

Table S6. List of Nested CRISPR Universal Sequences

Reagent	Sequence (5' to 3')
EGFP Step 2 crRNA	CGTCGAGCTCGACGGAGTCA
mCherry Step 2 crRNA	GTTTCATGCGTTTCAAGGCCG
wrmScarlet Step 2 crRNA	CATGGAGGGATCCATGACCG
2xTY1::EGFP::3xFLAG Step 2 crRNA	CACAAACCAAGATCCACTCG
2xTY1::EGFP::3xFLAG Step 2 crRNA^a	TCTCCATCGTGGTCTTTGAG
EGFP 1-3 block	TCCAAGGGAGAGGAGCTCTTCACCGAGTCGTCCCAATCCTCGT CGAGCTCGACGGAGTCA AGG AGTTCGTACCGCTGCCGGAATC ACCCACGGAATGGACGAGCTCTACAAG
mCherry 1-3 block	TCCAAGGGAGAGGAGGACAACATGGCCATCATCAAGGAGTTCA TGCGTTTCAAGGCCG AGG GACGTCCTCCACCGAGGAATGGA CGAGCTCTACAAG
wrmScarlet 1-3 block	GTCAGCAAGGGAGAGGCAGTTATCAAGGAGTTCATGCGTTTCA AGGTCCACATGGAGGGATCCATGACCG AGG GACGTCCTCCAC CGGAGGAATGGACGAGCTCTACAAG
2xTY1::3xFLAG block	GAAGTGCATACCAATCAGGACCCGCTGGATGAAGTCCACACAA ACCAAGATCCACTCG AGG AGACTATAAAGATCATGACATTGACT ACAAGGATGACGACGACAAG
2xTY1::3xFLAG block^a	GAAGTGCATACCAATCAGGACCCGCTGGATGAAGTCCACACAA ACCAAGAT CC ACTCAAAGACCACGATGGAGACTATAAAGATCA TGACATTGACTACAAGGATGACGACGACAAG
Fwd primer to amplify EGFP 1-2-3	CCAAGGGAGAGGAGCTCTTCA
Rev primer to amplify EGFP 1-2-3	CTTGTAGAGCTCGTCCATTC
Fwd primer to amplify mCherry 1-2-3	TCCAAGGGAGAGGAGGACAA
Rev primer to amplify mCherry 1-2-3	CTTGTAGAGCTCGTCCATTC
Fwd primer to amplify mScarlet 1-2-3	GTCAGCAAGGGAGAGGCAGTTAT

Rev primer to amplify mScarlet 1-2-3	CTTGTAGAGCTCGTCCATTCCT
Fwd primer to amplify 2xTY1::EGFP::3xFLAG	ATACCAATCAGGACCCGCTG
Rev primer to amplify 2xTY1::EGFP::3xFLAG	TGTCGTCGTCATCCTGTAGT
Internal Fwd primer to genotype EGFP Step 2	CATGCCAGAGGGATACGTCC
Internal Rev primer to genotype mCherry Step 2	TTGGGTTCCCTCGTATGGAC
<i>dpy-10</i> co-CRISPR crRNA	GCTACCATAGGCACCACGAG

Nucleotides in bold represent PAM sequences. Two designs are provided for the 2xTY1::EGFP::3xFLAG construct and step 2 crRNA: the ones used in this study and a hypothetical improved design based on *in silico* predictions. ^aThese are the recommended designs predicted to have a more efficient guide sequence.