

GW2T2 on Genome A

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AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAECTCGGCCCTGCTACCCGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
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GW2T2 on Genome B

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AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAECTCGGCCCTGCTACCCGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
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GW2T2 on Genome D

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AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAECTCGGCCCTGCTACCCGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
AGGGCTGTACGAGCACAGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAAGCTCGGCCCTGCTACCGGGGGCTGACGACGCCCGG;G
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LPX1T2 on Genome B

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CGCACCTACGTCGACACCACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCA-CCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGT--ACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGT--CACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--GACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
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LPX1T2 on Genome D

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CGCACCTACGTCGACACCACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCA-CCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGT--ACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--GACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--CACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACG--ACCCCGGCGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
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MLOT1 on Genome A

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TCTCGCTGCTGCTCGCCGTACAGCAGGACCCAATCTCCGGGATATGCATCTCCAGAAGG
TCTCGCTGCTGCTCGCCGTCA-GCAGGACCCAATCTCCGGGATATGCATCTCCAGAAGG
TCTCGCTGCTGCTCGCCGTCA--CGGACCCAATCTCCGGGATATGCATCTCCAGAAGG
TCTCGCTGCTGCTCGCCGT----GAACCCAATCTCCGGGATATGCATCTCCAGAAGG
TCTCGCTGCTGCTCGCCGTCA----CCCAATCTCCGGGATATGCATCTCCAGAAGG
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SUPPLEMENTARY FIG. S6. Comparison of the gene editing efficiency of single and multiplex gene editing CRISPR-Cas9 constructs using the protoplast transient expression assay. The mutations were detected by the NGS of the PCR amplicon libraries generated from the protoplast DNA. Representative reads with mutations in the target sites GW2T2, LPX1T1, and LPX1T2 induced by single gene editing construct pBUN421-GW2T2, pBUN421-LPX1T2, and pBUN421-MLOT1, respectively, are shown. The first rows correspond to the wild-type genomic sequences from the A, B, or D genomes. The targeted regions are shown within the red rectangles; the PAM sequences are underlined. The genome specific nucleotide bases are shown within the black rectangles.