

A Novel Algorithm to Test Significant Differences in Microarray Experiments

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1 Introduction

We present a novel algorithm to detect significant gene expression differences in microarray experiments. The algorithm was examined with the reference to the well-known t-test with duplicate control experiments and duplicate chemical-stimulation experiments. These methods are shown to be comparable. The standard deviation (SD) estimates of expression which is used to judge the significant differences is derived from the repeated experiments in the t-test, but in our algorithm, the estimate is given *a priori* as a function of expression levels. Although our algorithm requires the probabilistic model of SD, it is also applicable to a single pair of experiments (one control and one treatment).

2 Materials and Methods

The cell lines used were human hepatocellular carcinoma cell line (HepG2), human acute promyelocytic leukemia cell line (HL60) and rat microglia. The combinations with DNA chips (Affymetrix GeneChip) were: HepG2 (U95A); HepG2 (U95B); HL60 (U95A); rat microglia (U74A).

The biotin-labeled cRNA for each cell line was stocked and later used for the repeated experiments ($n = 6$) which began with the hybridization (six arrays for each cell line).

The HL60 cells were stimulated with 20 nM 12-O-tetradecanoyl-phorbol-13-acetate (TPA) for one hour and the biotin-labeled cRNA was prepared and stored as a stock solution. A total of four U95A arrays were used (two with the TPA-stimulated stock solution and two with the control stock solution).

Scanned microarray data were handled by Microarray Suite 5.0 (Affymetrix) and the values of “Signal” were used as measurements for the *t*-test and algorithm using *a priori* SD.

3 Results and Discussion

Figure 1 shows the precision plot for human HepG2 using U95A chips. The X axis is the average of 6 expression levels (measurements: “signal”) for each gene (total 12559 genes). The Y axis denotes the SD values estimated statistically from the 6 measurements each. The SD estimates (●) are not randomly scattered, and seem to increase with increasing expression level. The least squares fitting (—) to the above relation was used as the SD model, and hereinafter is called the *a priori* SD. The *a priori* SD was in good agreement with the SD profiles for other cell lines and other GeneChips (human HL60, U95A; human HepG2, U95B; rat microglia, U74A). From this fact, we can conclude that the SD model is general to the diverse conditions examined here and that the variability in measurements is independent of the sequence of the bases in the chips. In terms of the *a priori* SD, we could create an algorithm to evaluate the significance of gene expression changes probabilistically.

The algorithm put forward here was examined with the reference to the well-known t-test with two controls and two TPA stimulations. The results from these methods are observed to be comparable. In general, average estimates are less variable than SD estimates. Therefore, our algorithm based on the *a priori* SD which is a function of the average estimates can provide more stable judgment than the statistical method.

Moreover, the validity of the algorithm for a single pair of experiments was examined in all possible four combinations of the control and TPA stimulation results. The results for the four combinations were nearly equal to each other, and our algorithm selected some genes which are known to be regulated on treatment with TPA. So, the algorithm for a single pair of experiments is useful for gene screening.

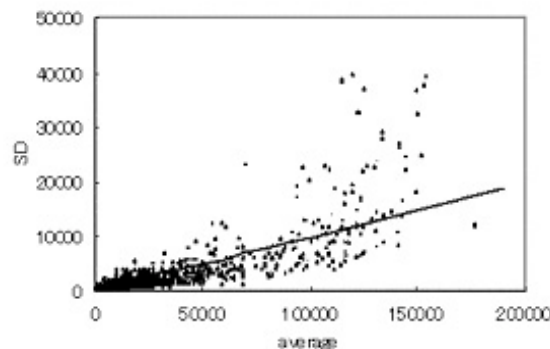


Figure 1: Precision plots for microarray measurements. Six DNA chips are used for a condition. The average and SD estimate of 6 measurements for a gene lead to the values of X and Y axes, respectively. ●: the SD estimates from six replicates; —: the fitted line (*a priori* SD). Sample is human HepG2 and GeneChip is U95A (Affymetrix).