

REPLI-g® Single Cell RNA Library Kit

For RNA library construction from single cells, for Illumina® sequencing applications

Single cell analysis enables researchers to gain novel insights across a diverse set of applications in fields such as developmental biology, systems biology, cancer and stem cell research. NGS-based transcriptome analyses often require large amounts of RNA. Whole transcriptome amplification (WTA) overcomes limited RNA availability and enables analysis of single cells. The REPLI-g Single Cell RNA Library Kit allows reliable investigation of the transcriptome from a single cell, with minimal bias. The kit leverages QIAGEN's unique Multiple Displacement Amplification (MDA) technology and efficient GeneRead™ library construction technology to prepare RNA libraries from single cells with high fidelity and minimal bias, while retaining the sample's unique transcriptional profile. The innovative lysis buffer included with the kit effectively stabilizes cellular RNA, ensuring that the resulting RNA accurately reflects the in vivo gene expression profile. All enzymatic steps have been developed to enable efficient processing of RNA for accurate amplification of cDNA, which is achieved with negligible sequence bias using innovative MDA technology. The streamlined, PCR-free library construction procedure delivers high-quality libraries optimized for use on Illumina sequencing instruments.

The REPLI-g Single Cell RNA Library Kit provides:

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

One cell, one kit, one day – that's all you need for NGS-ready libraries

Investigating transcription regulation in single cells – rather than analyzing a larger number of cells and basing result interpretation on their average behavior – is of increasing scientific interest. The REPLI-g Single Cell RNA Library Kit is specifically designed to reliably investigate gene expression patterns and its regulation at the single cell level. The kit provides enzymes and buffers required to uniformly amplify all transcripts from single cells and generate a

library compatible with Illumina sequencing instruments. The resulting RNAseq library accurately represents the transcription pattern of a single cell with minimal amplification bias. Due to the high yield of MDA-amplified cDNA, in combination with the high ligation efficiency of the GeneRead library construction technology, sufficient amounts of sequencing library are generated, without the need for a library amplification step, thereby saving time. ▷

With the REPLI-g Single Cell RNA Library Kit, reaction setup is straightforward and handling time is greatly reduced, allowing reverse transcription, WTA and library preparation to be completed in a single working day. Co-optimization of WTA and library construction processes enables a highly streamlined and efficient workflow. The kit combines all the reaction steps for WTA in a one-tube protocol and all the reaction steps for library construction in a second one-tube protocol, greatly reducing hands-on time and minimizing starting material loss and cross-contamination risk. Optimized enzyme and buffer compositions ensure generation of high-quality, NGS-ready libraries in just one working day (Figure 1).

sequencing reactions. REPLI-g SensiPhi DNA Polymerase, a newly developed, high-affinity enzyme that binds cDNA more efficiently, has up to 1000-fold higher fidelity compared to normal PCR polymerases, which reduces the risk of costly false-positive or -negative results. The REPLI-g Single Cell RNA Library Kit combines the advantages of REPLI-g Single Cell technology with the superior ligation efficiency of GeneRead technology, delivering high-quality libraries ready for NGS, without the need for any library enrichment – further minimizing amplification bias.

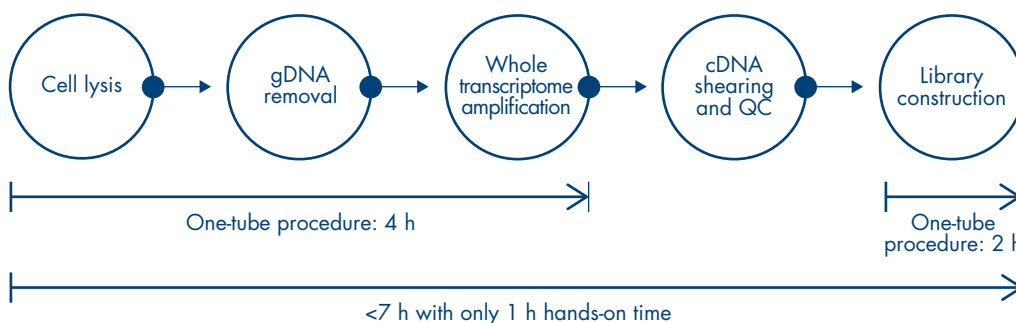


Figure 1. A time-saving, streamlined protocol delivers RNA libraries – ready for use on Illumina NGS platforms. The REPLI-g Single Cell RNA Library Kit provides a complete WTA workflow – from cell lysis, gDNA removal and cDNA synthesis to highly uniform amplification across the entire transcriptome in a one-tube protocol – with negligible sequence bias. This is followed by fast, one-tube library construction without cleanup steps between different enzymatic reactions.

Minimal bias due to PCR-free library construction

In standard PCR amplification procedures, regions of cDNA with high GC or AT content can result in little or no amplification, leading to unreliable NGS results. The REPLI-g Single Cell RNA Library Kit employs high-fidelity MDA technology to provide accurate amplification of all transcripts with negligible sequence bias. The kit includes novel REPLI-g SensiPhi DNA Polymerase, which, together with its proprietary buffer formulation, ensures uniform amplification of cDNA regions that contain highly variable GC content, thereby ensuring even coverage in subsequent

Reliable detection of all transcripts

The REPLI-g Single Cell RNA Library Kit combines several innovative technologies to reliably generate sequencing libraries from just a single cell. The Lysis Buffer contains RNA stabilization agents that effectively preserve RNA integrity, following cell lysis. gDNA Wipeout Buffer removes gDNA prior to the WTA process to ensure accurate measurement of transcript levels. Quantiscript® RT Enzyme Mix enables sensitive cDNA synthesis from single cells, while the novel REPLI-g SensiPhi DNA Polymerase uniformly amplifies cDNA across the entire transcriptome with negligible sequence bias. The sequencing library prepared

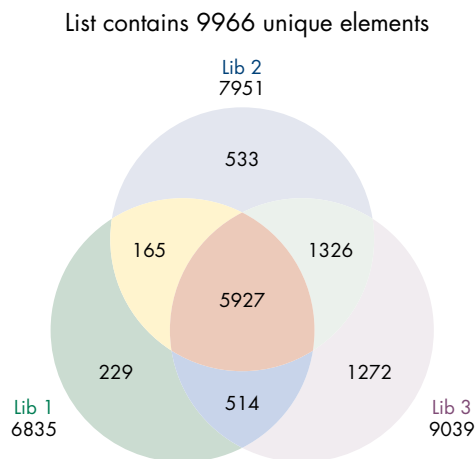


Figure 2. Single cell RNA sequencing reveals cellular heterogeneity. Sequencing libraries were generated from 3 individual HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit and sequenced on an Illumina sequencer. The plot shows the number of genes detected in each single cell.

using the kit preserves the unique gene expression profile of each individual cell and reveals cellular heterogeneity (Figure 2). Single cell analysis can be challenging when transcript abundance varies greatly within a cell. For accurate results, it is essential that the WTA procedure reliably amplifies all transcripts, regardless of their levels within the cell. The sequencing library generated by the REPLI-g Single Cell RNA Library Kit includes a high number of genes – detected even from single cells – providing a comprehensive picture of the transcriptome at the single cell level (Figure 3).

Significant number of mapped reads belong to protein-coding RNA

The ability to selectively amplify mRNA (polyA+ RNA) from total RNA preparations, makes the REPLI-g Single Cell RNA Library Kit particularly suitable for the investigation of effects

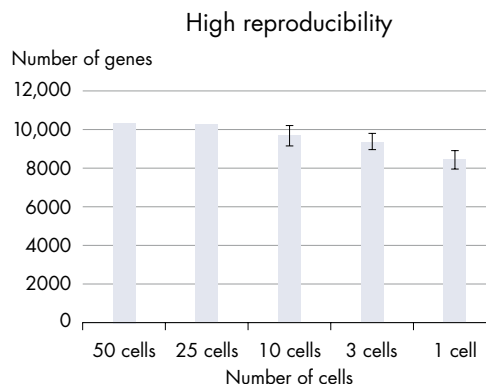


Figure 3. High number of genes is detected, independent of input cell number. RNAseq libraries were prepared from 1, 3, 10, 25 or 50 HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit and sequenced on an Illumina platform.

on transcription regulation at the single cell transcriptome level. Amplification of ribosomal RNA, which makes up >90% of the total cellular RNA population, is virtually eliminated, allowing generation of meaningful mRNA-Seq data. Following the REPLI-g Single Cell RNA Library procedure, >80% of mapped reads belong to protein-coding RNA (Figure 4).

A highly sensitive solution for multiple applications

The REPLI-g Single Cell RNA Library Kit offers an efficient, PCR-free method for RNA library construction from single cells for RNAseq applications on NGS instruments from Illumina. Fields of application include developmental biology, systems biology, characterizing bulk tumor heterogeneity, circulating tumor cell (CTC) analysis and stem cell research.



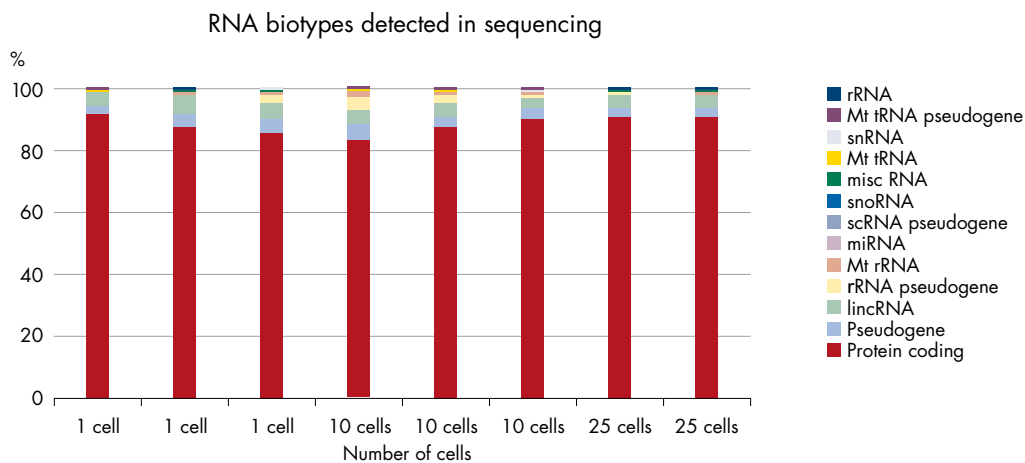


Figure 4. Superior RNAseq library quality and reproducibility with a high percentage of protein-coding reads. RNAseq libraries were prepared in triplicate from 1 or 10 HeLa S3 cells and in duplicate from 25 HeLa S3 cells using the REPLI-g Single Cell RNA Library Kit, and sequenced on an Illumina platform. The percentage of RNA biotypes detected in each library is plotted.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Single Cell RNA Library Kit (24)	REPLI-g SensiPhi DNA Polymerase, buffers and reagents for 24 x 60 µl whole transcriptome amplification reactions and subsequent end-repair, A-addition and NGS adapter-ligation; for use with Illumina NGS instruments	150073

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/goto/REPLI-g-sc-RNA for more information!

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