Evaluation of a novel microarray-based assay for gene expression profiling on formalin-fixed, paraffin embedded (FFPE) tissue samples

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Motivation

The importance of microarrays in cancer research has been well recognized since a large scale overview of cell transcriptional activity, in a complex diseases as cancer, is useful to unveil the underlying molecular mechanisms. One limitation of microarray applications to clinical studies is the need of very high quality RNA, which in the case of tumor biopsies can not be easily found. FFPE samples, by contrast, are widely available since it has been long practice in hospitals to collect and store tumor samples embedded in paraffin blocks along with the whole clinical history of the patient. The problem is the poor quality of RNA extracted from this samples, which makes it impossible to analyze them by microarray standard techniques. The cDNA-mediated Annealing, Selection, extension and Ligation (DASL) assay protocol has recently been introduced [1] specifically for FFPE RNA samples and we decided to test it on cancer tissues and cell line samples to verify its reproducibility and ability to assay partially degraded RNA.

Methods

The assay uses the Illumina Universal multiple arrays [2] available in two formats: Sentrix Array Matrix (SAM, 96 arrays) and BeadChip (16 arrays). On each array, up to 1536 probe sequences can be assayed; we have used the DASL Human Cancer Panel gene set of 502 genes, each represented by 3 probe sequences. In a pilot study, we have assayed two RNA samples from the human estrogen-responsive breast cancer cell line MCF-7, cultured in presence (+E2) or absence (-E2) of 17beta-estradiol. These samples were first assayed by standard microarray analysis and then exposed to high temperature for increasing time intervals (15', 30', 60' and 90') to cause progressive RNA fragmentation, which was quantitated by capillary electrophoresis before multiple DASL analysis. In subsequent experiments, 6 tumour samples, including samples of the same tumor derived from different paraffin blocks, were tested by repeated RNA extraction from different slices of the same block and by hybridization of the same RNA on multiple arrays (technical replicates). DASL assays were performed in different days, using both BeadChips and a SAM chip formats. Data analysis was performed using Illumina BeadStudio software, Genomatix tools for data mining [3] and R scripts generated on purpose.

All R functions developed for this study have been collected in a package that will be soon available on Bioconductor.

Results

In each case, technical replicates clustered together with a high correlation index.

In the MCF-7 degradation experiment, RNA samples degraded for up to 30' of heat exposure clustered correctly regardless of degradation level or chip format, while highly degraded samples made a separate cluster. Differential expression between -E2 vs. +E2 samples was consistently detectable even after 15 or 30 heat-mediated degradation, where genes expected to be regulated by E2 could still be identified.

A second experiment comprised 3 FFPE tumour samples, two mammary and one bladder carcinoma, whose RNAs were analyzed both separately and mixed in various mammary:bladder proportions. We selected a list of genes which showed a statistically significant higher expression in bladder than in mammary samples and which did show a positive correlation in their pattern of expression with the relative concentration of bladder RNA in the mixed sample. With a bibliographic data mining analysis we found that the tissue most significantly associated with this list in the literature was indeed bladder cancer.

Finally, a blind survey was performed also on three breast cancer samples comparing data obtained from FFPE tissues (DASL) to gene expression data obtained from frozen tissue of the same lesion (FF). Overall comparison of DASL vs FF gene expression data from the same biopsy produced remarkably good correlation. In conclusion, in our experience the DASL assay shows a surprisingly good reproducibility, reliability and consistency with biological identity of the starting sample.

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