

GW2T2 on Genome A

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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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GW2T2 on Genome B

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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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GW2T2 on Genome D

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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
AGGGCTGTACGAGCACATGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCCGGGGCTGACGACGCGCGG
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LPX1T2 on Genome B

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CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
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LPX1T2 on Genome D

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CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
CGCACCTACGTCGACACCAACCCCGGGGAGTTTCGACTCCTTCCAGGACATCATCAACCTC
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MLOT1 on Genome A

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TCTCGTGTGCTGCTCGCCGTCACAGGACCCCAATCTCCGGGATATGCATCTCCCAGAAGG
TCTCGTGTGCTGCTCGCCGTCACAGGACCCCAATCTCCGGGATATGCATCTCCCAGAAGG
TCTCGTGTGCTGCTCGCCGTCACAGGACCCCAATCTCCGGGATATGCATCTCCCAGAAGG
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SUPPLEMENTARY FIG. S5. 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 the MGE construct pBUN421-GLM 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.