

Gene expression profiling of fixed, paraffin-embedded tumor specimens, using a 192 gene panel RT-PCR assay.

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The ability to profile gene expression using formalin-fixed, paraffin-embedded (FPE) biopsy specimens will enable development of new prognostic and predictive tests. Although RNA isolated from FPE tissue is fragmented, we and others have reported that TaqMan® RT-PCR can measure the expression of diagnostically important genes such as ER, PR and HER2, and that the measured mRNA levels are concordant with the results of standard immunohistochemistry-based diagnostic assays. With a TaqMan assay optimized to detect FPE RNA, we have recently measured the levels of 192 mRNA species in 146 FPE breast tumor biopsy specimens, using three 10 μ thick sections cut from each specimen. Overall, the success rate for identifying useful probe/primer sets amongst the different genes was greater than 95%, and the success rate across the sample set for recording an informative expression profile was greater than 90%. Numerous sets of genes known to co-express were identified, for example: GRB7 and HER2 (which lie within 70kB on chromosome 17 and are known to frequently co-amplify) that co-expressed with a correlation coefficient (R) > 0.9; an “ER cluster” of ER, PR, GATA3, and HNF3 α , ($R=0.78$); a basal epithelial cytokeratin cluster of KRTs 5, 14 and 17, ($R=0.87$); and, a “growth cluster” of KI-67, thymidine kinase, thymidylate synthase, survivin, topoisomerase II α , and MCM2, ($R>0.80$).

We have also profiled gene expression in six gastrointestinal stromal tumors, a cancer that responds to the drug Gleevec™. The five Gleevec tyrosine kinase targets were found to express with extraordinary homogeneity across these tumors ($R>0.97$), consistent with recently reported evidence that this cancer exhibits remarkable genetic and genomic stability [S.V. Allander et al., *Cancer Res.* 61:8624-28 (2001)].

These results predict that RT-PCR assays of FPE tumor tissue will validate candidate marker genes identified in microarray experiments, and provide sensitive and quantitative multi-analyte diagnostics.