Clinical Laboratory Experience of Gene Expression Profiling of 2,384 Solid Tumors
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Abstract

Methods: Solid tumor samples are assessed by board-certified pathologists to ensure the presence of at least 20% tumor nuclei. Total RNA is extracted from tumor tissues (FFPE, frozen and RNAlater-preserved tissues). In addition, extracted RNA is verified to be of sufficient quality (using spectrophotometric metrics and qPCR results on a housekeeping gene RPL13a). The RNA sample is then subjected to a whole-genome (29,285 transcripts) microarray analysis using Illumina DASL-mediated annealing, selection, extension and ligation (DAL) process with the HumanHT-12 v4 beadChip (Illumina Inc., San Diego, CA). After direct hybridization and scanning of the bead array, the expression of a subset of 88 transcripts are then compared to expression data from previously scanned normal tissues using the Human Ref-8 bead chip.

Results: The median Ct value from the RPL13a qPCR results for both frozen samples (N=1463) and RNA later preserved samples (N=851) was 24. The median Ct value for FFPE samples (N=1463) was 27. The ability of this assay to detect expression changes is directly proportional to the amount of tumor nuclei present in the patient sample. Dilution experiments showed that samples with 20% tumor nuclei are >50% concordant for gene-expression information compared to samples with >95% tumor nuclei with an R-squared value >0.99. Our intra-assay variability (same chip, same operator) was extremely low and the R-squared value was 0.99. The inter-assay variability (different chips, different operators) was somewhat lower with R-squared value of 0.93. The expected variability with internal proficiency (different chip; different operators) was somewhat lower with R-squared value of 0.93. The expected variability with internal proficiency (different chip; same operator) was 0.96. The expected variability with inter-assay variability (same chip; same operator) was extremely low and the R-squared value was 0.99. The inter-assay variability (same chip; different operators) was extremely low and the R-squared value was 0.99. The inter-assay variability (different chips, different operators) was extremely low and the R-squared value was 0.99. The inter-assay variability (different chips, different months, different operators) was >0.8.

Conclusions: This microarray assay has been a reliable and robust method to perform gene expression profiling on routine solid tumor samples, including FFPE, submitted for clinical molecular profiling.

Background: DNA microarrays for gene expression analysis rely on the use of nucleic acid polymer probes complementary to known gene transcripts that are immobilized on a glass surface. Direct hybridization of this probe array on glass is then used with patient tumor samples to ascertain the relative amounts of a given transcript. In 2009 we reviewed commercially available microarray platforms and decided that the Illumina whole genome DASL gene expression platform was most aligned with our intent to validate a gene expression array platform that was designed specifically for additional sample types such as FFPE, and a platform that was amenable to high-throughput production testing in a clinical diagnostic laboratory.

Results

Figure 1 – Gene Expression Profiling with the Whole Genome DASL Assay (Source: Figure 2 from Illumina Data Information Sheet 1)

The resulting PCR products are then hybridized to the Human HT-12 expression BeadChip to determine the presence or absence of specific gene transcripts (see Table 1 below).

Table 1

<table>
<thead>
<tr>
<th>Probes</th>
<th>Description</th>
<th>Number</th>
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<tbody>
<tr>
<td>NM</td>
<td>Coding transcripts, well established annotations</td>
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<tr>
<td>XM</td>
<td>Coding transcripts, provisional annotations</td>
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<tr>
<td>NR</td>
<td>Non-coding transcripts, well established annotations</td>
<td>1,540</td>
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<tr>
<td>XR</td>
<td>Non-coding transcripts, provisional annotations</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
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<td>29,285</td>
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</table>

Table 1 – RefSeq* Content of the Human HT-12 v4 BeadChip *Release 38. Source: http://www.ncbi.nlm.nih.gov/RefSeq/

After hybridization, the Human HT-12 Expression BeadChips are scanned using the iScan system from Illumina. This scanner incorporates high-performance lasers, optics, and detection systems for rapid, quantitative scanning of the bead chip array. Gene expression data from previously scanned normal tissues are used to assess relative gene expression changes in a given tumor sample. The normal reference samples are a pool of three individually quality controlled (RNA-quality, normal diagnosis from H&E slide by a board certified pathologist) normal reference tissue samples.

Figure 2 – Distribution of Ct values for the various sample types: A, FFPE samples; B, frozen samples; C, RNA later preserved samples. The red vertical dashed line indicates the median value observed for each distribution.

Figure 3 – Frequency distribution of 20 leading cancer types where microarray analysis was completed as part of routine molecular profiling of solid tumors.

Figure 4 – Concordance of gene expression data across 80 target genes versus percent tumor nuclei in dilution experiments with 7 independent tumor samples using the Human Ref-8 bead chip.

Figure 5 – Concordance of gene expression results inter-assay experiments (different chips, different operators, same lot of reagents). Each data point represents the relative fluorescence units (RFU) for a given marker (N=40) using the Human Ref-8 bead chip.

Table 2

<table>
<thead>
<tr>
<th>Source of variability</th>
<th>Scenario</th>
<th>R-squared value</th>
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<tbody>
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<td></td>
<td>intra-assay</td>
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<tr>
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<td>inter-assay</td>
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<tr>
<td></td>
<td>proficiency</td>
<td>&gt;0.8</td>
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Table 2 – Main sources of variability seen in Illumina microarray performance

Study Highlights

We validated the Illumina microarray platform with FFPE samples, frozen samples and RNA later preserved samples.

Both frozen and RNA later preserved tissue samples exhibited similar values of RNA quality as shown by the nearly identical Ct values. Since RNA later-preserved samples performed equivalent to frozen tissue samples we are able to use normal frozen tissue samples as the ‘reference’ samples for RNA later preserved tumors.

Conclusions

Specimen requirements for routine profiling of solid tumor samples include: RNA concentration of 40 ng/μl (200 ng total input), A260/A280 ratio > 1.5, Ct for RPL13a gene must be <38.

Despite showing larger Ct value than both frozen and RNA later preserved samples, RNA extracted from FFPE specimens that meet our stringent QC metrics routinely provide quality expression profiling results. This microarray assay has been a reliable and robust method to perform gene expression profiling on routine solid tumor samples, including FFPE, submitted for clinical molecular profiling.

References

1. Whole Genome DASL* HT Assay for Expression Profiling in FFPE Samples, Data Sheet RNA Analysis, Ipsos; Pub. No: 470-2010-005 as of 14 October 2010, Illumina Inc., San Diego, CA.