The use of CRISPR/Cas9 scarless system to generate *Drosophila melanogaster* line with point mutations

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Aim:

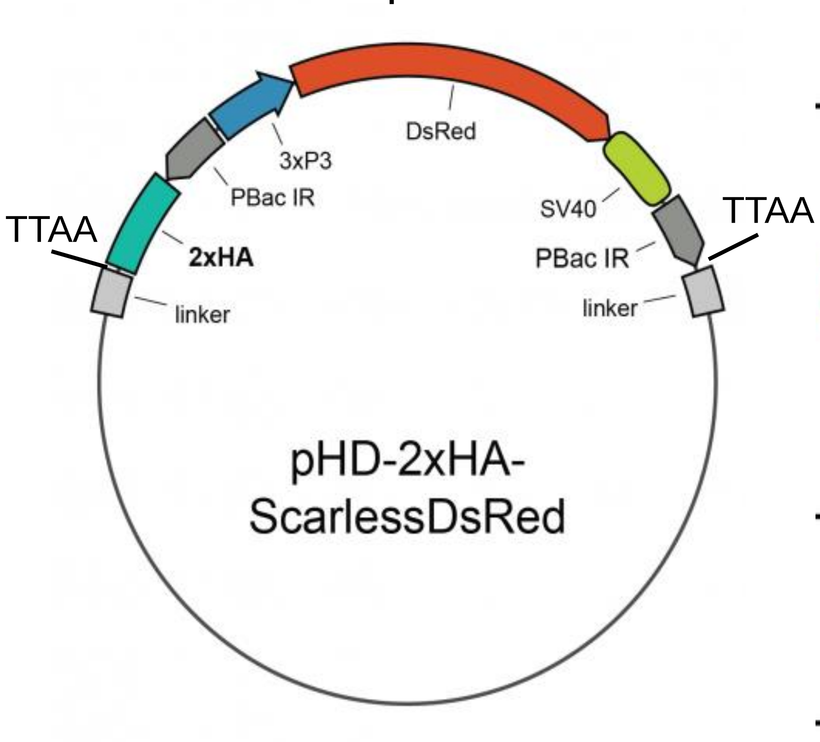
To obtain *Drosophila melanogaster* line with a single nucleotide substitution in the *orb* gene, leading to the appearance of a stop codon and synthesis of the truncated protein.

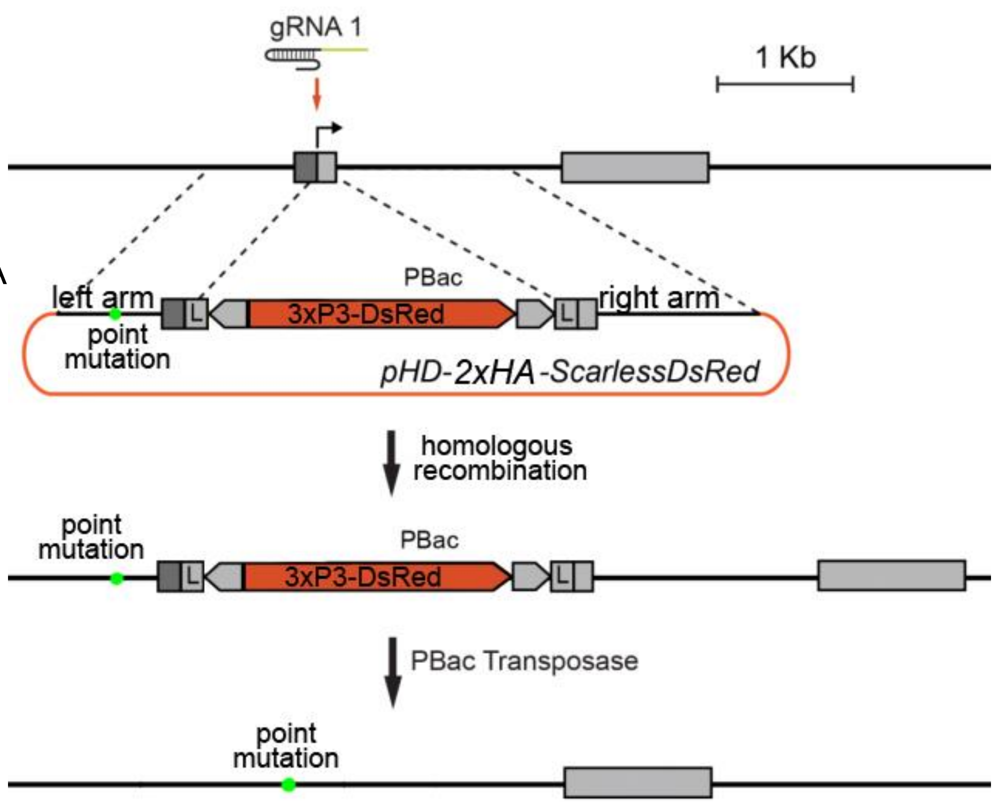
Materials and methods:

The CRISPR/Cas9 scarless system is based on the traceless excision of selective markers using piggyBac (PBac) transposase. PBac transposons target TTAA sites for insertion. TTAA sites are duplicated during transposon insertion and remain in a single copy after transposon excision by PB transposase.

Scheme of CRISPR/Cas9 genome editing

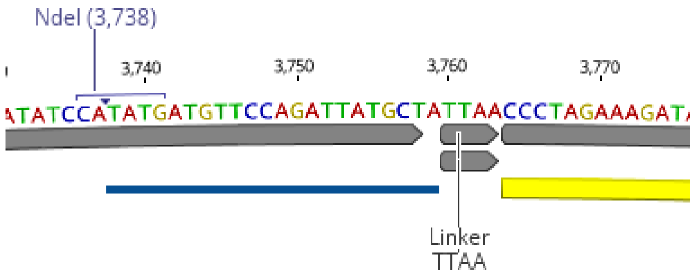
Donor plasmid

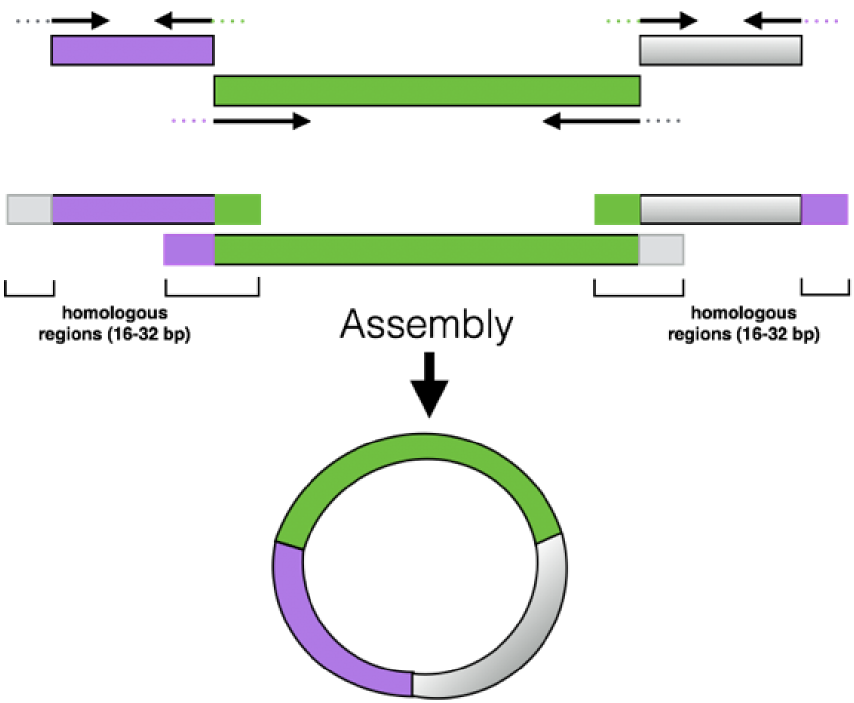




Donor plasmid design

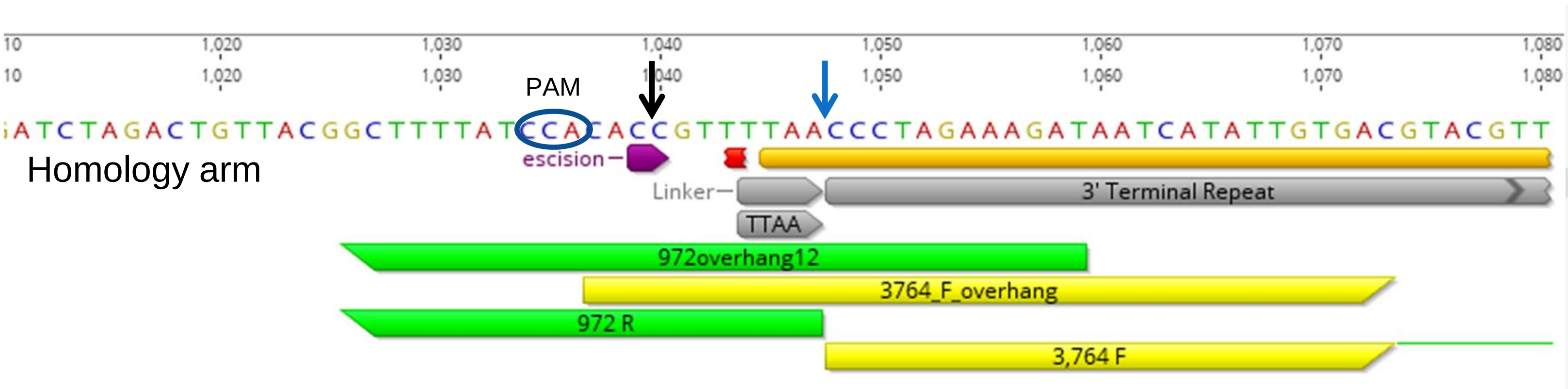
If we insert homology arms in the donor plasmid using restriction sites, a sequence between the restriction site and TTAA will be inserted in the genome





We used a stitching PCR to fuse homology arms with the selective marker, then inserted this construction in the donor plasmid

Guide RNA and donor plasmid design



Homology arms are bordered by TTAA sequence, not by the excision site.
gRNA is not complementary to donor plasmid (to prevent cleavage by Cas9), because cleavage site is close to the border of a homology arm (3' terminal repeat begins).

Cas9 cleavage site was 252 nt away from the point mutation. Homology arm with the point mutation was obtained using site-directed mutagenesis.

Excision of the selective marker DsRed

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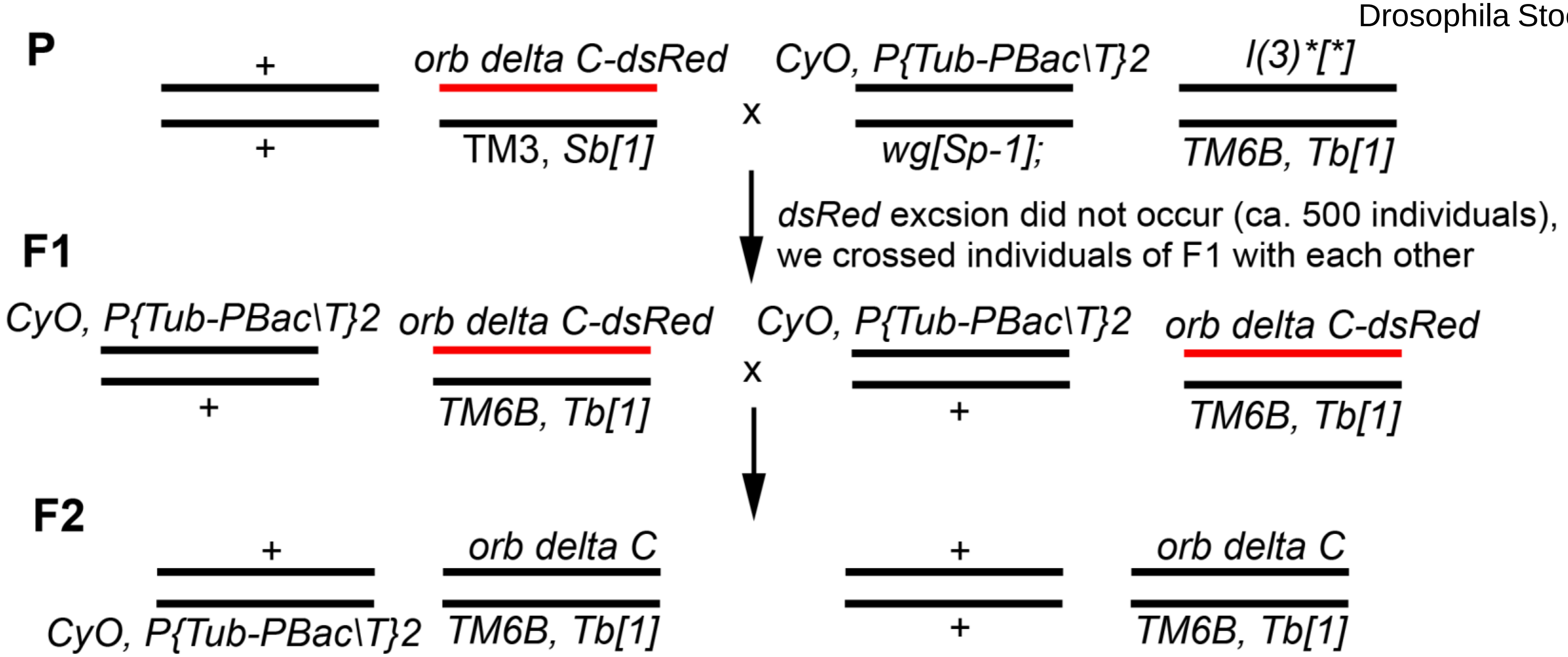

Tub-Pbac: expresses piggyBac transposase driven by the *alphaTub84B* promoter.

Conclusions:

The most problematic stage of the scarless CRISPR/Cas9 genome editing was excision of the selective marker, which was not very efficient in our case.

We used stitching PCR to connect homologous arms with a selective marker to avoid insertion of additional nucleotides.

The approach can be used not only to obtain lines with point mutations in other genes, but also for other types of mutations, such as deletions and insertions.

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