



Title: DRAGEN Single Cell RNA v4.2.7 for NextSeq 1000/2000

Customer Release Notes

Part Number: N/A

Document Number: 200052833, Version: 00

Effective Date: 27-FEB-2024

Page 1 of 8

Customer Release Notes

DRAGEN Single Cell RNA v4.2.7

for NextSeq 1000/2000



Table of Contents

INTRODUCTION	3
NEW FEATURES.....	3
NOTES	4
REFERENCE GENOME USAGE RECOMMENDATIONS	4
HOW TO UPDATE INSTRUMENT REFERENCE GENOMES	5
FIXED ISSUES	5
INSTALLATION INSTRUCTIONS	6
ONLINE INSTALLATION OF WORKFLOWS	6
OFFLINE INSTALLATION OF WORKFLOWS	7
RELEASE HISTORY	8

INTRODUCTION

These Release Notes detail the key changes to the DRAGEN Single Cell RNA Workflow onboard the NextSeq 1000/2000 instrument, since the v3.10.12 release. If you are updating from an older version, please see all intermediate release notes.

NEW FEATURES

- DRAGEN™ v4.2 generally offers significant improvements in accuracy, added features for a more comprehensive solution, and efficiency improvements. For full extensive details on each feature of pipeline, please consult the latest Illumina DRAGEN™ Bio-IT Platform User Guide available on the support website at <https://support.illumina.com/downloads/illumina-dragen-bio-it-platform-user-guide.html>
- **DNA and RNA Alignment updated.**
 - DRAGEN mapper implemented a much more rigorous method for determining split-read alignments and influencing primary alignments and MAPQs with split-read analysis.
 - This sophisticated new method can support up to a maximum of 4095 secondary alignments and 4095 supplementary alignments per read.
- **Single Cell RNA**
 - Variable size blocks in single-cell barcodes are now correctly processed.
 - Memory usage during single cell RNA processing reduced by upwards of 20%.
 - Single-cell barcodes can now be marked as reverse complemented.
 - Cell barcodes of up to 40bp are now supported.
 - A filtered version of the cell-by-gene matrix containing only valid barcodes is now also generated.
 - New Outputs:
 - Cell-by-peak matrices in mtx format
 - Full matrix with all cell barcodes
 - Filtered matrix with cell barcodes corresponding to called cells
 - Per-sample metrics
 - Per-barcode metrics
- Output file differences vs 3.10:
 - The output BAM/CRAM is no longer named in pattern <sample_name>.cram. The filename is now <sample_name>.scRNA.cram. Note the extra ".scRNA". This applies to the cram/bam and their md5sum and index files.
 - Temporary files "ref_bitmask.bin" and "sjdb.annotations.bin" are removed (same as RNA)
 - "scRNA.metrics.csv" has been renamed to "scRNA_metrics.csv". Note the "." replaced with "_"
 - "scRNA.matrix.mtx" is now gzipped to "scRNA.matrix.mtx.gz", AND a new file "scRNA.filtered.matrix.mtx.gz" is added.
 - A new file "scRNA.filtered.barcodes.tsv.gz" is added.
 - "scRNA.genes.tsv" is now gzipped to "scRNA.genes.tsv.gz"
 - "scRNA.barcodes.tsv" is now gzipped to "scRNA.barcodes.tsv.gz"
- scATAC is not enabled for the on-board workflow.

NOTES

Reference Genome Usage Recommendations

Germline, Enrichment Germline, Amplicon Germline Workflows

- Use ALT-Masked mapping.
- Use Graph genomes.
- Use the Homo sapiens [1000 Genomes] hg38 Alt Masked Graph v3 reference genome.
- If not using hg38, the Homo sapiens [UCSC] hg19 Alt Masked Graph v3 or Homo sapiens [NCBI] hs37d5 v3 Graph are recommended.
- Non-graph is supported, but has reduced accuracy

Enrichment Somatic, Amplicon Somatic, RNA and scRNA Workflows

- Use ALT-Masked mapping.
- Use non-Graph genomes.
- Use the Homo sapiens [1000 Genomes] hg38 Alt Masked v3 reference genome or Homo sapiens [UCSC] hg19 Alt Masked v3 reference genome.

Table 1 v4.2 Reference Support and Recommended Use for Human Data

Human		hg19	hs37d5	hg38	chm13	Recommended Reference Type
Germline	SNV	Yes	Yes	Yes	Yes	Graph
	CNV	Yes	Yes	Yes	Yes*	Graph
	SV	Yes	Yes	Yes	Yes*	Graph
	Expansion Hunter	Yes	Yes	Yes	No	Graph
	Targeted Callers	Yes	Yes	Yes	No	Graph
	RNA	Yes	Yes	Yes	Yes*	Non-Graph
	De Novo	Yes	Yes	Yes	Yes*	Graph
	Joint Genotyping	Yes	Yes	Yes	Yes*	Graph
	Biomarkers (HLA)	Yes	Yes	Yes	Yes*	Graph
	Gvcf Genotyper	Yes	Yes	Yes	Yes*	Graph
Somatic	SNV	Yes	Yes	Yes	Yes*	Non-Graph
	UMI SNV	Yes	Yes	Yes	Yes*	Non-Graph
	CNV	Yes	Yes	Yes	Yes*	Non-Graph
	SV	Yes	Yes	Yes	Yes*	Non-Graph
Methylation	Methylation	Yes	Yes	Yes	No	Non-Graph
Annotation	Nirvana	Yes	Yes	Yes	No	n/a

(*) DRAGEN™ supports the component execution; however, the component's accuracy has not been established.

Table 2 v4.2 Reference Support and Recommended Use for Non-Human Data

Non-Human		Supported	Recommended Reference Type
Germline	SNV	Yes	Non-Graph

	CNV	No	n/a
	SV	Yes	Non-Graph
	Expansion Hunter	No	n/a
	Targeted Callers	No	n/a
	RNA	Yes	Non-Graph
	De Novo	Yes	Non-Graph
	Joint Genotyping	Yes	Non-Graph
	Biomarkers (HLA)	No	n/a
	Gvcf Genotyper	Yes	Non-Graph
Somatic	SNV	No	n/a
	UMI SNV	No	n/a
	CNV	No	n/a
	SV	No	n/a
Methylation Annotation	Methylation	No	n/a
	Nirvana	Yes	n/a

How to Update Instrument Reference Genomes

You can only import new reference genomes from the administrator account.
 Steps:

1. Download the desired genome package tar.gz from the NextSeq 1000/2000 Software Downloads page, OR
2. Create a reference genome using the Reference Builder for Illumina Instruments BaseSpace Sequence Hub app. For more information, refer to Reference Builder for Illumina Instruments v1.0.0 App Online Help.
3. Select the control software menu, and then select **Process Management**. Make sure that there are no sequencing runs or on-instrument secondary analyses in progress.
4. From the control software menu, select **Minimize Application**.
5. Log into ilmadmin.
6. Select the control software menu, and then select **DRAGEN**.
7. In the Genome section, select **View Installed Genomes** to view a list of all currently installed genomes.
8. Close the window.
9. Under Import New Reference Genomes, select Choose.

Navigate to the reference genome file (*.tar.gz) on the portable or mounted network drive, and then select **Open**.

FIXED ISSUES

Fixed issues of the DRAGEN™ v4.2.7 Single Cell RNA Workflow

Component	Defect ID	Issue Description
scRNA	DRAGEN-20879	Fix for Single cell scRNA.barcodeSummary.csv reporting incorrect UMI counts

scRNA	DRAGEN-24741	Fix to allow pre-signed URLs for UMI files in FASTQ list.csv
scATAC	DRAGEN-22854, SET-4565	Fix for Feature/Peak ID missing in scRNA/scATAC output
QC Metrics, scRNA	DRAGEN-15950	Fix a run-to-run variation in scRNA on bdbio
scRNA	DRAGEN-16360	Fix for DRAGEN on NextSeq 2000: Single Cell RNA hang-up
scRNA	DRAGEN-15329	Fix the number of non-null entries in the matrix.mtx file (feature counting)

INSTALLATION INSTRUCTIONS

DRAGEN v4.2.7 workflows are compatible with Control Software 1.7.x. For information regarding compatibility with other Control Software versions, please reference the NextSeq 1000/2000 Compatible Products page on the Illumina support site. If you would like to update the control software, please follow the steps detailed in the NextSeq 1000/2000 Control Software Suite v1.7.x Release Notes on the Illumina Support Site.

Online Installation of Workflows

If the instrument is connected to the internet, you can install DRAGEN workflows directly from the Control Software. Online installation of workflows is available since Control Software v1.3 or later.

Steps:

1. Make sure that you have the password to the *ilmnadmin* account.
2. Log in to the *ilmnadmin* account:
 - a. If you are logged in as *ilmnuser*, and in control software, select the control software menu, and then select **Exit Application** to access the desktop.
 - b. Select the power button icon in the upper right corner and log out of *ilmnuser*.
 - c. After you are on the login screen, select *ilmnadmin*, and then enter the password to log in.
 - d. The control software automatically launches once you are logged in.
3. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
4. On the control software menu, select **DRAGEN**. Under Version, the Available Workflows section lists the workflows currently installed on the system.
5. Select **Check Online**. Not all DRAGEN versions and workflows are compatible with online installation. Use offline installation for additional workflows.
6. Select the checkbox for the workflows that you would like to install. **NOTE: For Online Updates, DRAGEN BCL Convert must be installed before or with other workflows of the same DRAGEN Version.** You can view information about the latest version of a workflow in the release notes.
7. Select Install to start installation.

8. Enter *ilmnadmin* for the system password, and then select Authenticate.
9. After installation is complete, you will be navigated back to the DRAGEN screen and can view the updated list of installed DRAGEN workflows.

Offline Installation of Workflows

Steps:

1. When a DRAGEN workflow update is available, download the installer (*.tar.gz) from the NextSeq 1000/2000 Sequencing System support page. Save the installer to a local or portable drive.
2. If you saved the installer to a portable drive, plug the drive into a USB 3.0 port, located on both the side and back of the instrument. Gently move the instrument as needed to access the back.
3. Follow steps 1-3 above to log in to *ilmnadmin*.
4. Select the control software menu, and then select **DRAGEN**.
5. Under Version, select **Browse for New Version** to navigate to the installer.
6. Select Install to start installation.
7. Enter *ilmnadmin* for the system password, and then select Authenticate.
8. After installation is complete, you will be navigated back to the DRAGEN screen and can view the updated list of installed DRAGEN workflows.

Note that starting with control software v1.5, it is possible to uninstall previous versions of DRAGEN workflows.

RELEASE HISTORY

Revision	Release Reference	Originator	Description of Change
00	CN 1103441	Yi Lian	Initial release