

Introduction

Analyzing genomic DNA at the level of the single cell will provide the ultimate level of variation analysis. Unfortunately, amplifying the picogram quantities of DNA in a single cell has been difficult. The recent advent of commercial kits for whole genome amplification have provided scientists the means to amplify the information from ~3000 cells, affording amplification that is a complete and faithful 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. The **GenomePlex® Single Cell Whole Genome Amplification Kit** was created to address these limitations.

GenomePlex is a whole genome amplification (WGA) method that allows the researcher to generate a representative amplification of genomic DNA. The kit utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR amplifiable OmniPlex Library molecules flanked by universal priming sites. The OmniPlex® library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles. The Single Cell WGA kit has been optimized to amplify the genome of a single cell. WGA from a single cell often results in a million-fold amplification yielding microgram quantities of gDNA. After purification, the Single Cell WGA product can be analyzed in a manner similar to any genomic or chromosomal DNA sample.

This poster shows data generated from single cell WGA. We show the utility of Genomeplex Single Cell WGA kit over a variety of single cell samples and numerous downstream applications.

WGA of Flow-Sorted Single Cells

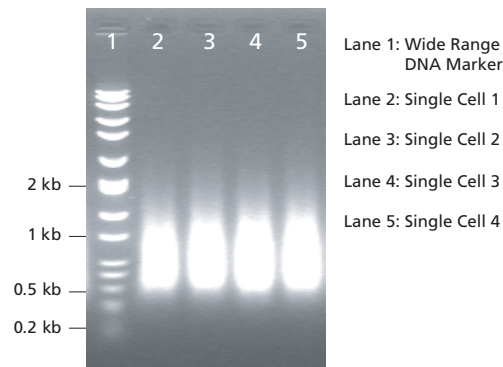


Figure 1: Human leukemia U937 cells were isolated using Flow Cytometric Analysis and sorting (FACS), lysed and WGA amplified using the GenomePlex® Single Cell WGA Kit. The DNA was then purified with the GenElute™ PCR Cleanup Kit. An estimated million-fold amplification from the WGA process resulted in 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 Figure 6.

WGA from Laser Captured Single Cells

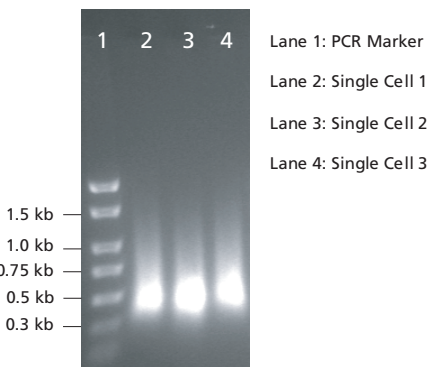


Figure 2: WGA was performed on single human leukemia U937 cells isolated by laser capture. As visualized on the agarose gel, yield and size are consistent from cell to cell.

Diluted Single Cell Tobacco Protoplasts

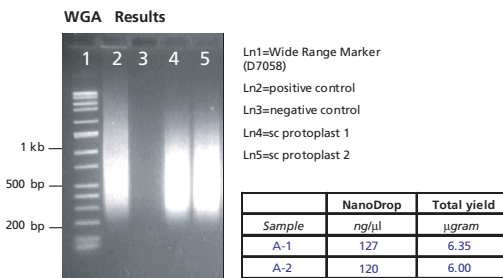


Figure 3: Tobacco protoplasts were diluted to single cell concentration. Cells were lysed and amplified using the GenomePlex Single Cell WGA Kit. Amplification resulted in approximately 6 µg of DNA per cell. Amplified DNA was used for gene expression analysis via qPCR using SYBR® Green. Relatively low allelic dropout was observed.

qPCR Gene Expression Results		
	sc1	sc2
Primer 1	16.7	18.0
Primer 2	20.4	18.0
Primer 3	20.7	17.7
Primer 4	no ct	17.9
Primer 5	16.3	17.4
Primer 6	19.4	18.1
ADO	2/6	1/6
No gene representation based on melt curve analysis		

SNP Analysis of Single Cell Clones using MGB Eclipse Probes

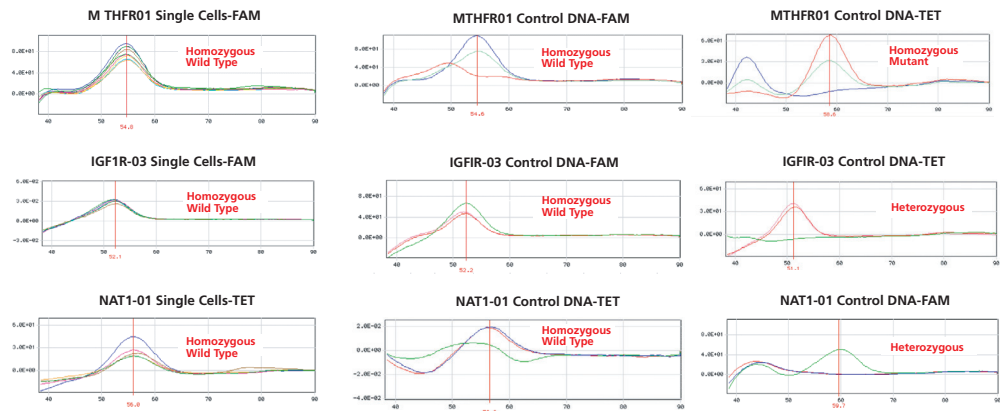


Figure 5: U937 Leukemia single cells were amplified using the GenomePlex® Single Cell Whole Amplification procedure. Ten nanograms of the cleaned single cell WGA DNA was then utilized in a MGB Eclipse™ SNP (Single Nucleotide Polymorphism) assay by quantitative PCR on the ABI 7700. The MGB Eclipse™ Probe 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, IGFIR-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.

Comparative Genomic Hybridization

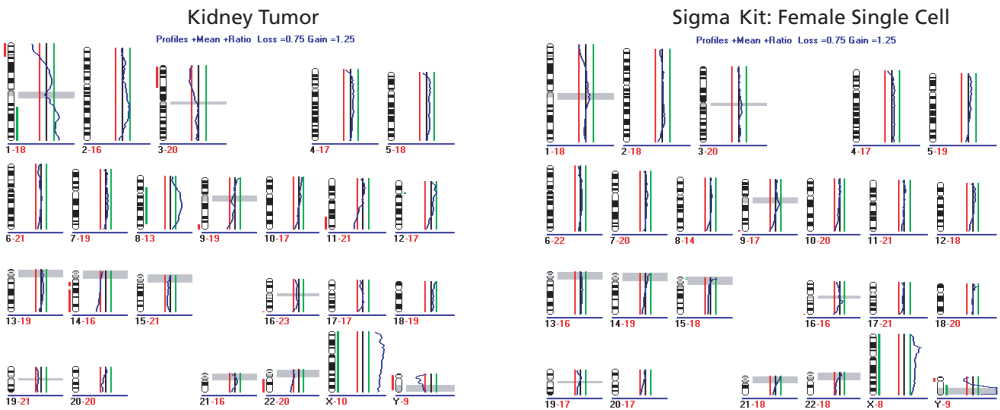


Figure 7: Two DNA samples of a known genotype were amplified using the GenomePlex Single Cell WGA Kit. Each library of amplified DNA was labeled and then hybridized to normal human metaphase spreads. The results were compared to the known genotype for each sample. In the first sample, ten kidney tumor cells were subjected to the single cell protocol. A control was also performed using a single cell from a normal female. The expected results are shown as a cartoon where regions of chromosome deletion or amplification are indicated by red or green lines respectively. The genotyping data is shown as a black line and measured CGH representation is measured as underrepresented (below the red line) or over represented (above the green line). In all cases the WGA DNA had representation values identical to the expected, previously determined values. (Data generated by Dr. Michael Speicher.)

Product Offerings

GenomePlex® Single Cell Whole Genome Amplification Kit (WGA4)

GenomePlex® Whole Genome Amplification Kit (WGA1)
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)

qPCR Plots and Melts for Single Cell WGA samples

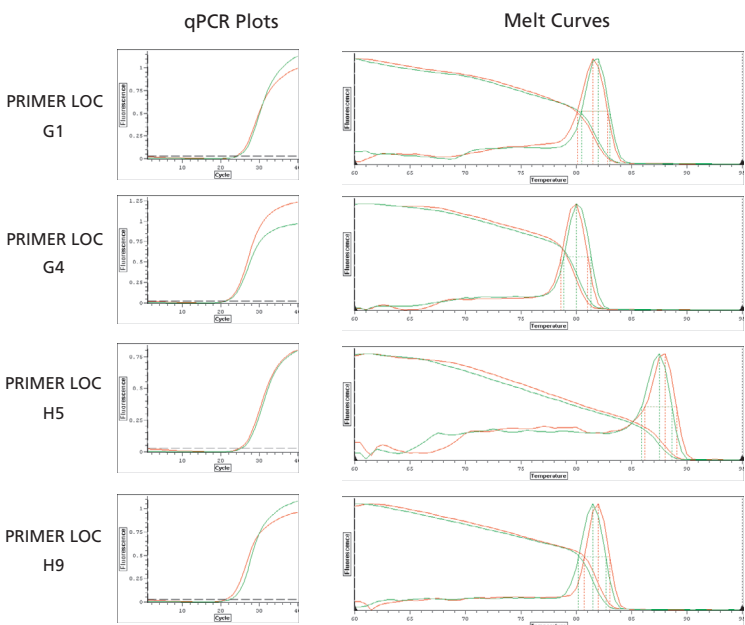


Figure 6: SYBR Green qPCR plots and melt curves for four examples of the human UniSTS primers demonstrate representative amplification of WGA product (green) when compared to unamplified gDNA (red).

Conclusions

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 GenomePlex® Single Cell Whole Genome Amplification Kit opens the door to the world within the single cell. This process amplifies the DNA from a single cell a million fold, allowing the genetic analysis of the ultimate biological unit and opening the secret to maturation, regeneration, and genetic diseases. Genomeplex is compatible with cells isolated by dilution protocols, flow sorting, and laser capture as shown in the data above. This DNA can be analyzed by SNP analysis (Figure 4), gene expression studies (Figure 6), and comparative genomic hybridization (CGH, Figure 5).

References

- Barker, D. L., *et al.* "Two methods of whole-genome amplification enable accurate genotyping across a 2320-SNP linkage panel." *Genome Research*, Vol 14 (2004): 901–907.
- Bergen A, *et al.* "Comparison of Yield and Genotyping Performance of Multiple Displacement Amplification and OmniPlex (trade mark) Whole Genome Amplified DNA Generated from Multiple Sources." *Human Mutation*, Vol 26 **2005**: 262–270.
- Gribble, S., *et al.* "Chromosome paints from single copies of chromosomes." *Chromosome Research*, Vol 12 **2004**: 143–151.
- Hughes S, Lasken R. Whole Genome Amplification. Scion, **2005**.
- Hughes S, *et al.* "The Use of Whole Genome Amplification in the Study of Human Disease." *Prog Biophys Mol Biol*, Vol 88 **2005**: 173–189.
- Kiechle F, *et al.* "The –Omics Era and its Impact." *Arch Pathol Lab Med*, Vol 128 **2004**: 1337–1345.

Acknowledgments

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