Profiling formalin-fixed, paraffin-embedded (FFPE) samples on three Agilent microarray platforms

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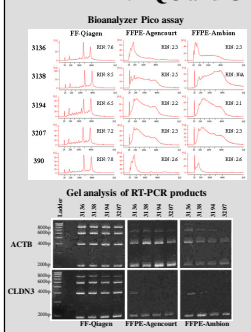
Introduction

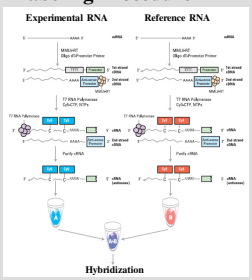
Thousands of formalin-fixed, paraffin-embedded (FFPE) samples from clinical archives are available for retrospective studies. Such samples could provide crucial information for drug target discovery and diagnostics of various diseases. Here we used FFPE samples and their matched fresh-frozen (FF) counterparts to examine their performance on three types of microarrays including whole genome expression, comparative genomic hybridization (CGH) and microRNA. Despite lower quality of nucleic acids from FFPE samples, the microarray data have proven to be useful. Advances of new methodologies for handling FFPE samples are expected to further improve the quality of microarray data, thus, enabling their routine use and performance comparable to that of FF samples.

Gene Expression (GE) Array Technology

- Agilent Whole Human Genome arrays used to identify differentially expressed genes
- 60-mer oligos interrogate the transcriptome with over 44K probes
- 1 ug total RNA input is required for labeling

RNA QC and GE Labeling Procedure





FF samples provide better templates for RT-PCR. However, amplification is also possible to various degrees in FFPE samples.

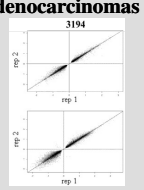
GE Array Quality Control

Various QC parameters are used for overall array quality assessment. Signal to Background ratios (STB), % passing features and number of down- and up-regulated genes are some of the metrics used to assess array performance. Z-score analysis, unsupervised clustering and intensity plots are also part of array QC.

Comparison of microarray performance of matched FF and FFPE ovarian adenocarcinomas

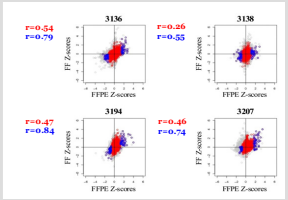
Passing probe intensities display high reproducibility between replicate arrays; high correlations are observed for replicates of both FF and FFPE samples.

	Sample	r	# probes
FF	3136	0.988	19049
	3138	0.989	19298
	3194	**0.990**	**21294**
	3207	0.991	20259
	390	0.989	20727
FFPE	3136	0.984	17363
	3138	0.985	16872
	3194	**0.985**	**19757**
	3207	0.983	16164
	390	0.983	14189



Cy5 signal ≥1000
Top 100 FFPE z-scores

Top 100 FFPE Z-scores for each of the 4 samples correlate well with their FF counterparts.



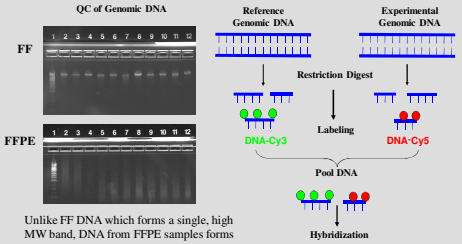
GE Summary

- RNA can be successfully extracted from FFPE samples using Agencourt FormaPure kit or Ambion Optimum kit
- FFPE samples can be processed on gene expression microarrays
- Known and novel markers can be identified using FFPE samples (data not shown)
- For more details, see **Fedorowicz G, Guerrero S, Wu TD, Modrusan Z. BMC Med Genomics 2:23, 2009.**

CGH Array Technology

- **Agilent CGH (Comparative Genomic Hybridization) arrays can identify and quantify DNA copy number changes**
- **60-mer oligos offer a high degree of specificity covering coding and non-coding regions with 244K probes/array**
- **500 ng genomic DNA input is required for labeling FF or FFPE samples**

DNA QC and CGH Labeling Procedure



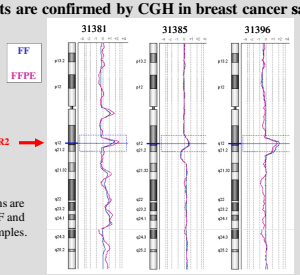
Unlike FF DNA which forms a single, high MW band, DNA from FFPE samples forms smears. The higher the MW range of the smear, the better the FFPE sample quality.

CGH Array Quality Control

Multiple QC parameters are measured and used for overall array quality assessment. Some of the metrics reviewed are: Derivative Log Ratio (DLR) spread, Signal to Noise ratios (S/N) for both channels, % passing features and % outliers.

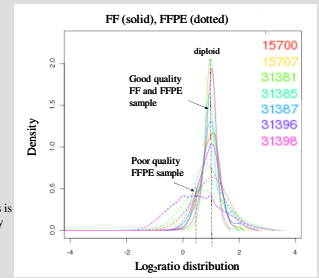
Comparison of matched FF and FFPE breast tumors

***Her2+* IHC results are confirmed by CGH in breast cancer samples**



Her2 amplifications are detected in both FF and matched FFPE samples.

Distribution of log2ratios in matched FF and FFPE samples



Compression of sample/reference ratios is observed in low quality FFPE samples.

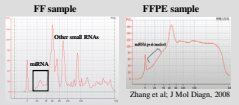
CGH Summary

- CGH arrays have been used to determine chromosome copy number variations across the genome in both FF and FFPE samples
- FFPE data show higher level of noise; however, larger copy number changes are still detected
- Compression of $\log_2$ratios occurs in poor quality FFPE samples and is likely due to the use of FF reference

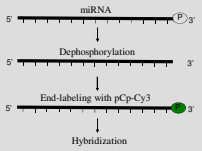
miRNA Array Technology

- MicroRNAs (miRNAs) are small single-stranded non-coding RNAs that serve as regulators of post-transcriptional gene silencing
- 866 miRNAs are represented by multiple probes and each probe is printed multiple times on the latest version of the Agilent Human 15K miRNA array
- 100 ng total RNA input is required for labeling

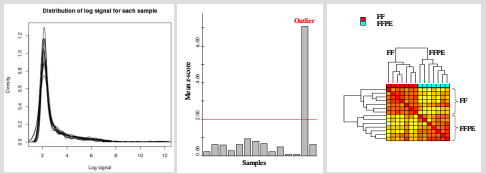
miRNA QC and Labeling Procedure



- Ambion's mirVana kit (FF samples) or RecoverAll kit (FFPE samples) is used for isolation of total RNA enriched for miRNA and other small RNAs
- Small RNA Bioanalyzer assay, used to characterize FF RNA, is not helpful for QC of FFPE samples

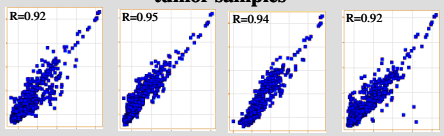


miRNA Array Quality Control



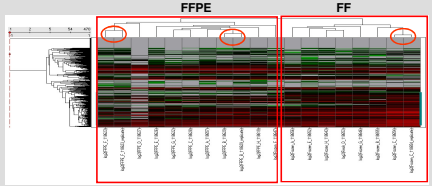
Cy5 Signal to Background ratio (STB), % passing features and outliers are some of the metrics used to assess overall array performance. Z-score analysis, unsupervised clustering and intensity plots are also part of array QC.

Comparison of $\log_{10}$signal of matched FF and FFPE tumor samples



High correlations between matched pairs are observed as shown on these Spotfire plots.

Unsupervised hierarchical clustering



Samples cluster according to prep method, not by matched pairs (marked by red box). Duplicate samples cluster together (marked by red circles).

miRNA Summary

- Ambion RecoverAll kit allows extraction of small RNAs from FFPE samples
- End-labeling procedure and probe design are optimized for short targets
- High correlations between FF and FFPE samples are observed; FFPE samples seem sufficient for generating data on differential expression of miRNAs

Acknowledgements

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