Customer Release Notes

DRAGEN RNA v4.2.7

for NextSeq 1000/2000

Table of Contents

INTRODUCTION

These Release Notes detail the key changes to the DRAGEN 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.
- **Gene Fusion**
  - Gene fusion scoring model is updated based on an improved truth set and aligner.
  - Fusions involving chrM genes are filtered by default.
  - Fusion candidates with multiple overlapping genes are reported as separate entries by default.
  - Added PSPH to the list of repetitive genes prioritized over paralogs for gene fusion calling.
  - Fixed a bug on GTF parser to handle whitespaces in attributes - they are not treated as attribute delimiters when surrounded by quotations.
- **Enhanced Bulk-RNA QC metrics**
  - Added Mapping/Aligning Summary metrics to account for detected abundant sequences.
  - Added Quantification metrics to check RNA-seq coverage quality.
- Metrics enhancements details
  - Added mapping/aligning summary metrics to account for detected abundant sequences. New metrics are in `mapping_metrics.csv` file.
    - rRNA filtered metrics:
      ```
      Filtered rRNA read count and % of total reads:
      Filtered rRNA reads,n,%
      ```
    - Excluded ChrM read count and % of total reads:
      ```
      Mitochondrial reads excluded,n,%
      ```
    - Mapped reads adjusted by adding rRNA & ChrM counts:
      ```
      Mapped reads adjusted for filtered mapping,n,%
      Mapped reads adjusted for excluded mapping,n,%
      Mapped reads adjusted for filtered and excluded mapping,n,%
      ```
    - Unmapped reads adjusted by subtracting rRNA & ChrM counts:
      ```
      Unmapped reads adjusted for filtered mapping,n,%
      Unmapped reads adjusted for excluded mapping,n,%
      ```

```
Unmapped reads adjusted for filtered and excluded mapping,n,%
```

NOTE: If optional CONTIG name not specified, the default for current reference will be used if possible

- Added Quantification metrics to check RNA-seq coverage quality. New metrics below are in `quant_metrics.csv` file.
  - Gene counts (previously only had transcript counts)

```
Total Genes
Coding Genes
Number of genes with coverage > 1x,n,%
Number of genes with coverage > 10x,n,%
Number of genes with coverage > 30x,n,%
Number of genes with coverage > 100x,n,%
```

  - Fold coverage of genomic regions

```
Fold coverage of all exons (i.e all biotypes)
Fold coverage of introns
Fold coverage of intergenic regions
```

`Fold coverage of coding exons` (excludes non-coding genes and pseudogenes)

  - Transcript end-coverage biases (*)

```
Median 5' coverage bias
Median 3' coverage bias
```

- Added Fusion and new Trimming metrics for functional QC. New metrics below are added to `fusion_metrics.csv` and `trimmer_metrics.csv` files respectively.
  - Gene fusion fusion statistics

```
All fusion candidates (unfiltered)
Final fusion candidates (passing filter)
Unique passing gene fusions
```

  - Poly-A (X) soft-trimmer 5’ statistics (additional to 3’ stats)

```
Poly-X soft trimmed reads unfiltered R1/R2 5prime,n,%
Poly-X soft trimmed reads filtered R1/R2 5prime,n,%
Poly-X soft trimmed bases unfiltered R1/R2 5prime,n,%
Poly-X soft trimmed bases filtered R1/R2 5prime,n,%
Poly-A (X) hard-trimmer 5’ statistics (additional to 3’ stats)
Poly-X trimmed reads unfiltered R1/R2 5prime,n,%
Poly-X trimmed reads filtered R1/R2 5prime,n,%
Poly-X trimmed bases unfiltered R1/R2 5prime,n,%
Poly-X trimmed bases filtered R1/R2 5prime,n,%
```

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	Methylation	No	n/a
Annotation	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 RNA Workflow

Comp	ID	Summary	Resolution/Workaround
Gene Fusion	DRAGEN-29181	RNA filter info and candidate output has a minor run-run variation on Azure Cloud.	No workaround. Does not affect the accuracy. Not present on AWS or Local.
RNA Quant	DRAGEN-24824	RNA quant - SJ.saturation.txt has minor differences with different num-threads value	No workaround. Negligible impact.

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