

Customer Release Notes

DRAGEN Amplicon v4.2.7

for NextSeq 1000/2000

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INTRODUCTION

These Release Notes detail the key changes to the DRAGEN Amplicon 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 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>
- **Reference Genome**
 - Enhanced multigenome (graph) reference v3 improves small variant calling accuracy.
 - DRAGEN v4.2 workflows require the installation of new reference genome Hash Tables (v9).
 - Pre-built instrument reference genomes can be downloaded from the NextSeq 1000/2000 Sequencing System Software Downloads page, for offline installation.
 - Please reference the NextSeq 1000/2000 Compatible Products page on the Illumina support site, for the list of available reference genomes and recommended genomes for different workflows.
 - New reference updates for hg38 improves variant calling in the Challenging, Medically Relevant Genes (CMRG) regions.
 - GRCh38 reference includes 34 sequences from chm13 and hs37d5 as decoys.
 - 29 decoys identified as missing segmental duplications.
 - 5 decoys identified in acrocentric arms of chromosomes 13, 14, 15, and 22 of CHM13.
 - Yield accuracy improvements in the CMRG genes: FANCD2, MAP2K3, KCNJ18, and KMT2C, as well as in the Y chromosome.
- **Mapper/Aligner**
 - 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.
 - New skew normal insert size model and pairing penalty function
 - Skew normal insert model better fits observed real-world asymmetric insert size distributions.
 - New PDF-based pairing penalty to avoid excessively penalizing larger insert size proper pairs.
 - Enabled by default for DNA pipelines, resulting in increased proper pairing rate.

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	Illumina Annotation Engine (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	Methylation	No	n/a
Annotation	Illumina Annotation Engine (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 ilmnadmin.
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**.

KNOWN ISSUES

Known issues of the DRAGEN™ v4.2.7 Amplicon Workflow

Comp	ID	Summary	Resolution/Workaround
Amplicon	DRAGEN-29540, SET-7374	In Amplicon analysis, a validated deletion at the end of the read was not present in alignment	No workaround. A fix has been made to future version.
Amplicon	DRAGEN-29572	In Amplicon analysis, there is an FN at the edge of amplicon targets if "vc-remove-all-soft-clips" is false.	Enable "vc-remove-all-soft-clips" to remove reads supporting the event.
SNV Germline	DRAGEN-26359	Small regression in INDEL sensitivity in v4.2 compared to v4.0	Single sample SNV has major accuracy improvements due to graph and reference and machine learning updates. In some cases, such as INDEL, there are very minor changes where precision may improve at cost of recall, and vice versa. For information only.
SNV Somatic	DRAGEN-22241	Some T/N and T/O samples have >5% runtime regression relative to v4.0	No workaround
SNV Somatic	DRAGEN-24060	Small regression in INDEL FP across most T/O WGS and WES test datasets	Single sample SNV has major accuracy improvements in v4.2 due to graph mapper and reference updates. In some cases, such as INDEL, there are very minor changes where precision may improve at cost of recall, and vice versa. For information only.
SNV Somatic	DRAGEN-29580	Padding an interval does not always produce the same results as running with larger interval, for somatic VC.	Specific to Somatic VC. Use larger regions instead of padding. A fix has been made to future version.
SNV VC	DRAGEN-25905	Hang observed on high depth samples, when target BED is used to run the SNV caller over regions which are close to the end of a chromosome.	Have more BED regions throughout the chromosome or increase bin memory.
SNV VC	DRAGEN-25933	VCF GQ values may not match VCF specification	In most positions, the probability that the position is a variant is very close to 1 and the impact is negligible. In corner cases where p(0/0) is not negligible, we have the wrong value in the GQ field. For information only.

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