

Supplementary File S1

Comparison of CRISPR-MAD7 and CRISPR-Cas9 for gene disruptions in *Komagataella phaffii*

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S1: *DsRed* nucleotide sequence (5' - 3')

ATGGTTTCTAAGGGTGAGGAAGTTATCAAGGAGTTCATGCGTTTCAAGGTCAGAATGGAAGGT
TCTATGAACGGTCATGAGTTCGAGATTGAAGGAGAAGGTGAAGGAAGACCATATGAGGGTACT
CAAACCGCAAAGTTGAAGGTTACTAAAGGAGGTCCTTACCATTTCGCTTGGGATATCCTGTCTC
CACAATTCATGTATGGTTCTAAGGCATACGTTAAGCATCCTGCAGACATTCCTGACTACAAGAA
GTTGTCCTTTCTGAGGGTTTCAAGTGGGAAAGAGTCATGAACTTCGAAGACGGTGGATTGGTG
ACTGTCACCTCAAGACTCTTCCCTTCAAGACGGTACTTTGATCTACAAGGTCAAGATGCGTGGTA
CCAACCTCCACCAGATGGTCTGTATGCAGAAAAAGACTATGGGATGGGAAGCTTCTACTGA
GAGATTGTATCCAAGAGATGGTGTGTTTGAAGGGTGAGATTCACCAAGCTTTGAAGCTTAAAGA
TGGAGGTCACACTTGGTTGAGTTCAAGACCATTTACATGGCTAAGAAACCAGTTCAACTTCCT
GGATACTATTACGTTGACACTAAGCTGGACATTACCTCTCACAACGAAGACTACACCATCGTTG
AGCAATACGAGAGATCCGAAGGTAGACACCACTTGTTCTTGACGGTATGGACGAGCTTTATAA
GTAA

S2: *Sh ble* nucleotide sequence (5' - 3')

ATGGCTAAACTCACCTCTGCTGTTCCAGTCCTGACTGCTCGTGATGTTGCTGGTGCTGTTGAGTT
CTGGACTGATAGACTCGGTTTCTCCCGTGACTTCGTAGAGGACGACTTTGCCGGTGTTGTACGT
GACGACGTTACCTGTTTCATCTCCGCAGTTCAGGACCAGGTTGTGCCAGACAACACTCTGGCAT
GGGTATGGGTTTCGTGGTCTGGACGAAGTACGCTGAGTGGTCTGAGGTCGTGTCTACCAACTT
CCGTGATGCATCTGGTCCAGCTATGACCGAGATCGGTGAACAGCCCTGGGGTCGTGAGTTTGCA
CTGCGTGATCCAGCTGGTAACTGCGTGCATTTTCGTGCGAGAAGAGCAGGACTAA

S3: sgRNA construct sequences (5' - 3')

With gRNA scaffold *E. coli* (CRISPR-MAD7 backbone):

AATTCCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCGGAATTTCTACTCTTGTAGAT
GCTAGCGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGC
GAATGGGAC

With gRNA scaffold Yeasts (CRISPR-MAD7 backbone):

CCAGACCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCGTCTGGCCCCAAATTCTAATT
TCTACTGTTGTAGATGCTAGCGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAAC
ATGCTTCGGCATGGCGAATGGGAC

With gRNA scaffold FAQ (CRISPR-MAD7 backbone):

TTTGACCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCGTCAAAAGACCTTTTAAATTT
CTACTCTTGTAGATGCTAGCGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACA
TGCTTCGGCATGGCGAATGGGAC

sgRNA cassette (CRISPR-Cas9 backbone):

AAAAAAGCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCAAAAAACGAGACGACGAGTA
GGTAGCTCAACTAGTTGTGAGGCTGACCGTCTCTGTTTTAGAGCTAGAAATAGCAAGTTAAA
ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTGGCCGGCATGGT
CCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCGAATGGGAC

Reverse complementary sequence, Hammerhead ribozyme, gRNA scaffold, a site for gRNA insertion, HDV ribozyme

S4: CRISPR vector backbones nucleotide sequences (5'-3')

CRISPR-MAD7 backbone vector:

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AGATCCAATTCCCGCTTTGACTGCCTGAAATCTCCATCGCCTACAATGATGACATTTGGATTG
GTTGACTCATGTTGGTATTGTGAAATAGACGCAGATCGGGAACACTGAAAAATACACAGTTATT
ATTCATTTAAATGACCCTTGTGACTGACACTTTGGGAGTCCCTATTCTACTTAGTCTCATATCG
CATGAAACTTTTGATAAATTATTTTCTGATAGGAATTTTTCATCAGATATTATCATCGCGGCTT
ACGTAATAACAAAAAAATTGATGGAGTCTATACTAGGCTAACATAAACTAAGTTATTAATTA
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 TTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGGTTTCGCCACCTCTGACTTGAGCG
 TCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCTTT
 TTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGGTACCC

CRISPR-Cas9 backbone vector:

TCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGCAGGCTTCATTTTTGATACTTTTTTAT
 TTGTAACCTATATAGTATAGGATTTTTTTTTGTGCTATTTTGTCTTCTCGTACGAGCTTGCTCCT
 GATCAGCCTATCTCGCAGCAGATGAATATCTTGTGGTAGGGGTTTGGGAAAATCATTCGAGTTT
 GATGTTTTTCTTGGTATTTCCCACTCCTCTTCAGAGTACAGAAGATTAAAGTGAGACCTTCGTTT
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S5: *MAD7* nucleotide sequences (5' - 3') without NLS:

PpMAD7:

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EcMAD7:

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HsMAD7 1:

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HsMAD7 2:

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AAATGGTGCGTATTGTATTGCATTAAGGGTTATATGAAATTAAACAAATTACCGAAAATTG
GAAAGAAGATGGTAAATTTTCGCGCGATAAACTCAAAATCAGCAATAAAGATTGGTTCGACTT
TATCCAGAATAAGCGCTATCTCTAA

HsMAD7 3:

ATGAACAATGGAACAAACAACTTCCAAAATTTTCATAGGAATATCCTCTCTGCAGAAAACATTGC
GGAATGCTCTCATCCCAACTGAGACAACCCAGCAGTTCATAGTCAAAAACGGGATCATCAAAGA
AGACGAACTGAGAGGCGAGAACCGCCAGATTTTGAAGGACATAATGGATGATTACTATCGGGG
GTTTATTTCCGAGACTTTGAGTAGCATCGACGATATCGACTGGACCTCCTTGTTTCGAGAAAATG
GAGATTGAGCTCAAGAATGGGGACAACAAAGATACCCTCATCAAGGAACAGACCGAATACAGGA

AGGCTATCCATAAAAAGTTTGCTAACGACGATCGCTTTAAGAACATGTTTGTAGTGCCAAACTGAT
TTCTGATATTCTGCCCCGAGTTTGTATTTCATAATAACAATTACTCTGCCAGTGAAAAAGAAGAG
AAGACTCAGGTTATTAAGCTGTTTTCTCGGTTCCGCGACTAGTTTCAAAGACTATTTTAAGAATA
GGGCTAATTGCTTCAGCGCCGATGACATCTCATCCAGCAGCTGCCATAGGATAGTCAACGACAA
TGCTGAAATTTTCTTCAGCAATGCCTTGGTCTACCGGAGAATCGTCAAGTCCCTGAGTAACGAC
GACATCAATAAAATCTCAGGCGATATGAAGGACTCATTGAAGGAAATGAGTCTCGAAGAAATC
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GTGGCAAAGTTAACTCATTCATGAATCTGTATTGCCAGAAGAACAAGGAGAACAAGAACCTGTA
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CTACATTGTTTCTAAATTCTACGAATCAGTCTCCCAAAAGACCTACAGAGACTGGGAAACCATT
AATACCGCCTTGAGATCCACTACAATAACATTCTCCAGGGAATGGTAAAAGCAAGGCCGACA
AGGTAAAGAAGGCAGTGAAAAATGATCTTCAGAAATCAATTACGGAAATCAATGAATTGGTGT
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CATCCTCAACAATTTTGAAGCTCAGGAGCTGAAGTATAACCCAGAGATTCACCTGGTGGAATCT
GAACCTAAGGCGTCCGAACCTGAAAAATGTGCTGGATGTGATCATGAACGCCTTCCATTGGTGCA
GTGTGTTTCATGACTGAGGAGCTGGTGGATAAAGACAATAATTTCTATGCTGAGCTGGAAGAGA
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GAAGGGGCAAGACAGATCGCTAGAAAAGAGTGGAAGAGATTGGAAAAATCAAAGAGATTAAG
GAGGGGTACCTGTCTCTCGTTATACACGAAATCAGCAAAATGGTCATAAAATACAACGCAATTA
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TCCGCATCAAACGACGCTTCGTGAATGGGCGCTTCAGTAATGAAAGCGACACCATTGACATTAC
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ATGCGTAACTCCTTGTCTGAACTGGAGGACCGTGATTACGATCGTCTCATTTACCTGTACTGA
ACGAAAATAACATTTTTTTATGACAGCGGAAAGCGGGGGATGCACTTCCTAAGGATGCCGATGC

AAATGGTGCGTATTGTATTGCATTAAGGGTTATATGAAATTAAACAAATTACCGAAAATTG
GAAAGAAGATGGTAAATTTTCGCGCGATAAACTCAAAATCAGCAATAAAGATTGGTTCGACTT
TATCCAGAATAAGCGCTATCTCTAA

HsMAD7 4:

ATGAACAACGGGCTAACAATTTTCAGAACTTTATCGGAATATCCTCCCTGCAGAAAACCTTA
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GGATGAGCTGAGAGGAGAAAACCGGCAGATACTCAAGGACATCATGGATGATTACTACAGAGG
CTTCATTTCTGAGACCCTGAGCAGTATAGATGATATTGACTGGACATCCCTTTTTGAAAAGATG
GAGATCCAGTTGAAAAATGGCGATAACAAGGACACCCTCATAAAGGAGCAGACAGAGTACCGA
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TCTCTGATATCCTCCCCGAGTTTGTTCATCCACAACAATTATAGCGCTAGTGAAGAGGAGGA
GAAGACACAGGTTATCAAGCTGTTTCAGTAGATTTGCAACATCTTTCAAAGACTACTTCAAAAT
CGGGCCAAGTCTTTTCAGCTGATGACATCAGTAGCAGTAGTTGTTCATCGCATTTGTGAATGATA
ACGCCGAGATATTTTTCAGTAATGCTCTGGTTTATCGGAGGATTGTCAAGAGCCTGTCTAACGA
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GATATTTTTCAGAAAGAGCAGCATCAAGAACCCCATCATCCATAAAAAGGGTAGTATTCTCGTA
AATAGAACCTACGAAGCTGAGGAAAAGGATCAATTCGGAAATATCCAAATCGTTAGGAAAAAT
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GGACTACAGGTACACTTATGACAAATACTTTCTGCATATGCCTATAACAATTAACCTCAAAGCC
AATAAGACCGGCTTCATAAATGATCGCATTTCTGCAATATATCGCTAAGGAGAAAGACCTGCACG
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 ACGTGGGCCACCAGTGTGGGTGCATCTTCTATGTGCCAGCTGCCTACACTTCTAAGATTGACCC
 ACAACCGGCTTTGTGAACATCTTTAAGTTTAAAGATCTTACCGTTGACGCCAAGCGCGAATTCA
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 GTTCGCATAAAACGCAGGTTTGTGAACGGACGCTTTTCTAATGAATCCGATACAATCGACATTA
 CGAAGGACATGGAGAAAACCCCTTGAGATGACAGACATCAACTGGCGGGACGGGCATGATCTTAG
 ACAGGATATCATCGACTATGAGATTGTGCAGCACATCTTTGAGATTTTCAGACTGACCGTGCAA
 ATGCGTAACTCCTTGTCTGAACTGGAGGACCGTGATTACGATCGTCTCATTTACCTGTACTGA
 ACGAAAATAACATTTTTTTATGACAGCGCGAAAGCGGGGGATGCACTTCCTAAGGATGCCGATGC
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 GAAAGAAGATGGTAAATTTTCGCGCGATAAACTCAAAATCAGCAATAAAGATTGGTTCGACTT
 TATCCAGAATAAGCGCTATCTCTAA

S6: Primers/ssDNA Oligonucleotides

Name	Sequence 5' - 3'
Primer 1	CGTTTCGGAATTCTGTTGTAGTT
Primer 2	CTCGAGACGGGAAGTCTTTAC
<i>OCH1</i> fwd	ATGGCGAAGGCAGATGGC
<i>OCH1</i> rev	TTAGTCCTTCCAACCTCCTTCAAATG
<i>PMT2</i> fwd	ATGACAGGCCGTGTCGACC
<i>PMT2</i> rev	CTATGGGTCTGATACTCTCCAACCTAGGTAG
<i>PMT2</i> sequencing primer	GATTCAATGCTGCTGCTATTTCACTG
<i>TP11</i> fwd	ATGGTACGTGCATTGAGTCTCATAAG
<i>TP11</i> rev	CTAGTTTCTGGAATTAATAATGTCCACGAATTC
<i>RFP</i> fwd	ATGGTTTCTAAGGGTGAGGAAGTTATC
<i>RFP</i> rev	TTACTTATAAAGCTCGTCCATACCGTAC
<i>GUT1</i> fwd	ATGGGAAAAGACTATACACCACTAG
<i>GUT1</i> rev	TTAAGCAGTGTCTTAAGCCAG
Sh blefwd	ATGGCTAAACTCACCTCTGCTGTTC
Sh blerev	TTAGTCCTGCTCTTCTGCGACGAAATG
gRNA <i>GUT1</i>	GTCGGAATTTCTACTCTTGTAGATCAACGGTATTGTGTGGAACGAGG CCGGCATGGTCCC
gRNA TTTA 1	GTCGGAATTTCTACTCTTGTAGATGTCACCATGGAGAACAAGAACGG CCGGCATGGTCCC
gRNA TTTA 2	GTCGGAATTTCTACTCTTGTAGATCCACTTGACTAACGAAAAATCGG CCGGCATGGTCCC
gRNA TTTG 1	GTCGGAATTTCTACTCTTGTAGATGTCCAAGAAGACAGGAAAGCCGG CCGGCATGGTCCC

gRNA TTTG 2	GTCGGAATTTCTACTCTTGTAGATGTACTATTGACTCTTGGTTGAGG CCGGCATGGTCCC
gRNA TTTC 1	GTCGGAATTTCTACTCTTGTAGATCTCAGCCAGGCTGGGTGAATGG CCGGCATGGTCCC
gRNA TTTC 2	GTCGGAATTTCTACTCTTGTAGATTGCAACCAAGTTCAGATGGTTGG CCGGCATGGTCCC
gRNA TTTT 1	GTCGGAATTTCTACTCTTGTAGATGACTACCACGGTCAGGAAGTGGG CCGGCATGGTCCC
gRNA TTTT 2	GTCGGAATTTCTACTCTTGTAGATGAAATTCTGGGACGTCGATACGG CCGGCATGGTCCC
gRNA CTTA 1	GTCGGAATTTCTACTCTTGTAGATCCTTACTGATGACGCCCTTGCGGC CGGCATGGTCCC
gRNA CTTA 2	GTCGGAATTTCTACTCTTGTAGATCTGATGACGCCCTTGCTCTTCGGC CGGCATGGTCCC
gRNA CTTG 1	GTCGGAATTTCTACTCTTGTAGATGAAGTTGAGTCCGTTGACAATGG CCGGCATGGTCCC
gRNA CTTG 2	GTCGGAATTTCTACTCTTGTAGATTGTGGTTGTCCAATCTCCACCGGC CGGCATGGTCCC
gRNA CTTC 1	GTCGGAATTTCTACTCTTGTAGATCGAAGGTATTTCCCTGACAGTGG CCGGCATGGTCCC
gRNA CTTC 2	GTCGGAATTTCTACTCTTGTAGATCAGAAATCAGATCTTCCGCAGGG CCGGCATGGTCCC
gRNA CTTT 1	GTCGGAATTTCTACTCTTGTAGATACAACGGTATTGTGTGGAACGGG CCGGCATGGTCCC
gRNA CTTT 2	GTCGGAATTTCTACTCTTGTAGATGGCTGGATGTCTGGGTGACCAGG CCGGCATGGTCCC
gRNA <i>RFP</i> 1	GTCGGAATTTCTACTCTTGTAGATAGTACCCTCATATGGTCTTCCGGC CGGCATGGTCCC
gRNA <i>RFP</i> 2	GTCGGAATTTCTACTCTTGTAGATAAGTGGGAAAGAGTCATGAACGG CCGGCATGGTCCC
gRNA <i>Sh ble1</i>	GTCGGAATTTCTACTCTTGTAGATTCCCGTGACTTCGTAGAGGACGG CCGGCATGGTCCC
gRNA <i>Sh ble2</i>	GTCGGAATTTCTACTCTTGTAGATCCGGTGTGTACGTGACGACGGG CCGGCATGGTCCC
gRNA <i>TPI1</i> 1	GTCGGAATTTCTACTCTTGTAGATAAGTTTCCTCCTACGAAAAATGG CCGGCATGGTCCC
gRNA <i>TPI1</i> 2	GTCGGAATTTCTACTCTTGTAGATCGTAGGAGGAACTTCAAATGG CCGGCATGGTCCC
gRNA <i>TPI1</i> 3	GTCGGAATTTCTACTCTTGTAGATTGCGGAGACGTAAACAGTCTTGG CCGGCATGGTCCC
gRNA <i>TPI</i> 4	GTCGGAATTTCTACTCTTGTAGATAAGGACTTGGGTGTCCCTTACGG CCGGCATGGTCCC
gRNA <i>OCH1</i> 1	GTCGGAATTTCTACTCTTGTAGATTACGGACCCTCACAACAATTAGG CCGGCATGGTCCC
gRNA <i>OCH1</i> 2	GTCGGAATTTCTACTCTTGTAGATATTTTCCTTACCGCAGTTACGGGC CGGCATGGTCCC
gRNA <i>OCH1</i> 3	GTCGGAATTTCTACTCTTGTAGATTGATACCCGATGATGCAGCATGG CCGGCATGGTCCC
gRNA <i>PMT2</i> 1	GTCGGAATTTCTACTCTTGTAGATAGGAAGAACCAGTCACTTCGAGG CCGGCATGGTCCC

gRNA <i>PMT2</i> 2	GTCGGAATTTCTACTCTTGTAGATGCTCCTACTATCTCAGACACGGGC CGGCATGGTCCC
gRNA <i>PMT2</i> 3	GTCGGAATTTCTACTCTTGTAGATATATCAAGACAACCTGGCTATGG CCGGCATGGTCCC
gRNA <i>PMT2</i> 4	GTCGGAATTTCTACTCTTGTAGATAGCCATTTCCACCATTTGCGAGGC CGGCATGGTCCC
gRNA <i>PMT2</i> 5	GTCGGAATTTCTACTCTTGTAGATTTCGTAAGAAATATGCTGCTCAGG CCGGCATGGTCCC
gRNA <i>PMT2</i> 6	GTCGGAATTTCTACTCTTGTAGATGGATCTGGTCTGTTACATTCCGG CCGGCATGGTCCC

S7: gRNAs

Name	Sequence 5' - 3'
<i>GUT1</i>	CAACGGTATTGTGTGGAACGA
TTTA 1	GTCACCATGGAGAACAAGAAC
TTTA 2	CCACTTGACTAACGAAAAATC
TTTT 1	GACTACCACGGTCAGGAAGTG
TTTT 2	GAAATTCTGGGACGTCGATAC
TTTC 1	CTCAGCCAGGCTGGGTGAAT
TTTC 2	TGCAACCAAGTTCAGATGGTT
TTTG 1	GTCCAAGAAGACAGGAAAGCC
TTTG 2	GTACTATTGACTCTTGGTTGA
CTTA 1	CCTTACTGATGACGCCCTTGC
CTTA 2	CTGATGACGCCCTTGCTCTTC
CTTT 1	ACAACGGTATTGTGTGGAACG
CTTT 2	GGCTGGATGTCTGGGTGACCA
CTTC 1	CGAAGGTATTTCCCTGACAGT
CTTC 2	CAGAAATCAGATCTTCCGCAG
CTTG 1	GAAGTTGAGTCCGTTGACAAT
CTTG 2	TGTGGTTGTCCAATCTCCACC
<i>RFP</i> 1	AGTACCCTCATATGGTCTTCC
<i>RFP</i> 2	AAGTGGGAAAGAGTCATGAAC
Sh ble 1	TCCCGTGACTTCGTAGAGGAC
Sh ble ^{BLE} 2	CCGGTGTGTACGTGACGACG
<i>TPI1</i> 1	AAGTTTCCTCCTACGAAAAAT
<i>TPI1</i> 2	CGTAGGAGGAACTTCAAAAT

<i>TPI1 3</i>	TGCGGAGACGTAAACAGTCTT
<i>TPI1 4</i>	AAGGACTTGGGTGTCCCTTAC
<i>OCH1 1</i>	TACGGACCCTCACAACAATTA
<i>OCH1 2</i>	ATTTTCCTTACCGCAGTTACG
<i>OCH1 3</i>	TGATACCCGATGATGCAGCAT
<i>PMT2 1</i>	AGGAAGAACCAGTCACTTCGA
<i>PMT2 2</i>	GCTCCTACTATCTCAGACACG
<i>PMT2 3</i>	ATATCAAGACAACTTGGCTAT
<i>PMT2 4</i>	AGCCATTTCCACCATTTGCGA
<i>PMT2 5</i>	TCGTAAGAAATATGCTGCTCA
<i>PMT2 6</i>	GGATCTGGTCTGTTACATTCC