

## A. pHsp70-Cas9

DmHsp70 promoter and 3'UTR

3x Flag

NLS

Cas9

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ATCCCCCTAGAATCCCAAACAAACTGGTTATTGTGGTAGGTCATTTGTTTGGCAGAAAGAAAA
CTCGAGAAATTTCTCTGGCCGTTATTCGTTATTCTCTCTTTTTCTTTTTGGGTCTCTCCCTCTCT
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AGAGAACTCTGGGGGATCTGATCGATATGGACTATAAGGACCACGACGGAGACTACAAGGATCA
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GAGGCCACCCGGCTGAAGAGAACC GCCAGAAGAAGATACACCAGACGGAAGAACC GGATCTGCT
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GACAGCCTGACCTTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGC  
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GAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTTCGAGCTGGAAAACGGCCGGAAG  
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GGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACC  
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GATGATTTCTTTAGCTAGTAATACATTTTAATTATATTCGGCTTTGATGATTTTCTGATTTTTT  
CCGAACGGATTTTCGTAGACCCTTTCGATCTCATAATGGCTCATTTTTATTGCGATGGACGGTCA  
GGAGAGCTCCACTTTTGAATTTCTGTTTCGACAGACACCGCATTTGTAGCACATAGCCGGGACATC  
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CGCTCTATATCCTCCGAACGGCGCTGGTTGACCCTAGCATTACATAAGGATCAGCAGCAAAAT  
TTGCCTCTACTTCATTGCCCGGAATCACAGCAATCAGATGTCCCTTTTCGGTTACGATGGATATT  
CAGGTGCGAACCGCACACAAAGCTCTCGCCGCACACTCCACACTGATATGGTTCGCTCGCCCGTG  
TGCGCGCCGATATGGATCTTAAGGTCGTTGGACTGCACAAAGCTCTTGCTGCACATTTTGCAGG  
AGTACGGCCTTTGACCCGTGTGCAATCGCATGTGTGCGCCAGCTTGTTCTGCGAAATAAACTA  
ACGGGAATTCCTGCAGCCCCGGGGATCCGCGGCCGC

## B. chiRNA expression vector pU6-BbsI-chiRNA

Dm snRNA:U6:96Ab promoter

BbsI sites for inserting guide sequence

crRNA repeat-derived sequence

tracrRNA

U6 terminator

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GTTTCGACTTGCAGCCTGAAATACGGCACGAGTAGGAAAAGCCGAGTCAAATGCCGAATGCAGAG
TCTCATTACAGCACAATCAACTCAAGAAAACTCGACACTTTTTTACCATTTGCACTTAAATCC
TTTTTTATTTCGTTATGTATACTTTTTTTGGTCCCTAACCAAACAAAACCAAACCTCTCTTAGTC
GTGCCTCTATATTTAAAACCTATCAATTTATTATAGTCAATAAATCGAACTGTGTTTTCAACAAA
CGAACAATAGGACACTTTGATTCTAAAGGAAAATTTGAAAATCTTAAGCAGAGGGTTCTTAAGA
CCATTTGCCAATTCTTATAATTCTCAACTGCTCTTTCCTGATGTTGATCATTATATAGGTATG
TTTTCTCAATACTTCGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAAT
AAGGCTAGTCCGTTATCAACTTGAAAAAGTGGACCCGAGTCGGTGC
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## C. yellow ssODN

5' and 3' homology arms

PAM

Target sequence corresponding to the nt that remain after cleavage

attP

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GTTGTCTTTAAATATTCTTTACATCAATCGAGTGTGTGAGAATATACCCAAGTACCGTGTCTAC
GCCCCAACTGAGAGAACTCAAAGGTTACCCAGTTGGGGCACTACATCAGGTGGCTTATGCTG
TTCCCATAGATCGCCAACCTTTCGTTTTGTCTTCCATGATT
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**Figure S2** Sequences of CRISPR RNA/Cas9 constructs

Annotated sequences of (A) codon-optimized Cas9 (Cong *et al.* 2013) under the control of the *Drosophila* *hsp70* promoter and 3' UTR. (B) chiRNA expression vector for generating site-specific, U6-driven chiRNAs (based on Cong *et al.*, 2013), and (C) the ssODN donor used to replace *yellow* with attP.