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UMI Second Strand Synthesis Module  
for QuantSeq FWD (Illumina, Read 1)

# User Guide

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

081UG366V0102

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### **LITERATURE CITATION**

For any publication using this product, please refer to it as Lexogen's QuantSeq™ 3' mRNA-Seq V2 Library Prep Kit FWD with UMI Second Strand Synthesis Module.

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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 V2 Library Prep Kit FWD. 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 dual indices (UDI). For more detailed information about this protocol, please refer to the complete QuantSeq FWD User Guide available at [www.lexogen.com/docs/quantseq](http://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) 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 during second strand synthesis. The UMI sequence is located between the partial P5 adapter and the random priming sequence (Fig. 1).

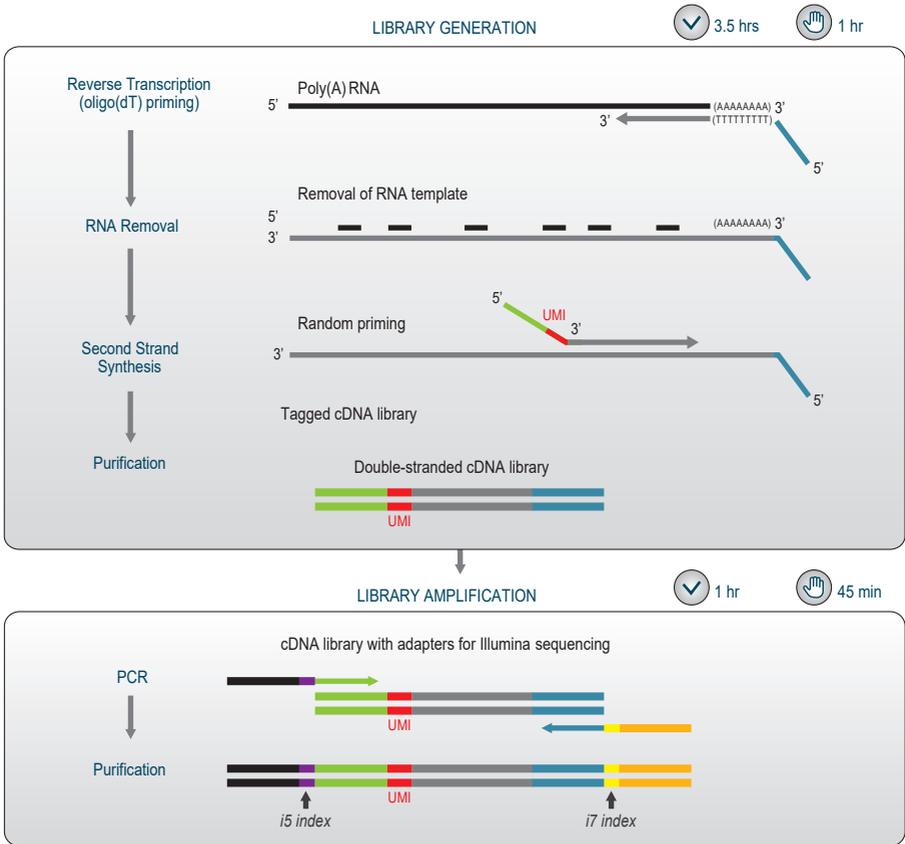


Figure 1. Schematic overview of the QuantSeq FWD workflow including UMIs. Using the UMI Second Strand Synthesis Mix (USS ●) instead of the regular Second Strand Synthesis Mix 1 (SS1 ●) in the QuantSeq FWD workflow introduces the 6 nt long UMI between the partial P5 adapter and the library insert.

## 2. Kit Components and Storage Conditions

UMI Second Strand Synthesis Mix Module (-20 °C)

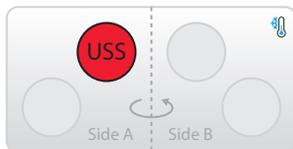


Figure 2. Location of kit components.

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.
- This UMI module is only compatible with the QuantSeq FWD. It is not compatible with QuantSeq REV or QuantSeq-Pool.
- Please refer to the QuantSeq FWD User Guide (191UG444) 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.
- 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 FWD User Guide.

# 3. Protocol

**ATTENTION:** QuantSeq generated first strand cDNA (FWD) 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 FWD User Guide (191UG444). 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 FWD User Guide (191UG444).

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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.

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Proceed to step 9 of the detailed protocol of the QuantSeq FWD User Guide (191UG444).

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## 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 insert sequence (see below). No custom sequencing primers are required.

We recommend adding a minimum of 5 - 30 % PhiX spike-in when sequencing QuantSeq FWD-UMI libraries in a pure lane mix. For more information, please check the QuantSeq online FAQs at [www.lexogen.com](http://www.lexogen.com).

```
5'-(Read 1 Sequencing Primer)-3' UMIn
5' AATGATACGGCGACCACCGAGATCT-i5-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNN -(Insert...
3' TTAATGATACGGCGACCACCGAGATCT-i5-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNN -(Insert...

5'-(Index 1 (i7) Sequencing Primer)-3'
...Insert)-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-i7-ATCTCGTATGCCGTCTTCTGCTTG 3'
...Insert)-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-i7-TAGAGCATAACGGCAGAAGACGAAC 5'
3'-(Read 2 Sequencing Primer)-5'
```

\*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 QuantSeq FWD-UMI data analysis pipeline available on Kangaroo - Lexogen's web-based data analysis platform (<https://kangaroo.com/home>).

With each purchased QuantSeq FWD kit, you receive a voucher code for free data analysis. The voucher code can be found on a label located on the cardboard above the microtube holder (or one of the holders, if the kit contains more than one). Each provided code allows for the same number of data analysis pipeline runs as the number of reactions included in the library prep kit, including differential expression (DE) analysis. The maximum amount of total data that can be uploaded is 1.5 GB for each sample included in the kit, which is sufficient for most standard QuantSeq experiments. The voucher code covers the download of the majority of the final results. If you are interested in downloading all data, including pre-processing data like trimmed FASTQ files or BAM files, or if you need to analyze bigger files, please contact us at [sales@lexogen.com](mailto:sales@lexogen.com) to purchase additional codes.

**ATTENTION:** The UMI Second Strand Synthesis Modules do not include codes for data analysis. The codes are provided with the QuantSeq FWD kits.

Alternatively, to perform UMI deduplication, 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. For more information on how to analyze data obtained from QuantSeq FWD-UMI libraries, check the QuantSeq online FAQs at [www.lexogen.com](http://www.lexogen.com).

For further inquiries, please contact [support@lexogen.com](mailto:support@lexogen.com).

## 6. Appendix C: Revision History

Publication No. / Revision Date	Change	Page
081UG366V0102 Jul. 22, 2024	Information updated according to the QuantSeq V2 (FWD) release.	All
	Updated data analysis information.	9
	Updated PhiX spike-in recommendations.	8
081UG366V0101 Jan. 25, 2023	Updated Kit Components Figure 2 and Table to reflect current packaging and storage requirements.	6
081UG366V0100 Aug. 16, 2021	Initial Release.	

Associated Products:

008 (SPLIT RNA Extraction Kit)  
022 (Purification Module with Magnetic Beads)  
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)  
070, 071 (Globin Block Modules for QuantSeq)  
167 (BC1 Block Module for QuantSeq)  
191 - 196 (QuantSeq 3' mRNA-Seq V2 Library Prep Kit FWD with UDI)  
208 (PCR Add-on and Reamplification Kit V2)

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