Accurate genome analysis of single cells

Christian Korfhage, Evelyn Fisch, Evelyn Fricke, Silke Baedker, Ulla Deutsch, and Dirk Loeffert

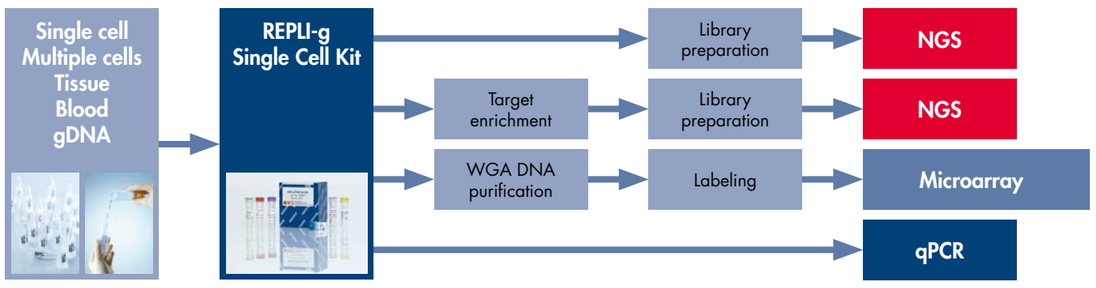
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

Whole genome analysis can be performed by next-generation-sequencing (NGS) techniques, microarrays, or parallel real-time PCR addressing multiple genomic regions. These analyses require a minimal amount of genomic DNA in the range of 100 to 1000 ng, which corresponds to 16,000–160,000 cells (e.g., human cells). The use of a high number of cells is not appropriate to analyze single-cell variations of the genome.

For analysis of genomic differences between individual cells, accurate replication of the single-cell genome is required. Here, we describe the reliability of single-cell whole genome amplification (WGA) and its application in NGS and real-time PCR. For this analysis, the QIAGEN® REPLI-g® Single Cell Kit was used for multiple-displacement-amplification (MDA) utilizing:

- An optimized formulation of Phi29
- High proofreading activity
- High processivity



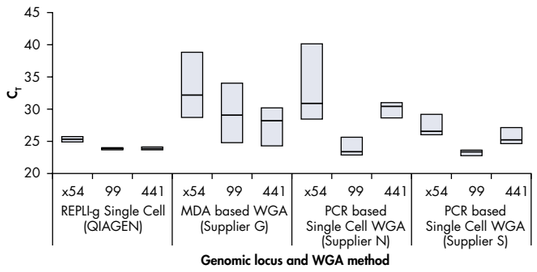
From tiny samples to analysis of the complete genome. DNA samples can be used directly for WGA using the REPLI-g Single Cell Kit. The WGA product can be used in the same way as genomic DNA is used for further genome analysis (e.g., in NGS).

Comparison of different methods

Experiment: 4 different WGA methods (QIAGEN and suppliers G, N, and S) were compared. Each method was applied to 5 individual human cells according to the supplier's protocol. After single-cell WGA, real-time PCR was used to analyze 3 markers (x54, 99, 441) to identify loss or variability in the amount of genomic loci. A box plot was created from C_T values. A C_T value of >40 was considered as an absence of the analyzed region after WGA.

Result: Unbiased amplification was obtained using the REPLI-g Single Cell Kit, indicated by equivalent C_T values for each marker and a far narrower box plot width. No locus dropouts were detected after REPLI-g Single Cell WGA. In contrast, the variation of individual WGA reactions is much higher and locus dropouts were found with the other WGA methods.

A



B

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

Real-time PCR results. A Box plot of real-time PCR C_T values and B dropout rates of single-cell WGA DNA. 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.

Method

WGA: Single cells were obtained by picking cells under the microscope (human cells) or by dilution (bacterial cells). Cells were stored in 4 µl PBS until use. After single cells were lysed and DNA was denatured using Buffer D2 (REPLI-g Single Cell Kit, QIAGEN), amplification reagents (REPLI-g sc Reaction Buffer, REPLI-g sc DNA Polymerase) were added. Amplification was performed for 8 hours at 30°C. Yield was determined by double-strand–specific PicoGreen dye. Typically, up to 40 µg of WGA DNA was generated during the replication process.

WGA methods from other suppliers were applied to single-cell samples in parallel.

NGS: For NGS, 2 µg of WGA DNA or genomic DNA (for control) was used for shearing (Covaris Instrument) and library preparation using TruSeq® DNA Sample Preparation Kit (Illumina®). Library was quantified and sequenced (paired-end) on a MiSeq® Instrument.

Real-time PCR: For real-time PCR, 100 pg (bacterial cells) or 10 ng (mammalian cells) of WGA DNA was analyzed using QuantiTect® SYBR® Green PCR reagents (QIAGEN). Alternatively, RT2 qPCR Arrays were used for real-time PCR analysis.

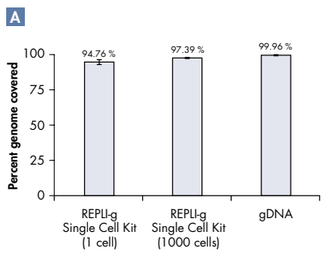
The process begins with lysis and denaturation of cells. REPLI-g Single Cell reagents are added to the lysate comprising denatured DNA. After amplification for 8 hours, up to 40 µg of DNA is generated.

Next-generation sequencing of a single cell

Single *Bacillus subtilis* cells or 10^3 cells were used for whole genome amplification using the REPLI-g Single Cell Kit. Whole genome sequencing of the *Bacillus subtilis* genome was performed on the Illumina MiSeq instrument from 2 µg of non-amplified genomic DNA or DNA amplified by REPLI-g Single Cell WGA from cells.

Comparable sequence coverage was observed for gDNA and REPLI-g Single Cell amplified DNA*. A comparison of non-amplified and REPLI-g amplified DNA revealed error rates in a similar, very low, percentage range†. The representation of regions of different GC content matches the representation of the genome.

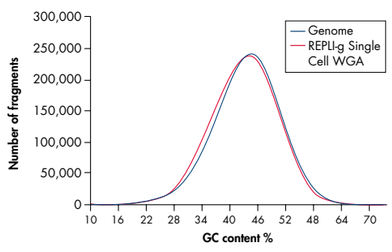
A



B

	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 %

C



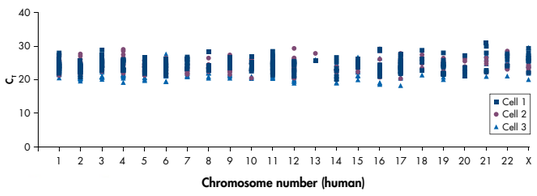
Next-generation sequencing analysis of single *Bacillus subtilis* genomes. A Genome coverage. B Error rates, chimeras, and indel rate. C GC bias of single-cell WGA, as determined by NGS. **Genome**: distribution in genomic DNA; **REPLI-g Single Cell WGA**: distribution as obtained by sequencing REPLI-g single-cell WGA from a single cell.

* 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).

Genomewide real-time PCR analysis

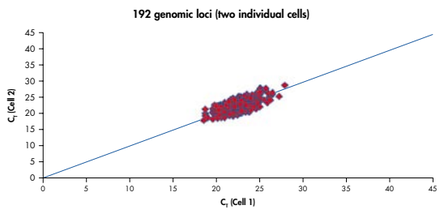
Three individual single cells were used for individual REPLI-g single-cell reactions. After WGA, a real-time PCR analysis of 267 loci across the entire genome was performed using 10 ng of WGA DNA for each primer assay. The results show low and consistent C_T values in real-time PCR for all loci, with no dropout from any marker, indicating that DNA was successfully amplified from all areas of the genome and is highly suited for single-cell genomics.



Complete genome coverage. Comprehensive analysis of 267 loci across the entire genome 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.

Variation of scWGA reactions

192 different loci were tested using real-time PCR with Quanti-Tect SYBR Green chemistry. Two DNA samples were amplified from individual human cells using the REPLI-g Single Cell Kit together with two individual scWGA reactions of human cells. C_T values for each WGA were spread on the X or Y axis respectively (scatter plot). Low cell-to-cell variations were obtained.



Scatter plot of real-time PCR. C_T values of two individual scWGA reactions from human cells are shown.

Conclusion

REPLI-g single-cell WGA offers:

- Effective lysis of cells and complete DNA denaturation
- Reliable amplification of the whole genome of asingle cell.
- Optimized strand-displacing REPLI-g sc DNA Polymerase with proofreading activity
- Increased accuracy during a single-cell WGA
- Minimized amplification bias
- Maximized sequence coverage

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.

The applications presented here are for molecular biology use. These products are not intended for the diagnosis, prevention, or treatment of a disease.

1074980 02/2012