

Introduction

Most biological analyses require microgram quantities of DNA. Even the best extraction methods are unable to produce sufficient DNA when starting with difficult or limited sources. Alternatively, samples that are able to provide sufficient DNA for analytical purposes become exhausted upon subsequent testing.

Whole Genome Amplification (WGA) provides the means to immortalize the genomic DNA from fresh, frozen, archived and chemically treated samples. We show that one particular method, GenomePlex® WGA, produces 500–1000 fold unbiased amplification of genomic DNA via a PCR-based sequence-independent approach. Single-cell WGA yields 1,000,000-fold amplification. Extensive research has demonstrated the versatility of this methodology in its ability to amplify DNA from a diverse array of both plant and animal tissues. Isolated single cells and formalin-fixed, paraffin-embedded (FFPE) tissue are examples of difficult templates due to the quality and quantity of genomic DNA. GenomePlex can reliably recover and amplify these challenging samples.

Results

Consistent Representation Over a Variety of Sources

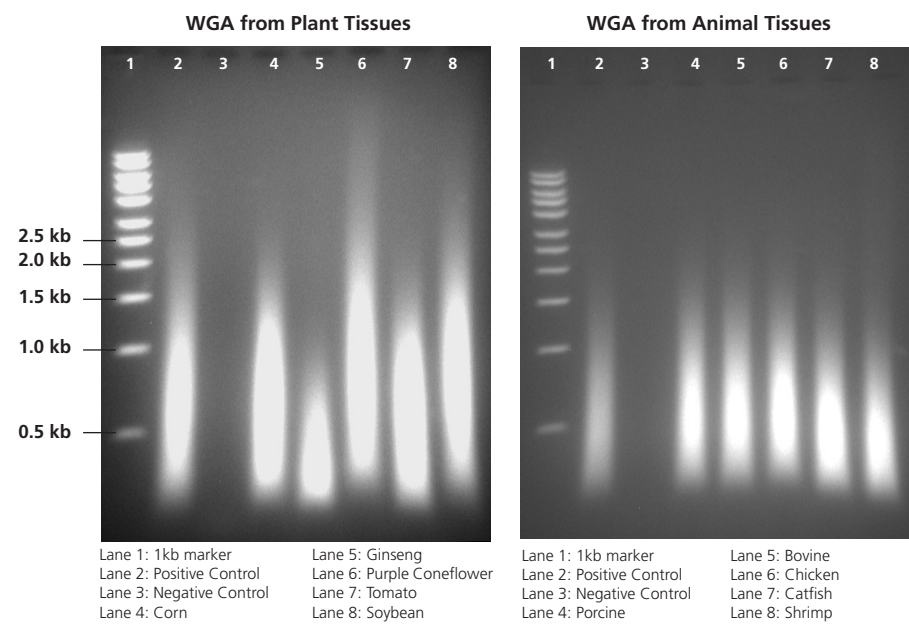


Figure 1: Multiple plant sources were WGA amplified, cleaned up with GenElute™ PCR Cleanup Kit, and resolved on a 1% agarose gel. An initial input of 10 ng genomic DNA was used for each sample, which resulted in yields between 1.9–3.9 µg.

Figure 2: Multiple animal sources were WGA amplified, cleaned up with GenElute™ PCR Cleanup Kit, and resolved on a 1% agarose gel. An initial input of 10 ng genomic DNA was used for each sample, which resulted in yields between 2.5–7.1 µg.

Resolution Equal to Purified Genomic DNA
Agilent 44K CGH Array

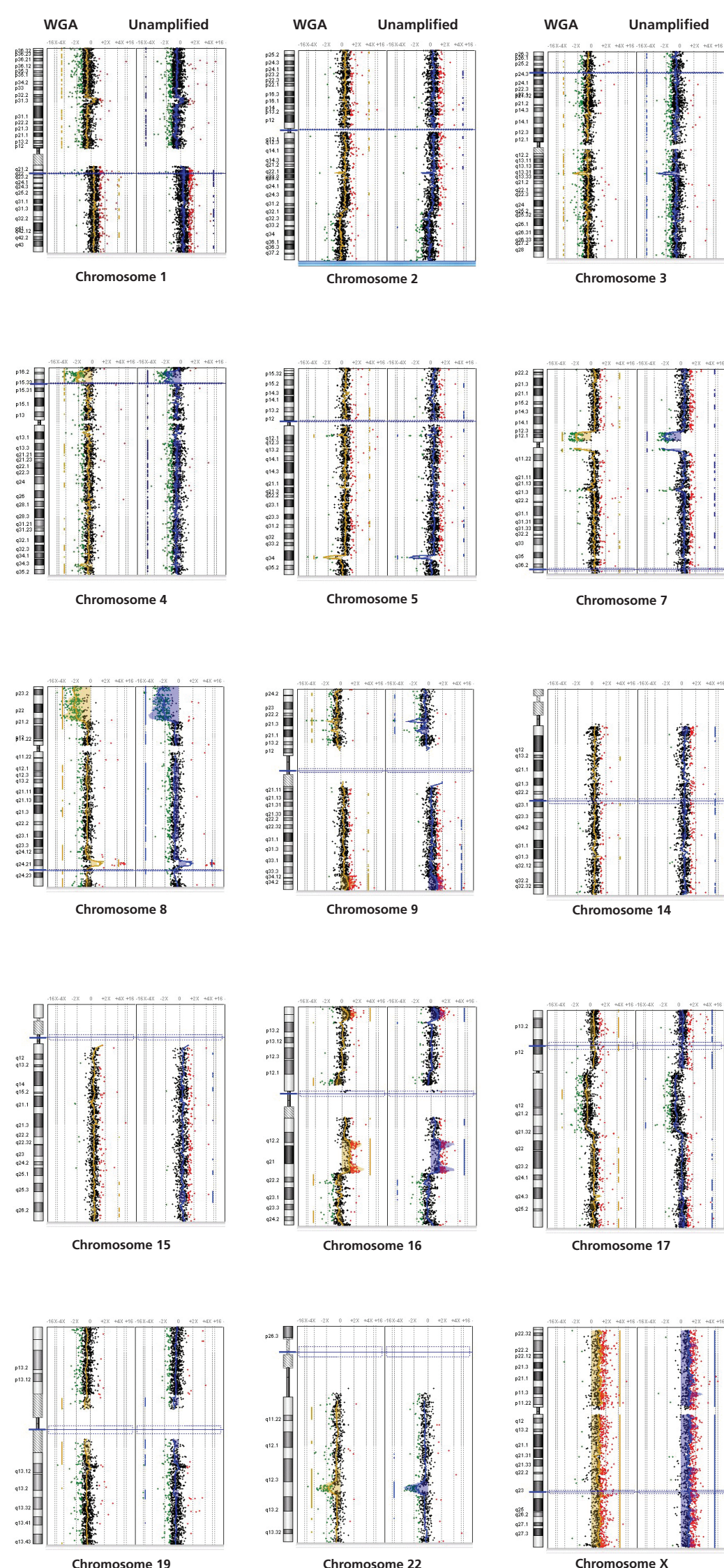


Figure 3: Treated and untreated human cells were run through the GenomePlex WGA Kit. Agilent 44K Human CGH array compared the WGA-amplified DNA to the original unamplified genomic DNA. WGA DNA was Cy3/Cy5 labeled by Kreatech's ULS cis-platinum method. The original unamplified DNA was Cy3/Cy5 labeled via labeled nucleotide incorporation as recommended by Agilent. Chips were loaded with 750 ng WGA DNA and 2000 ng original unamplified DNA and allowed to hybridize for 40 hours. After washing, the chips were analyzed on Agilent Scanner. The WGA process did not produce detectable bias when compared to original unamplified DNA.

Successfully Amplifies Limited and Degraded Samples

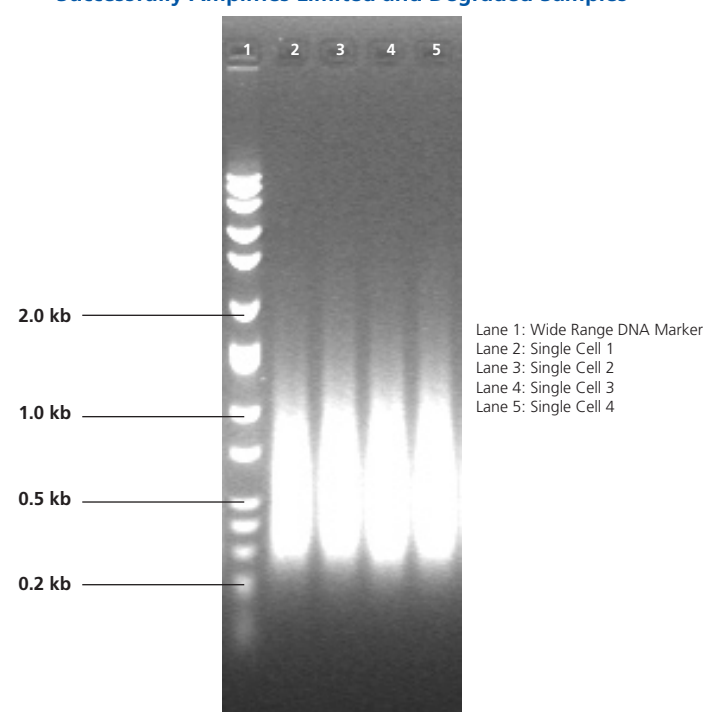


Figure 4: Fluorescence-Activated Cell Sorting (FACS)-isolated human leukemia U937 cells were WGA amplified and cleaned up with GenElute™ PCR Cleanup Kit. The picograms of genomic DNA that were released from a single cell were amplified a million-fold by WGA to a final yield of 5.4–6.2 µg. The Single Cell WGA Kit produces consistent yield and range as visualized by a 1% agarose gel.

Single Cell qPCR Analysis

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

Table 1: Four human leukemia U937 single cells were FACS-isolated and amplified with GenomePlex Single Cell WGA Kit. This table represents a melt curve analysis of 95 UniSTS qPCR loci spread across the human genome. The melt curves from WGA-amplified single cells were compared to unamplified human genomic DNA on an MJ Opticon™ 2. Allelic Dropout (ADO) results when melt curves from WGA-amplified single cells do not match unamplified human genomic DNA. Separately, the four single cells demonstrated 29–35% ADO. When evaluated cumulatively, the four cells show only 2% ADO. 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.

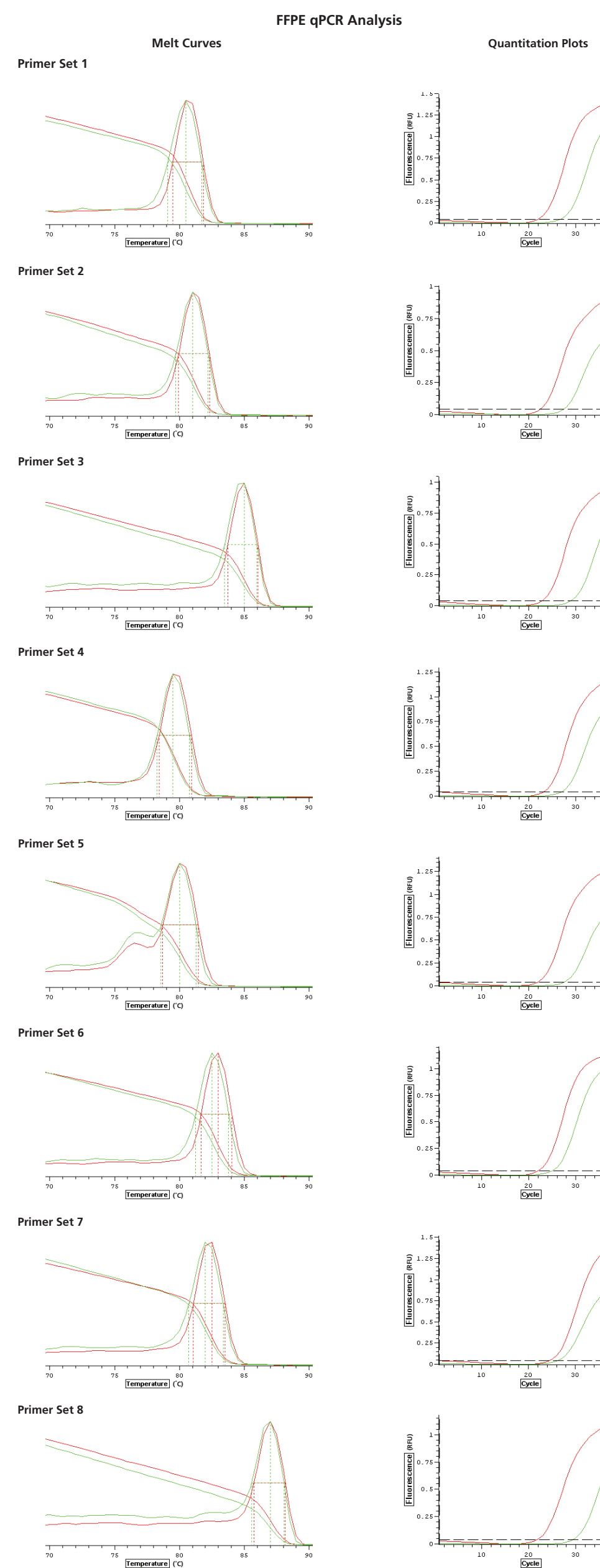


Figure 5: Genomic DNA from a FFPE human pancreas section was purified with GenElute™ Mammalian Genomic DNA kit and subsequently WGA amplified. After cleanup, WGA DNA (green) was compared to unamplified control DNA (red) via SYBR Green® qPCR across eight human primer sets on an MJ Opticon 2. There was less than a five-cycle difference between WGA DNA and control DNA. Melt curves for WGA DNA and control DNA were identical to one another, which demonstrates the desired product was amplified.

Conclusions

GenomePlex WGA has been applied to various starting sources of DNA material. The technology has proven successful and robust amplification of both plant and animal genomic DNA with increased yields by 500–1000 fold. The amplification process was product specific, resulting in no detectable signal for negative controls. Difficult templates, such as FFPE tissue, were equally amplified resulting in high quality amplicons. Moreover, the novelty of this system was demonstrated in its ability to amplify a single cell genome a million fold. Allele bias has been tested by qPCR and Agilent CGH arrays, demonstrating that the genome sequence integrity was maintained. GenomePlex Whole Genome Amplification technology has proven to be a reliable molecular tool that can accurately amplify the genomic DNA from almost any research specimen, no matter its difficulty or limitations.

References

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Product Offerings

- GenomePlex® Whole Genome Amplification Kit (WGA1)
- GenomePlex® Complete Whole Genome Amplification Kit (WGA2)
- GenomePlex® WGA Reamplification Kit (WGA3)
- GenomePlex® Single Cell Whole Genome Amplification Kit (WGA4) *Coming Soon!*
- GenElute™ PCR Clean-Up Kit (NA1020)
- GenElute™ Mammalian Genomic DNA Miniprep Kit (G1N10)
- SYBR® Green JumpStart™ Taq ReadyMix™ (S4438)

Acknowledgments

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