xml • If supplied, only lanes specified in the column will be converted from BCL to FASTQ <p>For MiSeq i100, values must be in the range of lanes specified in RunInfo.xml</p> <ul style="list-style-type: none"> • <ul style="list-style-type: none"> ○ 5M, 25M Flow Cells: 1-1 ○ 50M Flow Cell: 1-2 ○ 100M Flow Cell: 1-4
Index	Yes	<p>The Index 1 (i7) index sequence. Format is a sequence using ACTG. Required if index cycles are specified for the sample in the OverrideCycles.</p> <p>Must adhere to the following requirements:</p> <ul style="list-style-type: none"> • Can only contain A, C, G, or T • Length of string must match number of first index cycles in RunInfo.xml or the number specified in OverrideCycles <p>A value of na must be used if:</p> <ul style="list-style-type: none"> • No indexes are specified in OverrideCycles for a sample
Index2	No	See description of Index, applied to Index2.



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3 Modes of Operations

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 requeues, the following pre-requisites are necessary in Local Mode:

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

4 Output Files

4.1 Toplevel DRAGEN Output

<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
 - Etc.
- BCLConvert
 - fastq files and FastQC results for samples configured for BCL Convert (see <Workflow> Details below)
- RunInstrumentAnalyticsMetrics
- logs
 - high-level logs
 - nextflow.log
 - Etc.

4.2 <Workflow> Details

<run_id>/Analysis/<no>/Data/BCLConvert

- <workflow>
 - AggregateReports
 - *Links to workflow specific sample level reports*
 - fastq (or ora_fastq)
 - <sample_ID>.S0_L00n_Rm_001.fastq.gz or *.fastq.ora (n=1-8, m=1-2)
 - *Additional samples*
 - Reports

- *Adapter_Metrics.csv*
- *Quality_Metrics.csv*
- *Additional metrics files*
- *<sample_ID>*
 - *<workflow>_seq*
 - *<workflow>-specific logs*
 - *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