Enabling RNA-Seq With Limited RNA Using Whole Transcriptome Amplification

Ioanna Andreou, Isabell Haupt, Annika Piotrowski, Nan Fang
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

RNA-Seq was developed to perform transcriptome profiling and provides a highly precise measurement of expression levels of transcripts and their isoforms. Normally, RNA-Seq analysis requires at least 500 ng –1 µg of total RNA. When working with small biopsies, single cells (such as circulating tumor cells), or other limited material, whole transcriptome amplification (WTA) is normally required. Various WTA methods overcome limited RNA availability and enable transcriptome analysis from limited material or even single cells. In standard PCR-based WTA procedures, however, bias from uneven coverage of cDNA regions with high GC or AT content or amplification errors can lead to the loss of transcripts and wrong variant calling.

Here, we compare a standard RNA-Seq library preparation method and the REPLI-g® RNA library protocol. The REPLI-g procedure is a PCR-free protocol to efficiently generate RNA-Seq libraries from small amounts of RNA or a single cell in 6.5–7 hours. The REPLI-g protocol uses whole transcriptome amplification based on multiple displacement amplification (MDA), combined with an efficient library adaptor ligation procedure, to prepare RNA-Seq libraries from small RNA amounts. The procedure demonstrates high fidelity, minimal bias and retention of sample's transcriptional profile. Compared to standard RNA-Seq library prep, the REPLI-g protocol demonstrates similar reproducibility and sensitivity in transcript detection.

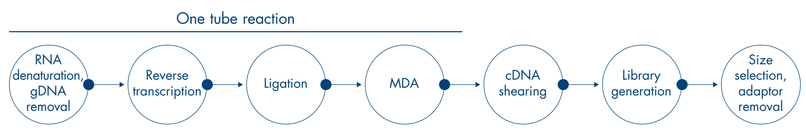
Generation of an RNA library from limited material

Sample type	Single cell, multiple cells, tissue, blood, gDNA, RNA
Application	Single-cell RNA analysis (1–1000 cells or 10 pg – 100 ng purified RNA)
QIAGEN's solution	REPLI-g Single Cell RNA Library Kit
Analytical technology	Next-generation sequencing

Experimental Workflows

RNA-Seq was performed using total RNA from 2 different cell types and 2 different methods. One method is based on MDA amplification of cDNA and the second is a strand-specific mRNA-Seq library preparation.

For WTA,10 ng total RNA was used. For the strand-specific mRNA-Seq library, 1 µg total RNA was used. Generated libraries were sequenced on an Illumina® MiSeq® instrument.

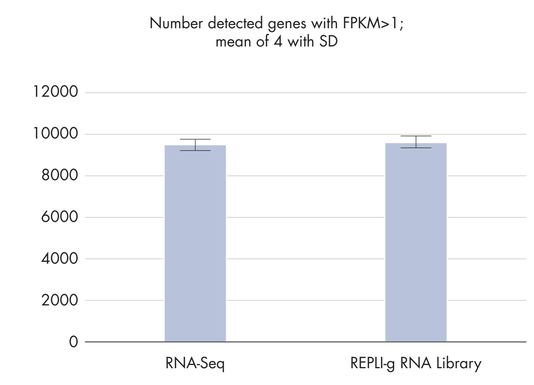


WTA workflow. mRNA is transcribed and ligated. Ligated cDNA was then amplified using the REPLI-g SensiPhi DNA Polymerase, included in the REPLI-g Single Cell RNA Library Kit. The polymerase leverages a high template affinity, high fidelity, and a high DNA strand displacement ability. The amplified cDNA was used to generate sequencing libraries using a completely PCR-free protocol.

RNA-Seq workflow. mRNA is hybridized on beads and transcribed using 2 types of primer to ensure strand-specific mRNA sequence. The transcribed fragments are ligated to the second primer before second strand synthesis is performed. The cDNA library is generated using PCR and then undergoes enrichment PCR and purification.

Reliable Detection of All Transcripts

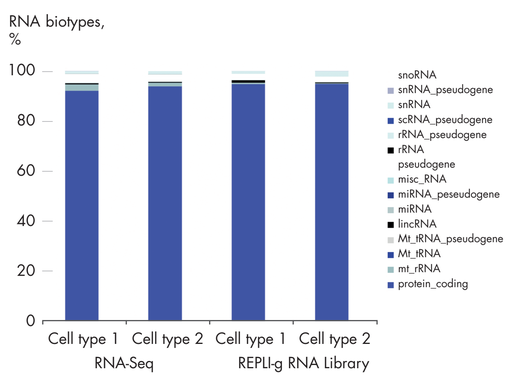
We compared the number of detected genes. The high number of expressed genes detected in sequencing enables accurate RNA expression studies. Using the REPLI-g Single Cell RNA Library Kit, we detected a comparable number of expressed genes with 10 ng total input RNA as with a standard RNA-Seq library prep kit with 1 µg input RNA. In the figure, we compare the number of detected genes with an FPKM (fragments per kb of transcript per million mapped reads) value of at least 1. In RNA-Seq, the relative expression of a transcript is proportional to the number of cDNA fragments that originate from it.



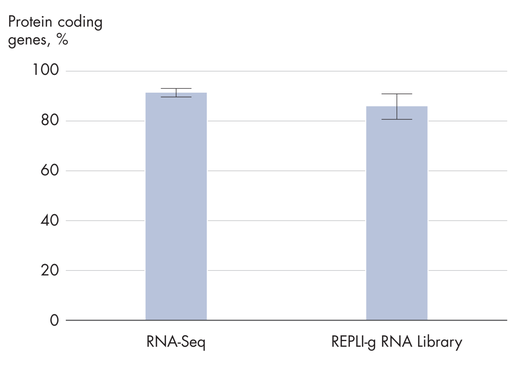
Comparison of number of detected genes obtained with RNA-Seq and REPLI-g RNA library Kit. Detected genes with FPKM>1.

Significant Number of Reads Belong to Protein-Coding RNA

We show that with the REPLI-g Single Cell RNA Library Kit, we obtained a high percentage of protein-coding genes, comparable with RNA-Seq. The efficient mRNA transcription and efficient genomic DNA wipeout step ensure a reliable and efficient reverse transcription and amplification of mRNA, maximizing the valuable information from the experiment.



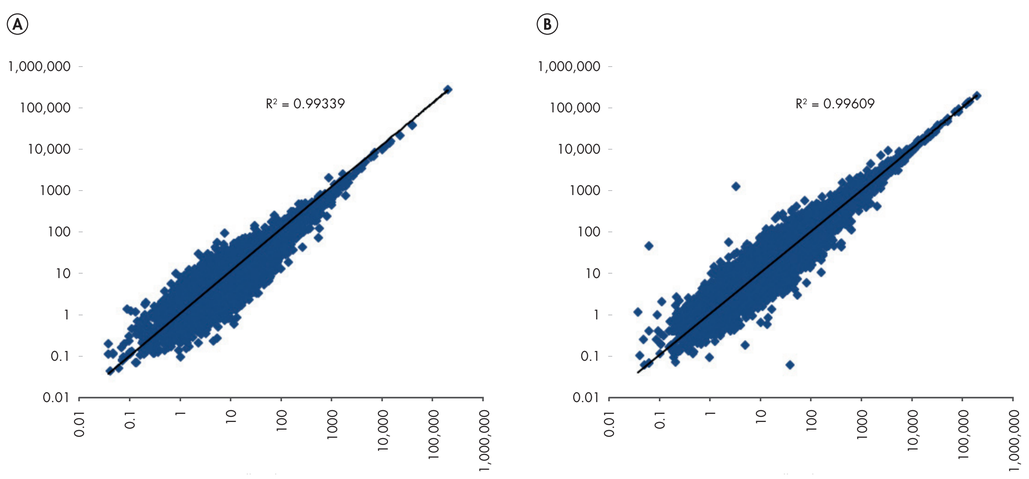
RNA biotype. Comparison of the percentage of RNA biotypes obtained with RNA-Seq and REPLI-g RNA library from 2 different cell types. With both methods, a comparable number of protein-coding genes were obtained.



Reproducibility of protocol. The REPLI-g RNA library protocol resulted in reproducible results. The percentage of protein coding genes (mean of 4 replicates with SD) is shown.

Reliable Expression Profiling

We compared expression levels of replicates in two different RNA library preparations generated with RNA-Seq and REPLI-g RNA library sequencing.



Expression levels of replicate experiments using Ⓐ RNA-Seq or Ⓑ the REPLI-g RNA Library Kit.

Conclusion

The REPLI-g Single Cell RNA Library Kit allows reliable investigation of the transcriptome from limited amounts of material. Our data demonstrate that MDA technology can be applied to amplify cDNA and result in expression analysis comparable to other RNA-Seq workflows.

The REPLI-g Single Cell RNA Library Kit provides:

- Fast time-to-result through a streamlined protocol
- Unbiased, PCR-free library construction
- Comprehensive and accurate transcriptome profiling of tiny samples
- Minimal bias and sensitive detection of low-abundance transcripts
- High-quality libraries, ready for use on any Illumina NGS platform

For more information, visit **www.qiagen.com**

1096386 08/2015

Sample to Insight