**Notes on the original *Hto* lines versus the new, recommended *Hto* *Starter* chromosome.**

After the introduction of the MiMIC system, we remodeled the original *Hto-WP* vector by adding flanking *attP* sites, so that it would be compatible with RMCE plasmids that are designed by the community for MiMIC (below).

We also modified the *Minos* right inverted repeat (IR). This region contains two natural polyA sites for *Minos* transposase, and thus a fraction of all transcripts proceeding through *Minos* will be truncated there. The truncated transcripts can express an unfused mCherry RFP that is uniformly distributed in the cytoplasm with a slightly higher concentration in the nucleus. The ratio of unfused RFP to the intended *Hto* fusion protein varies among the different inserts.

To address this, we mutated both polyA sites, changing AATAAA to AACAAA in each case. This greatly reduced the appearance of unfused RFP as judged by Western blot and by microscopy.

-------------

New version of *Hto* with cassette exchange and improved read-through:

****

--------------

The new construct was hopped, and two inserts on the X chromosome (*RENX05* and *RENX20*) were recovered that do not cause eye or wing phenotypes upon GAL4 expression. (***REN*** = **R**FP-positive, **e**yes **n**ormal with *GMR*). The two inserts were recombined to yield the *Starter* chromosome (*RENX05*, *X20*). This *Starter* was tested in a phenotypic screen, and yields new hops to autosomes at a rate of 21% after one heat shock induction of the standard Minos transposase construct.

*RENX05* lies at *X*: 7,076,138 in the minus orientation, within *CG9650*.

*RENX20* insertion site was not yet identified. We see roughly 14% recombination between *X05* and *X20*.

**Nomenclature:**

We named the original version of *Hto* as *Mi*[*Hto*-***WP***] for "**W**ild type **P**olyA". This is used in the GenBank record JN049642 and the Genetics paper Singari et al. 2014. This is the version present in all the lines submitted May 2014 (apart from the new *Starter* element).

For simplicity, the new version (with the attP sites and mutated PolyA signals) will just be called *Mi*[*Hto*], since we do not intend to make any more modifications.