Microarray Assay

Total RNA was isolated from the whole-blood lysate (Ovcharenko et al., 2005) followed by depletion of globin messenger RNA (Whitley et al., 2005). All samples passing quality control were then amplified and labeled by using the Illumina TotalPrep-96 RNA amplification kit. Amplified RNA was hybridized to Illumina HT-12 V3 beadchips (48,803 probes) and scanned on an Illumina Beadstation 500. Illumina’s BeadStudio version 2 software was used to generate signal-intensity values from the scans. After background subtraction, the average normalization recommended by the BeadStudio 2.0 software (Illumina, San Diego, CA) was used to rescale the difference in overall intensity to the median average intensity for all samples across multiple arrays and chips. For modular analysis, a set of 260 transcriptional modules was used as a preexisting framework for the analysis of this data set. The approach used for the construction of such framework was previously reported (Chaussabel et al., 2008). Briefly, genes with coordinate expression within or across nine whole-blood disease data sets where selected in multiple rounds of clique and paraclique clustering to form a 260 transcriptional module framework. Following the transformation of gene level data into module level activity scores, both unsupervised and supervised analyses of the complete data set were conducted.





