

**Table S1 Primers**

| <b><i>Drosophila melanogaster</i> primers</b> |                                       |                                                      |
|-----------------------------------------------|---------------------------------------|------------------------------------------------------|
| yar.spliced.Fw                                | AGA-CGC-CCT-TTA-ATT-TCA-CGC-TGC       | amplify spliced <i>yar</i> (Fig.1C)                  |
| yar.spliced.Rev                               | TCC-GTC-TGC-CTT-TGT-GTT-TAG-ACG       |                                                      |
| yar.intron.Fw                                 | GCT-CCG-TAT-TCC-ACT-TCT-CTG-AGG       | amplify <i>yar</i> intron (Fig.1C)                   |
| yar.intron.Rev                                | GAT-TGC-GTG-TGC-CTC-GTG-TGC-TG        |                                                      |
| Ras64B.spliced.Fw                             | GGC-AAG-TCA-GCG-ATA-ACG-ATA-CAG-TTC   | amplify spliced <i>Ras64B</i> (Fig.1C)               |
| Ras64B.spliced.Rev                            | CGA-GTC-TTC-AAT-GGT-GGG-ATC-GTA-GTC   |                                                      |
| Ras64B.intron.Fw                              | AAT-CAT-CAT-CAT-CAT-CAT-TGG-CAG       | amplify <i>Ras64B</i> intron (Fig.1C)                |
| Ras64B.intron.Rev                             | ATA-TAC-ACG-AAA-GAT-GTG-CAG-ATG-ACG   |                                                      |
| yar.genomic.Fw                                | CGG-ATC-CAA-GGT-TAA-GAG-TTT-ACG-C     | amplify <i>yar</i> genomic region to generate rescue |
| yar.genomic.Rev                               | CGG-ATC-CTA-ATT-TCA-ATG-CCG-TAT-CAT-C | construct, contain BamHI cut sites (Fig. 3)          |
| yellow.Fw                                     | TGG-AGT-CGA-ACA-CTT-TGG-CAA-TCG-G     | amplify <i>yellow</i> (Fig.4)                        |
| yellow.Rev                                    | CCC-ACA-GCC-GAC-CAC-ACT-CAT           |                                                      |
| yar.Fw                                        | GTA-TAT-CCA-CAT-CAC-CAG-ACC-TCA-GGA-C | amplify <i>yar</i> (Fig.4)                           |
| yar.Rev                                       | ACA-TCC-TCG-AAT-CAC-TAT-GCA-AGT-CG    |                                                      |
| achaete.Fw                                    | CTT-CGG-CCT-TTA-ATG-GAC-CCT-CTG-TT    | amplify <i>achaete</i> (Fig.4)                       |
| achaete.Rev                                   | AAT-CGT-CGA-TGT-TGC-TGG-CTT-GC        |                                                      |
| scute.Fw                                      | CTA-TCG-CCT-GGT-TCC-TCG-CC            | amplify <i>scute</i> (Fig.4)                         |
| scute.Rev                                     | TCC-ACC-AGA-TCC-TGA-AGC-CTC-C         |                                                      |
| RpL32.Fw                                      | AAG-ATG-ACC-ATC-CGC-CCA-GCA-TAC       | amplify <i>RpL32</i> (housekeeping gene              |
| RpL32.Rev                                     | ACG-CAC-TCT-GTT-GTC-GAT-ACC-CTT-G     | control (Fig.4)                                      |
| Ras64B.Fw                                     | AGG-AAG-TGC-TGC-CTG-ATG-TAG-AAG       | amplify <i>Ras64B</i> (housekeeping gene             |
| Ras64B.Rev                                    | TTA-TAT-GTT-GGC-TCC-TGC-TTC-CGC       | control (Fig.4)                                      |
| <b><i>Drosophila virilis</i> primers</b>      |                                       |                                                      |
| Dvir.achaete.Fw                               | GCA-CGA-ATT-GTT-GGG-ATA-ATC-TTC       | amplify <i>D. virilis achaete</i> (Fig.2)            |
| Dvir.achaete.Rev                              | AAC-GCC-TCC-AAC-TCC-AGC-AGC           |                                                      |
| Dvir.yellow.Fw                                | AAT-AGG-CAA-CAG-CAT-CAT-CGC-AGC       | amplify <i>D. virilis yellow</i> (Fig.2)             |
| Dvir.yellow.Rev                               | TTT-GAT-CTG-ACC-ACA-AAC-ACA-AGA-AT    |                                                      |
| Dvir.RpL32.Fw                                 | AGC-TCA-CGC-ACA-TTG-TGT-ACG-AGG       | amplify <i>D. virilis RpL32</i> (Fig.2)              |
| Dvir.RpL32.Rev                                | ACA-GAG-TGC-GTC-GTC-GCT-TCA-AG        |                                                      |
| Dvir.RpL32-CDS.Fw                             | TGA-GAA-CGC-AGA-CGA-CCG-TTG-G         | amplify <i>D. virilis RpL32</i> cDNA (used to        |
| Dvir.RpL32-CDS.Rev                            | CAC-CAA-AAT-GAC-CAT-TCG-TCC-AGC       | make probe in Suppl.Fig.2A)                          |
| Dvir.GS-ex1.Fw <sup>A</sup>                   | AAG-GCG-AAA-GCA-ACA-GCA-GCA-GC        | amplify fragments of <i>D. virilis y-ac</i>          |
| Dvir.1-3.Fw <sup>B</sup>                      | TCC-ACT-TGC-CAG-GTG-AGA-ATT-GAC       | intergenic region (Fig.2, Suppl.Fig.2B).             |
| Dvir.1-2.Fw <sup>D</sup>                      | GAG-CAC-ACT-TTT-GCC-TGC-GGT           | Primer pairs that generated cDNAs A, B,              |
| Dvir.2-1.Fw <sup>C</sup>                      | TCG-TTG-TGG-TTG-TCA-TTA-GTC-CGC-TG    | C and D are indicated.                               |
| Dvir.2-4.Fw                                   | GAT-CGT-GAG-TGC-CAG-AAG-CTG           |                                                      |
| Dvir.1-1.Rev <sup>A</sup>                     | CTT-TTG-GCG-TTA-AGT-GTT-TCC           |                                                      |

|                             |                                       |
|-----------------------------|---------------------------------------|
| Dvir.1-4.Rev                | CAG-CTT-GAT-ATT-AAC-GCC-GTC-TAG       |
| Dvir.GS-ex2.Rev             | TTC-ACG-CGT-GTC-TTG-TCA-TAT-CGC-CAG-G |
| Dvir.1-3.Rev                | GTC-AAT-TCT-CAC-CTG-GCA-AGT-GGA       |
| Dvir.1-2.Rev <sup>B</sup>   | ACC-GCA-GGC-AAA-AGT-GTG-CTC           |
| Dvir.2-3.Rev                | TTC-TCA-TTG-ATT-AAT-TGC-CAT-CAT-TAG-C |
| Dvir.2-4.Rev <sup>C,D</sup> | CAG-CTT-CTG-GCA-CTC-ACG-ATC           |
| Dvir.GS-ex5.Rev             | TCT-TCG-ACA-AGG-GTC-GCT-CTT-GGC-AG    |
| Dvir.2-5.Rev                | GCA-GCA-GGC-GCT-ATC-ATT-ATC-TGA-CAC   |
| Dvir.GS-ex6.Rev             | GCA-ATA-GAA-ATT-CGC-TCT-GGG-GTG-AAG-G |

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