

Table S5. List of ssODNs used for Nested CRISPR Step 1

Allele	ssODN repair template (5' to 3')
<i>gtbp-1::EGFP 1-3</i> (sense)	CGGTTCCGGGTGGTGTCTCCACGAGGTGGTATGCGCTCCAAGGGAGAGGAGC TCTTACC CGGAGTCGTCCCAATCCTCGTCGAGCTCGACGGAGTCAAGGAGT TCGTCACCGCTGCCGGAATCACCCACGGAATGGACGAGCTCTACAAGAGC GGTTTCCAAAATGCGGGACAAAAT TAG AAG
<i>pgl-1::EGFP 1-3</i> (sense)	CGTGGACGTGGTGGTTACGGGGGTCGTGGTGGACGIGGCGGCTT TCCAAG GGAGAGGAGCTCTTACC CGGAGTCGTCCCAATCCTCGTCGAGCTCGACGGA GTCAAGGAGTTCGTACCGCTGCCGGAATCACCCACGGAATGGACGAGCT CTACAAG TAA ACTCCAATATTGAATGTTTAATTGTTTTTAAG
<i>prpf-4::EGFP 1-3</i> (antisense)	TTAGAGATCACGAAAAAATTTGGGAAATGT TTA CTTGTAGAGCTCGTCC ATTCCGTGGGTGATTCCGGCAGCGGTGACGAACCTCTTGAATCCGTCGAGC TCGACGAGGATTGGGACGACTCCGGTGAAGAGCTCCTCTCCCTTGGACTTG ATAGGTATGGTGAAGAATGGGTGTTTGAGAGCCT
<i>ubh-4::EGFP1-3</i> (sense)	AAGAAAAATCCAAGCTGAATACAGACATAACCAAGTCCAAGGGAGAGGAG CTCTTACC CGGAGTCGTCCCAATCCTCGTCGAGCTCGACGGAGTCAAGGAG TTCGTACCGCTGCCGGAATCACCCACGGAATGGACGAGCTCTACAAGCTT GAATTGAAGAGAAAAACAAT TAG ATATTGCATTCT
EGFP 1-3::<i>nfki-1</i> (sense)	GTTTTCCAAAATTACGTCGTTTGTTCAGCCATGTCCAAGGGAGAGGAGCT CTTACC CGGAGTCGTCCCAATCCTCGTCGAGCTCGACGGAGTCAAGGAGTT CGTACCGCTGCCGGAATCACCCACGGAATGGACGAGCTCTACAAGGCAA CCGTTGCCCCCAAGGAAAATGCCTTGTGCT
<i>prpf-4::</i> 2xTY1::EGFP::3xFLAG (antisense)	TTA GAGATCACGAAAAAATTTGGGAAATGT TTA CTTGTCTCGTCATCCT TGTAGTCAATGTCATGATCTTTATAGTCTCT TC CGAGTGGATCTTGGTTGTGT GGACTTCATCCAGCGGGTCTGATTGGTATGC ACTTC CTTGATAGGTATGGT GAAGAATGGGTGTTTGAGAGCCT
<i>gtbp-1::mCherry 1-3</i> (sense)	CGGTTCCGGGTGGTGTCTCCACGAGGTGGTATGCGCTCCAAGGGAGAGGAGG ACAACATGGCCATCATCAAGGAGTTCATGCGTTTCAAGGCCGAGGGACGTC ACTCCACCGGAGGAATGGACGAGCTCTACAAGAGCGGTTTCCAAAATGCG GGACAAAAT TAG AAG
<i>pgl-1::mCherry 1-3</i> (sense)	CGTGGACGTGGTGGTTACGGGGGTCGTGGTGGACGIGGCGGCTT TCCAAG GGAGAGGAGGACAACATGGCCATCATCAAGGAGTTCATGCGTTTCAAGGC CGAGGGACGTCACTCCACCGGAGGAATGGACGAGCTCTACAAG TAA ACTC CAACTATTGAATGTTTAATTGTTTTTAAG
<i>prpf-4::mCherry 1-3</i> (antisense)	TTAGAGATCACGAAAAAATTTGGGAAATGT TTA CTTGTAGAGCTCGTCC ATTCTCCGGTGGAGTGACGTCCCTCGGCCTTGAAACGCATGAATCCTTGA TGATGGCCATGTTGTCTCTCTCCCTTGGACTTGATAGGTATGGTGAAGAA TGGGTGTTTGAGAGCCT
mCherry 1-3::<i>sftb-1</i> (sense)	CTTCCATATAATCAATATTGATTGTAGATCGATGTCCAAGGGAGAGGAGGA CAACATGGCCATCATCAAGGAGTTCATGCGTTTCAAGGCCGAGGGACGTCA CTCCACCGGAGGAATGGACGAGCTCTACAAGTCAAGATCTGGCGAGGCTTA TGCGCAGGAGTTGAACCGCAAAAG
K12C11.3p::mCherry 1-3 (sense)	GCCTGACTATACTTATTTTTATGAGCAGAAAATGTCCAAGGGAGAGGAGG ACAACATGGCCATCATCAAGGAGTTCATGCGTTTCAAGGCCGAGGGACGTC ACTCCACCGGAGGAATGGACGAGCTCTACAAG TGA ATCGATCGATAATGTT TATATAATTTTTGTTT
F27C1.2::wrmScarlet 1-3	TGATGGAGAAGTGACGGATTGTTCCAAACCGATGCTTGCCAGCTCAGCAA GGGAGAGGCAGTTATCAAGGAGTTCATGCGTTTCAAGGTCCACATGGAGG GATCCATGACCGAGGGACGTCACTCCACCGGAGGAATGGACGAGCTCTAC AAG TAA TAATTGCGATCTCCAATCTCAATCCTCAAACA

Green, red, and dark red nucleotides represent EGFP 1-3, mCherry 1-3, and wrmScarlet 1-3 sequences, respectively; whereas purple and orange nucleotides represent FLAG and TY1 fragments, respectively. Nucleotides in blue and bold represent start and stop codons, respectively; and underscored nucleotides represent silent mutations.