

Introduction

There is a growing desire to profile FFPE samples via array comparative genomic hybridization (aCGH), however many of these samples are unsuitable for microarray analysis due to nucleic acid damage introduced during the fixation process. We apply a simple, gel-based, multiplex PCR assay to FFPE tissues prior to whole genome amplification (WGA) and aCGH analysis to assess DNA quality and predict aCGH feasibility. In addition, there are currently two commercially available technologies for the application of WGA. One is Sigma's GenomePlex[®] WGA product line that is based on a modified DOP-PCR technology (1), and the other is multiple displacement amplification (MDA) WGA technologies (2). Several groups have applied MDA-based WGA to FFPE samples, but the requirement of full-length template DNA appears to preclude this method from use with archived samples (3, 4, 5). Here we show that Sigma's GenomePlex WGA platform and PerkinElmer's SpectralChip[™] 2600 BAC array platform work reasonably well for generating high quality aCGH data from archival samples when starting with 10ng FFPE genomic DNA or ~1mg FFPE tissue.

Methods

FFPE tissues were WGA amplified directly using Sigma's GenomePlex Tissue WGA kit (Cat. No. WGAS), where as purified genomic DNA was WGA amplified using Sigma's GenomePlex WGA kit (Cat. No. WGA2) per manufacturer's recommendations. BAC aCGH profiles were generated using 1µg WGA DNA on PerkinElmer's SpectralChip 2600 array platform. BAC data was analyzed using PerkinElmer Spectralware[™] BAC Array Analysis Software using default settings. Oligo aCGH was performed using Agilent's Human Genome CGH Microarray 44K platform and Agilent's CGH Analytics version 3.4 software per manufacturer's recommendations. Agilent arrays were performed by an Agilent certified service provider (UHN Microarray Center, Toronto).

FFPE DNA Quality Check

Gel-based multiplex PCR assay for FFPE DNA quality assessment

Figure 1:

A single reaction, multiplex PCR assay was used to determine FFPE DNA quality and WGA/aCGH outcome. High quality FFPE DNA produces all five amplicons, whereas lower quality DNA produces no amplicons, or a subset of the amplicons.

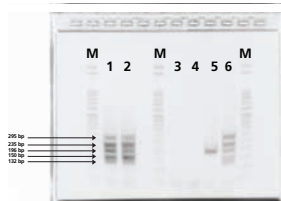
Primer information for multiplex PCR assay

UniSTS number	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Size (bp)	Chr
STB39112.SP6	GCAAAATCCATACCCTTTCTGC	TCITTCCTCTACAACCCTCTAACCC	132	4
STSG50529	GCTGTAGAGCTTTATTGCGC	CTAGAAATTTCTGCATAAACCAACC	150	22
CSNPHARP	CATGGCTCACTGGCTTACAA	TTGCCTTACAGAGGAGCAG	196	2
SHGC147491	TTTGATGTTAGGACACGCTGAAA	AAAAACGGAAGAAGTCTTTGGC	235	12
SHGC105883	GTCAGAAGACTGAAAACGAAGCC	GCTTGCCACACTCTCTCAAGT	295	13

(A)

Primer sequences were derived from the UniSTS database, and the UniSTS accession numbers, primer sequences, amplicon sizes, and chromosomal locations of the amplicons are provided.

Multiplex PCR assay results using FFPE tissue lysates

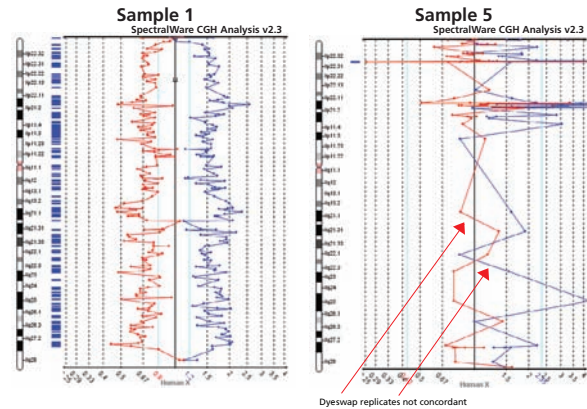


M: Invitrogen 100 bp DNA ladder
 1: male colon FFPE tissue lysate (~2yrs old)
 2: female uterus FFPE tissue lysate (~2yrs old)
 3: female DNA tissue lysate
 4: female DNA tissue lysate
 5: female DNA tissue lysate
 6: 10 ng female genomic control DNA

(B)

Approximately 1mg of tissue was collected from five FFPE tissue samples followed by processing with the GenomePlex Tissue Whole Genome Amplification Kit (Sigma Cat. No. WGAS) as outlined in the technical bulletin. 5 µl of undiluted WGAS tissue lysate was subjected to multiplex PCR amplification as described at http://www.sigmaaldrich.com/img/assets/3090/Advisor_FFPE_DNA_diagnostic.pdf, and the amplicons were resolved on an agarose gel. All five bands were amplified in lanes 1 and 2 indicating these samples contain high quality FFPE DNA, whereas lanes 3, 4 and 5 contain low quality DNA since all, or most, of the PCR fragments were not amplifiable. Similar results were observed when purified DNA or amplified WGA product derived from these FFPE tissues were used in this assay (data not shown).

aCGH analysis shows good correlation with multiplex PCR assay results (male/female comparison)



(C)

1µg of WGA FFPE DNA were hybridized against 1µg WGA normal control DNA from Promega and subjected to BAC aCGH. BAC aCGH outcome correlated well with the multiplex PCR results.

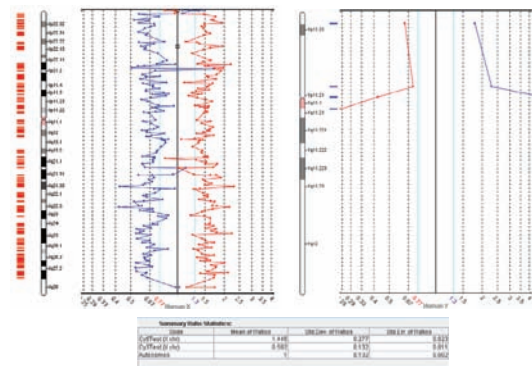
Validation of GenomePlex and SpectralChip

Validation of WGA amplified DNA on BAC aCGH arrays using a male/female model system

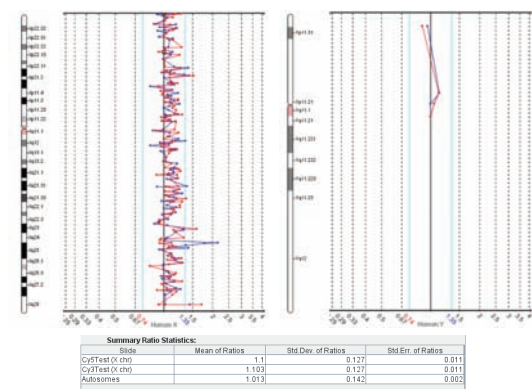
Figure 2:

We generated a series of GenomePlex amplified BAC aCGH profiles from male and female FFPE tissues and female DNA known to contain gains on chromosome 23, and hybridized these against GenomePlex amplified normal female control DNA from Promega.

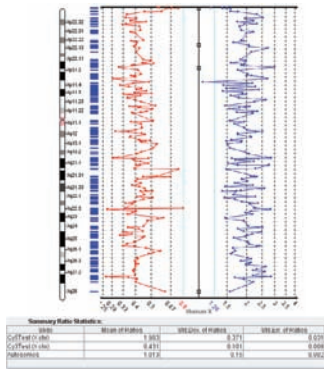
(A) GenomePlex Amplified XY vs. XX (1:2 loss of X; 1:0 gain of Y)



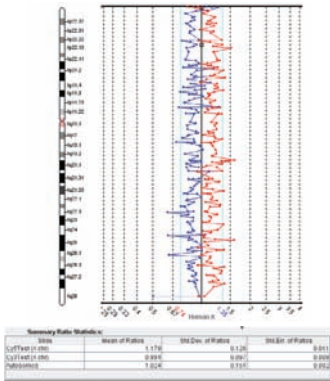
(B) GenomePlex Amplified XY vs. XY (1:1 ratio of X & Y)



(C) GenomePlex Amplified XXX vs. XX (3:2 gain of X)



(D) GenomePlex Amplified XY+XX vs. XX (1.5:2 Dilution of X)

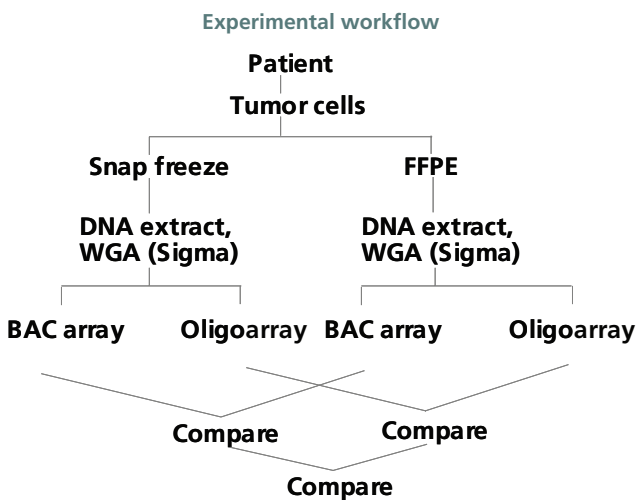


(A-D) The ideograms generated show varying X and Y chromosome dosage effects in amplified BAC aCGH. The genotypes for each sample and reference are given above each plot, where as array metrics are provided in the tables below each plot. Mixed male and female DNA samples (1:1) are indicated as XY+XX, where as chromosome trisomy X DNA is indicated as XXX.

FFPE aCGH Application: BAC arrays vs. oligo arrays

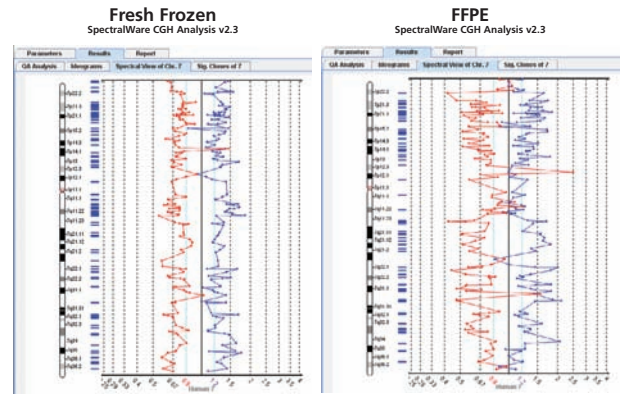
FFPE DNA analysis shows better results with BAC aCGH relative to oligo aCGH

Figure 3:



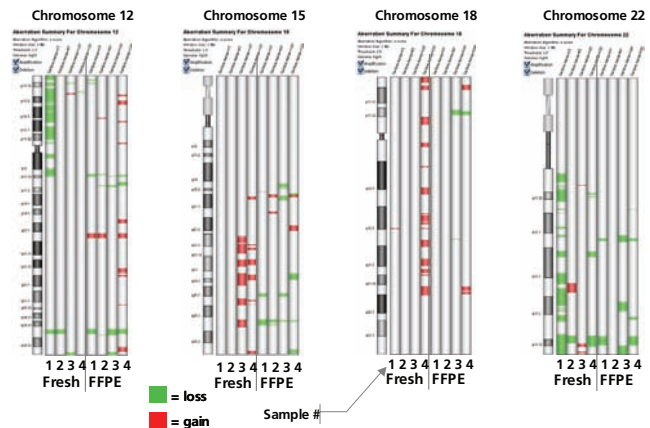
(A) Diagram of the experimental workflow comparing BAC aCGH and oligo aCGH analysis of GenomePlex amplified paired fresh frozen and FFPE myeloma samples and GenomePlex amplified control DNA (Promega Corp.). Hybridizations were performed against control DNA of the opposite sex. Each hybridization was performed in quadruplicate.

SpectralChip 2600 ideograms of chromosome 7 showing reasonable correlation between paired frozen and FFPE myeloma samples



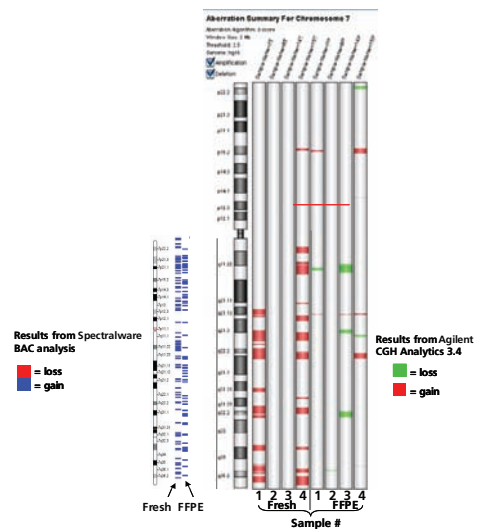
(B) Comparison of SpectralChip™ 2600 BAC aCGH analysis of frozen and FFPE myeloma samples. These whole genome results of chromosome 7 are from 1 representative sample, and they show reasonable concordance between fresh frozen and FFPE samples.

Agilent Human Genome CGH Microarray 44K analysis showing effectively no correlation between frozen and FFPE myeloma samples



(C) Agilent Human Genome CGH Microarray 44K analysis of frozen and FFPE myeloma samples. Representative ideograms showing data from quadruplicate hybridizations of chromosome 12, 15, 18 and 22 are depicted here. There is effectively no correlation between replicates.

Comparison of BAC aCGH and oligo aCGH ideograms of chromosome 7



(D) Agilent and PerkinElmer ideograms of chromosome 7 comparing results from frozen and FFPE myeloma samples.

Conclusion

- FFPE samples can now be a valuable source of archival material for genomic data analysis
- DNA is extracted and WGA amplified directly from tissue with Sigma's optimized WGA5 kit
- DNA quality is predicted with a simple, gel-based, multiplex PCR test and linked to aCGH success
- SpectralChip 2600 BAC array data correlates well when comparing fresh frozen samples and FFPE samples
- Oligo-probe aCGH data did not correlate well when comparing fresh frozen samples and FFPE samples prepared in the same manner.

Reference

1. E. Mueller. Genomic analysis of formalin-fixed paraffin embedded (FFPE) tissues through the use of whole genome amplification (WGA). <http://www.sigmaaldrich.com/sigma/general%20information/ffpewhitepaper>. October, 2007.
2. F.B. Dean et al. Rapid Amplification of Plasmid and Phage DNA Using Phi29 DNA Polymerase and Multiply-Primed Rolling Circle Amplification. *Genome Res*, 2001, 11:1095-1099.
3. S.E. Little et al. Array CGH using whole genome amplification of fresh-frozen and formalin-fixed paraffin-embedded tumor DNA. *Genomics*, 2006, 87:298-306.
4. K. Iwamoto et al. Evaluation of whole genome amplification methods using postmortem brain samples. *J. Neuroscience Methods*, 2007, 165:104-110.
5. M.J.L. Sjöholm et al. Comparison of archival plasma and formalin-fixed paraffin-embedded tissue for genotyping in hepatocellular carcinoma. *Cancer Epidemiol. Biomarkers Prev*, 2005, 14:251-255.

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