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# Local Run Manager RNA Amplicon Analysis Module v3.0.1 Release Notes

Effective Date: 15-MAY-2024



# Introduction

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These Release Notes detail notable items for the Local Run Manager RNA Amplicon Analysis Module v3.0.1 release.

Please note that the RNA Amplicon Analysis Module v3.0.1 requires Local Run Manager Framework v3.0 or higher and is not compatible with Local Run Manager Framework v2.4 or lower.

For more information on module version compatibility per instrument platform, refer to Local Run Manager Module Selector

(https://customprotocolselector.illumina.com/selectors/LRM-moduleselector/Content/Source/FrontPages/LRM-module-selector.htm).

For more information about this analysis module and how to use it, refer to the Local Run Manager RNA Amplicon Analysis Module Workflow Guide, available from the Local Run Manager Support Page, Documentation & Literature.

(https://sapac.support.illumina.com/sequencing/sequencing software/local-runmanager.html)

#### **NEW FEATURES:**

- From RNA Amplicon Analysis Module v3.0.0.26 onwards: Illumina Library Prep Kits (LPK) and Index Adapter Kits (IAK) are decoupled
  - Users now able to choose both the LPK and IAK options from Local Run Manager interface.
  - Sample sheet export from Local Run Manager will now include the Index Kits field aside from the Library Prep Kits.
  - o Added support for custom Library Prep Kits and Index Kits.
  - Added UMI Anchor Kit toggle for applicable Index Kits.
  - Added separate directories for Illumina Library Prep Kits and Index Kits.

#### **DEFECT REPAIRS:**

Fixed the intermittent issue of Analysis being stuck at checkpoint 05 and checkpoint 06.

#### **KNOWN ISSUES:**

- MiSeg Suite v4.0: When importing sample sheet onto the MiSeg Control SW, unclear error message might be displayed. Check sample sheet if error is seen.
  - Common issues include formatting, ensure headers are specified, ensure workflow field is exactly as Local Run Manager interface display, etc.
- Display: Gene Normalization Dropdown list is truncated.



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### **PREVIOUS RELEASE NOTES**

# RNA Amplicon Analysis Module v2.0.2

# Introduction

These Release Notes detail notable items for the Local Run Manager RNA Amplicon Analysis Module v2.0.2 release.

Please note that the RNA Amplicon Analysis Module v2.0.2 requires Local Run Manager Framework v2.0.0 or higher and is not compatible with Local Run Manager Framework v1.3.1 or lower.

For more information about this analysis module and how to use it, refer to the Local Run Manager RNA Amplicon Analysis Module Workflow Guide, available in the Documentation section of Local Run Manager Support Page.

http://support.illumina.com/sequencing/sequencing\_software/local-run-manager/documentation.html

#### **NEW FEATURES:**

• Added ability to perform analysis with additional Advanced Settings (see table below) for Ampliseq Panel.

Setting Name	Value Options	Description
Clipping Penalty	The default value is 50.	BWA MEM clipping penalty.
Maximum Fusion Indel Size	Any value >=0 The default value is 100	Maximum indel size allowed in an alignment to a fusion target.
Short-read Length Threshold	Any value 1-35 The default value is 30.	The threshold determines whether an alignment is short.
Maximum Short-read Substitution Fraction	Any value 0-1 The default value is 0.1.	Maximum substitution fraction allowed for a short alignment.
Maximum Substitution Fraction	Any value 0-1 The default value is 0.04.	Maximum substitution fraction allowed for a non- short alignment.
Maximum Soft-clip Fraction	Any value 0-1 The default value is 0.1.	Maximum soft-clip fraction allowed for an alignment in the target region (excluding probes).
Minimum Mapping Quality	Any value >=0 The default value is 1.	Minimum mapping quality allowed for an alignment.
Require Both Reads Mapped	0 or 1 Default to 0.	Require that both read 1 and read 2 are mapped.
Minimum Singleton Coverage Fraction	When the Require Both Reads Mapped is  set to 0, any value 0 and 1. Default to 0.75. set to 1.	Minimum target coverage fraction required for a singleton alignment.

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#### **DEFECT REPAIRS:**

- Fixed "Analysis aborts early with error" issue when Resources requested are greater than maximum allowed in iSeq.
- Fixed "Aggregated Large Log files causing secondary analysis failure" in MiSeq

#### **KNOWN ISSUES:**

• Display: Gene Normalization Dropdown list is truncated.

## RNA AMPLICON ANALYSIS MODULE V2.0.1

#### **NEW FEATURES:**

- Added ability to perform analysis of External RNA Controls Consortium (ERCC) spikein RNAs as part of settings on user interface.
  - ERCC targets are part of the reporting files output.
  - Merged manifests are now always encrypted with ERCC targets defined in the manifest.
- Lowered the detection threshold for RNA fusion events.
- Added the option to enable Gene Based Normalization. (option is disabled by default)
- Now, the output for RNA fusions are tabix-indexed VCF and CSV files and exons are skipped.
- Added a column of "Number of detected exon variants" to the summary CSV file.

#### **DEFECT REPAIRS:**

- Fixed "array index out of bound" error when writing fusion output files.
- Fixed intermitted issue with the report links.
- Fixed app crashing when the first segment of a fusion target aligns to the reference with a deletion or skipped region.
- Ensure that there are two entries present in fusions.vcf for each entry in fusions.csv.
- Overlapping target regions are now merged to calculate total length.

#### **KNOWN ISSUES:**



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- Analysis for TruSeq Targeted RNA Expression Library Prep Kit are not supported when used with the associated panel manifests. List: https://support.illumina.com/downloads/truseq-targetd-rna-expression-manifestfiles.html
- Display: Index Column Dropdown Arrow Missing for TruSeq Targeted RNA Expression Kit
- Display: Library Prep Kit dropdown does not scale in size to match longer library prep kit names.
- Display: Report footer information contains the RNA Amplicon Analysis Worker version instead of the RNA Amplicon Local Run Manager module version.
- In rare instances, the Sample Sheet file generated during analysis may not reflect the same ordering of samples as what was entered on the "Run Setup" page.
- Report incorrectly lists PUMA Metrics version, which was replaced with bammetrics