File S1

Supplementary results

Array hybridization quality was evaluated for individual probe signals by examining kernel density distributions across modules and detection above background (DABG) within modules. Similar kernel density distributions were observed for all hybridizations, although the signal intensities for time-point zero, replicate one were slightly dimmer. This was reflected in the proportion of probes detected above the median of the GC band control signals, where DABG ranged from 80.5-86.3% in the 3'IVT module and 84.1-89.3% in the exon module, and 78.8% and 82.3% in the 3'IVT and exon modules respectively for the dimmer slide. All hybridizations were well in the expected range for *D. melanogaster* given that an average of 72% DAGB was reported for heterologous hybridizations in *D. simulans* (Yang, Graze et al. 2011). Despite the dimmer slide, Kappa statistics indicated good agreement between biological replicates at the individual probe level, and excellent agreement at the probeset and gene level when modules were considered separately (Table 1.). Within a slide, signals for probesets corresponding to the same gene in the 3'IVT and exon modules were in good agreement indicating inter-slide reliability of gene expression between modules (Table 1.). Normalised signal agreement between the *heat shock* genes (*Hsps)* was excellent within and between modules (Table 1.), in addition average signal intensities of seven early responding *Hsps* correlate strongly **(**R 0.77-0.99, P <0.05- 0.0001, Fig S1).

Overall expression patterns at the level of sampling temperature and time-point were visualised using hierarchical clustering. Average linkage clustering was applied to the average normalised signals of 1114, 1285, and 1173 differentially expressed genes from the 3'IVT, exon constitutive and exon modules respectively (Fig S2). The modules clustered similarly, with the dendograms revealing two main expression clusters; 1) the early time points plus 48 hours, and 2) the mid time-points (Fig S2). For cluster 1, the high temperature points clustered closely with time zero in the 3'IVT module (Fig S2a), while these were more similar to 4 hours recovery in the exon module (Fig S2b,c). In all cases, expression tended to return to basal levels by 48 hours recovery indicated by the grouping of time zero and 48 hours. Cluster 2 comprised the 8 and 12 hour recovery points, while 36 hours recovery grouped independently (Figure S2).