

OmniPlex Amplification for Genetic, Epigenetic, and Expression Analysis – Applications in Genomics, Pharmacogenomics, Diagnostics, Biosurveillance, and Forensics

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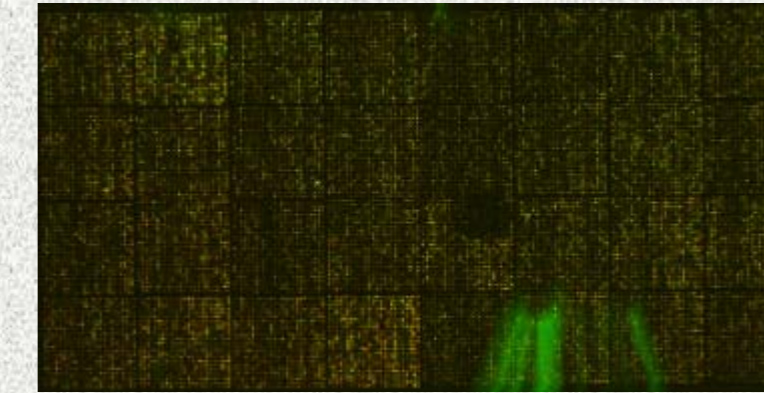
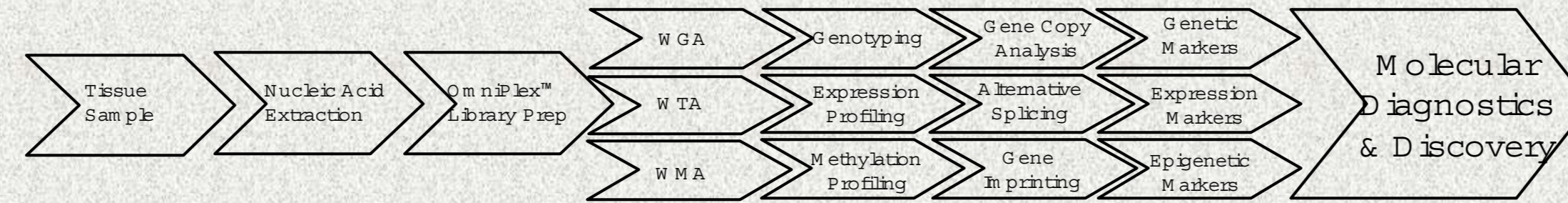
Abstract

Many genetic studies and diagnostic tests are limited by the amount of DNA or RNA that is available for study. Common factors that lead to inadequate amounts of nucleic acid are small initial sample size, degradation at the source or in handling/storage, cost, depletion of existing samples by unanticipated numbers of tests or collaborators, and difficulties of recontacting subjects. Cell transformation and growth is not a viable solution to these problems.

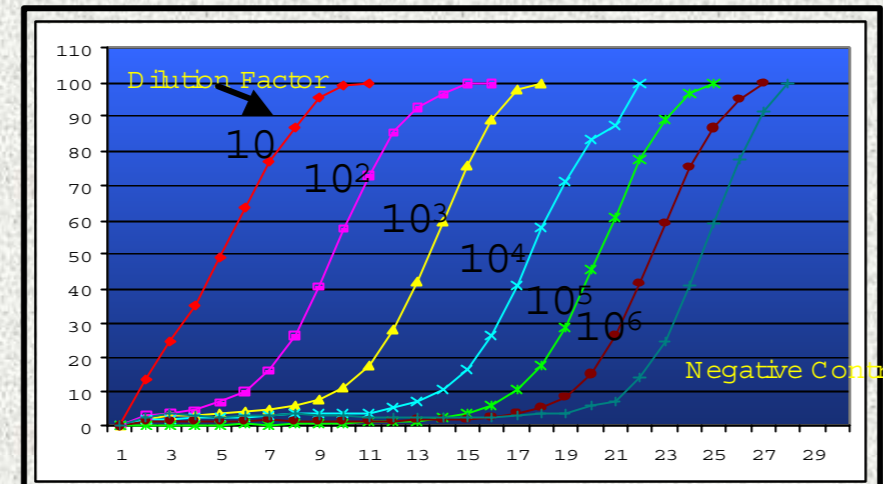
We have developed a simple method for whole genome amplification, called OmniPlex WGA, that can accurately and robustly amplify sub-nanogram amounts of total human DNA using common reagents. The process is a random, non-enzymatic fragmentation of genomic DNA followed by addition of adaptor sequences to both ends to form an in vitro molecular library that is amplified using PCR. Library preparation and amplification takes less than three hours, is automatable, and can be repeated multiple times to produce microgram amounts of DNA. OmniPlex WGA data will be shown from whole blood, blood spots, buccal swabs, serum, fixed or frozen tissue, hair follicles, degraded archived DNA, single cells, and single sorted chromosomes. OmniPlex has been used in academic, government, and commercial projects for SNP and STR genotyping, mutation discovery by sequencing and heteroduplex analysis, for chromosome painting and CGH, and for methylation and expression analysis. Genotype concordance between the gDNA and the WGA DNA is >99.7% on high throughput single base extension, ligation, and exonuclease assays. By enabling large-scale genotyping or resequencing studies to be done with blood spots, hair, or buccal swabs, WGA allows genetic resources for large-scale population studies to be collected, archived, and shared more rapidly and economically than by other methods.

The very low background, insensitivity to DNA breakage, and high sequence accuracy of the amplification process make WGA an attractive method for more accurate and sensitive genetic and epigenetic diagnostics, and human and pathogen identification.

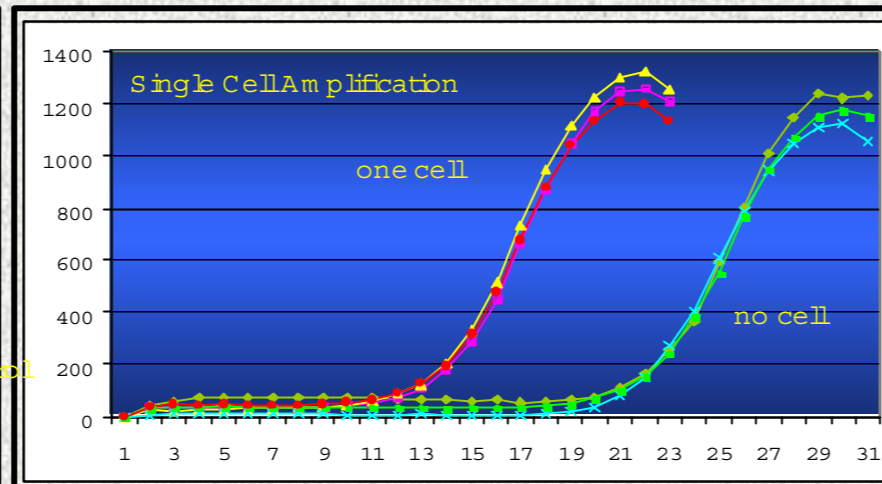
OmniPlex™ WGA Library Preparation



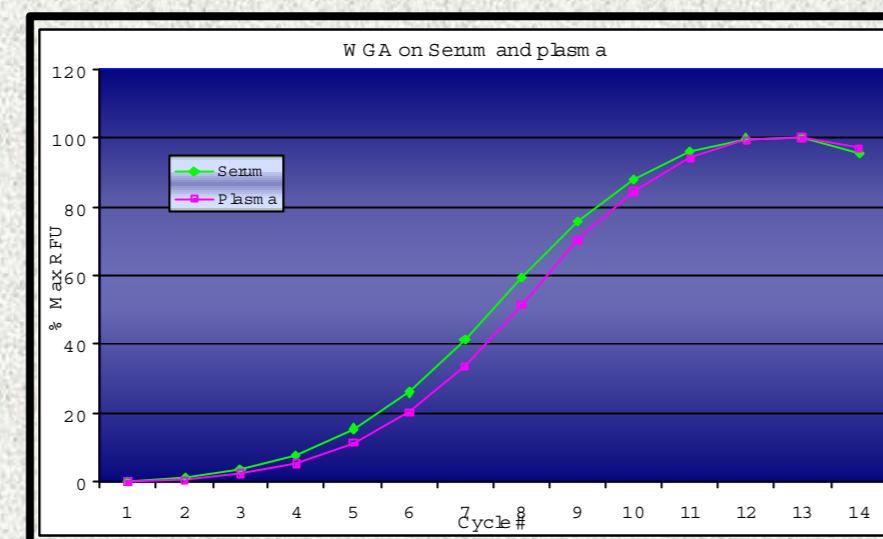
WGA



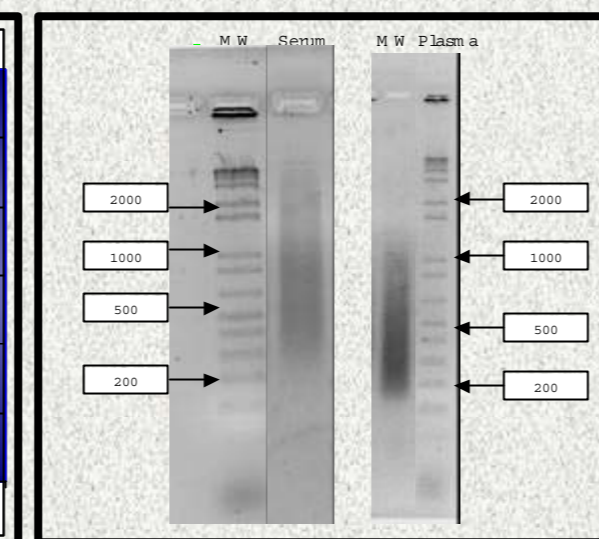
WGA from dilutions of a single human hair follicle



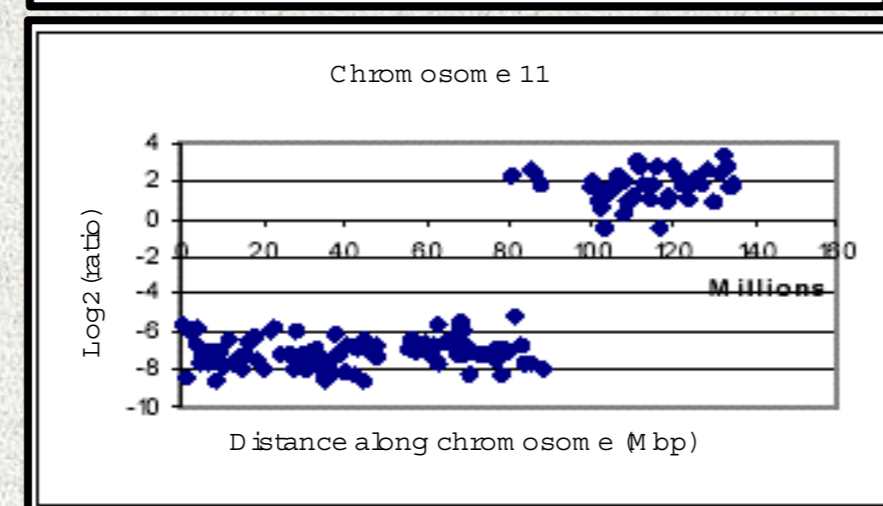
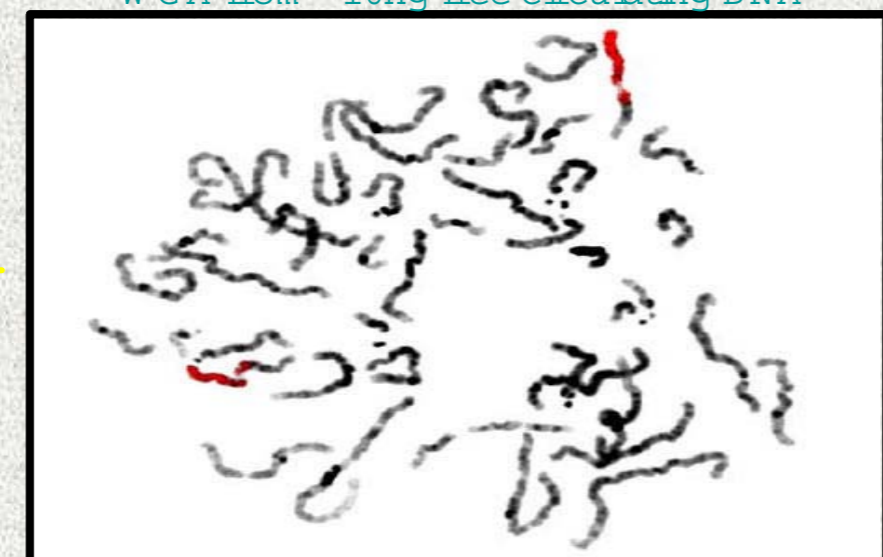
WGA from a single human leukocyte



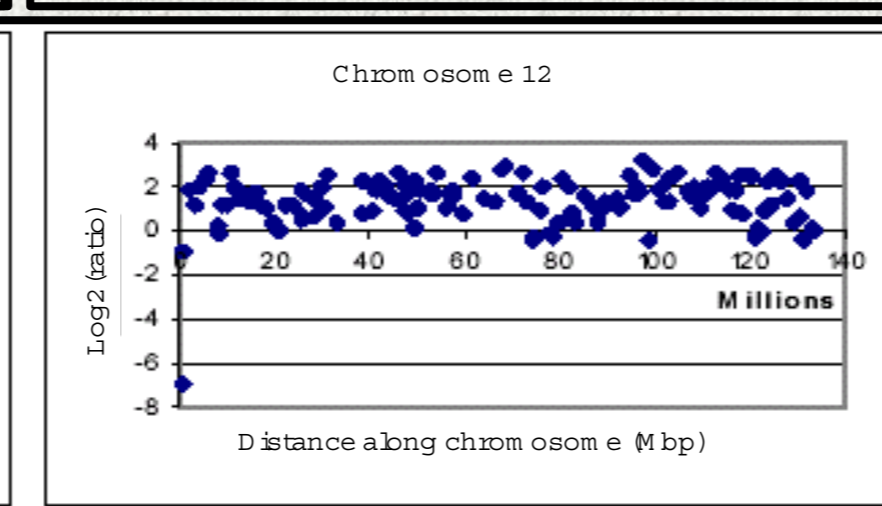
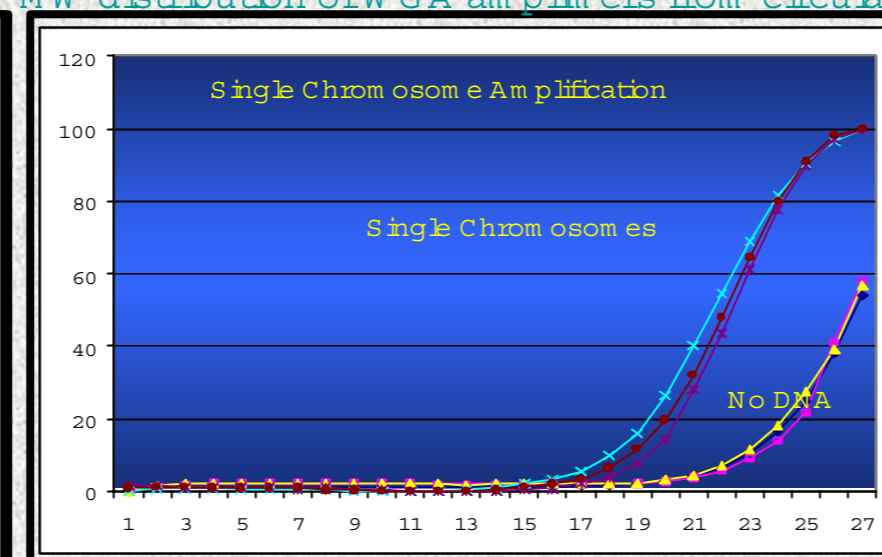
WGA from ~10ng free circulating DNA



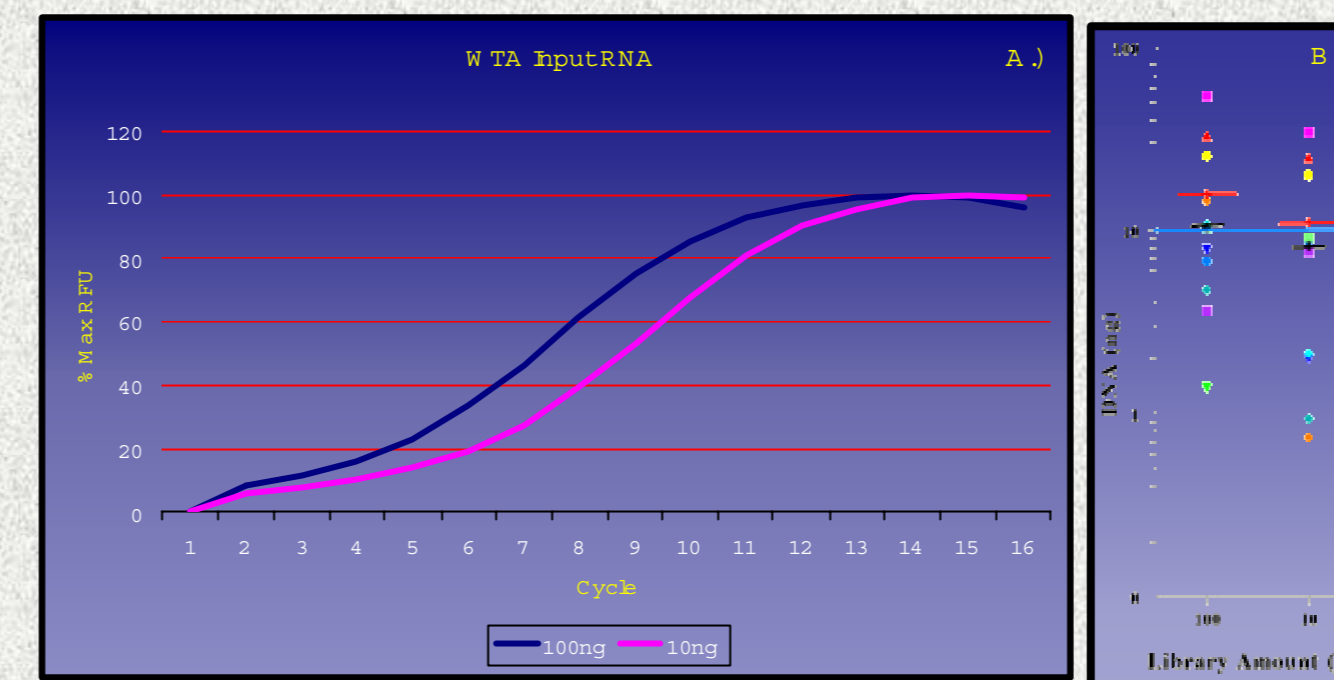
MW distribution of WGA amplicons from circulating DNA



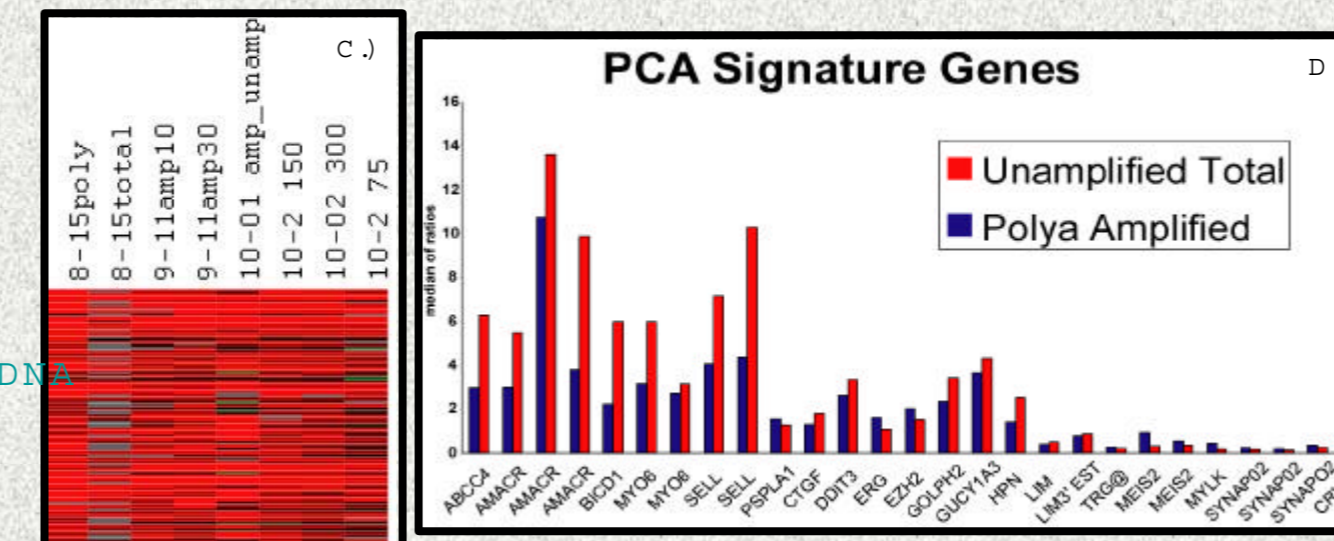
Array painting of the derivative chromosomes from a cell line carrying a t(11;12)(q21;p13.33) translocation. Probes were generated from OmniPlex™ libraries constructed from single copies of the derivative chromosomes. (Data provided courtesy of Nigel Carter, Wellcome Trust/Sanger Institute)



WTA



WTA Library Amplification and Representation



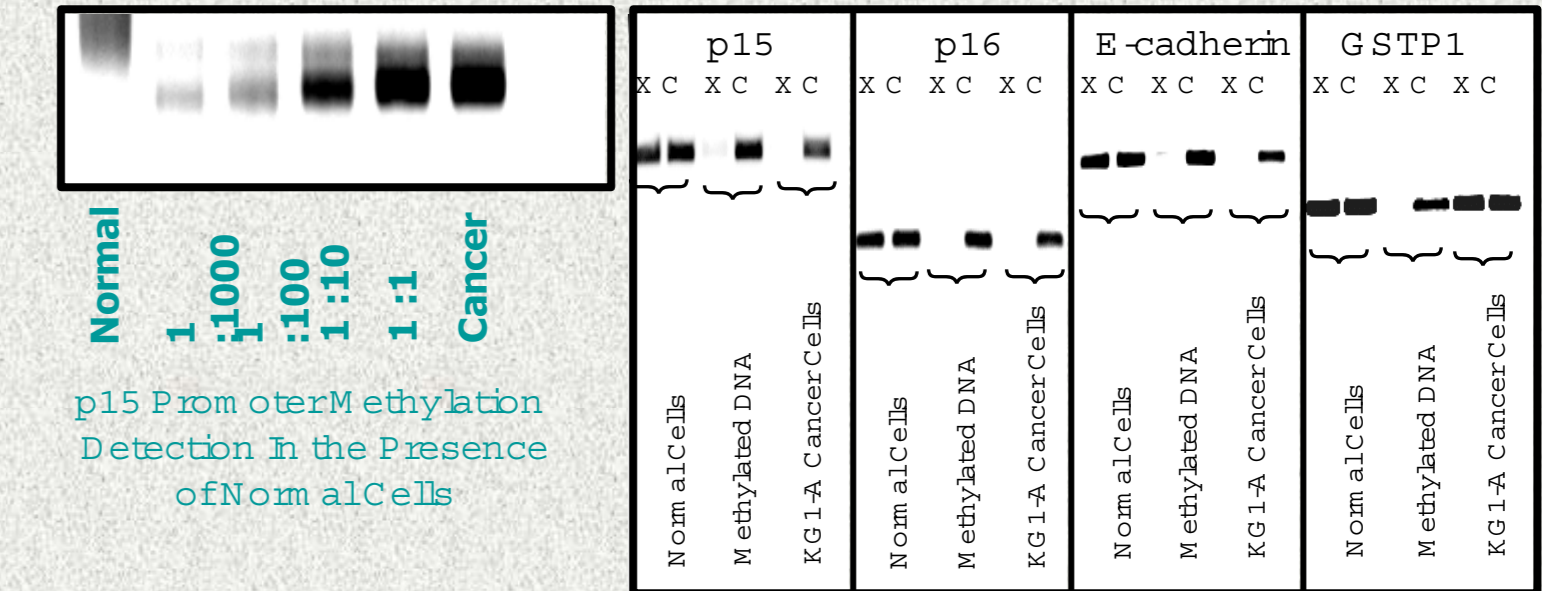
Correlation Coefficient = 0.907

Rubicon WTA technology yields robust amplification over a wide range of RNA input template (A). WTA can be applied to mRNA or directly to total RNA. Libraries show uniform amplification of RNA as demonstrated by STS analysis across a set of differentially expressed genes in control tissue samples (B).

Prostate cancer marker discovery demonstrates a powerful application of WTA. Normal pooled prostate vs. prostate cancer RNA were WTA processed from a range of input amounts from both polyA+ selected and total RNA. Differentially labeled normal and cancer WTA samples were applied to a 18.5K cDNA microarray hybridization and compared to unamplified. The correlation for the top 509 (3 fold) over and under expressed genes show a 91% correlation with respect to unamplified RNA (fig. C). The PCA signature genes with the most frequently altered gene expression in prostate cancer are shown as a median of their ratios (fig. D).

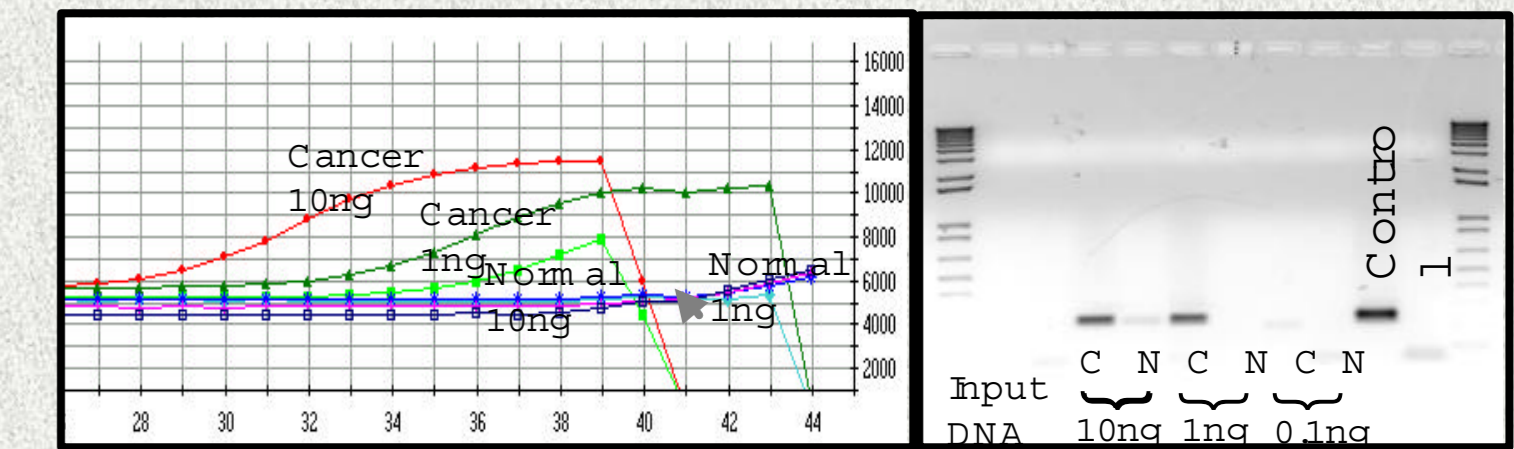
(Prostate array data provided courtesy of Anil Chinnaiyan M.D., Ph.D., Department of Pathology, University of Michigan Medical School)

WMA



p15 Promoter Methylation Detection in the Presence of Normal Cells

Screening for CpG Methylation in Promoter Regions Normal vs Cancer Cells



Sensitivity of p16 Promoter Methylation Detection in Cancer Cells

OmniPlex™ Solutions

Rubicon genomics OmniPlex™ presents an integrated solution to discovery and diagnostics of genetic, epigenetic, and expression markers.

Sensitivity, speed, and accuracy of OmniPlex DNA and RNA amplification are unsurpassed, with samples ready for analysis in less than 3 hours.

OmniPlex Whole Genome Amplification has been validated in commercial genotyping, sequencing, CGH, and cytogenetics projects.

Amplified OmniPlex DNA and RNA can be analyzed on a variety of widely-available instrumentation.

Non-invasive cancer detection and monitoring become feasible using OmniPlex WGA, WTA, and WMA from degraded DNA/RNA in serum.

OmniPlex enables retrospective studies from fixed tissue.

OmniPlex facilitates the study of genetic, epigenetic, and expression markers from a unified platform. (www.rubicongenomics.com)