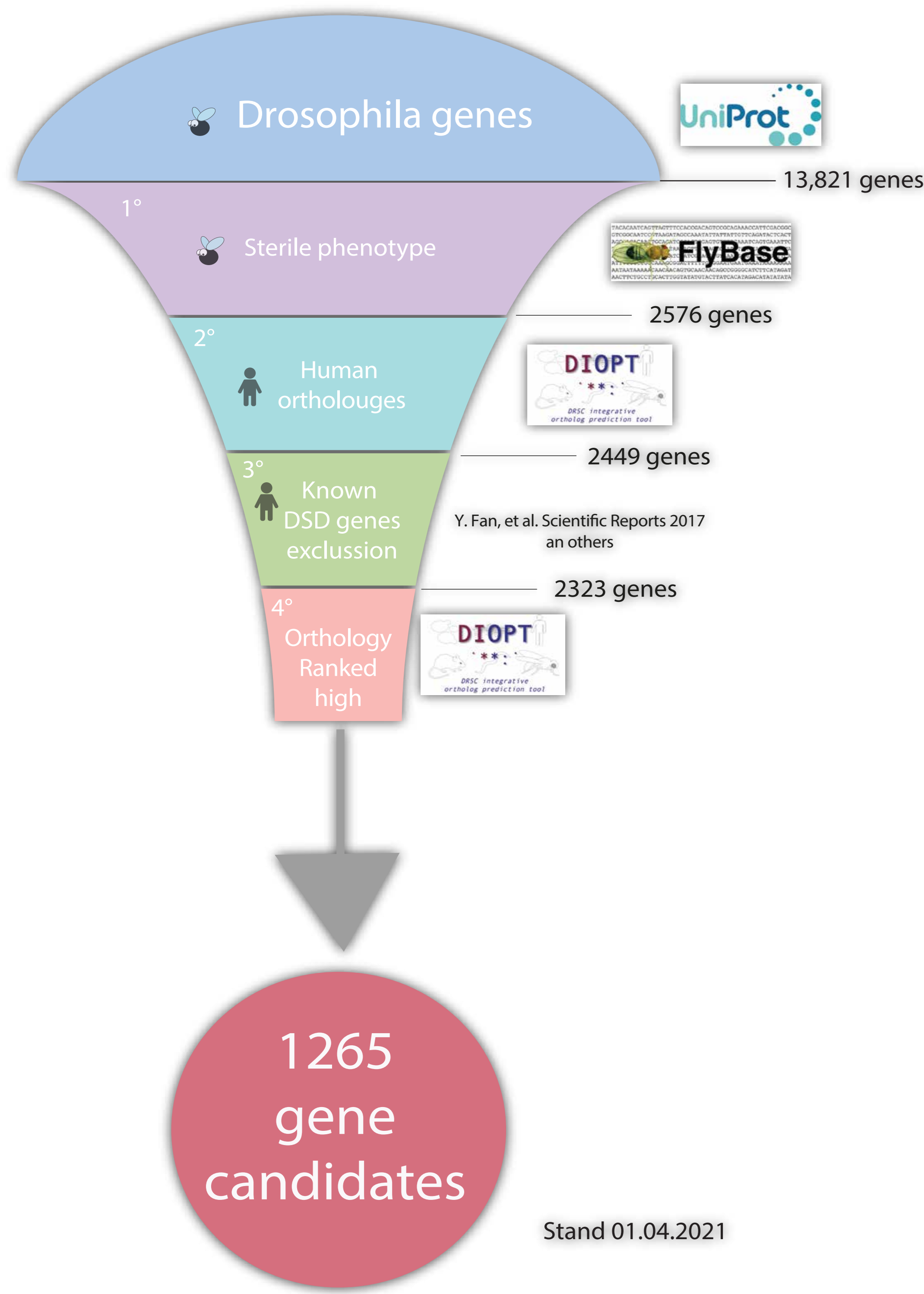


# High throughput screening of DSD candidate genes with the help of powerful model *Drosophila melanogaster*

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## Background

*Drosophila melanogaster* as a study model has already significantly contributed to the understanding of the mechanisms of many human diseases. So far *D. melanogaster* has rarely been exploited as a model for human sex development. Nanda et.al already demonstrated in 2009 that the *Drosophila* orthologue of SOX9, Sox-100B is essential for testis development in *Drosophila*. Similarly, we could demonstrate that the fly homologue of STARD8, *cv-c*, that is suspected to be the cause of sex reversal in two 46 XY sisters, has similar location and function in the fly as its mammalian counterpart. *cv-c* mutant male flies had defects in formation of embryonic gonads and germ cells (GC) evading from their niche. These results indicate that the molecular mechanisms regulating dimorphic gonad development may be much more conserved over phyla than previously suspected. We thus established the fruit fly as a new alternative model to filter for new genes involved in gonadal development in flies and humans.



## High throughput filtering for new DSD candidate genes

All genes that are known to cause sterility in flies (2576 genes) are predicted with the "DIOPT-DRSC Integrative Ortholog Prediction Tool" to have homologs and/or orthologues in humans (2449 genes) (duplicates are removed). After the already known DSD genes are excluded (126 genes), all other genes then orthologs that are predicted to rank "high" are excluded. The remaining 1265 genes are subsequently knocked down in the germ cells (*Nos-Gal4*) or the somatic gonadal tissue (*mtd-Gal4*) of the fly. The consequences on sex development will be rated by the female/male ratio of the F1 generation (Figure 1).

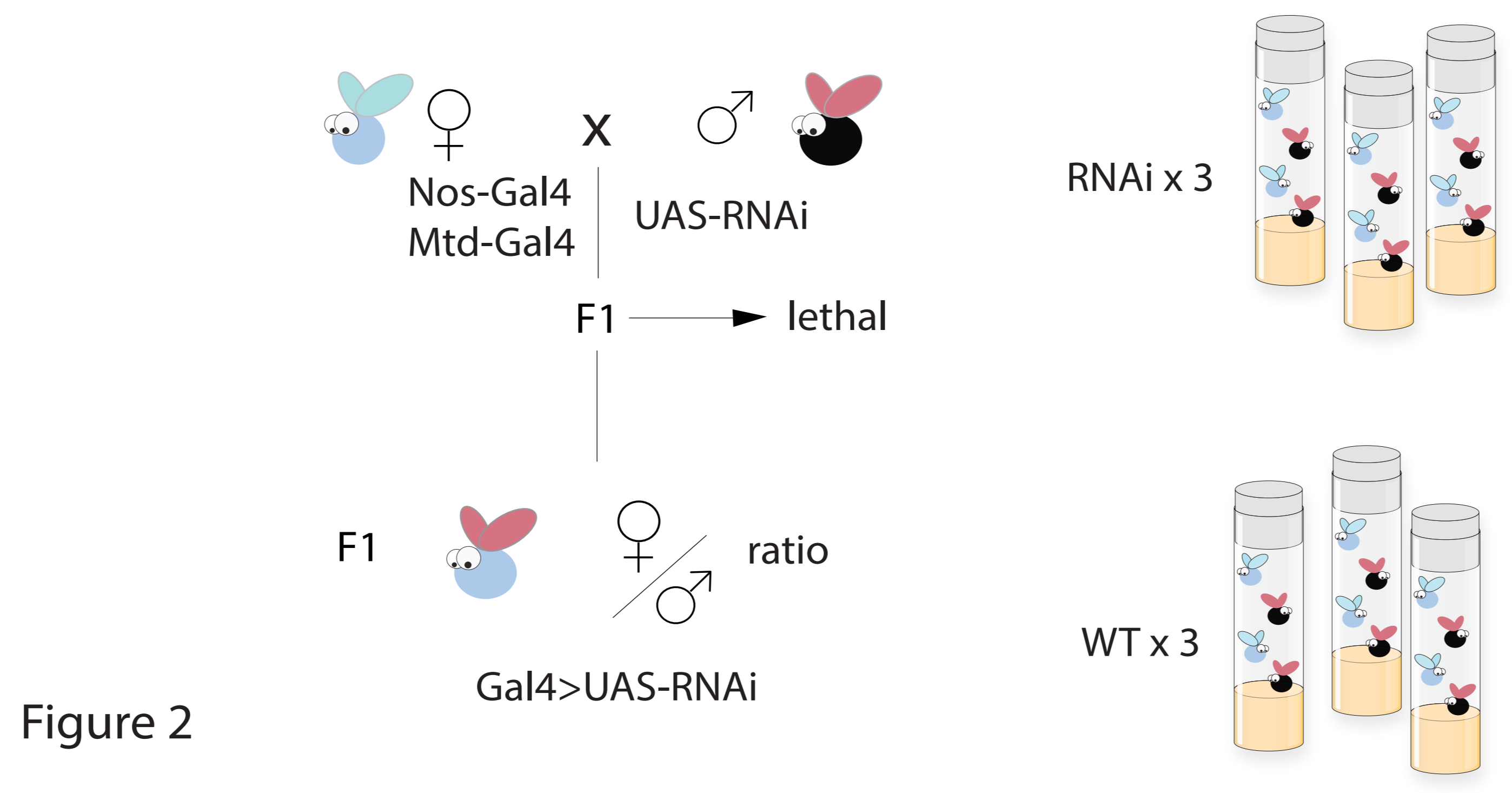


Figure 2 RNAi knockdown screening

All 1265 genes will be knocked down with the GAL4-UAS RNAi system in flies. The consequences of the knock down of the genes in the gonads (*Nos-Gal4*) and in the somatic gonadal tissue (*Mtd-Gal4*) will be evaluated in triplicates by comparing the female to male ratio in the F1 generation of wild type and the RNAi lines. 15 virgins of *Nos-Gal4* or *Mtd-Gal4* are crossed with 5 males containing RNAi-UAS for a specific gene.

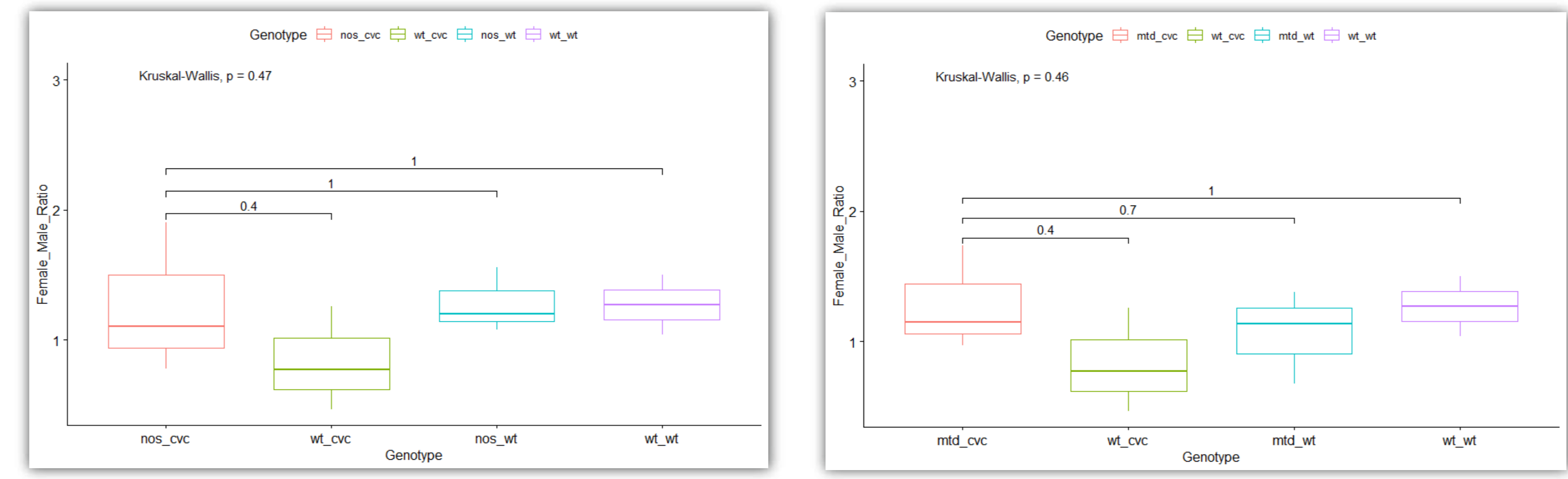


Figure 3 Female to male ratio of *Nos-Gal4/cv-c-UAS* and *Mtd-Gal4/cv-c-UAS* compared to three control crosses.

## Results and Discussion

Until now RNAi knockdown were performed on 4 different genes (*bnl*, *da*, *cv-c*, *hsp83*) out of the list of 1265 genes. We could not detect any consequences of the knock downs that hint to an involvement of those 4 genes in gonadal development (*Nos-Gal4/cv-c-UAS* and *Mtd-Gal4/cv-c-UAS* shown in Figure 3). We could previously show that *cv-c* inactivation in the mesoderm in the fly embryos lead to severe developmental defects of the head and the gonads. We also showed that its human orthologue STARD8 showed similar function in flies and was able to rescue the phenotype of flies which inactive *cv-c* (Figure 4).

These data showed that *cv-c* mutant male flies had defects in formation of embryonic gonads and germ cells evading from their niche. However, the *cv-c* RNAi knock down fail to show any effect, raising the question if the knock down with the *Nos-Gal4* is sufficient enough to trigger a phenotype. We therefore plan to vary the degree of *cv-c* reduction by qPCR. Further studies are necessary to clarify this apparent discrepancy. Implementing automated embryo counting will speed up and accurate further screening.

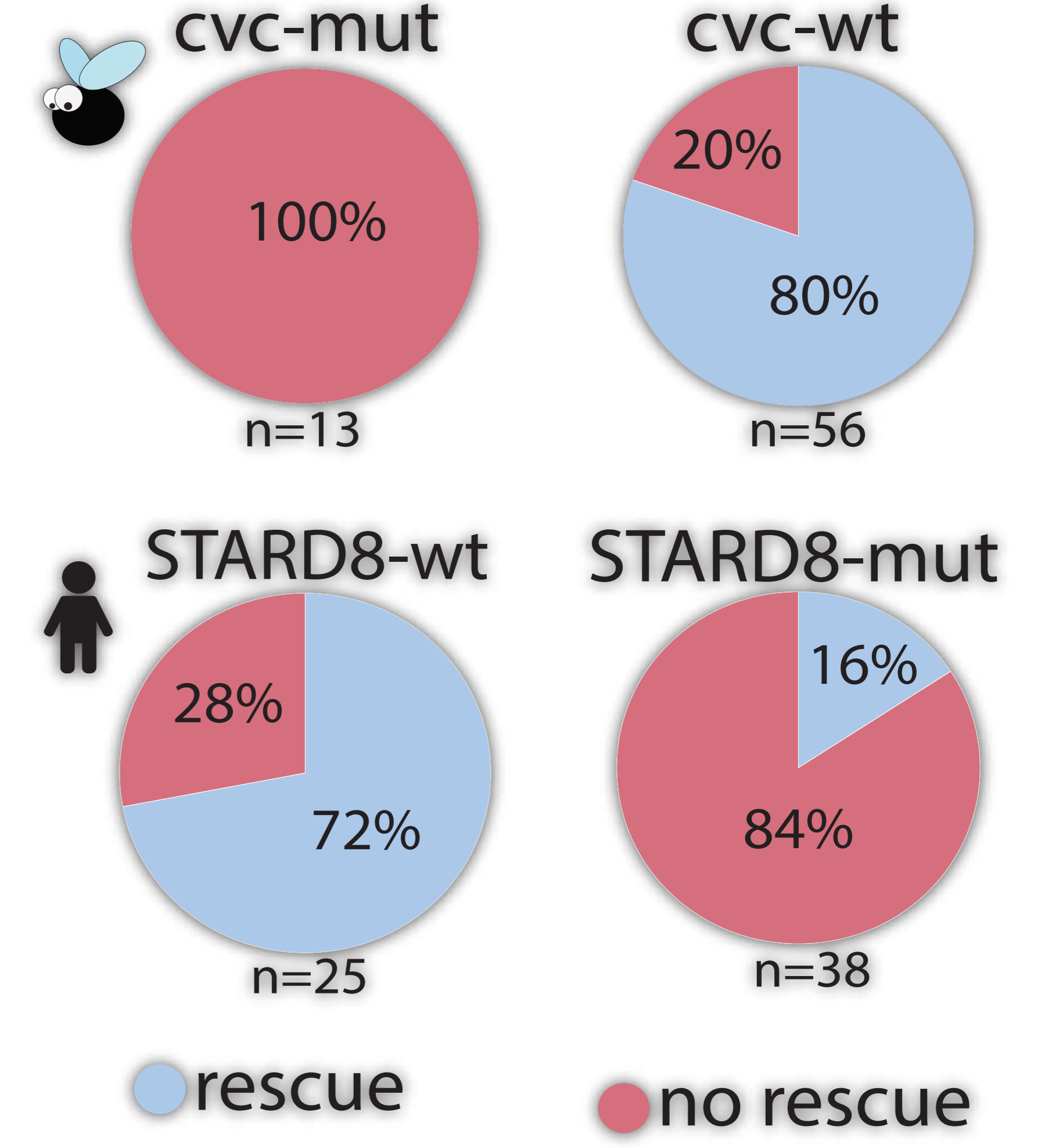


Figure 4

## Outlook

The immediate impact of the work will be provision of unconventional means for stratifications of genome-wide analysis and the identification of networks and biomarkers. This approach will be a more rapid screening of possible candidates for DSD and limit the usage of other animal models that usually pose more profound ethical issues and need more complex and expensive infrastructures.

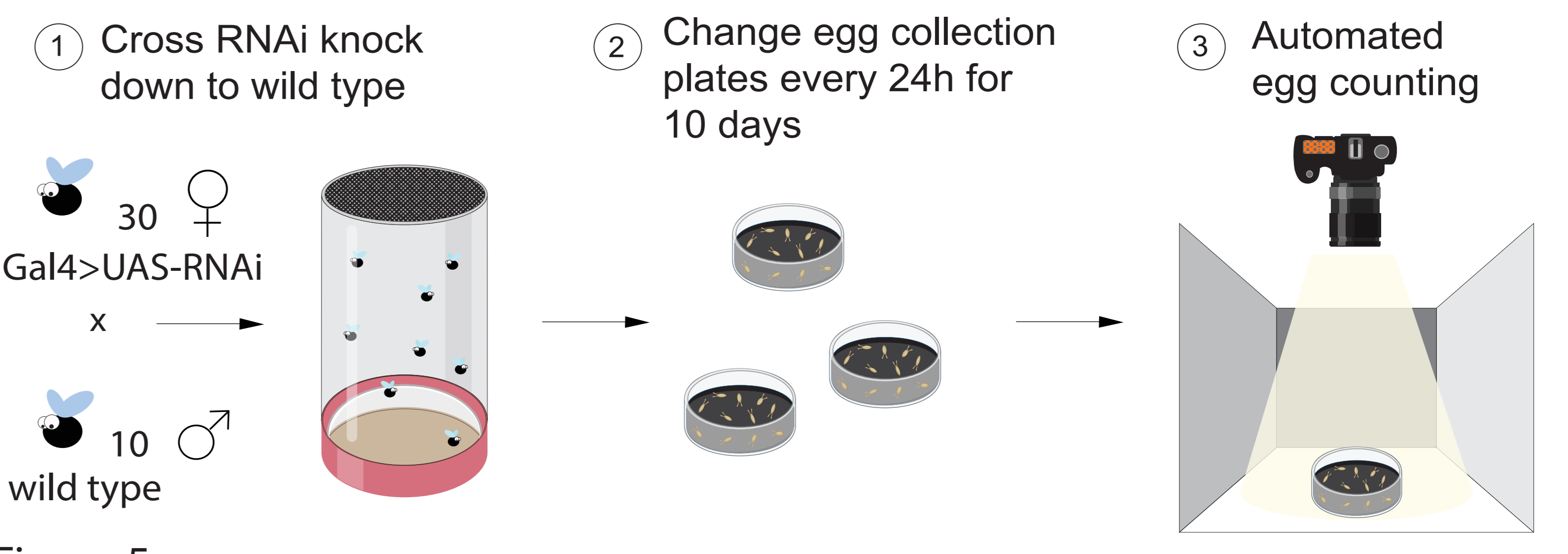


Figure 5

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