Product Profile

QIAseq™ Stranded RNA Library Kits

For precise transcriptomics insights powered by a new generation of RNA-seq chemistry

QIAseq Stranded RNA Library Kits enable researchers to generate RNA-seq libraries which retain critical strand-specific transcript information. This is accomplished by either enriching for mRNA to facilitate comprehensive gene expression profiling or using total RNA sequencing for whole transcriptome analysis (WTA). Kits include all the reagents required to build an RNA-seq library for sequencing on any Illumina® platform.

Benefits of QIAseq Stranded RNA Library Kits

- New CleanStart™ protocol with Hi-Fidelity PCR amplification and contamination control
- Fast and easy protocol, compatible with fresh and FFPE samples
- No need for Actinomycin D and dUTP digestion for stranded library prep
- Dual-barcoded sample adapter plates and QIAseq magnetic beads included
- mRNA enrichment or total RNA optimized protocols

Unique, fast workflow for high-quality RNA-seq libraries

QIAseq Stranded RNA Library Kits provide an efficient and time-saving protocol leveraging new chemistry (Figure 1). After an optional RNA fragmentation step, the reverse transcription (RT) step generates first strand cDNA. Strand specificity is ensured due to optimized combinations of RT enzyme and buffer, eliminating the need to use toxic reagents such as Actinomycin D. In the second strand synthesis step, a special combination of enzymes with carefully adapted buffer formulations allow the degradation of RNA, the generation of a second cDNA strand and the generation of blunt DNA ends and A-base addition necessary for efficient ligation of the Illumina-compatible adapters. QIAseq Stranded RNA Library Kits allow fast, efficient and superior NGS library construction for samples of various origins and species. The resulting highly complex and strand-specific libraries, in combination with the decontaminating properties of the CleanStart PCR Mix, allow the analysis of even low-abundance transcripts with high sensitivity. The streamlined, 4–5 hour protocol permits library preparation and the start of an NGS run in just one working day.

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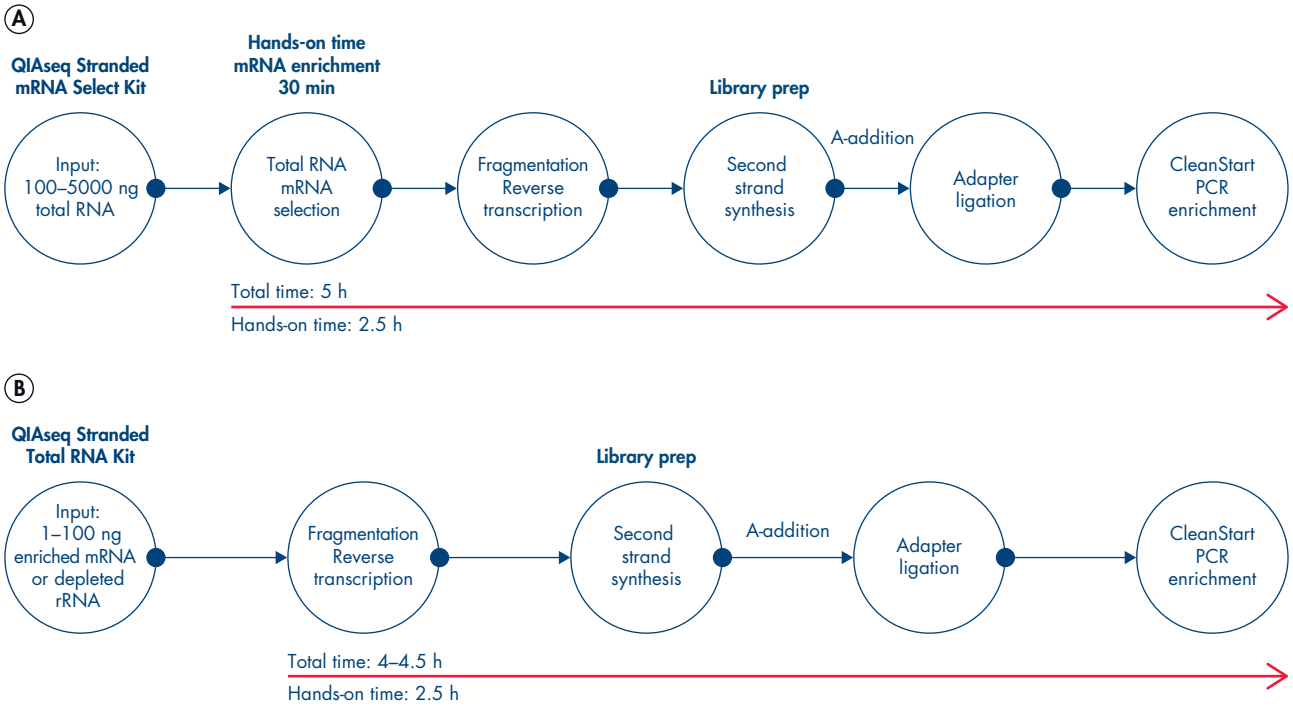

Figure 1. Preparation of stranded RNA-seq libraries in 1 day. Stranded RNA-seq libraries can be prepared in just 4–5 h using the QIAseq Stranded Total RNA and QIAseq Stranded mRNA Select Kits, with only 3 h of hands-on time. The compressed total protocol time allows subsequent library QC steps and start of an NGS run on any Illumina NGS instrument in just one working day.

The special, strand-specific ligation step in the QIAseq Stranded RNA protocols ensures strand specificity without the need for additional reagents or laborious and time-consuming protocol steps. The included ready-to-use adapter plates allow the sequencing of up to 96 different samples by the combination of a unique 8 nucleotide i5 and i7 index barcode. Finally, the new proprietary CleanStart PCR Mix not only efficiently and uniformly amplifies the RNA-seq library, irrespective of high GC or AT content, but also degenerates contaminating material, such as previously generated NGS libraries. When comparing treated and untreated samples, even a slight mixture of library material can lead to false results, especially for low-abundance transcripts. The paramagnetic QIAseq Beads included with kits provide rapid and effective reaction cleanup between protocol steps.

Superior library quality and reproducibility with a high percentage of protein-coding reads

In whole transcriptome NGS applications, it is highly important to maximize the amount of information received from a sequencing run. Due to the low proportion of mRNA in the total cellular RNA pool, reducing the amount of rRNA saves valuable sequencing resources, which

means that fewer reads are required compared to other methods. QIAseq Stranded mRNA Select Library Kits enable researchers to enrich for mRNA and build RNA-seq libraries which retain strand-specific transcript information. The result is superior library quality and reproducibility with a high percentage of protein-coding reads (Figure 2).

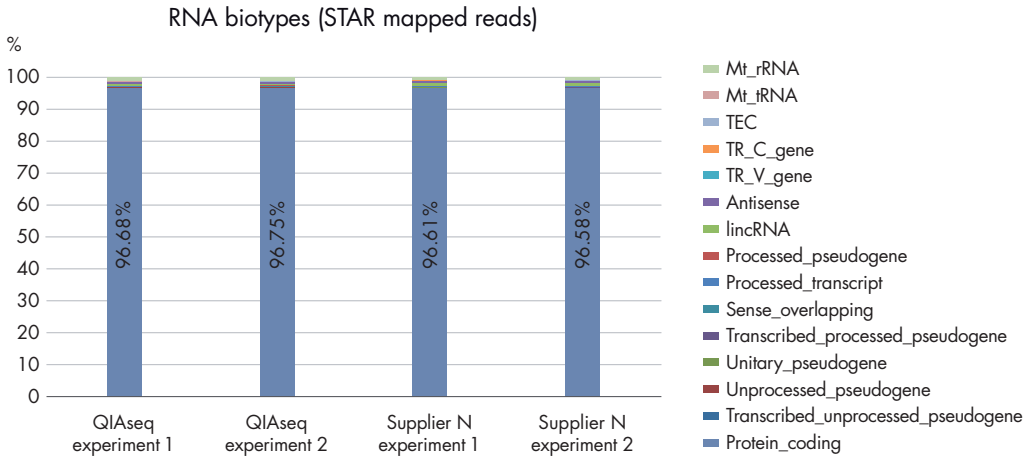

Figure 2. RNA biotype distribution: High efficiency of mRNA enrichment protocol. UHRR RNA (Agilent®) was used to prepare RNA-seq libraries with the QIAseq Stranded mRNA Select Kit or with a stranded RNA-seq kit from Supplier N. RNA-seq libraries were generated with 50 ng mRNA as starting material, sequenced on the Illumina MiSeq® instrument and reads were aligned to the GRCh38 human reference genome. The RNA biotype distribution of uniquely aligned reads was analyzed with the HTSeq-count tool. Supplier N and the QIAseq Stranded RNA libraries show a very high fraction of reads, with more than 96% of reads mapped to protein coding regions. These results are indicative of the high efficiency and specificity of the QIAseq mRNA enrichment and library construction steps.

QIAseq Stranded mRNA Select Library Kits are typically used when starting with 100 ng or more of total RNA and combine the convenient handling of magnetic particles with the specificity of oligo-dT hybridization. Oligo-dT probes are covalently attached to the surface of Pure mRNA Beads. mRNA binds rapidly and efficiently to the oligo-dT probes on the Pure mRNA Beads in the presence of a unique buffer. mRNAs bound to the magnetic particles are then efficiently washed through two steps, which considerably improve the purity. The resulting highly pure poly(A)+ mRNA is ready for stranded NGS library preparation utilizing the QIAseq Stranded Total RNA Library Kit. QIAseq Stranded RNA-seq libraries show a high degree of correlation (R2 >0.99) between technical replicates, as well as excellent dynamic range with different amounts of starting RNA (Figure 3). ▷

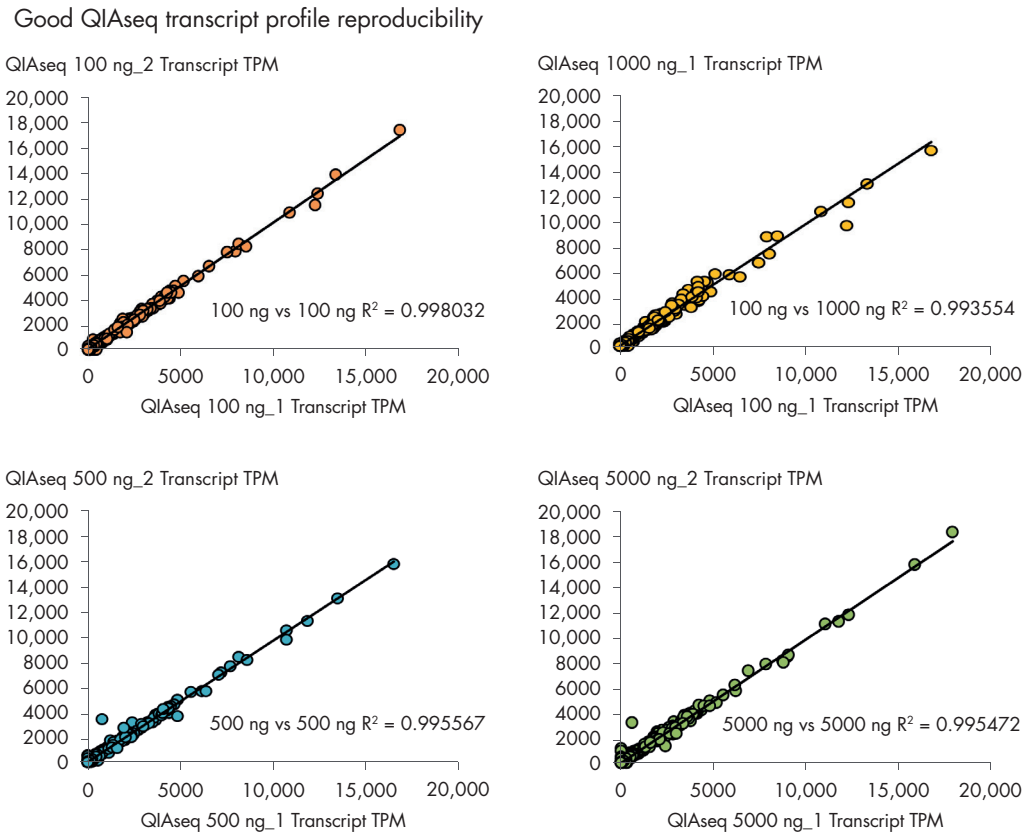

Figure 3. Reproducible transcript profiles with different RNA input amounts. mRNA was captured from different amounts of UHRR Reference RNA (Agilent) (100, 500, 1000 or 5000 ng) and used to generate QIAseq Stranded mRNA-seq libraries for sequencing on the Illumina MiSeq instrument. After the alignment of the RNA-seq NGS reads to the GRCh38 human reference genome using CLC Genomics Workbench, the expression values for annotated transcripts were calculated using the TPM (Transcripts Per Million) normalization method. Pearson correlation coefficients were calculated for technical replicates with similar or different RNA input amounts of UHRR RNA. QIAseq Stranded RNA-seq libraries show a high degree of correlation (R^2 >0.99) between technical replicates, as well with different amounts of starting RNA.

High-performance RNA-seq libraries: Low duplication rates and greater complexity

New-generation enzymology coupled with optimized protocols result in RNA-seq libraries with high complexity and minimal duplication. This ensures that QIAseq Stranded RNA Library Kits deliver the maximum amount of data from each sample, even when starting with low amounts of RNA (Figure 4).

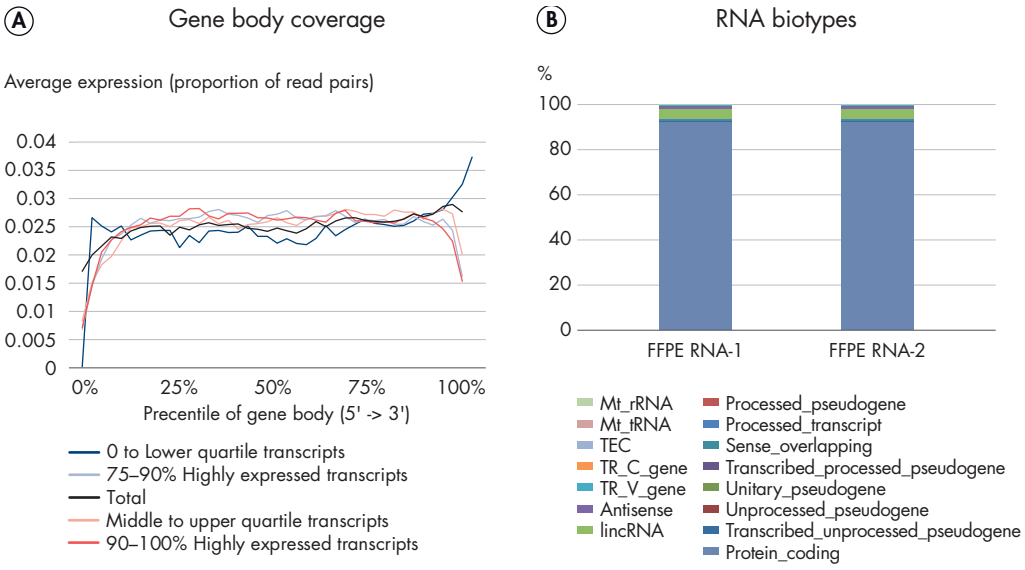

Figure 4. RNA-seq data from highly degraded RNA. RNA was isolated from human non-small cell lung cancer FFPE samples using QIAGEN's RNeasy® FFPE Kit. Ribosomal RNA was depleted from the isolated RNA using the Ribo-Zero Gold rRNA Removal Kit from Illumina. Depleted rRNA samples were checked for ribosomal integrity numbers (RIN) using the Agilent Bioanalyzer. To construct an RNA-seq library using the QIAseq Kit, 10 ng of rRNA depleted FFPE RNA with a RIN of 2 was used. RNA-seq libraries were sequenced on the Illumina MiSeq instrument and reads were mapped to the GRCh38 human reference genome using CLC Genomics Workbench v10. **A** Even exon coverage of FFPE RNA was observed. Normalized expression data (uniquely mapped read pairs to exons) for different transcript expression levels were plotted against the exon regions (transcript size normalization). Very low bias was detected across the gene body. **B** A high proportion of protein-coding RNA was mapped from FFPE samples. Highly degraded FFPE RNA samples show a high proportion of reads mapped to protein coding regions, which can be used to determine transcript expression, as well as lincRNA (long intergenic noncoding RNA).

Ordering Information

Product	Contents	Cat. no.
QIAseq Stranded Total RNA Lib Kit (24)	For strand-specific RNA-seq library preparation of 24 samples: Kits include magnetized reaction cleanup beads and sample index adapters for Illumina NGS platforms	180743
QIAseq Stranded Total RNA Lib Kit (96)	For strand-specific RNA-seq library preparation of 96 samples: Kits include magnetized reaction cleanup beads and sample index adapters for Illumina NGS platforms	180745
QIAseq Stranded mRNA Select Kit (24)	For mRNA enrichment and strand-specific RNA-seq library preparation of 24 samples: Kits include magnetized reaction cleanup beads and sample index adapters for Illumina NGS platforms	180773
QIAseq Stranded mRNA Select Kit (96)	For mRNA enrichment and strand-specific RNA-seq library preparation of 96 samples: Kits include magnetized reaction cleanup beads and sample index adapters for Illumina NGS platforms	180775
QIAseq CleanStart PCR Kit (100)	For 100 reactions: Includes reagents for amplification of NGS libraries using Hi-Fidelity DNA polymerase	180795

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com/QIAseq-Stranded-RNA** for more information!