

## Product Profile

# QIAseq FX Single Cell RNA Library Kit

Obtain robust and sensitive RNA-seq data from single cells

The proliferation of technologies that enabled the analysis of single, isolated cells has transformed research in neuroscience, cancer, developmental biology and more. Because of technological breakthroughs such as single cell isolation, whole transcriptome amplification (WTA) and NGS library preparation, experiments using single cells are now possible – opening a wealth of exciting new insights for you to discover. Today's new solutions for single cell RNA-seq can help you achieve a deeper understanding of the transcriptomes in your research. However, understanding gene expression and its regulation is limited by the quality and amount of data that you can generate. The QIAseq FX Single Cell RNA Library Kit allows you to uncover more of the transcriptome with the same sequencing depth, and delivers data including both mRNA and lincRNA, enabling the analysis of this important class of regulatory RNAs. With exceptional ease of use and affordability, the QIAseq FX Single Cell RNA Library Kit lets you expand your study size and increase both the statistical power of your dataset and confidence in your conclusions. Take advantage of the QIAseq FX Single Cell RNA Library Kit to increase the resolution of your data and gain better insights for your research!

The QIAseq FX Single Cell RNA Library Kit delivers:

- An all-in-one, cell-to-library solution incorporating robust whole transcriptome amplification and highly efficient QIAseq FX library preparations
- Completely PCR-free cell-to-library protocol to minimize bias and maximize transcript detection
- Libraries from single cells in under 6 hours using a streamlined protocol
- RNA-seq data includes both mRNA and lincRNA
- Fewer sequence errors – perfect for viral genome sequencing
- NGS libraries and amplified cDNA samples that can be archived for follow up experiments or secondary analysis

## PCR-free RNA libraries from single cells in under 6 hours

The QIAseq FX Single Cell RNA Library Kit generates robust, PCR-free RNA-seq libraries from single eukaryotic cells in as little as 6 hours. The kit combines optimized reverse transcription reagents with a high fidelity, phi29-based cDNA amplification method, unbiased enzymatic cDNA fragmentation and a proprietary single-tube end-polishing and ligation reaction to deliver a robust, single-kit solution for single cell mRNA-seq. In addition to delivering an Illumina-compatible NGS library, the protocol generates large amounts of amplified cDNA that can be stored for follow up studies (Figure 1).

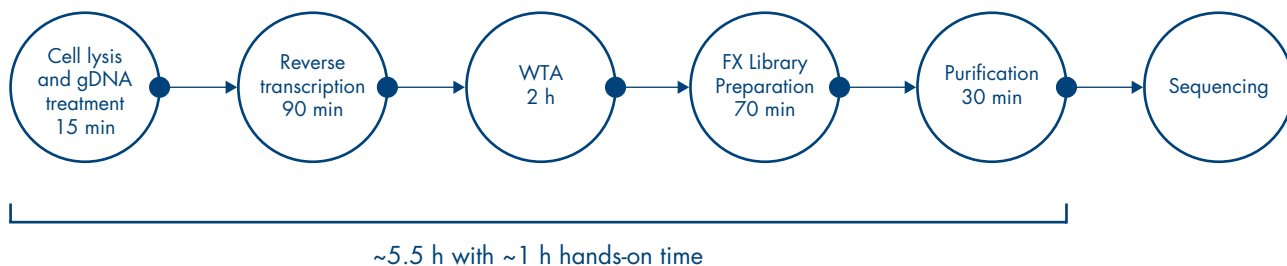
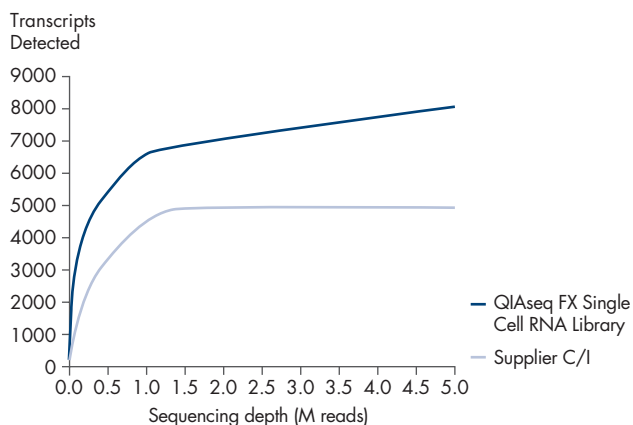
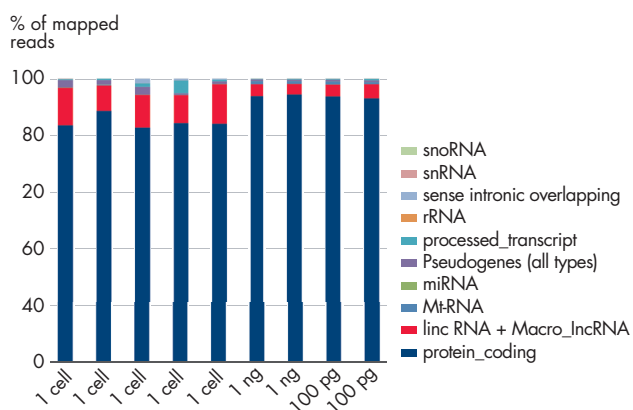


Figure 1. QIAseq FX Single Cell RNA workflow.



**Figure 2. Comparison of transcript detection versus sequencing depth.** The QIAseq FX Single Cell RNA Library Kit detects a greater number of transcripts at the same sequencing depth. To account for cell-to-cell differences in transcript abundance, libraries were produced from 100 pg of reference RNA from PBMCs. After sequencing, quality control and mapping, annotated transcripts with >1 TPM were quantified from either the full dataset or rarified sub-fractions. Saturation curves are from different sample preparation methods. Each point on the curve was generated by randomly selecting a number of raw reads from each sample library and then using the same alignment pipeline to call genes with mean TPM>1.



**Figure 3A. Number of detected transcripts.** Single cell libraries were prepared from PBMCs or toRNA from PBMCs using the QIAseq FX Single Cell RNA Library Kit and sequenced on NextSeq. Plotted is the % of reads that map to different RNA biotypes.

## Discover more insights with greater sensitivity

The number of quantifiable transcripts in a given library is heavily influenced by the sensitivity of the library preparation method and the efficiency with which this method captures the full complexity of the transcriptome. The QIAseq FX Single Cell RNA Library Kit delivers greater library diversity, providing a broader and more detailed view of the transcriptome through the quantification of a greater number of transcripts at the same sequencing depth (Figure 2).

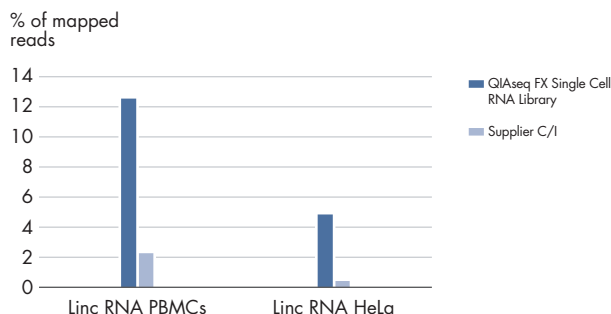
## Unlock the full potential of your RNA-seq experiment for both lncRNA and mRNA

While the QIAseq FX Single Cell RNA Library Kit is intended for mRNA-seq and delivers a high proportion of reads mapping to annotated protein-coding genes, it also unlocks non-coding lncRNA and lincRNA (Figure 3A). These long, regulatory RNAs serve key roles in modulating gene expression and epigenetic modification, but are not often observed due to difficulties capturing long transcripts with some library preparation methods (Figure 3B). A combined single cell dataset containing both mRNAs and long non-coding RNAs can deliver an additional level of insight.

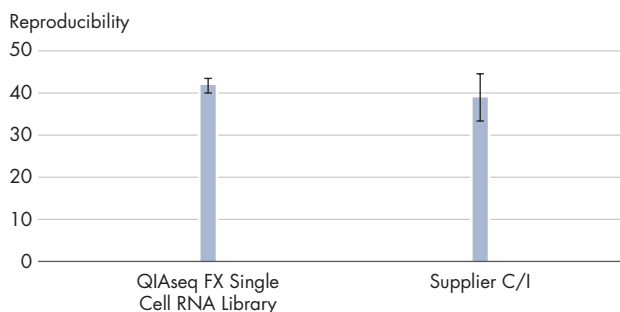
## Achieve reproducible results with less bias

The QIAseq FX Single Cell RNA Library Kit provides a number of key advantages to help you make critical discoveries by minimizing research artifacts. In addition to delivering greater transcript discovery in experiments using both low and high sequencing-depth and capturing effectively both mRNA and lncRNA, the kit does not compromise on reproducibility (Figure 4A), meaning that you can have confidence in these additional results.

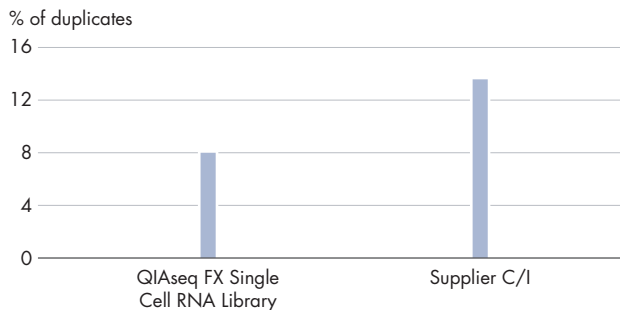
Finally, the completely PCR-free workflow eliminates the possibility of producing duplicate reads. In RNA-seq experiments such reads are indistinguishable from reads arising from unique molecules, and can skew expression values while at the same time reducing sensitivity. By focusing on sequencing actual expressed transcripts and not PCR duplicates, the kit delivers higher library complexity and allows you to evaluate your data with greater quantitative accuracy while also achieving more biologically informative data in your research (Figure 4B).



**Figure 3B. Higher percentage of detected long non coding intergenic RNA.** Single Cell RNA-Libraries from PBMCs and HeLa Cells were generated using QIAseq FX Single Cell RNA Library kit and a kit from Supplier C/I. Plotted are the percentage of reads that map to linc RNA detected in PBMC and HeLa preparations. QIAseq detects a significantly higher percentage of long regulatory RNAs compared to Supplier C/I.



**Figure 4A. Reproducibility of single cell RNA-seq protocols.** 4 Individual HeLa cells were isolated from the same cell culture and libraries were prepared with either the QIAseq FX Single Cell RNA Library Kit or a competing workflow. After sequencing to equal depth, quality control, alignment, and TPM calculation, pairwise comparisons of the number of common transcripts detected with >1 TPM divided by sum of all transcripts in both preps were made between all tested cells. The graphs represent mean of 6 pairwise comparisons with SD.



**Figure 4B. Duplication level.** Single Cell RNA-Libraries from PBMCs were generated using QIAseq FX Single Cell RNA Library kit and a Kit from Supplier C/I. Libraries were sequenced on Illumina NextSeq. Plotted is the % of duplicates, that was obtained from the Fast QC report of the sequenced libraries.

## Greater sequence fidelity and detection capabilities

The QIAseq FX Single Cell RNA Library Kit delivers best-in-class sequence fidelity, ensuring ensuring fewer sequence errors in your data. During standard transcript quantification and differential sequence analysis, sequence errors are usually not evaluated. However, the introduction of sequence errors is a key consideration when analyzing viral genomes. By minimizing sequence errors with a highly accurate WTA, the QIAseq FX Single Cell RNA Library Kit minimizes

false positive mutations and reduces experimental noise. Together, this allows you to confidently detect sequence polymorphisms in rapidly-evolving viral genomes when working with complex microbiome samples. For more information on current advances in single cell research, see our Single Cell Knowledge Hub under Knowledge Area at [www.qiagen.com](http://www.qiagen.com).

## Ordering Information

Product	Contents	Cat. no.
QIAseq FX Single Cell RNA Library Kit (24)	For 24 reactions: Buffers and reagents for cell lysis, reverse transcription, gDNA degradation, whole transcriptome amplification and library construction. Includes a plate containing 24 barcoded adapters for use with Illumina instruments.	180733
QIAseq FX Single Cell RNA Library Kit (96)	For 96 reactions: Buffers and reagents for cell lysis, reverse transcription, gDNA degradation, whole transcriptome amplification and library construction. Includes a plate containing 96 barcoded adapters for use with Illumina instruments.	180735

These products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

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Visit [www.qiagen.com/goto/QIAseq-FX-SC-RNA](http://www.qiagen.com/goto/QIAseq-FX-SC-RNA) for more information!

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