The R scripts that you sent worked perfectly. I think that I’ll try to use them to do the TE expression analysis. Do you think that reanalyzing Whiston’s data would be a good idea? The only reason I think so is that John will probably be a reviewer.

I have done a little analysis of the differentially expressed genes.

|  |  |  |
| --- | --- | --- |
|  | 48 h up | 48 h down |
| Bio Proc | |  |  | | --- | --- | | GO:0055114 | oxidation-reduction process | | GO:0000255 | allantoin metabolic process | | GO:0000256 | allantoin catabolic process | | GO:0055085 | transmembrane transport | | GO:0043605 | cellular amide catabolic process | | GO:0035434 | copper ion transmembrane transport | | GO:0006825 | copper ion transport | | |  |  | | --- | --- | | GO:0006928 | movement of cell or subcellular component | | GO:0007018 | microtubule-based movement | | GO:0007017 | microtubule-based process | | GO:0044282 | small molecule catabolic process | | GO:0016054 | organic acid catabolic process | | GO:0046395 | carboxylic acid catabolic process | | GO:0007165 | signal transduction | | GO:0023052 | signaling | | GO:0007154 | cell communication | | GO:0071900 | regulation of protein serine/threonine kinase activity | | GO:0000079 | regulation of cyclin-dependent protein serine/threonine kinase activity | | GO:0043549 | regulation of kinase activity | |
| Mol Fx | |  |  | | --- | --- | | GO:0046982 | protein heterodimerization activity | | GO:0046983 | protein dimerization activity | | GO:0022857 | transmembrane transporter activity | | GO:0016614 | oxidoreductase activity, acting on CH-OH group of donors | | GO:0016491 | oxidoreductase activity | | GO:0005215 | transporter activity | | GO:0005375 | copper ion transmembrane transporter activity | | GO:0016616 | oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | | GO:0003824 | catalytic activity | | |  |  | | --- | --- | | GO:0046982 | protein heterodimerization activity | | GO:0046983 | protein dimerization activity | | GO:0022857 | transmembrane transporter activity | | GO:0016614 | oxidoreductase activity, acting on CH-OH group of donors | | GO:0016491 | oxidoreductase activity | | GO:0005215 | transporter activity | | GO:0005375 | copper ion transmembrane transporter activity | | GO:0016616 | oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | | GO:0003824 | catalytic activity | |
| Component | |  |  | | --- | --- | | GO:0000786 | nucleosome | | GO:0044815 | DNA packaging complex | | GO:0032993 | protein-DNA complex | | GO:0000785 | chromatin | | GO:0031224 | intrinsic component of membrane | | GO:0016021 | integral component of membrane | | GO:0016020 | membrane | | GO:0044425 | membrane part | | GO:0044427 | chromosomal part | | GO:0005694 | chromosome | | |  |  | | --- | --- | | GO:0000786 | nucleosome | | GO:0044815 | DNA packaging complex | | GO:0032993 | protein-DNA complex | | GO:0000785 | chromatin | | GO:0031224 | intrinsic component of membrane | | GO:0016021 | integral component of membrane | | GO:0016020 | membrane | | GO:0044425 | membrane part | | GO:0044427 | chromosomal part | | GO:0005694 | chromosome | |

|  |  |  |
| --- | --- | --- |
|  | 8 d up | 8 d down |
| Bio Proc | |  |  | | --- | --- | | GO:0006928 | movement of cell or subcellular component | | GO:0007018 | microtubule-based movement | | GO:0007017 | microtubule-based process | | GO:0044282 | small molecule catabolic process | | GO:0046395 | carboxylic acid catabolic process | | GO:0016054 | organic acid catabolic process | | GO:0023052 | signaling | | GO:0007165 | signal transduction | | GO:0007154 | cell communication | | GO:0043549 | regulation of kinase activity | | GO:0045859 | regulation of protein kinase activity | | GO:1904029 | regulation of cyclin-dependent protein kinase activity | | GO:0071900 | regulation of protein serine/threonine kinase activity | | GO:0051338 | regulation of transferase activity | | |  |  | | --- | --- | | GO:0055114 | oxidation-reduction process | | GO:0000255 | allantoin metabolic process | | GO:0000256 | allantoin catabolic process | | GO:0055085 | transmembrane transport | | GO:0035434 | copper ion transmembrane transport | | GO:0043605 | cellular amide catabolic process | | GO:0006825 | copper ion transport | |
| Mol Fx | |  |  | | --- | --- | | GO:0008017 | microtubule binding | | GO:0003777 | microtubule motor activity | | GO:0048037 | cofactor binding | | GO:0046906 | tetrapyrrole binding | | GO:0020037 | heme binding | | GO:0003774 | motor activity | | GO:0015631 | tubulin binding | | GO:0005488 | binding | | GO:0043167 | ion binding | | GO:0004553 | hydrolase activity, hydrolyzing O-glycosyl compounds | | GO:0008092 | cytoskeletal protein binding | | GO:0005506 | iron ion binding | | GO:0016798 | hydrolase activity, acting on glycosyl bonds | | GO:0051537 | 2 iron, 2 sulfur cluster binding | | |  |  | | --- | --- | | GO:0046982 | protein heterodimerization activity | | GO:0046983 | protein dimerization activity | | GO:0022857 | transmembrane transporter activity | | GO:0016614 | oxidoreductase activity, acting on CH-OH group of donors | | GO:0016491 | oxidoreductase activity | | GO:0005215 | transporter activity | | GO:0005375 | copper ion transmembrane transporter activity | | GO:0016616 | oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | | GO:0003824 | catalytic activity | |
| Component | |  |  | | --- | --- | | GO:0071944 | cell periphery | | GO:0005618 | cell wall | | GO:0030312 | external encapsulating structure | | GO:0005886 | plasma membrane | | |  |  | | --- | --- | | GO:0000786 | nucleosome | | GO:0044815 | DNA packaging complex | | GO:0032993 | protein-DNA complex | | GO:0000785 | chromatin | | GO:0031224 | intrinsic component of membrane | | GO:0016021 | integral component of membrane | | GO:0016020 | membrane | | GO:0044425 | membrane part | | GO:0044427 | chromosomal part | | GO:0005694 | chromosome | |

The terms in these table are partial lists – all terms have p values less than 0.05, although most have less impressive adjusted p values. The 48 hour GO terms are similar to what I saw with my DESeq2 analysis – oxidation reduction, transport, protein dimerization up-regulated. The down-regulated analysis is new – microtubules, signal transduction, oxidation reduction, transporters and nucleosome. Presumably, some of the enzymes in each shared GO term are being turned on and others off. The nucleosome and chromatin GO terms suggest that chromosome remodeling might be happening (although I’ve not looked it up).

The up-regulated GO terms in the 8 day group include microtubules and signaling and iron binding among many others. The GO terms in the 8 day down-regulated group are very similar to those in the up-regulated 48 h samples.

The results suggests that many of the processes that were being turned on at 48 h were being turned off at 8 days. To see what the relationship between the two sets of genes, the groups of genes were compared.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 48h | 8 d up | 8 d unchanged | d 8 down |
| Up | 1128 | 1 | 14 | 1104 |
| Unchanged | 4496 | 11 | 4395 | 14 |
| Down | 1112 | 1 | 12 | 1 |

It is clear that essentially all the up-regulated genes at 48 h were down-related at 8 days. As would be expected the unchanged genes were consistently unchanged. The down-regulated genes at 48 h seem worrisome – I have checked the results they seem right, but it’s hard to figure out what’s going on.