

Accurate genome analysis of single cells



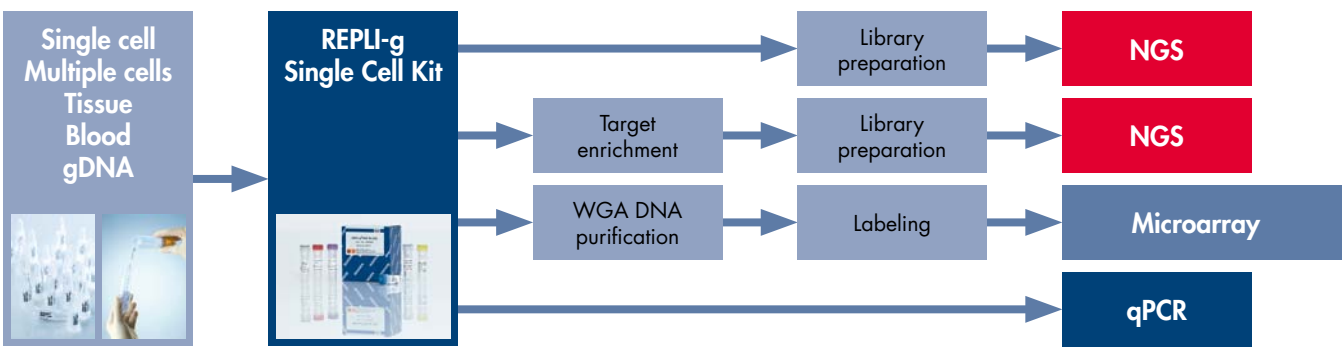
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Introduction

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).

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.

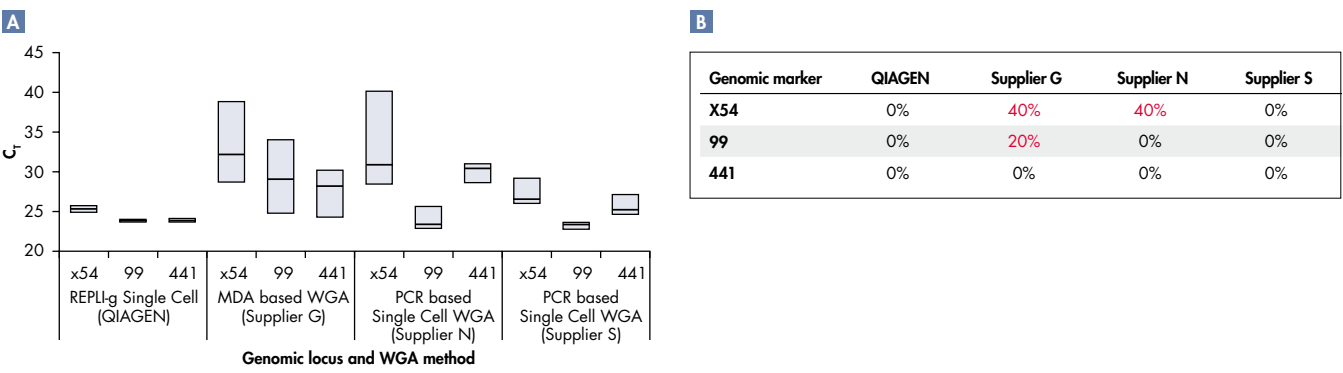


The process begins with lysis and denaturation of cells. REPLI-g Single Cell reagents are added to the lysate comprising denatured DNA. After amplification for 8 hours, up to 40 µg of DNA is generated.

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.

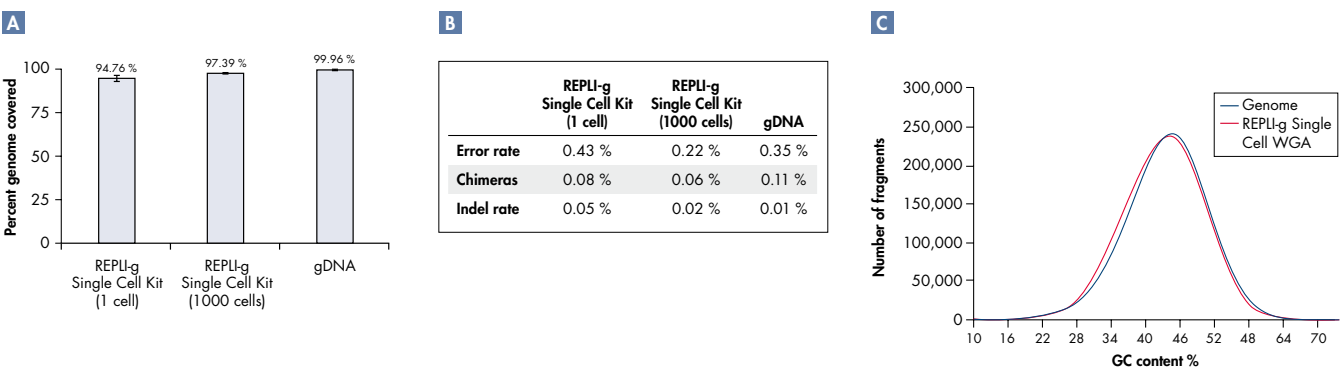


Real-time PCR results. A Box plot of real-time PCR C_T values and B dropout rates of single-cell WGA DNA. DNA amplified using the kits from Suppliers G and N demonstrated high dropout rates. For both kits, genomic marker x54 was not amplified in 2 of the 5 cells tested, and the kit from Supplier G did 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.

Next-generation sequencing of a single cell

Single *Bacillus subtilis* cells or 10³ 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.

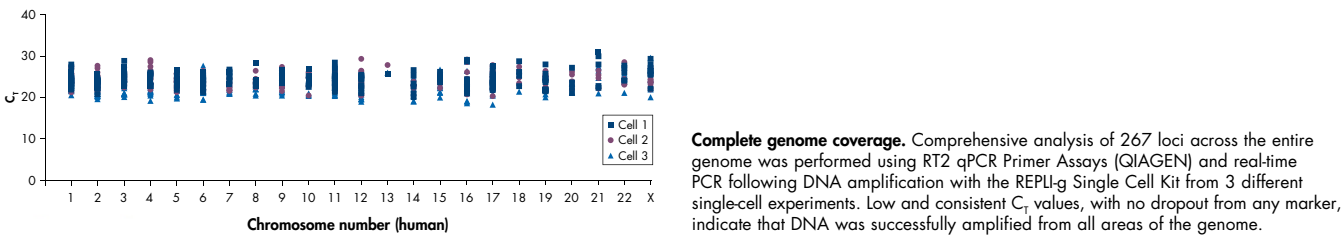


Next-generation sequencing analysis of single *Bacillus subtilis* genomes. A Genome coverage. B Error rates, chimeras, and indel rate. C GC bias of single-cell WGA, as determined by NGS. **Genome:** distribution in genomic DNA; **REPLI-g Single Cell WGA:** distribution as obtained by sequencing REPLI-g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

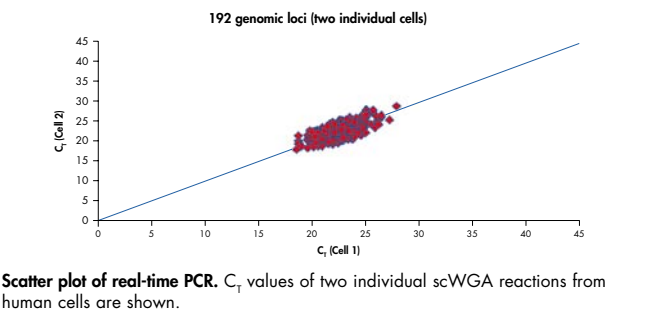
Genomewide real-time PCR analysis

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.



Variation of scWGA reactions

192 different loci were tested using real-time PCR with QuantiTect 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.



Conclusion

REPLI-g single-cell WGA offers:

- Effective lysis of cells and complete DNA denaturation
- Reliable amplification of the whole genome of a single 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

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

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