



b)

| Gene Ontology Term | Number of Genes | Enrichment Score |
|---|-----------------|------------------|
| Cluster 1: Genes Down-regulated in 20E 5hr midgut | | |
| Catabolic Processes | 11 | 4.39 |
| Cytoplasmic Vesicles | 9 | 3.1 |
| Nuclear Import | 8 | 2.1 |
| Cluster 2: Genes Up-regulated in 20E 5 hr midgut | | |
| Membrane Transport | 12 | 3.25 |
| Innate Immunity | 4 | 1.72 |
| Endopeptidase Activity | 4 | 1.47 |
| Cluster 3: Genes Up-regulated in 20E 18hr midgut | | |
| Ion Homeostasis | 8 | 1.88 |
| Protein Ubiquitination | 7 | 1.59 |
| Proline Metabolism | 5 | 1.53 |
| Cluster 4: Genes Down-regulated in 20E 18hr midgut | | |
| Proton Transport | 21 | 12.6 |
| Cytochrome-C Reductase | 4 | 2.98 |
| Heme-copper oxidase activity | 5 | 2.11 |

Gene expression in *Aedes aegypti* midguts treated with 20-hydroxyecdysone (20E). **a)** Heat expression map of transcriptome from dissected midguts of mosquitoes injected with either $\sim 1.25\mu\text{g}$ 20E or PBS. Guts were taken from both an early (5hr) and late (18hr) timepoint. **b)** Gene Ontology (GO) analysis performed using David Functional Annotation. Clusters of interest (1-4): those that are either up or down-regulated in response to 20E at 5hrs and 18hrs. Enrichment scores of over 1.3 are significant.