Uniform amplification of genomic DNA from 1 to 1000 cells using illustra Single Cell GenomiPhi DNA Amplification Kit

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Introduction

The analysis of genomes at the single cell level offers unprecedented biological insights in diverse fields such as cancer research, immunology, and microbiology. To enable single cell genomics, a technology for amplification of genomic DNA (gDNA) is required that provides utmost sensitivity, accuracy, and robustness. The new illustra Single Cell GenomiPhi DNA Amplification Kit has been specifically formulated to uniformly amplify genomic DNA from 1 to 1000 cells via a multiple strand displacement amplification (MDA) mechanism. New manufacturing processes, including UV and enzymatic reagent cleanup, help to ensure that all kit components are free from any detectable DNA contamination and enable sensitivity of amplification down to 1 fg of gDNA. An optional, proprietary, enzymatic cleanup step in the protocol ensures that any potential DNA contaminants introduced during setup are removed before each individual reaction.

Quality of the output DNA in terms of genome coverage, uniformity of amplification, and error rate is critical to obtain useful single cell data. Single Cell GenomiPhi DNA Amplification Kit has demonstrated robust and accurate genome amplification across multiple downstream applications.

Methods

Single Cell GenomiPhi DNA Amplification Kit amplification

Single Cell GenomiPhi DNA Amplification Kit provides a quick and simple amplification workflow (Fig 1) that can be completed in less than 3 h with less than 15 min of hands-on time.

Cells are gently lysed by adding 1 µl of lysis buffer to 1 µl of cells in PBS. The samples are then incubated for 10 min at 65°C before the addition of 1 µl of neutralization buffer to stop lysis. A reaction master mix is then added to initiate whole genome amplification (WGA). Optionally, amplification can be monitored in real time on a fluorescence plate reader or quantitative real-time PCR machine by the addition of SYBR™ Green I nucleic acid gel stain (10,000x, Sigma™, S9430) to the reaction master mix (diluted to 0.5x in final 20 µl reaction).

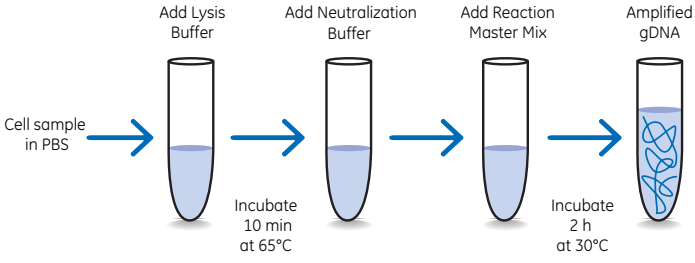

Fig 1. Single Cell GenomiPhi DNA Amplification Kit has a new, quick, and simple amplification workflow that can be completed in less than 3 h with less than 15 min of hands-on time.

An optional enzymatic cleanup protocol is also available to degrade any potential DNA contaminants introduced during setup. This step has been shown to decontaminate up to 10 pg of contaminating gDNA per reaction (Fig 2); it involves a simple 60 min incubation of reaction buffers at 30°C prior to amplification.

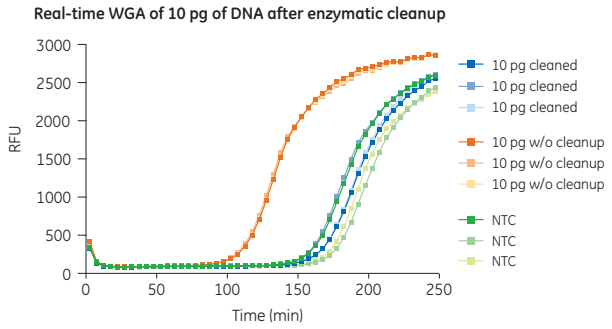

Fig 2. Six reactions containing Phi29 DNA Polymerase and Single Cell GenomiPhi Reaction Buffer were spiked with 10 pg of human gDNA as a simulated contaminant. Three reactions were incubated at 30°C for 60 min to degrade the contaminating DNA using Phi29 DNA Polymerase's exonuclease activity (10 pg cleaned). The other three reactions were left uncleaned (10 pg w/o cleanup). Real-time amplification was then performed at 30°C on a Tecan™ plate reader after the addition of nucleotides and SYBR Green I. "10 pg w/o cleanup" samples show the expected kinetics for this amount of input DNA whereas "10 pg cleaned" samples show delayed reaction kinetics similar to no template controls (NTC), indicating the absence of any amplifiable template DNA. (The increase in fluorescence observed in the NTC and cleaned-up samples is due to non-template-dependent primer extension).

Single nucleotide polymorphism (SNP) analysis

Samples containing five Jurkat cells each were obtained by dilution. Cells were lysed and gDNA was amplified using Single Cell GenomiPhi DNA Amplification Kit or a PCR-based WGA kit following the respective manufacturer's protocols. Amplified gDNA was purified as per the manufacturer's recommendations (ethanol precipitation for gDNA amplified using Single Cell GenomiPhi DNA Amplification Kit and GenElute™ PCR cleanup columns (Sigma) for PCR-amplified gDNA). Two hundred fifty nanograms of each sample was run on an Affymetrix™ Genome-Wide Human 6.0 SNP Array as per the manufacturer's instructions. Two hundred fifty nanograms of unamplified control gDNA extracted from the same cell line was run on the same type of arrays for comparison. SNP data was analyzed using Partek™ Genomics Suite™ 6.6.

Array comparative genomic hybridization (aCGH) (24sure™+ bacterial artificial chromosomal [BAC] arrays)

Samples containing five female HCC2218 human B-lymphoblast cells each were obtained by dilution. Cells were lysed, and the gDNA was amplified using Single Cell GenomiPhi DNA Amplification Kit or a competitor MDA-based WGA kit. Five hundred picograms of purified male gDNA control samples were amplified by the same WGA kits in parallel. Amplified test and control gDNA was purified by ethanol precipitation before 250 ng samples were labeled using BlueGnome™ Fluorescent Labelling System [dCTP] and run on BlueGnome 24sure+ BAC arrays. Data was analyzed using BlueFuse™ Multi Software (BlueGnome, an Illumina™ company).

Whole exome sequencing

Samples containing five female HCC2218 human B-lymphoblast cells each were obtained by dilution. Cells were lysed, and the gDNA was amplified using Single Cell GenomiPhi DNA Amplification Kit before being purified by ethanol precipitation. SureSelect™ All Exon Target Enrichment System (Human All Exon Kit V5) (Agilent™) was used to capture the exomes of amplified test and unamplified control samples (3 µg of input DNA was used for all samples). All samples then underwent sequencing on HiSeq™ 2500 platform (Illumina). DNA sequences were aligned to hg19 using BWA-0.6.1. All the parameters were set as default.

Results and discussion

Fast, consistent and sensitive amplification

Single Cell GenomiPhi is sensitive enough to consistently amplify gDNA from as little as 1 fg of starting template, as shown in Figure 3. Yields of 4 to 7 µg of gDNA are obtained from 1 pg templates in less than 2 h and from 1 fg templates in less than 4 h (data not shown).

Single Cell GenomiPhi DNA Amplification Kit has an optimized alkaline lysis method that promotes complete cell lysis and uniform denaturation of gDNA. This facilitates consistent and robust amplification from human cell samples resulting in final gDNA yields of 4 to 5 µg (Fig 4). Samples of five pooled cells with a calculated total DNA content of approximately 30 pg are amplified with very similar kinetics to control samples of 30 pg of purified gDNA.

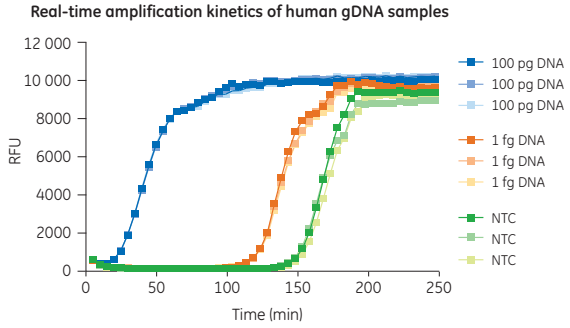

Fig 3. Human gDNA (100 pg and 1 fg) was amplified with Single Cell GenomiPhi DNA Amplification Kit. Amplification kinetics were monitored on a Tecan plate reader in real time by the addition of SYBR Green I. There is clear separation between 1 fg samples and no template control (NTC) samples, demonstrating the sensitivity of the system.

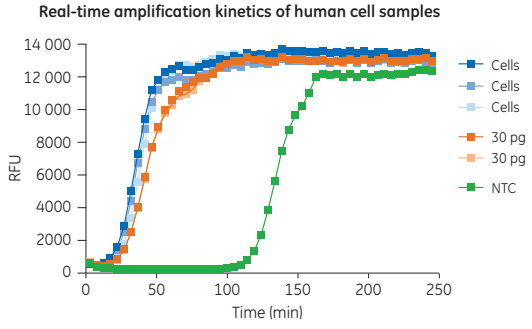

Fig 4. Three replicates of five Jurkat cells were amplified with Single Cell GenomiPhi DNA Amplification Kit. Amplification kinetics were monitored on a Tecan plate reader in real time by the addition of SYBR Green I.

SNP analysis of amplified gDNA samples

gDNA samples amplified from human cells with Single Cell GenomiPhi DNA Amplification Kit show greater than 99% genome coverage in a genome-wide SNP array (Fig 5).

Single Cell GenomiPhi DNA Amplification Kit produces minimal errors during amplification of human cell samples resulting in SNP calls that are highly concordant with the control gDNA, as shown in Figure 6.

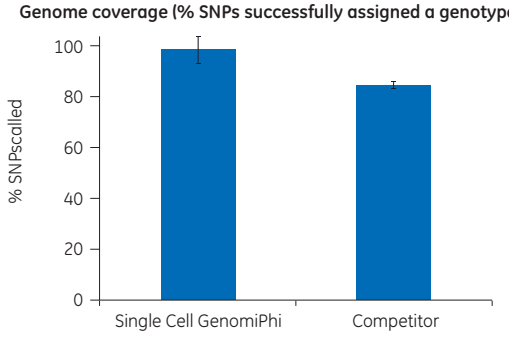

Fig 5. gDNA amplified from samples containing five Jurkat cells using Single Cell GenomiPhi DNA Amplification Kit (n=2) and a PCR-based WGA kit (Sigma GenomePlex™ Single Cell Whole Genome Amplification Kit, n=3) was run on an Affymetrix™ Genome-Wide Human 6.0 SNP Array and compared with unamplified control gDNA. Genome coverage was calculated by normalizing the percentage of SNPs successfully assigned a genotype in the amplified samples to the percentage of SNPs successfully assigned a genotype in the unamplified control samples. No statistical comparison was performed due to low sample numbers.

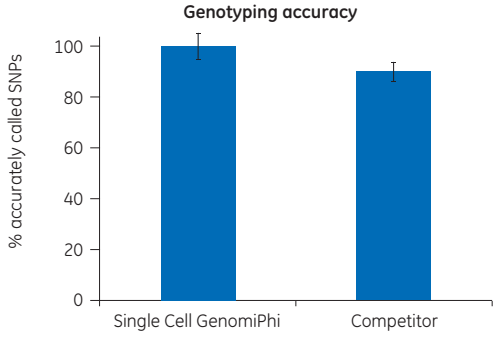

Fig 6. gDNA amplified from five cell Jurkat samples using Single Cell GenomiPhi DNA Amplification Kit (n=2) and a PCR-based WGA kit (Sigma GenomePlex Single Cell Whole Genome Amplification Kit, n=3) was run on an Affymetrix Genome-Wide Human 6.0 SNP Array and compared with unamplified control gDNA. Percentage genotyping accuracy is represented by the percentage of called SNPs that were assigned a genotype concordant to the unamplified control gDNA. No statistical comparison was performed due to low sample numbers.

Copy number analysis of SNP array data (Fig 7) demonstrates that Single Cell GenomiPhi DNA Amplification Kit produces minimal amplification bias during WGA of human cells.

Analysis of gDNA on 24sure+ BAC arrays

Single Cell GenomiPhi amplification results in DNA suitable for aCGH analysis on BlueGnome 24sure+ BAC arrays. Amplification bias, measured as Derivative Log Ratio (DLR) fused scores, falls below quality control limits (Fig 8), which allows copy number differences, such as sex mismatch calls, to be easily identified.

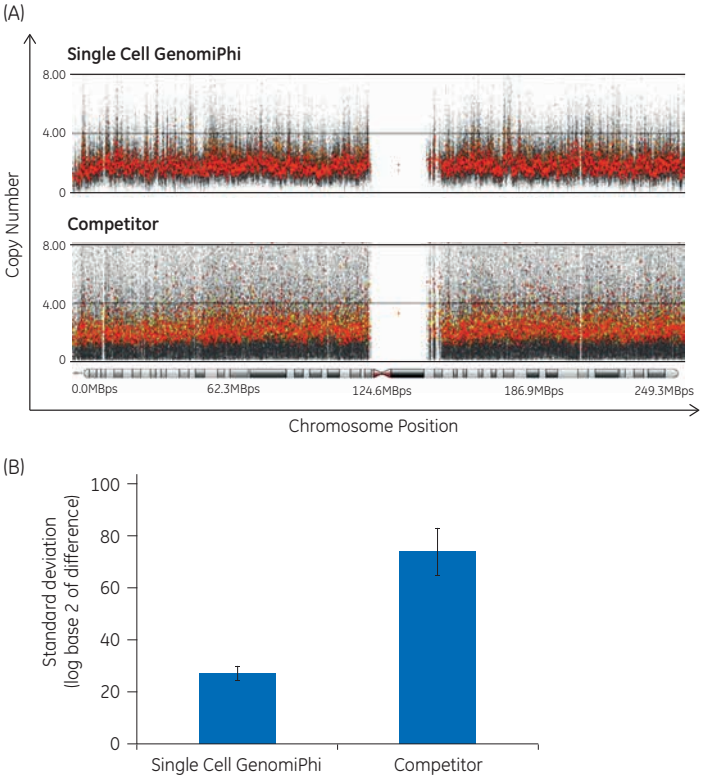

Fig 7. (A) Copy number graph showing WGA gDNA normalized to the unamplified bulk control gDNA samples. Graphs represent overlap of at least two separate samples. Colored dots = mean intensity of 30 probes; representative graph of chromosome 1 is shown. (B) Graph showing amplification bias of Single Cell GenomiPhi DNA Amplification Kit and a PCR-based WGA competitor (Sigma GenomePlex Single Cell Whole Genome Amplification Kit). Each spot intensity was compared with that same spot on one of the control slides. The standard deviation of each slide compared with that control is shown. No statistical comparison between Single Cell GenomiPhi DNA Amplification Kit and the competitor was performed due to low sample numbers.

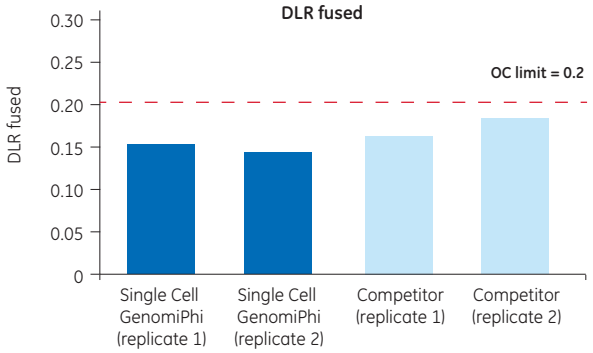

Fig 8. gDNA amplified from samples containing five female human cells using Single Cell GenomiPhi DNA Amplification Kit was run on BlueGnome 24sure+ arrays (BlueGnome) against 500 pg of purified male gDNA also amplified with Single Cell GenomiPhi DNA Amplification Kit. This was repeated using a competitor's MDA-based WGA kit (Qiagen™ REPLI-g™ Single Cell Kit). Single Cell GenomiPhi DNA Amplification Kit amplified gDNA produces low DLR fused scores that lie below the manufacturer's quality control test limits (BlueGnome Quality Measures for 24sure microarrays).

Whole exome sequencing

The percentage of sequencing data matching the human genome from human cell samples amplified using Single Cell GenomiPhi DNA Amplification Kit is equivalent to the percentage produced from unamplified gDNA control samples (Fig 9). This indicates that no contaminants are introduced and amplified during the workflow.

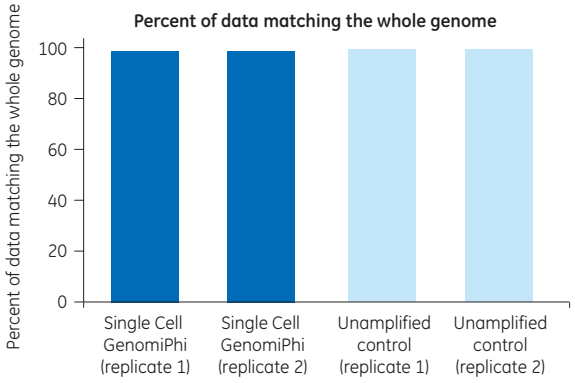

Fig 9. Greater than 98% of gDNA amplified from human cell samples using Single Cell GenomiPhi DNA Amplification Kit matches the human genome. This is equivalent to unamplified control samples.

Single Cell GenomiPhi amplified gDNA produces a high percentage of on-target exome sequence coverage, as shown in Figure 10.

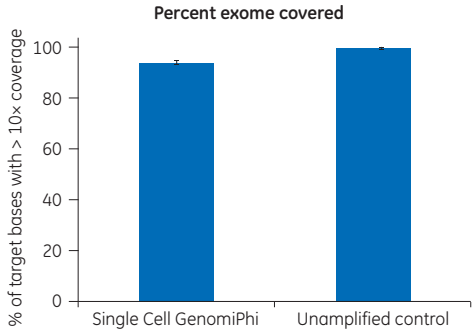

Fig 10. gDNA amplified with Single Cell GenomiPhi DNA Amplification Kit results in a high percentage of exome sequence coverage when run in whole exome sequencing (10x coverage).

Conclusions

Single Cell GenomiPhi DNA Amplification Kit provides a fast and robust method of amplifying whole genomes that is sensitive down to single cell levels. Contaminating DNA, which is a common problem in single cell experiments, is effectively eliminated. Amplified gDNA showed high genome coverage, low amplification bias, and a low error rate. The DNA was successfully used in aCGH, SNP analysis, and Next Generation Sequencing.

Ordering information

Product	Code number
illustra Single Cell GenomiPhi DNA Amplification Kit 25 reactions	29-1081-07
illustra Single Cell GenomiPhi DNA Amplification Kit 100 reactions	29-1080-39

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