Product Profile

REPLI-g® Single Cell DNA Library Kit

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

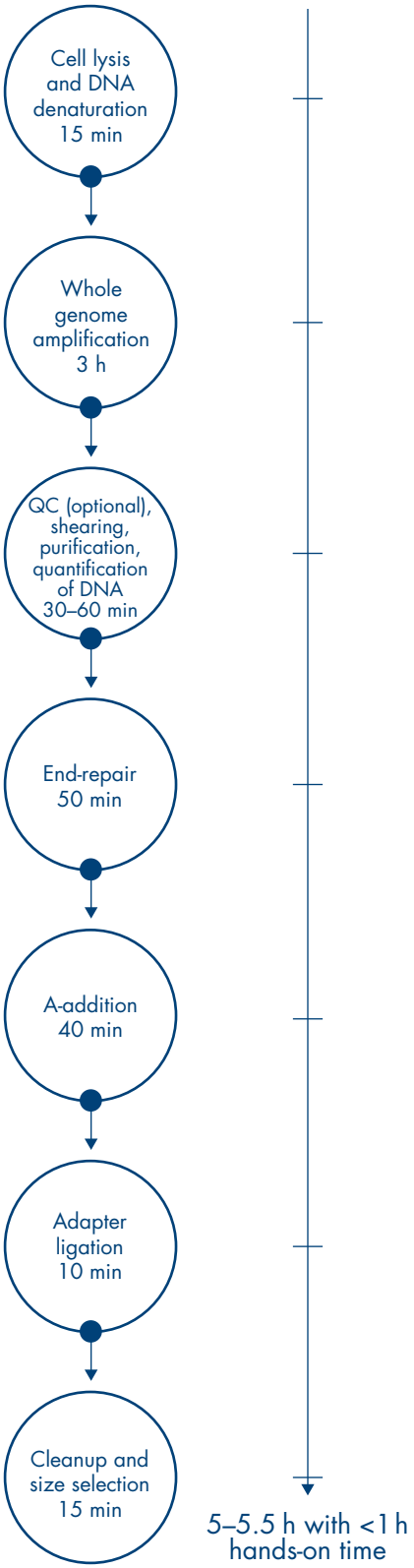
Single cell genomic analysis enables researchers to gain novel insights across a diverse set of applications, including, developmental biology, tumor heterogeneity and disease pathogenesis and progression. Conducting single cell genomic analysis using next-generation sequencing (NGS) methods has traditionally been challenging, since the amount of genomic DNA present in a single cell is very limited. PCR-based whole genome amplification methods normally have high error rates, low coverage uniformity, extensive allelic drop-outs and limited amplification yields. The new REPLI-g Single Cell DNA Library Kit leverages QIAGEN's unique multiple displacement amplification (MDA) technology and efficient GeneRead™ library construction technology to overcome these challenges by preparing a sequencing library with high fidelity and minimal bias, while retaining the sample's genomic diversity.

The REPLI-g Single Cell DNA Library Kit provides:

- Unbiased, PCR-free single cell library construction
- Complete and accurate sequence coverage from a single cell
- Fast time-to-result through a streamlined, PCR-free protocol
- 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

Genotyping and DNA sequence analysis of biological samples is limited by the small amount of sample available. Whether you want to characterize tumor cell heterogeneity, identify cytogenetic alterations in developmental biology or determine the genomic diversity of circulating tumor cells, the REPLI-g Single Cell DNA Library Kit offers a unique solution to expedite your analysis. Due to the high yield of MDA-amplified DNA, in combination with the high ligation efficiency of GeneRead library construction technology, sufficient amounts of sequencing library are generated, without the need for a library amplification step, thereby saving time and minimizing amplification bias. With the REPLI-g Single Cell DNA Library Kit, reaction setup is straightforward and handling time is greatly reduced, allowing whole genome amplification (WGA) and library preparation to be completed in a single working day. Co-optimization of WGA and library construction processes ▷



enables a highly streamlined and efficient workflow. The kit combines all the reaction steps for WGA 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).

Minimal bias due to a PCR-free workflow

In standard PCR amplification procedures, regions of DNA with high GC or AT content can result in little or no amplification, leading to misleading sequence data and NGS results. The REPLI-g Single Cell DNA Library Kit employs high-fidelity MDA technology to provide accurate amplification of genomes with negligible sequence bias and minimal genomic drop-outs. The REPLI-g Single Cell DNA Library Kit contains an optimized Phi29 DNA polymerase formulation, which, together with its proprietary buffer formulation, ensures uniform amplification of genomic regions that contain highly variable GC content, thereby ensuring even coverage in subsequent sequencing reactions. Costly false-positive or -negative results are minimized with REPLI-g technology due to Phi29 DNA polymerase, which has up to 1000-fold higher fidelity compared to normal PCR polymerases. Dedicated buffers and reagents undergo a unique, robust decontamination procedure to avoid amplification of contaminating DNA, ensuring high reliability (Figures 2 and 4).

High-quality sequencing libraries

The REPLI-g Single Cell DNA Library Kit combines the advantages of REPLI-g Single Cell technology with the high ligation efficiency of GeneRead technology, delivering high-quality libraries ready for NGS, without the need for any library enrichment – avoiding additional amplification bias. Due to the high yields achieved during the WGA step, as well as the high ligation efficiency of the library construction reagents, library preparation can be performed without PCR-based

Figure 1. A time-saving, streamlined protocol delivers ready-to-use libraries, suitable for use on Illumina NGS platforms. The REPLI-g Single Cell DNA Library Kit provides a complete workflow for highly uniform amplification across the entire genome, with negligible sequence bias, followed by fast, one-tube library construction.

library amplification, which can introduce coverage bias and reduce library diversity. The kit allows construction of complex libraries from single cells or limited DNA materials with a high percentage of mapped reads, uniform genome coverage, high sequence complexity and low error rates, and outperforms PCR-based single cell library construction products from alternative suppliers (Figures 3–5).

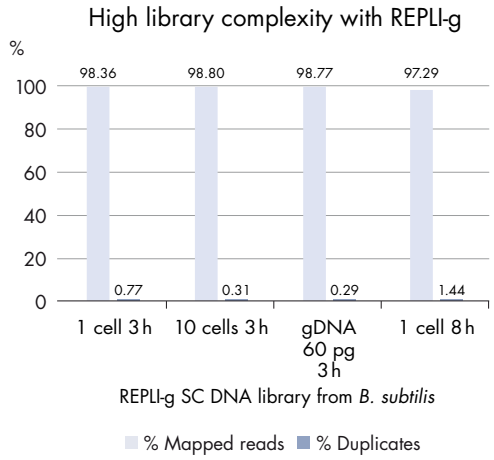


Figure 2. High-quality libraries with a high percentage of mapped reads and a very low percentage of duplicates. For analysis, 1 cell, 10 cells or gDNA were used as starting material. WGA was carried out for 3 h or 8 h. The complexity of libraries prepared using the REPLI-g Single Cell DNA Library Kit was very high, as indicated by the extremely low percentage of duplicates detected, enabling efficient use of sequencing capacity. Similar results were obtained independent of the amount of starting material and incubation time.

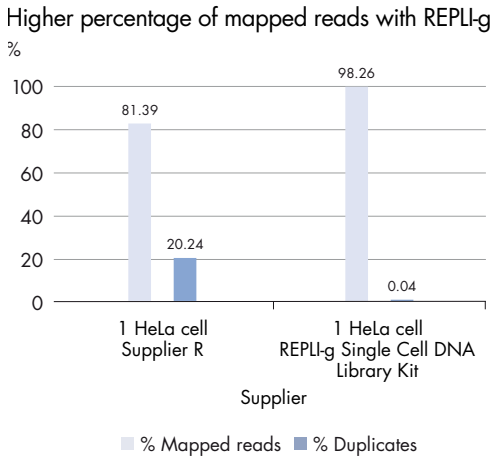


Figure 3. The REPLI-g Single Cell DNA Library Kit outperforms PCR-based single cell library prep. Data from libraries constructed from one HeLaS3 cell is shown. Compared to a PCR-based single cell library kit, the REPLI-g Single Cell DNA Library Kit provided a higher percentage of mapped reads and an extremely low percentage of duplicates.

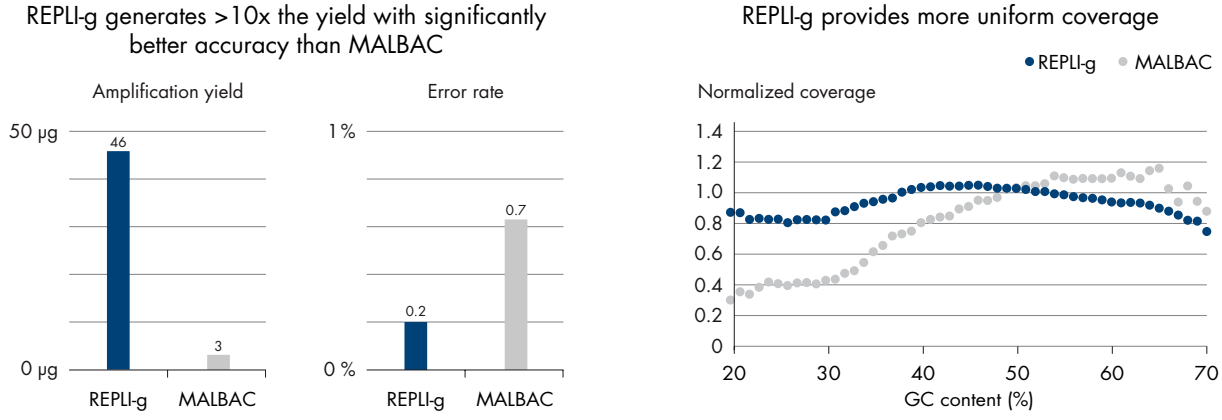


Figure 4. Superior performance, greater accuracy and more even coverage. For analysis, 1 pg *E. coli* DH10B DNA was amplified with either the REPLI-g Single Cell DNA Library Kit or by the MALBAC (Multiple Annealing and Looping Based Amplification Cycles) method (Supplier Y) and sequenced on a MiSeq® instrument (V2, 2x150 nt). The REPLI-g Single Cell DNA Library Kit generated >10x the yield, with significantly improved accuracy than the MALBAC method and ensured more even coverage and significantly better accuracy than MALBAC.

Uniform and complete sequence coverage – regardless of GC content

The REPLI-g Single Cell DNA Library Kit contains an optimized Phi29 DNA polymerase formulation, which, together with its unique buffer formulation, ensures uniform amplification of genomic regions that contain highly variable GC content. The kit allows construction of complex libraries with high sequence coverage and very low number of duplicate reads (% duplicates), and outperforms products from alternative suppliers (Figures 3–5). Libraries generated using the REPLI-g Single Cell DNA Library Kit provide uniform coverage, independent of GC content (Figures 4–5).

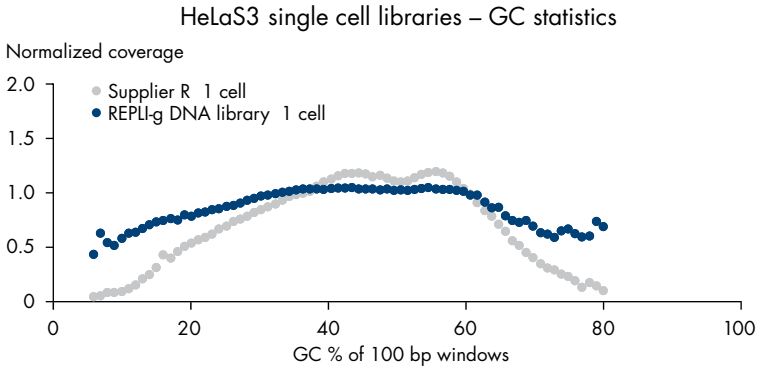


Figure 5. Uniform coverage over a wide range of %GC content. Compared to a product from Supplier R, libraries generated using the REPLI-g Single Cell DNA Library Kit provided greater and more uniform coverage over a wide range of GC content (%).

A highly sensitive solution for multiple applications

The REPLI-g Single Cell DNA Library Kit offers an efficient, PCR-free method for NGS library construction from single cells, which makes it highly suitable for applications in the fields of developmental biology and microbial research, as well as for characterizing tumor cell heterogeneity and studying the genomic diversity of circulating tumor cells.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Single Cell DNA Library Kit (48)	REPLI-g sc DNA Polymerase, Buffers and Reagents for 48 whole genome amplification reactions and subsequent end-repair, A-addition and NGS adapter-ligation; for use with Illumina instruments	150354

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-DNA** for more information!