

Introduction

Analyzing the genomic material in a single cell has long been desirable but heretofore unachievable due to the miniscule amount of DNA available for analysis. The recent advent of commercial kits for whole genome amplification has provided scientists the means to amplify the information from ~3000 cells, affording amplification that is a complete representation of the original DNA. While advancing the field of DNA variation analysis, these kits still have limitations in fields such as oncology, molecular pathology, and *in vitro* fertilization where analyzing the DNA from a single cell is the optimal choice.

We have used a new and sensitive method for whole genome amplification, GenomePlex® Single Cell WGA Kit, to perform genomic analysis on a single cell. This Whole Genome Amplification (WGA) utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR amplifiable molecules flanked by universal priming sites. This Single Cell WGA Kit has been optimized to amplify the genome of a single cell. This poster demonstrates the utility of GenomePlex and indicates future directions for growth as the method matures.

WGA from Laser Captured Single Cells

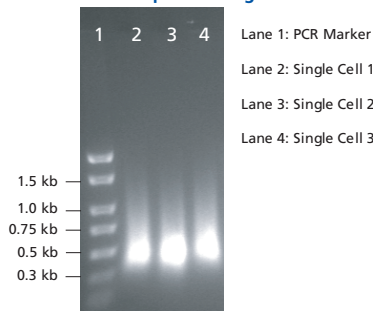


Figure 1A. WGA was performed on single human leukemia U937 cells isolated by laser capture. As visualized on the agarose gel, yield and product distribution are consistent from cell to cell.

WGA of Flow-Sorted Single Cells

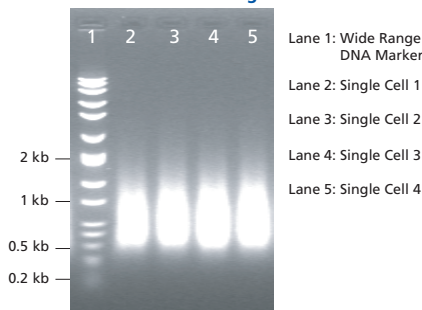


Figure 1B. Flow Cytometric Analysis and Sorting (FACS): Isolated human leukemia U937 cells were lysed and WGA amplified using the GenomePlex Single Cell WGA Kit. The DNA was then purified with the GenElute™ PCR Cleanup Kit. An estimated million-fold amplification from the WGA process resulted in a final yield ranging from 5.4–6.2 µg. The Single Cell WGA Kit produces consistent yield and size range as visualized by a 1% agarose gel. Further analysis regarding representation and allelic dropout can be viewed in Table 1 and Figures 2 and 3.

SNP Analysis of Single Cell Clones using MGB Eclipse™ Probes

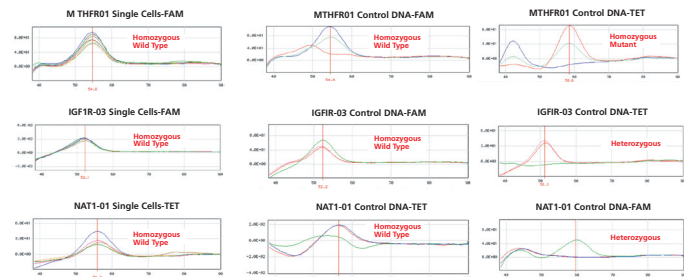


Figure 2. U937 Leukemia single cells were amplified using the GenomePlex® Single Cell Whole Amplification procedure. Ten nanograms of the cleaned single cell WGA DNA was then utilized in a MGB Eclipse™ SNP (Single Nucleotide Polymorphism) assay by quantitative PCR on the ABI 7700. The MGB Eclipse™ Probe System facilitates allelic discrimination due to its ability to allow post-PCR dissociation curves. Dissociation curves allow determination of the melting point (Tm). Different alleles will have a substantially different Tm value. The FAM and TET fluorescent signals help distinguish homozygous wild, heterozygous and homozygous mutant types. The WGA amplified single cell DNA was tested for 3 SNPs, MTHFR01, IGF1R-03 and NAT1-01. A positive, unamplified control was evaluated with each group. The data indicates that all of the single cells were the same allele for each SNP tested.

Single Cell qPCR Analysis

Chromosome	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10
1	X	0	X	X	X	X	X	0	X	X
2	0	0	X	X	0	X	X	0	0	X
3	0	0	0	0	0	0	0	0	0	0
4	X	0	X	0	0	0	X	X	X	0
5	0	0	0	0	0	0	0	0	0	0
6	X	0	X	0	0	X	X	0	0	X
7	0	0	0	0	0	0	0	0	0	0
8	X	0	X	0	0	0	X	0	X	0
9	X	0	X	0	0	0	X	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	X	0	X	0	0	X	X	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	X	0	X	0	0	0	X	0	0	0
15	X	0	X	0	0	0	X	0	0	0
16	X	0	X	0	0	0	X	0	0	0
17	X	0	X	0	0	0	X	0	0	0
18	0	0	0	0	0	0	0	0	0	0
19	X	0	X	0	0	0	X	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	X	0	X	0	0	0	X	0	0	0
22	0	0	0	0	0	0	0	0	0	0
Y	0	0	0	0	0	0	0	0	0	0

Table Key	
X	= Loci Detected
0	= Allelic Dropout

Percent Cumulative Loci Coverage by qPCR

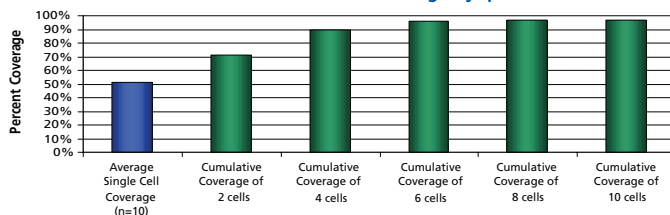


Table 1 and Figure 3. Ten human leukemia U937 single cells were FACS isolated and amplified with GenomePlex Single Cell WGA Kit. This table represents a melt curve analysis of 92 UniSTS qPCR loci spread across the human genome. The melt curves from WGA amplified single cells were compared to unamplified human genomic DNA on a Stratagene MX3000P®. Allelic Dropout (ADO) results when melt curves from WGA amplified single cells do not match unamplified human genomic DNA. Separately, the ten single cells demonstrated 33–67% ADO which suggests that no two cells are identical in this supposedly clonal sample. However, when evaluated cumulatively the ten cells show only 3% ADO, suggesting a more modest difference in the cell population. Whole genome amplification can recover the majority of a single cell's genome; nearly complete genomic coverage is obtained evaluating a small pool of cells.

Comparative Genomic Hybridization of HT29 WGA DNA to Normal WGA Human Genomic DNA, Chromosome 8

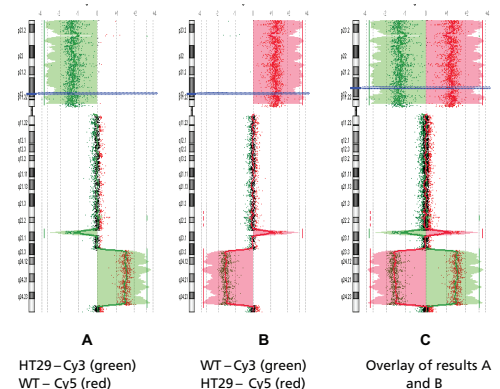


Figure 4. WGA was performed on genomic DNA isolated from HT29 colon carcinoma cells and from a healthy human male. 2.5 µg of WGA product was labeled with Cy3 or Cy5 dye using the Genomic DNA Labeling Kit PLUS (Agilent). The entire labeled sample was loaded onto an Agilent Human Genome CGH Microarray 105A. Specific activities were between 28 and 43 pmol dye/µg DNA for all samples, and always within 50% of samples being compared. The dye swaps (A and B) demonstrate that there was no bias in the DNA labeling and the aberrations detected are consistent with the HT29 karyotype reported previously.^{3,8} These CGH results are the starting point for future microarray analysis of DNA amplified from a single cell.

Conclusions and Future Work

The ability to analyze DNA at the single cell level is now available via the GenomePlex® Single Cell Whole Genome Amplification Kit. The process amplifies DNA from a single cell a millionfold increasing the researcher's power to analyze its genetic data. As demonstrated above, GenomePlex is compatible with cells isolated by flow sorting, laser capture and dilution protocols (data previously published). Data in this and other posters has demonstrated its use for SNP analysis, qPCR, and gene expression.

This work demonstrates that WGA DNA, when applied to a large pool of cells, gives positive results in Comparative Genomic Hybridization (CGH) arrays. This combined with successful single chromosome metaphase spread based CGH performed with GenomePlex single cell DNA (data by Dr. Michael Speicher) has laid the groundwork for analyzing DNA from a single cell via CGH arrays.

Additional References

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Product Offerings

- GenomePlex® Single Cell Whole Genome Amplification Kit (WGA4)**
- GenomePlex® Complete Whole Genome Amplification Kit (**WGA2**)
- GenomePlex® WGA Reamplification Kit (**WGA3**)
- GenElute™ PCR Clean-Up Kit (**NA1020**)
- GenElute™ Mammalian Genomic DNA Miniprep Kit (**G1N10**)
- SYBR® Green JumpStart™ Taq ReadyMix™ (**S4438**)

Acknowledgments

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The University of Albany's Center for Functional Genomics has kindly provided laser-captured microdissected cells to further validate the GenomePlex Single Cell WGA Kit.

- CGH Analysis performed by MOgene, LLC.
- GenomePlex is a registered trademark of Rubicon Genomics, Inc.
- GenomePlex WGA technology patent pending.
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