Microarray analysis of gene expression

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Method Article

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Abstract

Introduction

Below are the procedures we used for microarray analysis.

Procedure

1. Isolate total hippocampal RNA from individual animals using \(RNA Aqueous, Ambion). 2. Determine RNA quality by measuring optical density values \(260/280). Values should be consistently at or over 1.9. 3. Use five micrograms of total RNA from experimental and control animals \(e.g. 1 week exercise vs. sedentary mice) and reverse-transcribe into cDNA. 4. Indirectly label using a sensitive fluorescent labeling procedure \(Genisphere). 5. A two-step hybridization and labeling protocol was used where the chip was hybridized to cDNA overnight, washed stringently to remove nonspecifically bound probe, and then poststained with fluorescent dendrimers. 6. After posthybridization and washes, scan slides using a GenePix scanner \(Axon Instruments). Image analysis was performed using GenePix Pro 4.0 software. 7. Import the resulting files from GenePix 4.0 \(Axon Instruments) analysis into Genespring 5.0 \(Silicon Genetics) for additional visualization and data mining. 8. Only consider hybridization spots as positive if there is a signal intensity of twice the background or more in at least one channel of half of the replicates. 9. Perform per-chip normalization by dividing the expressed genes by the median of two housekeeping control genes, β-tubulin and cyclophilin, that were not regulated. 10. Determine gene regulation by taking the log ratio of the median experimental \(running) channel signal to the median control \(sedentary) channel signal. 11. Define up-regulated genes as having an average expression ratio of >1.3, and define the down-regulated genes as having an average expression ratio of <0.7. Determine these values by performing homotypic hybridizations where the same sample is hybridized in both channels \(cy3 & cy5). 12. These cutoff levels are also consistent with expected levels of gene regulation in brain tissue relative to cultured cell reports by others[1,2]. 13. Perform statistical analysis by an unpaired t test using the cross-gene pooled error method in, for example, Genespring software. Significance was set at $p < 0.05$. 14. Classify significantly regulated genes into relevant functional categories.

References

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