Heat fragmentation protocol for Collibri™ Stranded RNA Library Prep Kit for Illumina® Systems

For use with or without H/M/R rRNA Depletion Kit

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Collibri™ Stranded RNA Library Prep Kit for Illumina® Systems* User Guide (Pub. Nos. MAN0017584, MAN0025359). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

About the protocol

Heat fragmentation is an effiecient and adjustable alternative to the RNase III-based enzymatic digestion procedure described in the user guide for *Collibri™ Stranded RNA Library Prep Kit for Illumina® Systems*. The random nature of heat fragmentation reduces the amount of duplicates in sequencing data.

Required materials

Components from the Collibri™ Stranded RNA Library Prep Kit for Illumina® Systems
10X Fragmentation Buffer
RNA End Repair Enzyme

Fragment the RNA

1. Assemble reactions for each RNA sample on ice or a cooling rack. The volumes given in the following table are for a single 0.2-mL PCR tube or a single well in a PCR plate.

Component	Volume
RNA sample (rRNA-depleted or mRNA-enriched)	8 µL
10X Fragmentation Buffer (blue)	1 µL
Total volume	**9 µL**

2. Flick the tube or pipet up and down several times to mix, then centrifuge briefly to collect the liquid in the bottom of the tube.

3. Incubate the reaction mixture in a thermal cycler or heating block using conditions basaed on the desired insert length.

Desired insert length	Fragmentation reaction conditions
~150 bp	95°C, 8 minutes
~200 bp	95°C, 6 minutes
~350 bp	95°C, 3 minutes
~400 bp	95°C, 1 minutes

Note: After indexing, PCR adaptors will add ~130 bp to the insert size.

4. After the fragmentation, add 1 µL of RNA End Repair Enzyme and incubate at 37°C for 10 minutes.

5. Proceed to "*Purify the fragmented RNA*" as described in the user guide for *Collibri™ Stranded RNA Library Prep Kit for Illumina® Systems*.

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A.0	1 February 2022	Heat fragmentation protocol for Collibri Stranded RNA Library Prep Kit for Illumina with or without Human/Mouse/Rat rRNA Depletion Kit.