A PCR-free protocol for single-cell RNA library construction

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Introduction

Recent advances in single-cell RNA sequencing technologies have revealed high heterogeneity in gene expression profiles of individual cells. Various whole transcriptome amplification (WTA) methods overcome limited RNA availability and enable transcriptome analysis of single cells. In standard PCR-based WTA procedures, however, uneven coverage of cDNA regions with high GC or AT content and base-copying errors can lead to the loss of data from a subset of actively expressed transcripts.

We describe a PCR-free protocol to efficiently generate RNA-seq libraries from a single cell or as little as picogram amounts of RNA in 6.5–7 hours. This protocol uses innovative Multiple Displacement Amplification (MDA), combined with an efficient library adaptor ligation procedure, to prepare RNA-seq libraries that retain the unique transcriptional profile of a single cell. All enzymatic steps are optimized for the efficient processing of RNA and amplification of cDNA with high fidelity and highly uniform coverage. This streamlined, PCR-free library construction procedure delivers high-quality libraries that enable whole transcriptome analysis from single-cell input.

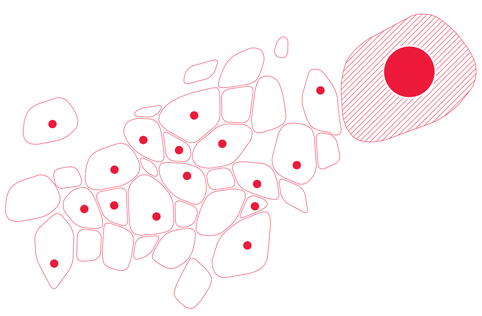

Figure 1. Gene expression patterns can vary significantly from cell to cell. Capturing an accurate representation of the transcriptome of a single cell can lead to novel insights about their contribution to tissue function, tumor development, disease progression and more.

Principle of the method

The protocol relies on the REPLI-g® Single Cell RNA Library Kit, which leverages QIAGEN's unique MDA technology and efficient GeneRead™ library construction technology to prepare a sequencing library with high fidelity and minimal bias.

Amplification occurs via isothermal amplification of the transcribed and ligated mRNAs or transcriptome amplification, constant-temperature strand displacement synthesis and additional priming events. Supported by a unique buffer/polymerase combination, it generates cDNA fragments up to 100 kb without sequence bias.

High-quality library preparation delivers NGS-ready libraries without need for any enrichment steps, thus avoiding additional amplification bias. The high WTA yields and high ligation efficiency of the library construction reagents remove the need for PCR-based amplification, thus avoiding reductions in library diversity.

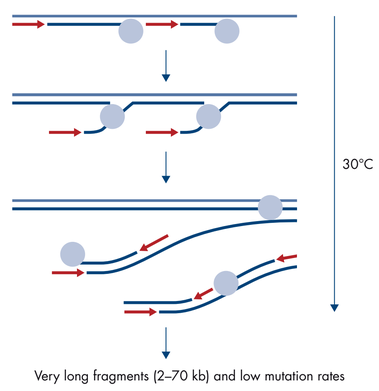

Figure 2. Multiple Displacement Amplification (MDA) technology amplifies cDNA with high uniformity and accuracy. Primers (arrows) anneal to the template and are extended at 30°C as the polymerase moves along the gDNA or cDNA strand, displacing the complementary strand while becoming a template itself for replication. In contrast to PCR amplification, MDA does not require different temperatures and ends in very long fragments with low mutation rates.

Simple, one-day procedure for amplification and library construction

In the first step of the procedure, the cell sample is lysed and the gDNA is removed. Reverse transcription is carried out for 60 min, followed by ligation of cDNAs (30 min). The isothermal amplification reaction then proceeds for 120 min, and can be preprogrammed in a thermal cycler. REPLI-g SC-amplified cDNA can be stored long-term at –20°C with no negative effects.

Following end-repair, platform-specific adaptors are ligated to both ends of the cDNA fragments. These contain sequences essential for binding library to a flow cell for sequencing, binding sequencing primer and allowing for PCR enrichment of adapter-ligated cDNA library.

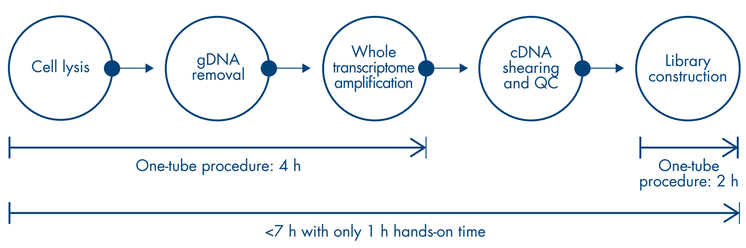

Figure 3. The time-saving, streamlined protocol for ready-to-use RNA library generation.

Results: high-quality libraries

This protocol, which uses the REPLI-g Single Cell RNA Library Kit, delivers high-quality libraries within one day. Independent of the amount of starting material and the incubation time, the libraries have a high number of detected expressed genes and a high percentage of protein-coding reads.

The results confirm that the yields of the WTA step and the ligation efficiency of the library construction reagents are sufficiently high, so that PCR-based library amplification, which can introduce coverage bias and reduce library diversity, is unnecessary.

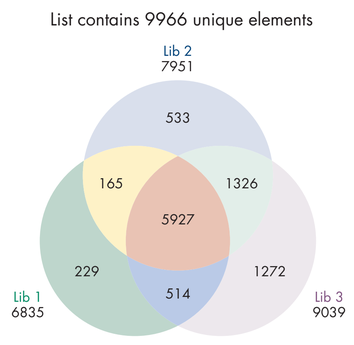

Figure 4. Single cell RNA sequencing reveals cellular heterogeneity. Sequencing libraries were generated from 3 individual HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit and sequenced on an Illumina® sequencer. The plot shows the number of genes detected in each single cell.

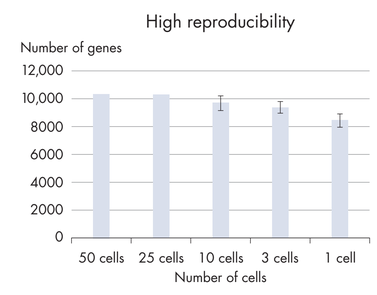

Figure 5. High number of genes is detected, independent of input cell number. RNAseq libraries were prepared from 1, 3, 10, 25 or 50 HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit and sequenced on an Illumina platform.

Results: Significant number of reads map to protein-coding RNA

Using this protocol, the REPLI-g Single Cell RNA Library Kit is able to amplify mRNA-enriched RNA (poly A+). Amplification of ribosomal RNA (rRNA), which makes up more than 90% of the total cellular RNA population, is virtually eliminated, allowing generation of meaningful mRNA-Seq data. Following this library prep procedure, >80% of mapped reads belong to protein-coding RNA.

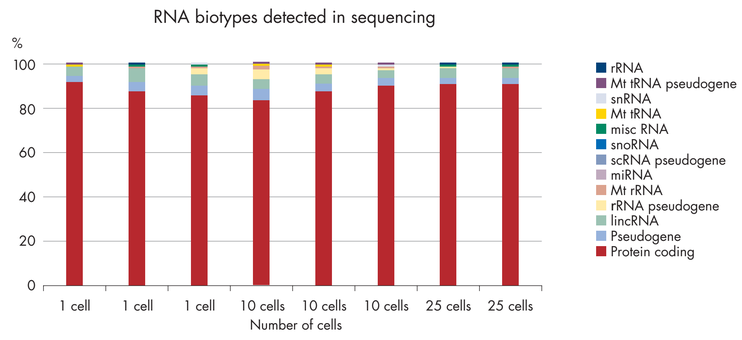

Figure 6. Superior RNAseq library quality and reproducibility with a high percentage of protein-coding reads. RNAseq libraries were prepared in triplicate from 1 or 10 HeLa S3 cells and in duplicate from 25 HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit, and sequenced on an Illumina platform. The percentage of RNA biotypes detected in each library is plotted.

Conclusion

The REPLI-g Single Cell RNA Library Kit offers an efficient, PCR-free method for next-generation sequencing library construction from single cells. The kit combines QIAGEN's unique multiple displacement amplification (MDA) technology and efficient GeneRead library construction technology to overcome the challenges of working with and analyzing single cells by allowing preparation of a sequencing library with high fidelity and minimal bias, while ensuring reproducible and accurate transcriptome analysis.

For more information, visit **www.qiagen.com/Single-Cell-RNA**

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