

REPLI-g® Single Cell Kit

For whole genome amplification (WGA) from single cells or limited sample material

DNA sequence analysis and genotyping of biological samples using innovative instrumentation is often limited by the small amount of sample available. With dedicated, UV-treated ingredients that eliminate any detectable contaminating DNA, the REPLI-g Single Cell Kit is specially designed to uniformly amplify genomic DNA from single cells (<1000 cells to as little as 1 bacterial or tumor cell) or purified genomic DNA, with negligible sequence bias and maximized genome coverage.

The REPLI-g Single Cell Kit provides:

- WGA from single cell material with maximized genome coverage
- Unbiased amplification of genomic loci due to MDA technology
- Optimized for use with new technologies like next-generation sequencing
- Consistent yields of up to 40 µg (average product length >10 kb)
- An innovative tool (for e.g., cancer research and metagenomics)

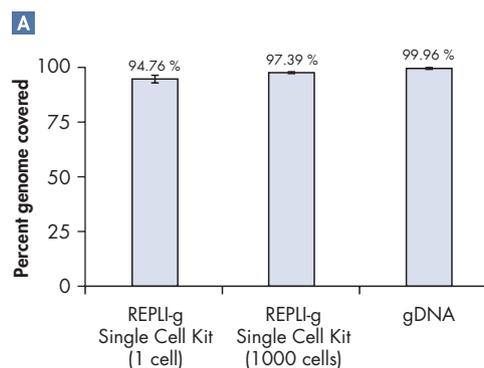
High yields from just a single cell, suitable for numerous applications

The REPLI-g Single Cell Kit is specifically designed to provide high yields of amplified DNA from single cells, such as isolated tumor cells or bacteria. It can also be used for a broad range of applications with various clinical and non-clinical research samples, including purified genomic DNA (gDNA), fresh or dried blood, and fresh or frozen tissue (Table 1). With an average product length of >10 kb and complete genome coverage, REPLI-g Single Cell amplified DNA is highly suited, and has been developed, for next-generation sequencing (NGS) (Figure 1), array-based comparative genomic hybridization (array CGH), or qPCR analysis (Table 2, Figure 3). ▶

Testimonial:

"I have tried the kit and successfully performed single-cell whole-genome amplification. The DNA obtained allowed me to reliably detect allele-specific point mutations in a colorectal adenocarcinoma cell line. The excellent yield and sequence representation of the amplified DNA really helped me to advance my work in single-cell genomics."

Dr. Masafumi Muratani, Research Associate, Cancer Therapeutics & Stratified Oncology, Genome Institute of Singapore



	REPLI-g Single Cell Kit (1 cell)	REPLI-g Single Cell Kit (1000 cells)	gDNA
Error rate	0.43 %	0.22 %	0.35 %
Chimeras	0.08 %	0.06 %	0.11 %
Indel rate	0.05 %	0.02 %	0.01 %

Figure 1. Comparable NGS results obtained using purified genomic DNA or REPLI-g Single Cell amplified DNA. Whole genome sequencing of the *Bacillus subtilis* genome was performed on the Illumina® MiSeq® instrument. For analysis, 2 µg of genomic DNA or DNA amplified from a single cell (three different single cell experiments) and 10³ cells, using the REPLI-g Single Cell Kit, was sheared into 300 bp fragments and 1 µg of each was used for library preparation.

A Comparable sequence coverage was observed for gDNA and REPLI-g Single Cell amplified DNA*. **B** Comparison of non-amplified and REPLI-g amplified DNA revealed error rates in a similar, very low, percentage range[†].

* Aligned using the Burrows-Wheeler Alignment program (cut-off: 10x coverage): bio-bwa.sourceforge.net.

[†] Comparison on non-amplified and REPLI-g Single Cell amplified DNA also revealed that sequences mapped to the genome with high percentage rates (data not shown).



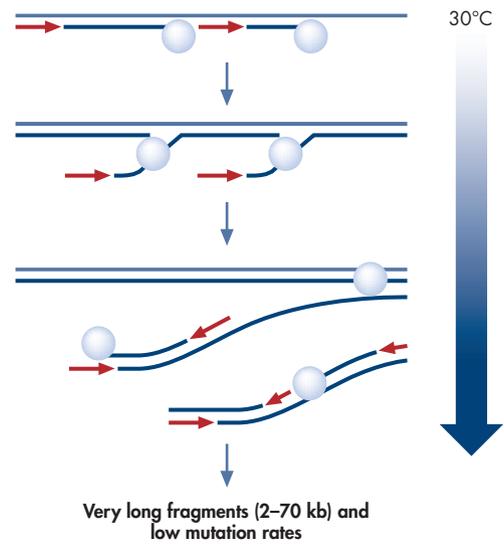


Figure 2. Multiple Displacement Amplification (MDA) technology delivers long read lengths with isothermal amplification. Primers (arrows) anneal to the template DNA and are extended at 30°C by Phi 29 polymerase, which moves along the DNA template strand, displacing the complementary strand while becoming 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.

Addressing single cell analysis requirements for NGS and array technologies

New, high-throughput NGS and array technologies have led to fast and versatile results in a number of research fields (Table 1). Today's NGS research requires the ability to accurately sequence DNA from a single cell — for example, to analyze somatic DNA mutations in tumor cells, or individual bacterial genomes. This relies heavily on technologies that yield sufficient amounts and quality of DNA for subsequent analysis. While a single cell comprises femtograms (bacteria) to picograms (eukaryotes) of DNA, micrograms of DNA are necessary for sequencing or microarray analyses. The REPLI-g Single Cell Kit has been specially developed and optimized to address these particular needs, allowing researchers to obtain trusted results when relying on modern platform technologies.

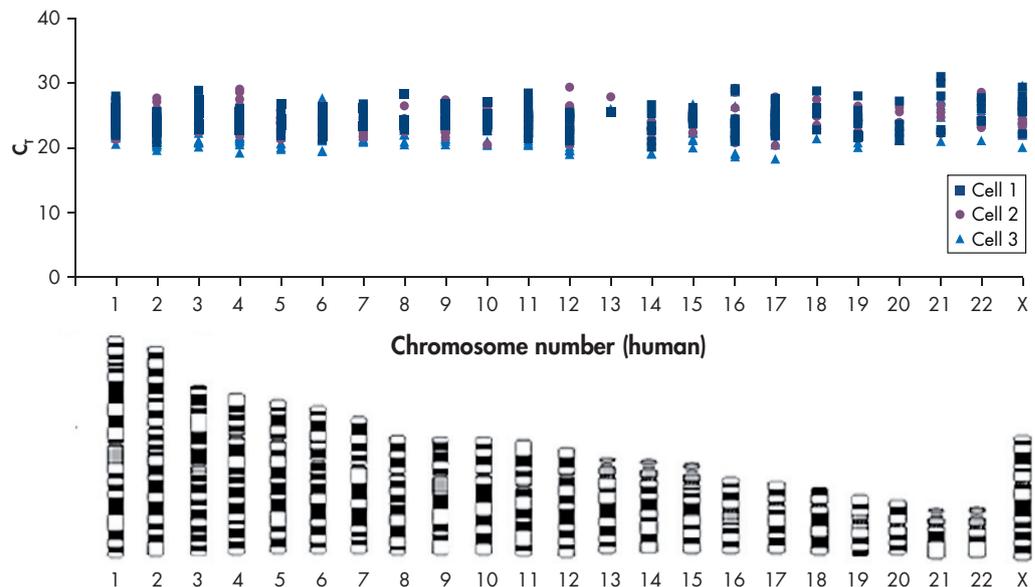


Figure 3. Complete genome coverage. Comprehensive analysis of 267 loci spread out over different chromosomes across the entire human genome (as indicated) was performed using RT² qPCR Primer Assays (QIAGEN) and real-time PCR following DNA amplification with the REPLI-g Single Cell Kit from 3 different single cell experiments. Low and consistent C_T values, with no dropout from any marker, indicate that DNA was successfully amplified from all areas of the genome and is highly suited for single-cell genomics.

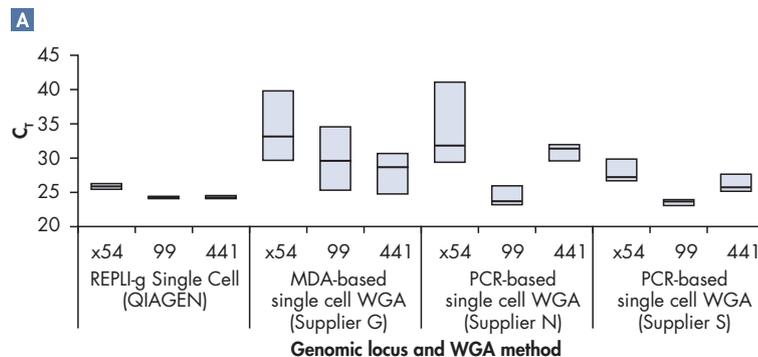
High-fidelity whole genome amplification using advanced MDA technology

REPLI-g single cell technology provides highly uniform DNA amplification across the entire genome. The REPLI-g sc Polymerase is an optimized formulation of the innovative, high-fidelity enzyme Phi29 polymerase, with strong displacement activity, to amplify complex genomic DNA by Multiple Displacement Amplification (MDA), combined with a gentle alkaline denaturation step to prevent DNA fragmentation and generation of abasic sites. Uniform amplification of genomic loci is ensured with Phi 29 polymerase, which replicates up to 70 kb without dissociating from the genomic DNA template and allows uniform amplification of genomic loci (Figure 2). In contrast to PCR-based WGA technologies, Phi 29 polymerase has 3'→5' exonuclease proofreading activity and maintains up to 1000-fold higher fidelity than *Taq* DNA polymerase during replication.

The REPLI-g Single Cell Kit outperforms the competition

PCR-based WGA methods, as generally used by other suppliers, can lead to short fragments and error-prone amplification that results in, for example, single base-pair mutations, STR contractions, and expansions, and also leads to biased or mutated loci due to the use of the low-fidelity enzyme *Taq* DNA polymerase.

In contrast, the REPLI-g Single Cell Kit provides highly uniform amplification across the entire genome, with minimal locus bias during amplification. We tested 4 WGA kits, using single-cell amplification protocols specific for each kit, for sequence representation and locus dropout. Unlike with the REPLI-g Single Cell Kit, single cells analyzed using kits from other suppliers often failed in complete and unbiased sequence representation (Figure 4).



B

Dropout rates

Genomic marker	QIAGEN	Supplier G	Supplier N	Supplier S
X54	0 %	40 %	40 %	0 %
99	0 %	20 %	0 %	0 %
441	0 %	0 %	0 %	0 %

Table 1. Range of sample material and research areas

Sample material (cells/DNA)	Research area
Human/animal	Biomarker research (SNPs, mutations, CNVs) Stem cell research Analysis of circulating fetal cells Mosaicism studies Genetic predisposition studies Typing of transgenic animals
Cancer	Somatic genetic variant analysis Tumor progression Tumor stem cells/evolution Analysis of circulating tumor cells
Bacteria	Metagenomic studies Pathogen analysis Microbial genotyping
Plants*	Stomata research Pollen analysis

* Cells without cell walls or purified genomic DNA.

Table 2. Downstream applications and instrumentation

Application	Research area
Whole genome sequencing Exome sequencing	Next-generation sequencing platforms†
SNP genotyping arrays Array CGH	Array platforms†
qPCR/PCR technologies	Real-time PCR/PCR cyclers†
Sanger sequencing	Capillary sequencers†
Pyrosequencing®	PyroMark® (QIAGEN)

† Various suppliers.

Figure 4. The REPLI-g Single Cell Kit delivers highly reliable, unbiased amplification of DNA from a single cell. The REPLI-g Single Cell Kit or single cell kits from Suppliers G, N, and S were used to individually amplify 5 human cells. Real-time PCR was used to analyze 3 markers to identify loss or variability in the amount of genomic loci. **A** Unlike kits from other suppliers, the REPLI-g Single Cell Kit delivered unbiased amplification of DNA in each of the 5 cells, indicated by equivalent C_t values for each marker. **B** Unlike with the REPLI-g Single Cell Kit, DNA amplified using the kits from Suppliers G and N demonstrated high dropout rates. For both kits, genomic marker X54 was not amplified in 2 of the 5 cells tested, and the kit from Supplier G did not amplify marker 99 in 1 of the 5 cells, indicating incomplete genome coverage and biased amplification that makes these kits unsuitable for reliable single cell research.

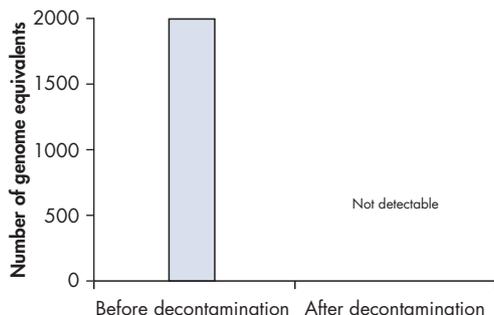


Figure 5. Innovative UV treatment eliminates any detectable trace of residual DNA in kit components. Bacterial DNA (2000 copies) was spiked into REPLI-g sc Reaction Buffer, which was then irradiated with UV using the standard procedure for all buffers and reagents provided with the REPLI-g Single Cell Kit (following UV treatment, the kits undergo stringent quality control to ensure complete functionality). In subsequent real-time PCR, no bacterial DNA was detectable following the UV decontamination procedure.

Innovative and convenient procedure — dedicated for single cell WGA

The REPLI-g Single Cell Kit uses dedicated buffers and reagents, along with easy reaction setup and a low handling time of just 15 minutes, to deliver highly pure whole genome amplified DNA from single cells, limited tissue material, and purified DNA with maximized sequence representation and unbiased amplification (Table 3). These unique components, along with an innovative and standardized UV treatment procedure to eliminate any detectable residual contaminating DNA, ensure high-quality results from just single cells (Figure 5).

Table 3. REPLI-g Single Cell Kit components

Kit component	Advantages
REPLI-g sc Polymerase	Long fragments up to 70 kb 1000-fold higher fidelity than <i>Taq</i> Maximized sequence representation Homogenous amplification for all loci
REPLI-g sc Reaction Buffer*	Optimized for unbiased amplification and representation of all loci
Buffer DLB (lysis and denaturation)	Efficient preparation for amplification Non-DNA damaging process
UV decontamination process	Ensures elimination of detectable residual DNA contamination

* Contains salt, primers, and dNTPs.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Single Cell Kit (24)	REPLI-g sc Polymerase, Buffers, and Reagents for 24 x 50 µl whole genome amplification reactions (yields up to 40 µg/reaction)	150343
REPLI-g Single Cell Kit (96)	REPLI-g sc Polymerase, Polymerase, Buffers, and Reagents for 96 x 50 µl whole genome amplification reactions (yields up to 40 µg/reaction)	150345

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/NGS or www.qiagen.com/goto/WGA for more information!

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