Expression Profiling of FFPE Specimens on Illumina BeadChips using WG-DASL®

This Technical Note compares Illumina BeadChip microarray analysis results for RNA specimens derived from FFPE with fresh-frozen using the Illumina WG-DASL assay.

Challenges with FFPE Specimens

The fixation of human tissue with formalin, and their subsequent embedding in paraffin, has been a routine method of collecting and preserving surgical specimens for many years. These formalin-fixed, paraffin-embedded (FFPE) tissues represent an extremely valuable resource for investigators interested in determining both the pattern of gene expression (RNA abundance) and chromosomal alterations (copy number and loss of heterozygosity) in archived tissue specimens. Microarray analysis of FFPE specimens has thus far proven to be technically challenging because nucleic acids isolated from these sources are typically degraded and modified. This issue becomes increasingly problematic with specimen ages.

Degraded RNA samples are not amplified and labeled well using conventional microarray procedures that generate hybridization targets by cDNA synthesis with oligo(dT) primers followed by *in vitro* transcription. Illumina's Whole-Genome DASL (WG-DASL) assay overcomes these limitations. The WG-DASL (c**D**NA-mediated **A**nnealing, **S**election, and **L**igation) assay combines the PCR labeling steps of the original DASL assay with transcript-based hybridization using a new whole-genome probe set designed for WG-DASL. This greatly increases the assay target set compared with the original DASL assay, while retaining the ability to accurately profile degraded RNA samples. We demonstrate that the WG-DASL assay presents a dramatic improvement in both the number of detected transcripts and the number of significant differentially expressed transcripts in RNA detected in FFPE.

RNA Isolation

A matched set of lung tissue specimens from a tumor and the normal adjacent tissue (NAT) was preserved as either fresh frozen or FFPE tissues. RNA was repeatedly isolated from the fresh frozen tissue using standard QIAGEN RNeasy procedures. RNA was isolated from replicate pools of 10

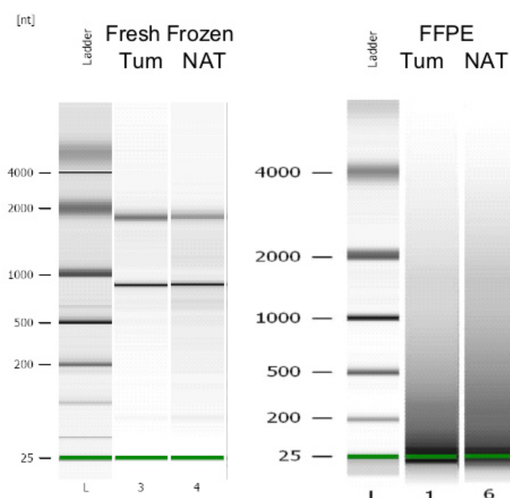

Figure 1: RNA isolated from FFPE sections and fresh-frozen tissue

µm sections from the FFPE tissues using Agencourt Bioscience's FormaPure isolation kit. RNA yields varied between 0.4 and 1.0 microgram per FFPE section. As expected the RNA from the FFPE sections is largely degraded compared to the RNA isolated from the matching fresh-frozen tissue (Figure 1).

BeadChip Hybridization and Probe Detection

Quadruplicate amplification and labeling reactions were performed for each of the four sample types using the Illumina DASL kit and 100 ng of input RNA for the fresh-frozen RNA and 200 ng for the FFPE RNA. Total RNA was converted to cDNA using both random nonamers and oligodT, followed by the DASL procedure.. For both of the tumor and the normal adjacent tissue, the number of probes detected in the FFPE specimens was approximately 88% to 94% of the number

of probes detected in the fresh frozen preparations (Figure 2). However, we should also note that typically 30% more probes were detected by the FFPE WG-DASL assay than were detected by 3' labeling methods using fresh frozen tissue[1]. In addition, roughly 160% more probes were detected by the FFPE WG-DASL assay than were detected by 3' labeling methods using FFPE tissue[1]. Therefore, the detection sensitivity of the WG-DASL assay using FFPE tissue is much greater than the sensitivity of the 3' amplification and labeling method with fresh frozen and FFPE.

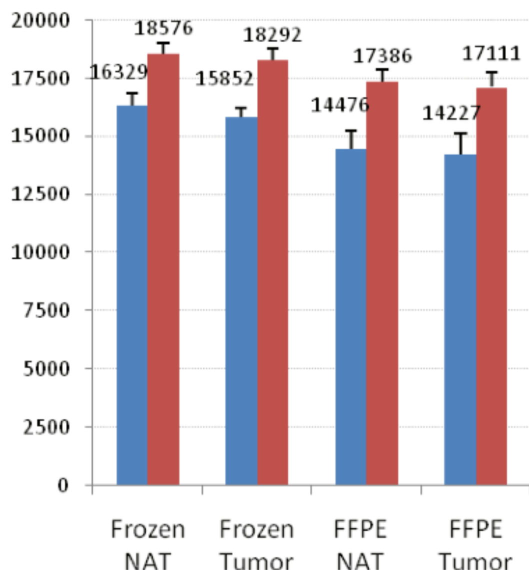

Figure 2: Mean number of detected probes across four replicates for both Fresh-Frozen and FFPE specimens using DASL. Presented at either the *P < .01 (blue) or P < .05 (red)* thresholds. Error Bars indicate observed standard deviations.

Gene List Comparison

To understand the comparability of the expression profiles, lists of differentially expressed (DE) transcripts between the tumor and normal adjacent tissue were compiled from both the set of FFPE tissues and fresh-frozen specimens using a permutation analysis[2] and an FDR of less than 5% (Figure 3). We see that size of the differential transcript list generated by the FFPE samples using WG-DASL is 45% of the list generated by the fresh frozen samples and 25% larger from the 3' labeling of FFPE samples[1]. In addition, roughly 74% of the WG-DASL FFPE DE transcripts are in common with the WG-DASL fresh frozen list. The agreement level is similar when using other methods such as joint fold-change/p-value rules. The single sample detection, differential detection and the absolute amount of agreement between FFPE and fresh-frozen provides a dramatic improvement over previous methods.

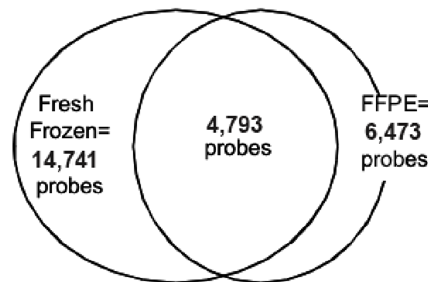

Figure 3: Differentially expressed transcript comparison between lung tumor and normal adjacent tissue for both the fresh -frozen and FFPE specimens using DASL.

Summary

RNA from FFPE tissue specimens can be used as substrate for genome-wide expression profiling on Illumina BeadChip microarrays using WG-DASL. Detection rates are higher than 3' labeling methods and near-equivalent between FFPE and fresh frozen tissue. In addition, there is a substantial improvement in transcript list size and agreement with results from traditional fresh frozen protocols

[1] Expression Profiling of FFPE Specimens on Illumina BeadChips, *Expression Analysis Technical Note, October 2007.*

[2] Two-Group Comparisons with Permutation Analysis for Differential Expression (PADE), *Expression Analysis Technical Note*, September 2005.