

Table S4. Multi-copy Y-linked genes across *Drosophila* species. Shown are the orthologous location of multi-copy Y genes in *D. melanogaster*, and their inferred molecular function and gene expression pattern in *D. melanogaster* (data from flybase.org).

| Species | Chromosome | Peptide ID | Gene ID | InterPro Domain | Molecular Function | Testes_expression | Highest_Expression_Tissue | Highest_Expression_Development | Name | Gene Snapshot |
|----------------------|------------|-------------|-------------|---|---|-------------------|---------------------------|--------------------------------|---------|--|
| <i>D. albomicans</i> | 3L | FBpp0091021 | FBgn0053725 | Protein of unknown function DUF1091 | NA | NA | NA | NA | NA | NA |
| <i>D. americana</i> | 2L | FBpp0080666 | FBgn0032715 | Thiolase | transferase activity, transferring acyl groups other than amino-acyl groups | Y | Digestive System | Larva | NA | NA |
| <i>D. athabasca</i> | 2R | FBpp0071874 | FBgn0045483 | 7TM chemoreceptor | taste receptor activity | NA | NA | NA | Gr59a | NA |
| <i>D. athabasca</i> | 2R | FBpp0085702 | FBgn0034429 | GNAT domain | N-acetyltransferase activity | N | Digestive System | Larva | NA | NA |
| <i>D. athabasca</i> | 2R | FBpp0085908 | FBgn0034328 | Immune-induced protein Dim | NA | N | Female Head | Adult Male | IM23 | NA |
| <i>D. athabasca</i> | 3L | FBpp0088483 | FBgn0036640 | Leucine-rich repeat | May be involved in the export of mRNA from the nucleus to the cytoplasm | Y | Ovary | Embryo,Adult Female | nxf2 | NA |
| <i>D. lummei</i> | X | FBpp0070717 | FBgn0011761 | Thioredoxin | female meiotic nuclear division, disulfide oxidoreductase activity | Y | Ovary | Embryo, Adult Female | dhd | Deadhead is a thioredoxin-like protein necessary for the initiation of embryonic development. |
| <i>D. melanica</i> | 3L | FBpp0075822 | FBgn0028668 | V-ATPase proteolipid subunit | proton-transporting ATPase activity, rotational mechanism | Y | Testes | Adult Male | Vha16-2 | NA |
| <i>D. melanica</i> | 3L | FBpp0088546 | FBgn0010424 | EF-hand domain | calcium ion binding | Y | Carcass of larva | Embryo,Larva,Pupae | TpnC73F | NA |
| <i>D. melanica</i> | 3L | FBpp0091046 | FBgn0053796 | Protein of unknown function DUF1091 | NA | NA | NA | NA | NA | NA |
| <i>D. miranda</i> | 2R | FBpp0071501 | FBgn0026582 | High mobility group box domain | nucleus | Y | Ovary | Embryo | Hmg-2 | NA |
| <i>D. miranda</i> | 2R | FBpp0072323 | FBgn0060296 | Ankyrin repeat | calcium channel activity, male courtship behavior | Y | Carcass of larva | Embryo,Larva,Pupae | pain | NA |
| <i>D. miranda</i> | 3L | FBpp0072616 | FBgn0004378 | Kinesin motor domain; Kinesin-associated microtubule-binding domain | ATP-dependent microtubule motor activity | Y | Ovary | Embryo | Kip61F | Kinesin-like protein at 61F (Kip61F) is a member of the kinesin-5 family of cytoskeletal motor proteins. Kip61F allows the crosslinking and sliding apart of adjacent microtubules. This 'sliding filament mechanism' is critical for many aspect of mitosis and chromosome segregation. |
| <i>D. miranda</i> | 2R | FBpp0085582 | FBgn0034491 | Hormone-sensitive lipase, N-terminal | lipase activity | Y | Ovary | Embryo | Hsl | Hormone-sensitive lipase is involved in lipid storage |
| <i>D. miranda</i> | 2R | FBpp0085619 | FBgn0005655 | Proliferating cell nuclear antigen, PCNA | DNA binding, mitotic spindle organization | Y | Ovary | Embryo,Adult Female | PCNA | NA |
| <i>D. miranda</i> | 2R | FBpp0085706 | FBgn0034435 | NA | spindle assembly involved in male meiosis I | Y | Testes,Imaginal Disc | Adult Male | fest | NA |
| <i>D. miranda</i> | 2R | FBpp0086004 | FBgn0003701 | NA | protein binding,mitotic sister chromatid segregation | Y | Ovary | Embryo | thr | Three rows (Thr) together with Sse forms the endoprotease separate complex, which cleaves a subunit of the cohesin complex, thereby allowing separation of the sister chromatids. |
| <i>D. miranda</i> | 2R | FBpp0086750 | FBgn0033860 | Peptidase M17, leucine aminopeptidase/peptidase B | aminopeptidase activity | Y | Testes | Adult Male | S-Lap5 | NA |
| <i>D. miranda</i> | 2R | FBpp0086788 | FBgn0033845 | SAPAP family | microtubule binding,chromosome segregation | Y | Testes,Ovary | Embryo | mars | NA |
| <i>D. miranda</i> | 2R | FBpp0086910 | FBgn0033788 | Transcription activator MBF2 | NA | Y | Digestive System | Larva | NA | NA |
| <i>D. miranda</i> | 2R | FBpp0087756 | FBgn0033354 | Fanconi anemia group I protein | DNA polymerase binding,mitotic G2 DNA damage checkpoint | Y | Ovary | Embryo | FANCI | NA |
| <i>D. miranda</i> | 2R | FBpp0087986 | FBgn0261385 | Pleckstrin homology domain; Anillin homology domain | actin binding,male meiosis cytokinesis | Y | Ovary | Embryo | scra | Scraps is a homolog of anillin, a conserved pleckstrin homology domain (PLEKH) containing protein that binds actin, nonmuscle myosin II and microtubules. It stabilizes the contractile ring and is required for completion of cytokinesis |

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|-------------------------|----|-------------|-------------|--|---|----|---------------------------------------|---------------------------|----------|--|
| <i>D. miranda</i> | 2R | FBpp0088003 | FBgn0033216 | Diacylglycerol acyltransferase | transferase activity | Y | Digestive System | Embryo,Adult Male | NA | NA |
| <i>D. miranda</i> | X | FBpp0290242 | FBgn0052666 | Protein kinase domain | protein serine/threonine kinase activity | Y | Ovary, Salivary gland of prepupae | Embryo,Pupae,Adult Female | Drak | Death-associated protein kinase related (Drak) is a protein serine/threonine kinase belonging to the Death-associated protein kinase family. Drak promotes phosphorylation of sqh at sites known to stimulate actomyosin contractility. Its main characterized function is to shape epithelial tissues during development. |
| <i>D. miranda</i> | 2R | FBpp0291585 | FBgn0054045 | NA | NA | NA | NA | NA | NA | NA |
| <i>D. nannoptera</i> | X | FBpp0077024 | FBgn0031103 | Protein of unknown function DUF4791 | NA | Y | Testes | Adult Male | NA | NA |
| <i>D. nigromelanica</i> | 2R | FBpp0085858 | FBgn0063491 | Glutathione S-transferase, N-terminal | glutathione transferase activity | Y | Head | Larva,Adult Male | GstE9 | NA |
| <i>D. nigromelanica</i> | 2R | FBpp0086954 | FBgn0011604 | ISWI family | DNA-dependent ATPase activity; nucleosome-dependent ATPase activity; protein binding; transcription factor binding; sperm chromatin condensation; spermatogenesis | Y | Ovary, Imaginal Disc and CNS of larva | Embryo | Iswi | NA |
| <i>D. pseudoobscura</i> | 3L | FBpp0076362 | FBgn0035915 | Peptidase M17, leucine aminopeptidase/peptidase B | aminopeptidase activity | Y | Testes | Adult Male | S-Lap1 | NA |
| <i>D. pseudoobscura</i> | 3L | FBpp0076363 | FBgn0052351 | Peptidase M17, leucine aminopeptidase/peptidase B | aminopeptidase activity | Y | Testes | Adult Male | S-Lap2 | NA |
| <i>D. pseudoobscura</i> | 3L | FBpp0076372 | FBgn0035916 | Rab-GTPase-TBC domain | GTPase activator activity | Y | Ovary | Embryo | GAPsec | NA |
| <i>D. pseudoobscura</i> | 3L | FBpp0112062 | FBgn0035690 | Zinc finger, AD-type; Zinc finger, RING/FYVE/PHD-type; Zinc finger C2H2-type | transcription factor activity, sequence-specific DNA binding | Y | Ovary | Embryo | NA | NA |
| <i>D. robusta</i> | 3L | FBpp0072987 | FBgn0010317 | Cyclin-like | cyclin-dependent protein serine/threonine kinase regulator activity; mitotic cell cycle | Y | Ovary | Embryo, Adult Female | Cyclin J | NA |
| <i>D. robusta</i> | 3L | FBpp0078102 | FBgn0046301 | Leucine-rich repeat domain | ubiquitin-protein transferase activity | Y | Testes | Embryo | NA | NA |
| <i>D. robusta</i> | 3L | FBpp0312385 | FBgn0053795 | Protein of unknown function DUF1091 | NA | NA | NA | NA | NA | NA |