Comparative transcriptome and genome analysis down to the sequence level for individual cells

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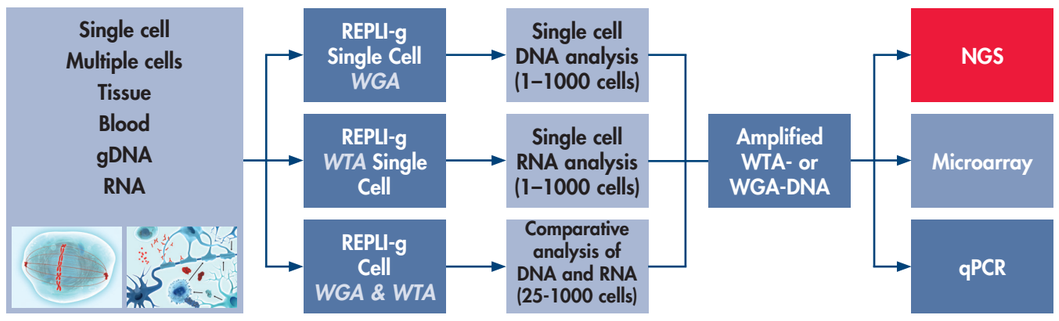
Introduction

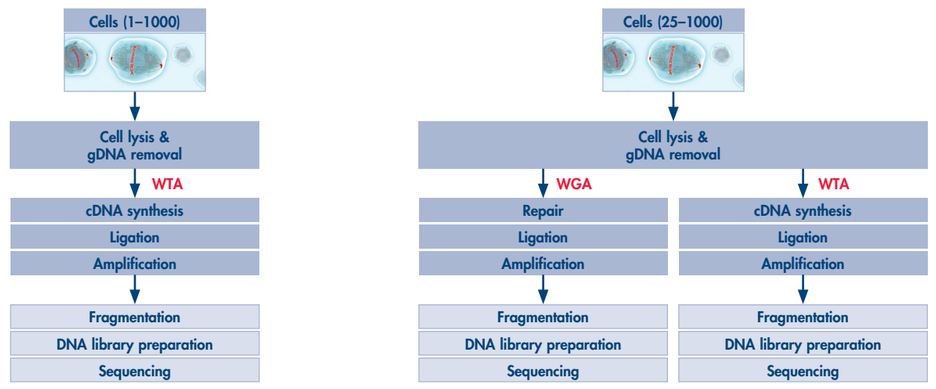
Deep genome and transcriptome analysis using next-generation sequencing (NGS), microarrays, or real-time PCR is often limited by the small amount of sample available (6 pg gDNA and 0.5 pg mRNA/human cell). A thorough analysis requires a few hundred nanograms up to micrograms of RNA or DNA. In order to overcome this, we developed new methods for whole genome amplification (WGA) and whole transcriptome amplification (WTA) from samples as small as a single cell. The methods include a new variation of Phi29 with high processivity, proofreading activity, and high affinity for a low template amount.

Here, we describe the streamlined methods for reliable RNA amplification from single cells and for parallel amplification of both DNA and RNA from a single sample for direct analysis. The method for genomic DNA amplification from single cells was previously described.



Workflow for WGA and WTA from tiny samples. Amplification is performed using the appropriate REPLI-g kit. RNA or DNA are successfully amplified from just a single cell. The product can be used in the same way as genomic DNA is used for further genome analysis (e.g., for NGS).

Whole transcriptome and genome amplification workflow



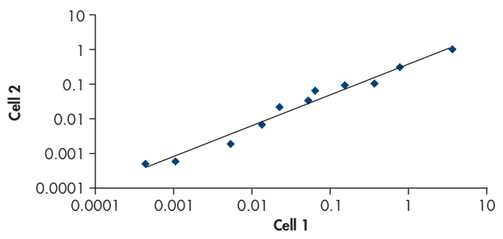
WTA from single or multiple cells. The REPLI-g WTA Single Cell protocols start with the lysis of 1–1000 cells. Up to 30 µg of cDNA is generated from a single cell. This can be used for NGS, real-time PCR, or microarray analysis. Two protocols can be applied: the amplification of mRNA with depleted rRNA or the amplification of total RNA.

Parallel WGA and WTA from a single sample. The REPLI-g Cell WGA & WTA protocols start with the lysis of 25–1000 cells. Up to 30 µg of gDNA and cDNA is amplified. This can be used for comparative genome and transcriptome analysis via NGS, real-time PCR, or microarray analysis.

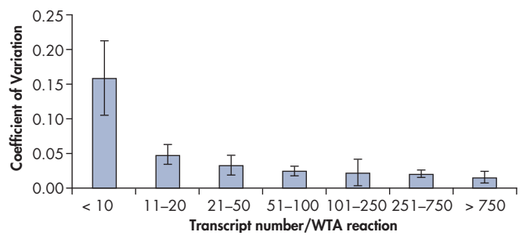
The two methods are based on a new ligation-mediated multiple displacement amplification that uses ligated cDNA or DNA as the input. The REPLI-g SensiPhi DNA Polymerase provides:

- Higher affinity for low template amounts
- Proof-reading activity for low amplification errors
- Strong DNA displacement activity.

WTA reproducibility and reliability



High reproducibility of WTA from a single cell. Using individual human cells, REPLI-g WTA Single Cell reactions were performed, including rRNA amplification. Real-time PCR of various transcripts (18S rRNA, 28S rRNA, ddx5, beta-actin, HPRT, GAPDH, PPIA, c-myc, RPS27a, BANF-1, abl-1) was performed using QuantiFast® SYBR Green PCR reagents and 1 ng of WTA cDNA. C_T values were normalized to the C_T value obtained with 18S rRNA after WTA.

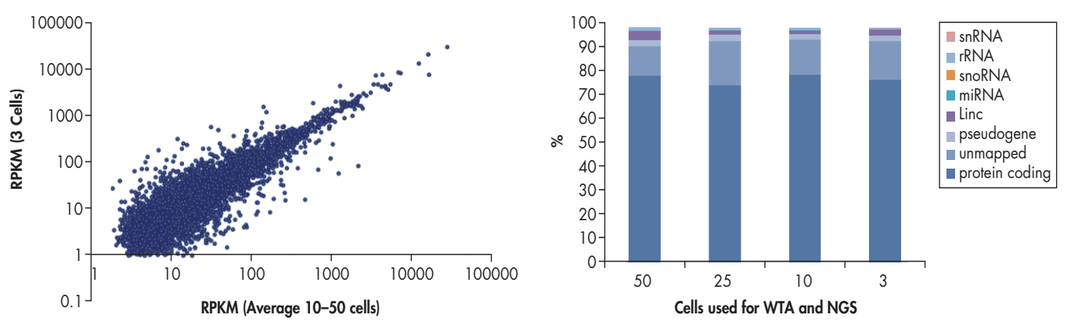


Technical noise of single-cell WTA. REPLI-g WTA Single Cell reactions were performed using 3 to 100 pg of human total RNA and the protocol for total RNA amplification (8 replicates per reaction). After WTA, 1 ng of WTA DNA was used for real-time PCR with QuantiFast SYBR Green PCR reagents to detect transcripts (28S rRNA, beta-actin, ddx5, abl-1, NFkB) of various transcript numbers per 10 pg total RNA. The coefficients of variation (CV) of the C_T values were calculated. Then the mean of the CVs for different transcript abundance classes was calculated.

The results demonstrate the high reproducibility of the REPLI-g WTA Single Cell reaction using individual cells. Normalized C_T values from two individual WTA reactions on single cells and a high R2 value (> 0.95) demonstrate a high level of concordance in RNA amplification between experiments.

If the copy number of a transcript is low (e.g., ~10 copies/sample), stochastic variability of amplification will occur due to Poisson distribution effects. The amplification of such low copy number transcripts will vary during WTA, so that the transcript copy number necessary for the limit of quantification is significantly higher than for the limit of detection.

Post-WTA transcriptome analysis by NGS

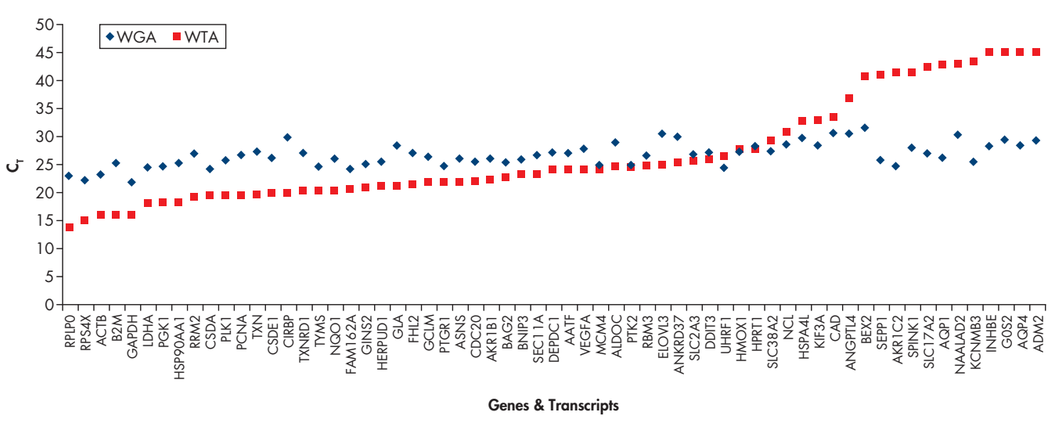


Transcriptome Analysis of single cells after WTA. REPLI-g WTA Single Cell reactions were performed on 3–50 cells in various replicates, using an mRNA (poly A+) enrichment protocol to reduce rRNA amplification. WTA cDNA was fragmented using a Covaris® S220 and an NGS sequencing library was prepared using the Gene Read Library Prep I Kit. Sequencing was done on an Illumina® MiSeq® Instrument and the RNA biotypes were mapped using Bowtie2. The reads per kilobase and million mapped reads (RPKM) were calculated.

A Results demonstrate comparable RPKM values for the 3-cell samples and the average 10–50 cell samples.

B Most of the sequence reads could be mapped to protein-coding regions. Less than 1% of reads mapped to rRNA sequences, indicating efficient enrichment for polyadenylated RNA sequences.

Analysis of genes and transcripts from the same sample



Comparative genome and transcriptome analysis after parallel WGA and WTA from the same limited cell sample. DNA and total RNA was amplified from the same 25-cell sample using the REPLI-g Cell WGA & WTA Kit. After parallel WGA and WTA amplification, 1 ng of amplified WGA DNA or WTA cDNA was used the same RT2 Profiler PCR Array to analyze over 60 genes and their corresponding transcripts, which were sorted according their expression levels. All of the genes derived from genomic DNA were detected with C_T levels between 20 and 30, while corresponding transcripts show variations due to different abundance and even undetectable transcript levels. The method is highly suited to comparative studies of the genome and transcriptome, linking the genome to the corresponding gene expression profile and to its phenotype.

Conclusion

The novel REPLI-g kit-based WGA and WTA protocols yield accurate, reliable, and repeatable results that support transcriptome and genome sequence analysis of individual cells. Using the novel proofreading REPLI-g SensiPhi DNA Polymerase gives high affinity binding to tiny template amounts. In terms of the workflow, the protocols provide:

- Effective lysis of cells without the need for a separate lysis kit
- Stabilization of all DNA and RNA without a separate isolation kit
- Reliable amplification of the whole genome or the whole transcriptome
- Flexibility for use on any detection platform, particularly next-generation sequencing
- Reliable and repeatable results from even individual cells
- Maximized transcript coverage after single cell-derived WTA

The protocols are suitable for use in comparative analyses of the genome sequence and transcriptome, e.g., in cancer analysis.

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