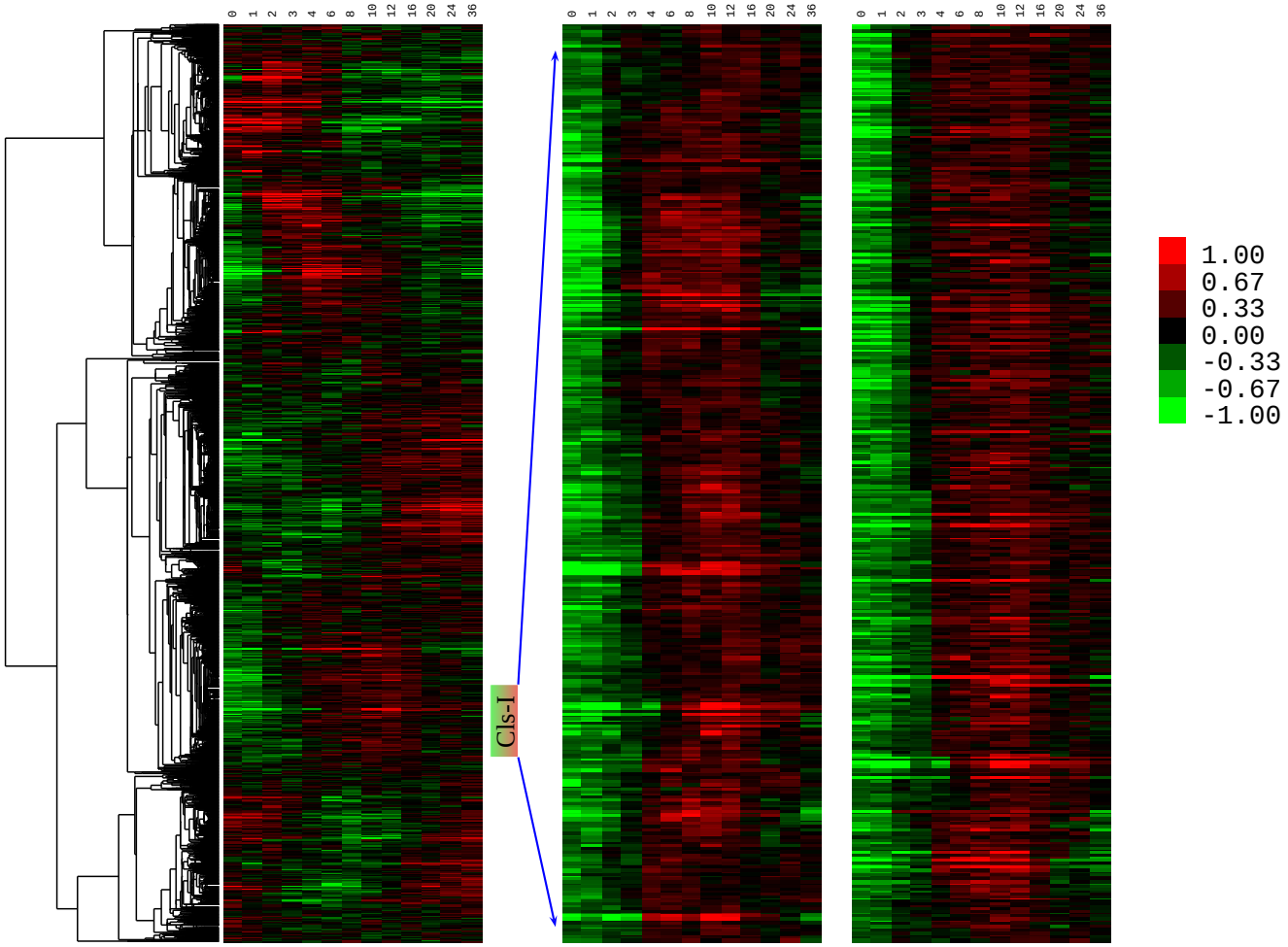
Comparing time-course analysis methods

For a more concrete view of the differences among tools, *StepMiner* and four other widely used publicly available programs were run on the same data, a publicly available microarray time course that traces the response of fibroblasts to the addition of serum [1, 2]. The goal is to determine whether these other tools can be used to find genes that transition at particular times, as *StepMiner* does. In several cases, multiple approaches were attempted; only the best results for each tool are reported.

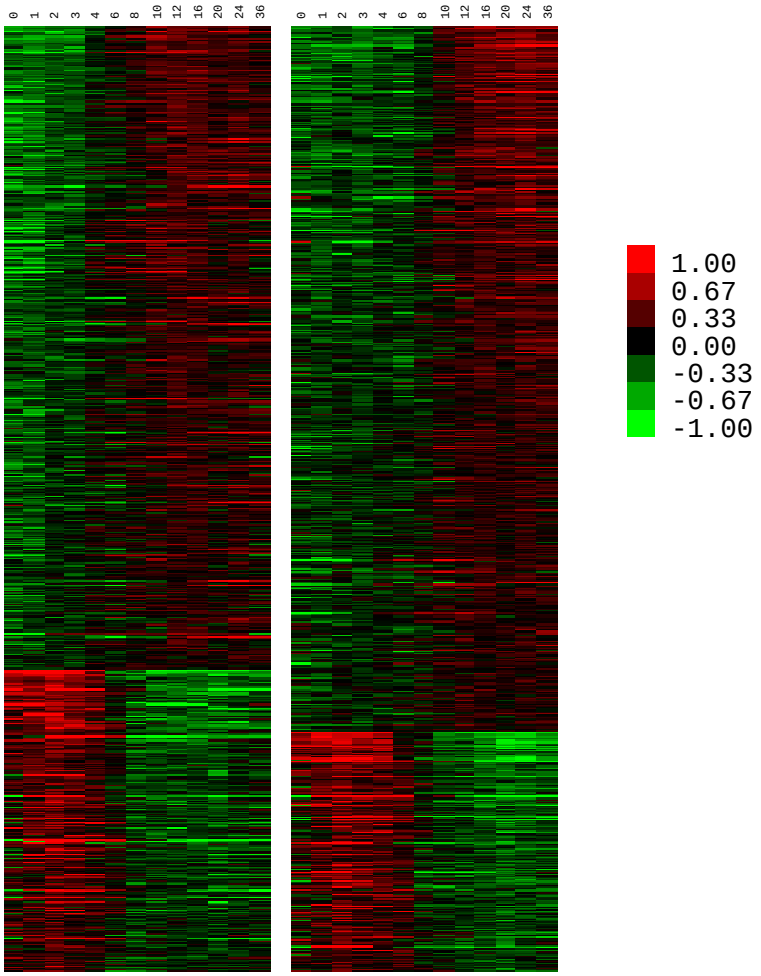
The time course consists of 13 arrays, taken at the times 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, and 36 hours. The data for all of the 5,289 genes that have missing time points were used. The time course was analyzed using hierarchical clustering [4], SAM [7], EDGE [8], STEM [5] and *StepMiner*.



(a) Hierarchical clustering (5289 genes) (b) Cls-I (377 genes) (c) Cls-I reordered (377 genes)

Figure 1: Comparison with hierarchical clustering. In the heatmaps, the expression levels for each gene are centered around the mean over the time course. (a) Hierarchical clustering with Pearson's correlation and centroid linkage was used in Gene Cluster 3.0[3] to cluster the genes in the time course. The heatmap was generated using Java Treeview [6]. (b) A cluster of genes (Cls-I) from the hierarchical clustering where the genes seem to be up-regulated at 4 hours. (c) The same cluster of genes (Cls-I), reordered using *StepMiner*, showing that the cluster contains genes that transition at several different times: 3 genes at 1 hour, 109 genes at 2 hours, 80 genes at 3 hours, 109 genes at 4 hours, and 22 genes at 5 hours. Also 37 genes have two transitions, and 17 genes are not significantly regulated.

Figure 1 shows the results of an attempt to use hierarchical clustering select genes that transition from down-regulated to up-regulated



(a) SAM 4 hr (1928 genes)(b) SAM 8 hr (1778 genes)

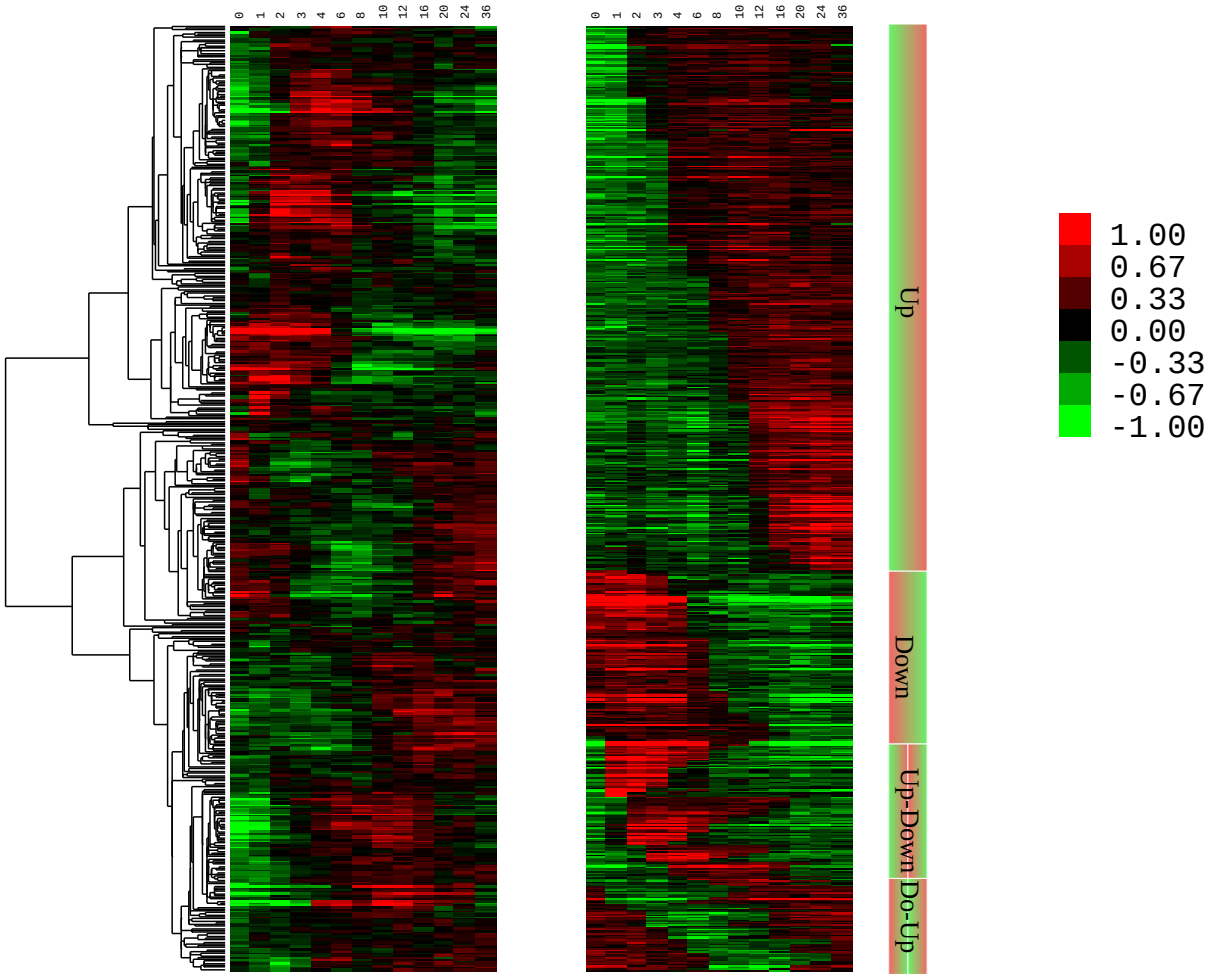
Figure 2: The expression levels for each gene are centered around the mean over the time course. (a) The 1928 differentially expressed genes before and after 4 hr were retrieved using SAM [7] at 5% FDR. (b) The 1778 differentially expressed genes before and after 8 hr were retrieved using SAM at 5% FDR. However, there are 1270 genes common between (a) and (b).

at 3 hours, by manually selecting the appropriate cluster. As shown in Figure 1 (b), there is, indeed, a cluster in the analysis (Cls-I) in which gene expressions appear to be up-regulated around 3 hour. However, when the cluster of genes (Cls-I) is reordered using *StepMiner*and displayed again in a heatmap in Figure 1 (c), it becomes evident that there are many genes that transition at other times, as well as some that transition twice and some that are not significant.

Figure 2 shows the an attempt to find genes that transition after 4 hours, using SAM. The heatmap shows the differentially expressed genes from SAM analysis. The experiment was to find genes that are significantly up-regulated after 4 hours, compared with their expression levels before 4 hours. SAM finds 1928 differentially expressed genes before and after 4 hours shown in Figure 2 (a). However, using SAM to find genes that are significantly up-regulated after 8 hours yields the 1778 genes shown in Figure 2 (b). These lists have 1270 genes in common, showing that genes that are differentially expression before and after a time point are very different from genes that *transition at that time point.* Differential expression requires only that the means of the expression levels before and after the time point be significantly different.

Figure 3 (a) shows the heatmap of differentially expressed genes after clustering in the serum response time course using EDGE. The purpose of EDGE is to identify genes whose expression systematically change over time and significantly different from the mean of the expressions over time. Clearly, this method doesn't provide the direction and position of significant change directly.

Figure 4 shows the analysis of the time course using STEM. It is not clear how to extract position and direction of change information from the results of these programs, even with significant manual effort.



(a) EDGE (488 genes) (b) *StepMiner* (780 genes)

Figure 3: The expression level of every gene is centered around the mean over the time course. (a) The differentially expressed genes over a time course were retrieved using EDGE [8]. The list of genes was clustered using hierarchical clustering. (b) *StepMiner* algorithm was applied to the serum time course and the list of ”up”, ”down”, ”up then down” and ”down then up” genes that are signifiant is shown in the heatmap.

References

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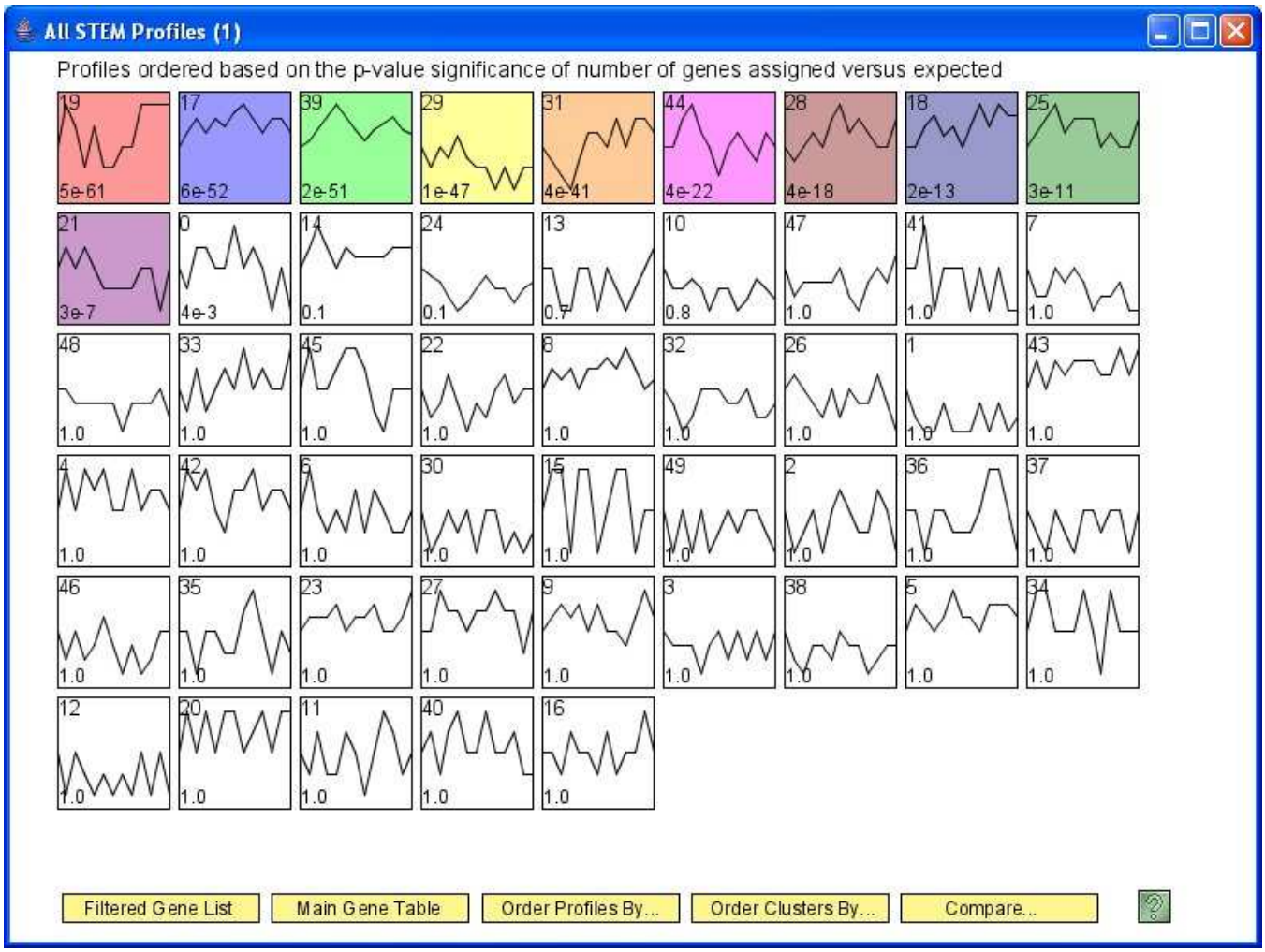

Figure 4: The serum response time course was analyzed using STEM [5] algorithm. The significance of all the model profiles are shown in the plot.