

A case study of the reproducibility of transcriptional reporter cell-based RNAi screens in *Drosophila*

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Supplemental Information

Additional data file 1 is a table listing the gene name, Curated Genes in the *Drosophila* genome based on gene predictions and previously characterized genes (CG#), and DRSC amplicon ID for all the new dsRNAs belonging to the DRSC-v library. The log-ratio of normalized luciferase units of experimental dsRNA (Nexp) with that of GFP dsRNA (Ngfp) is listed. Experiments were performed twice in triplicates (six data points for each gene tested). A consistent increase or decrease of at least 30% of the reporter activity with respect to the average of multiple negative controls (GFP dsRNA) was considered as a positive hit. Validation information for a second dsRNA is also provided for the genes that could be validated by the first amplicon.

Additional data file 2 is a table listing genes name and CG# provided for those dsRNAs that were reported to have multiple potential OTs in the previously published Wnt/wg screen of DasGupta *et al.* [3], but still pass the validation test with DRSV-v dsRNAs (first column). Also listed are genes/CG# representing dsRNAs that represent unique amplicons in the DasGupta *et al.* screen and still pass with validation dsRNAs of the DRSC-v library (column 2). Note that several dsRNAs of the DRSC1.0 library that were thought to have OTEs could be re-validated using unique DRSC-v amplicons. Moreover, not all unique dsRNAs of the DRSC1.0 library had reproducible effects on the modulation of the Wg reporter activity when a corresponding unique validation dsRNAs (DRSC-v) was used.

Additional data file 3 is a table listing the gene name, CG#, and DRSC amplicon number for all of the new dsRNAs tested in the Hh luciferase reporter assay. The number of potential off-targets calculated for the amplicon that was identified in the original Hh screen, based on a 19 bp window, is listed once for each gene. The average fractional change in reporter activity compared to GFP dsRNA controls (listed at the bottom) are presented, with scores between -0.25 and -0.50 highlighted in yellow, scores less than -0.50 highlighted in orange, and scores greater than or equal to + 0.50 highlighted in blue. At the bottom of the list, scores for GFP, Ci, Smo, and th dsRNA controls that were included in the assay plates are also indicated.