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# Unique Molecular Identifiers for QuantSeq
# User Guide

Catalog Number:
081 (UMI Second Strand Synthesis Module for QuantSeq FWD, 96 rxn)

081UG366V0100

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# Table of Contents



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# 1. Overview

This User Guide outlines the protocol for using the UMI Second Strand Synthesis Module with the QuantSeq 3′ mRNA-Seq Library Prep Kits for Illumina (FWD, Cat. No. 015, 113 - 115, 129 - 131). QuantSeq uses total RNA as input with oligo(dT) priming to generate first strand cDNA. RNA removal is then performed and second strand synthesis is initiated by random priming. Final library amplification by PCR adds complete Illumina-compatible sequencing adapters and unique indices. For more detailed information about these protocols, please refer to the complete QuantSeq 3′ mRNA-Seq User Guide available at www.lexogen.com/docs/quantseq.

Unique Molecular Identifiers (UMIs) can be included in QuantSeq FWD libraries to enable the detection and removal of PCR duplicates. The UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1) (Cat. No. 081) includes the UMI Second Strand Synthesis Mix (**USS** ●), which contains UMI-tagged random primers. The **USS** ● simply replaces the Second Strand Synthesis Mix 1 (**SS1** ●) from the standard QuantSeq FWD Kit. No other protocol changes are required. The UMIs are added between the partial P5 adapter and the random priming sequence during second strand synthesis (Fig. 1).

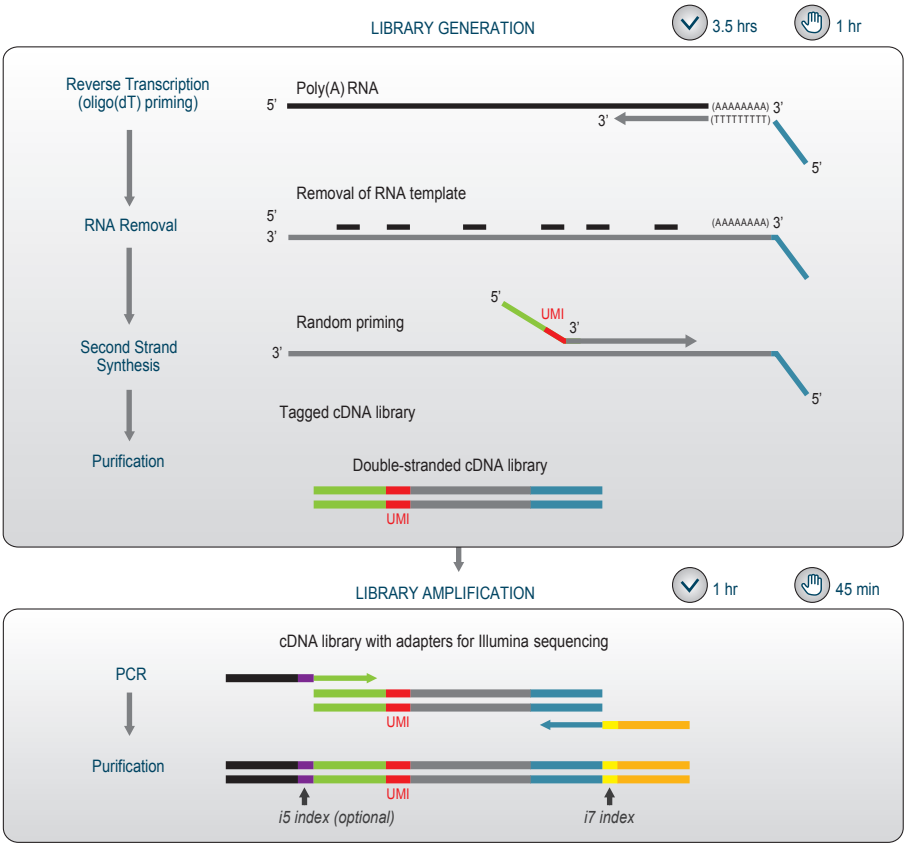



Figure 1. Schematic overview of the QuantSeq workflow including UMIs. Using the UMI Second Strand Synthesis Mix (USS ●) instead of the regular Second Strand Synthesis Mix 1 (SS1 ●) in the QuantSeq workflow introduces the 6 nt long UMI between the partial P5 adapter and the library insert.

# 2. Kit Components and Storage Conditions

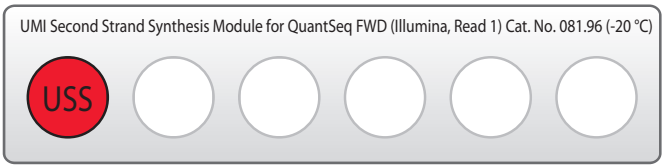



Figure 2. Location of kit components.

| Kit Component | Tube Label | Volume* | Storage |
|---|---|---|---|
| | | 96 rxn | |
| **UMI Second Strand Synthesis Mix** (Cat. No. 081) | **USS** ● | 1,056 µl | -20 °C |

*including ≥10 % surplus

**ATTENTION:**

- The UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1) **is not a stand-alone kit** and must be used in combination with the QuantSeq FWD Kits for Illumina (Cat. No. 015, 113 - 115, 129 - 131).
- This UMI module is only compatible with the QuantSeq 3′ mRNA-Seq Library Prep Kits FWD for Illumina (Cat. No. 015, 113 - 115, 129 - 131). It is not compatible with QuantSeq REV (Cat. No. 016) or QuantSeq-Pool (Cat. No. 139).
- Please refer to the QuantSeq User Guides (015UG009, 113UG227) for the full, detailed protocol and supporting information regarding library preparation, quality control, and sequencing.
- The UMI Module can also be used for libraries prepared with Lexogen's Globin / BC1 Block Modules for QuantSeq (Cat. No. 070, 071, 167) and are compatible with dual indexing.
- The UMI Second Strand Synthesis Mix (**USS** ●) replaces the Second Strand Synthesis Mix 1 (**SS1** ●) from the standard QuantSeq FWD Kit.
- The minimum recommended sequencing length for QuantSeq libraries containing UMIs is 75 bp (i.e., SR75 or longer).

**NOTE:** For user-supplied consumables and equipment needs, please refer to the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guides.

# 3. Protocol

**ATTENTION:** QuantSeq generated first strand cDNA (FWD, Cat. No. 015, 113 - 115, 129 - 131) after RNA removal is required as input for Second Strand Synthesis using the UMI Second Strand Synthesis Mix (**USS** ●), which contains UMI-tagged random primers.

## Second Strand Synthesis

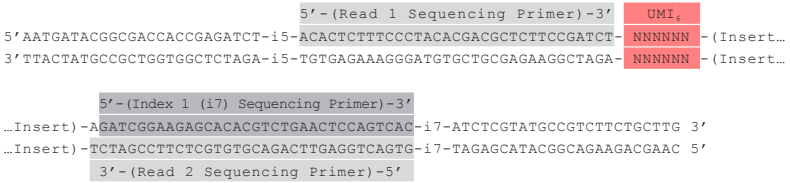
**NOTE:** This protocol replaces steps 7 and 8 of the detailed protocol of the QuantSeq User Guides (015UG009, 113UG227). Step 8 has not been changed for UMI libraries and is included here for ease of reference.

| | |
|---|---|
| ➡ | Follow steps 1 - 6 as indicated in the detailed protocol of the QuantSeq User Guides (015UG009, 113UG227). |
| 7 | Add 10 µl of UMI Second Strand Synthesis Mix (**USS** ●) to the reaction. Mix well by pipetting, seal the plate, and spin down. **REMARK:** Use a pipette set to 30 µl for efficient mixing. |
| 8 | Incubate the plate for 1 minute at 98 °C in a thermocycler and slowly cool down to 25 °C at a reduced ramp speed of 0.5 °C/second. Incubate the reaction for 30 minutes at 25 °C. Quickly spin down the plate at room temperature before removing the sealing foil. |
| ➡ | Proceed to step 9 of the detailed protocol of the QuantSeq User Guides (015UG009, 113UG227). |

# 4. Appendix A: Sequencing*

A minimum length of 75 bp (i.e., SR75 or longer) is recommended for sequencing QuantSeq FWD libraries that include UMIs. The 6 nt UMI is read-out at the beginning of Read 1, upstream of the random priming sequence (see below). No custom sequencing primers are required.

We recommend adding a minimum of 5 - 15 % PhiX spike-in when sequencing QuantSeq FWD-UMI libraries in a pure lane mix. For more information, please check our Frequently Asked Questions.




* Note: Some nucleotide sequences shown in "Sequencing" may be copyrighted by Illumina, Inc.

# 5. Appendix B: Data Analysis

## QuantSeq FWD-UMI Data Analysis

Sequencing data from QuantSeq FWD libraries prepared with the UMI Second Strand Synthesis Module can be analyzed using the FWD-UMI QuantSeq Data Analysis pipelines available on the BlueBee® Genomics Platform. Simply use the activation code included with your QuantSeq FWD Library Prep Kit and select the respective "FWD-UMI" pipeline when setting up your data analysis run. For further information regarding the pipeline workflow please refer to the QuantSeq 3' mRNA-Seq Integrated Data Analysis Pipeline on BlueBee® Platform User Guide, available online at https://www.lexogen.com/lexogen-data-analysis-solutions-on-bluebee-platform/

Alternatively, a Linux/Unix-compatible data analysis tool package for QuantSeq FWD-UMI libraries (collapse_UMI_bam) is available from Lexogen upon request. This package contains two analysis tools that can be integrated into existing QuantSeq Data Analysis pipelines: *umi2index* (input: raw fastq.gz file(s)), which trims the UMI (and spacer) from each read and adds the UMI to the read identifier; and *collapse_UMI_bam* (input: aligned .bam files), which collapses reads that have identical mapping coordinates and UMI sequences to remove duplicates, generating a filtered .bam file that contains de-duplicated, uniquely-mapped reads. For more information on collapsing reads using UMIs, please contact support@lexogen.com.

If you map to the transcriptome rather than to the genome, a publicly-available UMI-Tools package is available on GitHub: https://github.com/CGATOxford/UMI-tools. This package is available for command-line analysis and performs de-duplication of sequencing read counts for QuantSeq FWD-UMI data.

# 6. Appendix C: Revision History

| Publication No. / Revision Date | Change | Page |
|---|---|---|
| 081UG366V0100<br>Aug. 16, 2021 | Initial Release. | |

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Associated Products:

008 (SPLIT RNA Extraction Kit)
015 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (FWD))
020 (PCR Add-on Kit for Illumina)
022 (Purification Module with Magnetic Beads)
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)
070, 071 (Globin Block Modules for QuantSeq)
080 (Reamplification Add-on Kit for Illumina)
113 - 115, 129 - 131 (QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A1, A2, A3, A4, A1-A4, or B1)
167 (BC1 Block Module for QuantSeq)

# BC1 Block Module for QuantSeq · User Guide

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