

## **Efficient CRISPR/Cas9 Plasmids for Rapid and Versatile Genome Editing in *Drosophila***

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## ssODN donor sequences

The homology arms are in upper case, the central V5 sequence in lower case, and the stop codons underlined. The gRNA target sequences are in bold, and are followed by a PAM (NGG). Silent nucleotide changes were introduced in the standard V5 sequence to create a *HindIII* restriction site. Donor sequences were synthesized in the same orientation as the gRNA. For all cases presented here, the insertion of the V5 tag destroyed the gRNA target site so that V5-tagged chromosomes would not be recognized and cleaved again.

### *ham-V5*

GCAACATCAGCAACAACAACAGCAGCAGCGGCAACAACAACAACAGCTCCA**AGGCCATTACA**  
**ATAAGCTCT**ggtaagcctatacctaaccctcttcttggtctagatagcacg**TAGGAATCGTA**  
AGAGATTAGAGAGCTTGCTTTCCTCGTTGTAAATGATAAACTTACTTTAACTC

### *dpn-V5*

GCAATGAAATAAATTAACAATGTATAAACGTAACATAGAAGTGG**CCTGTGCGGTTGATCTA**c  
gtgctatctagaccaagaagaggggttaggtataggcttacc**CCAC**GGCCTCCAAGCGGAGCT  
CGACTTTTCGATGGCGCCAGCTAGGAATTTCTTGGACGAGGGC

### *m $\gamma$ -V5*

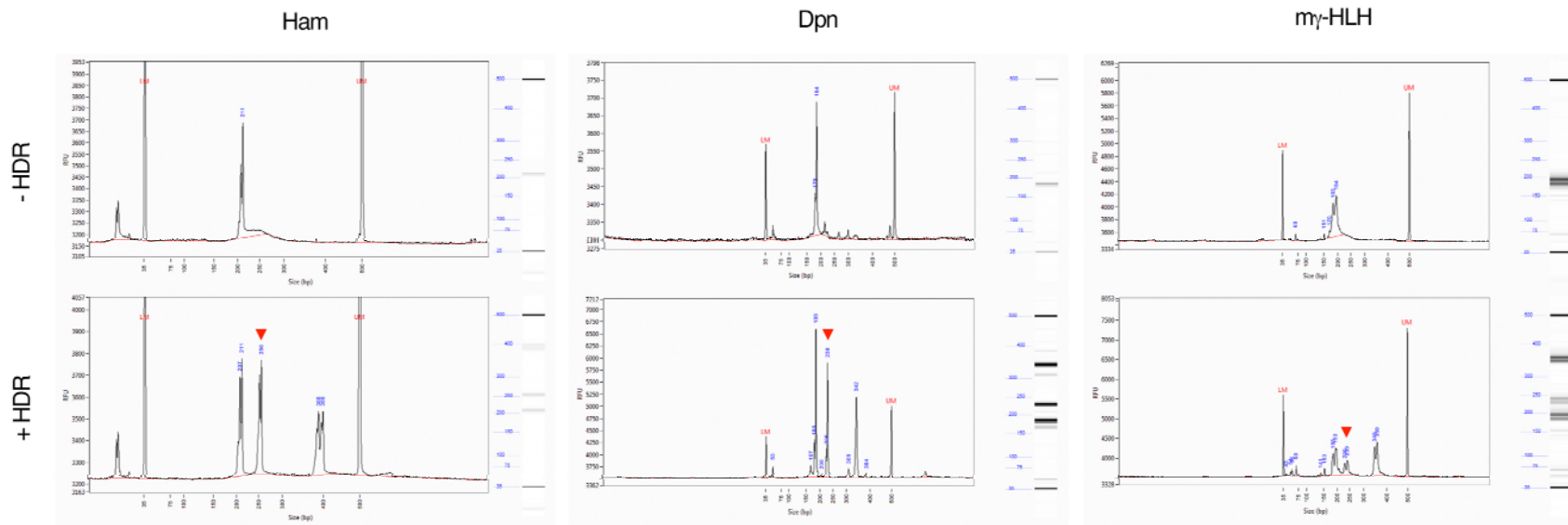
CTTTCTATGATCTCTCCTTCGTTTTTTTTTTTTTTCAGTTGTGGT**GTGAAGTAATCCTATCTA**  
cgtgctatctagaccaagaagaggggttaggtataggcttacc**CCAGGGACGCCAGACGTTCT**  
CCTCGTCCTCGCTGTCGTCCTCCATCTTGGTCACATCGATGGTGGTC

GCGCCCAATACGCAAACCGCCTCTCCCCGCGGTTGGCCGATTTCATTAATGCAGGCAACTCGTGAAAGGTAGGCG  
GATCAGCGGTTTCGACTTGCAGCCTGAAATACGGCACGAGTAGGAAAAGCCGAGTCAAATGCCGAATGCAGAGTCT  
CATTACAGCACAATCAACTCAAGAAAACTCGACACTTTTTTACCATTTGCACTTAAATCCTTTTTTATTTCGTTA  
TGTATACTTTTTTGGTCCCTAACCAAAACAAAACAACTCTCTTAGTCGTGCCTCTATATTTAAACTATCAA  
TTTTATTATAGTCAATAAATCGAACTGTGTTTTCAACAAACGAACAATAGGACACTTTGATTTTAAAGGAAATTTT  
GAAAATCTTAAGCAGAGGGTTCTTAAGACCATTTGCCAATTTCTTATAATTTCTCAACTGCTCTTTCTGATGTTGA  
TCATTTATATAGGTATGTTTTCTCAATACTTCggGTCTTCgaGAAGACctGTTTTAGAGCTAGAAAAGCAAGT  
TAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCCGGTGTCTTTTTGTTTTAGAGCTAGAAAT  
AGCAAGTTAAAATAAGGCTAGTCCGTTTTTAGCGCGTGCGCCAATTTCTGCAGACAAATGGCTCTAGAATCCCAAA  
ACAAACTGGTTATTGTGGTAGGTCATTTGTTTTGGCAGAAAGAAAACCTCGAGAAATTTCTCTGGCCGTTATTTCGTT  
ATTCTCTCTTTTTCTTTTTGGGTCTCTCCCTCTCTGCACATAATGCTCTCTCACTCTGTACACAGTAAACGGCATA  
CTGCTCTCGTTGGTTCGAGAGAGCGCGCCTCGAATGTTTCGCGAAAAGAGCGCCGGAGTATAAATAGAGGCGCTTC  
GTCTACGGAGCGACAATTC AATTC AAACAAGCAAAGTGAACACGTGCTAAGCGAAAGCTAAGCAAATAAACAAG  
CGCAGCTGAACAAGCTAAACAATCTGCAGCCAAGCTCGATAAGCTTGTTCGAATCTCGAGTGC GCGCTTCCGGAG  
GTATACACCTAGGCGGTACCCTGCAGTGAATTCGGAGCTCTACCGGTGCCACCATGGACTATAAGGACCACGCAC  
GGAGACTACAAGGATCATGATATTGATTACAAAGACGATGACGATAAGATGGCCCAAGAAGAAGCGGAAGGTC  
GGTATCCACGGAGTCCAGCAGCCGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGG  
GCCGTGATCACCGACGAGTACAAGGTGCCAGCAAGAAATTC AAGGTGCTGGGCAACACCGACCCGGCACAGCATC  
AAGAAGAACCTGATCGGAGCCCTGCTGTTTCGACAGCGGCGAAAACAGCCGAGGCCACCCGGCTGAAGAGAACC GCC  
AGAAGAAGATACACCAGACGGAAGAACC GGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCC AAGGTG  
GACGACAGCTTCTTCCACAGACTGGAAGAGTCTTCTGTTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATC  
TTCGGCAACATCGTGGACGAGGTGGCCTACCAGAGAAGTACCCACCATCTACCACCTGAGAAAAGAACTGGTG  
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CAGCTGTTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCGCAAGGCCATCTGTCTGCCAGACTGAGCAAG  
AGCAGACGGCTGGAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCGGAACCTGATTGCC  
CTGAGCCTGGGCTGACCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAG  
GACACCTACGACGACGACCTGGACAACCTGTGGCCAGATCGGCGACAGTACGCCGACCTGTTTCTGGCCGCC  
AAGAACCTGTCCGACGCCATCTGCTGAGCGACATCTGAGAGTGAACACCGAGATCACC AAGGCCCTCTGAGC  
GCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAAGCTCTCGTGC GGCAGCAGCTG  
CCTGAGAAGTACAAGAGATTTTTCTTCGACCAGAGCAAGAACGGCTACGCGGCTACATTTGACGGCGGAGCCAGC  
CAGGAAGAGTTTACAAGTTTCAAGCCCATCTGGAAAAGATGGACGGCACCGAGGAAGTCTCGTGAAGCTG  
AACAGAGAGGACTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAG  
CTGACGCCATCTGCGGCGG CAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGAAAAGATCGAGAAGATC  
CTGACCTTCCGCATCCCCTACTACTAGTGGGCCCTTGGCCAGGGGAAAACAGCAGATTCGCCTGGATGACCAGAAAG  
AGCGAGGAAACCATCACCCCTGGAACCTTCAGGAAAGTGGTGGACAAGGGCGCTTCCGCCAGAGCTTTCATCGAG  
CGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTC  
ACCGTGTATAACGAGCTGACCAAGTGAAATACGTGACCGAGGGAATGAGAAAAGCCCGCTTCTGAGCGGCGAG  
CAGAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAAGTGACCGTGAAGCAGCTGAAAAGAGGACTAC  
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GACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAAC  
GGCATCCGGGACAAGCAGTCCGGCAAGACAATCTGGATTTCTGAAAGTCCGACGGCTTCGCCAACAGAAACTTC  
ATGCAGCTGATACGACGACGACGCTGACCTTTAAAGAGGACATCCAGAAAAGCCAGGTGTCCGGCCAGGGCGAT  
AGCCTGCACGACACATCTGCCAATCTGGCCGGCAGCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTG  
TGGACGAGCTCGTGAAGTGTATGGCCGGCACAAGCCGAGAACATCGTGATCGAAATGGCCAGAGAAACCCAG  
ACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGC  
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CAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGCAAGAGCGAC  
AACGTGCCCTCGAAGAGGTCTGGAAGAAGTGAAGAACACTAGCCGCGCAGCTGTGAACGCCAAGCTGATTACC  
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CGGAAGATGATCGCAAGAGCGAGCAGGAAATCGCAAGGCTACCCCAAGTACTTCTTACAGACAACATCATG  
AACTTTTTCAAGACCGAGATTACCTTGGCCAACCGCGAGATCCGGAAGCGGCTCTGATCGAGACAAAACGGCGAA  
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CTGATCGCCAGAAAAGGACTGGGACCCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTG  
CTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCAAGAAACTGAAGAGTGTGAAAAGAGCTGCTGGGGATCACCATC  
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ATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCC  
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GACCCCAAGGACTTTCCCTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGC  
TTTGCTATTTTACACCACAAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATATCTGTAAACCTTT  
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CCTCCCCCTGAACTGAAACATAAAAATGAATGCAATTTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTA  
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TCGTAAGTGTTTTTCCCGAGGCCAGTGTCTTTAGCGTTATTGAAAAAGGAAGAGTATGAGTATTCACACATTTCCG  
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TGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTT  
TCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTAAAGTTCTGCTATGTGGCGCGGTATATCCCGTATTGA  
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AAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGTCTGCCATAACCATGAGTGATAACACTGCGGC  
CAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTGCACAACATGGGGGATCATGTAAC  
TCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAGATGCCTGTAGC  
AATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAAGTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTG  
GATGGAGGCGGATAAAGTTGAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGATAAAATC  
TGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGT  
TATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCTCACTGAT  
TAAGCATTGGTAACTGTGACACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTTAATTTAA  
AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAAATCCCTTAAAGTGGTGTTCGTTCCACTGAGC  
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AAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGCCTGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGG  
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AGCACCCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTAC  
CGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCC  
CAGCTTGGAGCGAACGACCTACACCGAAGTGAATACCTACAGCGTGAGCTATGAGAAAAGCGCCACGCTTCCCGA  
AGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTGGAAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG  
AAACGCCTGGTATCTTTATAGTCTGTGCGGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTTGTGATGCTCGTC  
AGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTTACGGTTCTTGGCCTTTTGTGCTGCTTTTGC  
TCACATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCAGCTTTTGTGAGTGGCTGATACCGC  
TCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAAGTGGAGCGGAAAGCGGAAGA

**Figure S1 Nucleotide sequence of pDCC6**

U6:96Ab promoter (83..483); sgRNA scaffold (502..577); hsp70Bb promoter (662..1004);  
3xFLAG (1105..1173); NLS (1174..1224); hSpCas9 (1225..5325); NLS (5326..5361); SV40 term  
(5414..6256); ampR (6353..7210)



**Figure S2** Detection of HDR-mediated V5-tag integration by high-resolution capillary electrophoresis.

PCR samples from heterozygous flies with integrated V5 tags (bottom row) contain an additional larger band/peak of predicted size (red arrowheads) that is not present in untagged chromosomes (top row).