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.

Post-WTA transcriptome analysis by NGS

*Transcriptome Analysis of single cells after WTA. WTA with REPLI-g WTA Kit was performed using 1-pg DNA isolated from single cells. A total of 1 ng of WTA DNA was used for real-time PCR. The bar graph shows the number of reads per gene from the MiSeq analysis. The reads from the different cell lines are normalized to the number of reads from 150 kbp of human DNA, which was calculated for each 25-cell sample. The data presented here are from a collection of 25-cell samples that were sequenced in parallel. The results demonstrate comparable RPKM values for the 3-cell samples and the single cell sample. The reads were mapped to the human genome using Bowtie. The reads per million and local and global genome-specific RNA reads are shown.**

Conclusion

The novel REPLI-g-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

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

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