

UPDATE: KK RNAi lines landing site – Jan. 14, 2016

The Vienna Drosophila Resource Center (VDRC) would like to inform you of a recent publication regarding the KK RNAi library and a new stock for control experiments.

Background information

During construction of the KK RNAi library, the hairpin constructs were targeted to a single genomic location. [Green et al \(2014\)](#) have previously shown that there are 2 potential genomic landing sites for the KK RNAi constructs: at 40D (the originally intended VIE-260B landing site, in the 5'UTR of the gene *tiptop*) and at 30B (the actual site). In 75% of the KK lines, the insertion is in the 30B landing site only and there are no problems. In ~25% of KK lines there is an additional insertion of the RNAi construct at the 40D landing site and this causes non-specific phenotypes when crossed to some Gal4 drivers.

New publication

A new publication investigating this issue further, from the lab of Kieran Harvey ([Vissers et al, 2016](#)), confirms that approximately 25% of VDRC KK RNAi lines (38 out of 150) can cause false-positive phenotypes. Whilst working on the hippo pathway and performing screens in the eye, Vissers *et al* noticed phenotypes which were due to the insertion site and not due to expression of the RNAi hairpin. They were able to attribute the false-positive phenotypes to enhancement of the Hippo pathway, owing to ectopic UAS-driven expression of the Tiptop (*tio*) transcription factor - a consequence of the 40D landing site being located in the *tio* 5' UTR.

New line for KK library control experiments

Vissers *et al* have separated the insertions in the 2 landing sites by meiotic recombination and created a line harbouring Gal4-responsive UAS repeats but no functional RNAi coding sequence at 40D and no transgene insertion at 30B (called *40D^{UAS}*). This line (available from VDRC soon) will enable you to test up-front, in a single cross, whether your screen of interest will be affected by ectopic *tio* expression.

We would like to emphasise that ~75% of KK lines (insertion at 30D only) are absolutely fine and that the remaining 25% (insertion at 40D) will only be a problem in cases where an assay is affected by misexpression of the *tio* gene.

If you are planning to use KK lines and are concerned that you will see non-specific phenotypes, we suggest that you cross your Gal4 driver of interest to the *40D^{UAS}* line and check whether the F1 progeny demonstrate a dominant phenotype. If your assay is not affected by *tio*

misexpression, you can use all 9,822 KK lines with confidence, but if you see an effect, then 25% of the KK lines will give misleading results in that particular screen.

Validation of KK lines

The VDRC have shipped more than 400,000 KK lines to the *Drosophila* research community. We would like to reassure researchers that use of KK lines has resulted in several hundred publications in which the KK line phenotypes have been validated with independent RNAi lines targeting a different region of the same gene and with other insertion sites. These publications include some large scale screens, e.g.:

Czech B *et al* (2013). A transcriptome-wide RNAi screen in the *Drosophila* ovary reveals factors of the germline piRNA pathway. *Mol Cell*. 2013 Jun 6;50(5):749-61. [[PubMed](#)].

Berns N *et al* (2014). A genome-scale in vivo RNAi analysis of epithelial development in *Drosophila* identifies new proliferation domains outside of the stem cell niche. *J Cell Sci*. 2014 Jun 15;127(Pt 12):2736-48. [[PubMed](#)].

Homem CC *et al* (2014). Ecdysone and mediator change energy metabolism to terminate proliferation in *Drosophila* neural stem cells. *Cell*. 2014 Aug 14;158(4):874-88. [[PubMed](#)].

Verify your results!

As is recommended for results obtained using any RNAi lines, we advise verifying your observed KK phenotype with an alternative RNAi line containing an independent RNAi hairpin construct targeting the same gene and a different genomic insertion site where possible.

Please note that the GD library was generated using P-element insertion and therefore the 40D landing site issue pertains to 25% KK lines only and does NOT affect GD lines.