# APPENDIX B

## SUPPLEMENTARY TABLES

Table B.1: PCR protocol used to amplify the sgRNA targeting the *MAT1-2-7* gene

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 20 µl |
| Forward Primer | 0.5 µM | 1 µl |
| Reverse Primer | 0.5 µM | 1 µl |
| Phusion DNA Polymerase Mastermix | 0.02 $U.\mu l^{-1}$ | 10 µl |
| Template DNA | 10 ng | 1 µl |

| Primer | Sequence [a] |
|---|---|
| T7_gRNA2_F | TAATACGACTCACTATAGGGGCTGATGGTATCAATACGCA |
| Scaff_gRNA_R | GCACCGACTCGGTGCCACTT |

[a] Red text indicates the region of the primer coding for the T7 promoter.

| | | | |
|---|---|---|---|
| Initial denaturation | 98 °C | 01:00 | |
| Denaturation | 98 °C | 00:10 | X 30 |
| Annealing | 55 °C | 01:00 | |
| Extension | 72 °C | 00:30 | |
| Final Extension | 72 °C | 10:00 | |

Table B.2: Sanger sequencing protocols used in this study

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 10 ul |
| Sequencing Buffer | 1X | 1 ul |
| Primer | 0.5 µM | 0.5 ul |
| BigDye | - | 2 ul |
| PCR Product | Variable | 2.5 ul |

| | | | |
|---|---|---|---|
| Initial denaturation | 96 °C | 02:00 | |
| Denaturation | 96 °C | 00:10 | X 25 |
| Annealing | 50 °C | 00:05 | |
| Extension | 60 °C | 04:00 | |

Table B.3: PCR protocol used to amplify the product used in the *in vitro* sgRNA tests

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 25 μl |
| 10 X PCR Buffer | 1 X | 2.5 μl |
| $MgCl_2$ | 0.5 mM | 0.5 μl |
| dNTPS | 200 μM each | 0.5 μl |
| Forward Primer | 0.2 μM | 0.5 μl |
| Reverse Primer | 0.2 μM | 0.5 μl |
| FastStart Taq DNA Polymerase | 1 U | 0.2 μl |
| Template DNA | 10 ng | 1 μl |

| Primer | Sequence |
|---|---|
| AF | GTCAGCCCTAAACCTTGAAAT |
| O127R | GAAATCCCCATAAAGCCT |

| | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 04:00 | |
| Denaturation | 95 °C | 00:30 | X 15 |
| Annealing | 55 °C | 00:30 | |
| Extension | 72 °C | 01:00 | |
| Denaturation | 95 °C | 00:30 | X 25 |
| Annealing | 55 °C | 00:30 + 5s/cycle | |
| Extension | 72 °C | 01:00 | |
| Final Extension | 72 °C | 10:00 | |

Table B.4: PCR protocol used to amplify the 5' region of the dDNA.

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 20 μl |
| Forward Primer | 0.5 μM | 1 μl |
| Reverse Primer | 0.5 μM | 1 μl |
| Phusion DNA Polymerase Mastermix | 0.02 $U.\mu l^{-1}$ | 10 μl |
| Template DNA | 10 ng | 1 μl |

| Primer | Sequence |
|---|---|
| dDNA_F | TGGCGTAATGGACATTGA |
| dDNA_HR | TTCAGCATCTTTTACTTTCACCAGCGTTGGAGATATCGATTTGGGGG |

| | | | |
|---|---|---|---|
| Initial denaturation | 98 °C | 01:00 | |
| Denaturation | 98 °C | 00:10 | X 30 |
| Annealing | 55 °C | 01:00 | |
| Extension | 72 °C | 00:30 | |
| Final Extension | 72 °C | 10:00 | |

Table B.5: PCR protocol used to amplify the 3' region of the dDNA.

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 20 μl |
| Forward Primer | 0.5 μM | 1 μl |
| Reverse Primer | 0.5 μM | 1 μl |
| Phusion DNA Polymerase Mastermix | 0.02 $U.\mu l^{-1}$ | 10 μl |
| Template DNA | 10 ng | 1 μl |

| Primer | Sequence |
|---|---|
| dDNA_HF | ACTTATTCAGGCGTAGCAACCAGGCGTCCACCGCCCTTTACAATG |
| dDNA_R | GAAATCCCCATAAAGCCT |

| | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 01:00 | |
| Denaturation | 95 °C | 00:20 | X 13 |
| Annealing | 53 °C | 01:00 | |
| Extension | 72 °C | 01:00 | |
| Denaturation | 95 °C | 00:20 | |
| Annealing | 52 °C | 01:00 | |
| Extension | 72 °C | 01:00 | |
| Denaturation | 95 °C | 00:20 | |
| Annealing | 51 °C | 01:00 | |
| Extension | 72 °C | 01:00 | |
| Denaturation | 95 °C | 00:20 | |
| Annealing | 50 °C | 01:00 | |
| Extension | 72 °C | 01:00 | |
| Final Extension | 72 °C | 10:00 | |

Table B.6: PCR protocol used to amplify the hygromycin resistance cassette of the dDNA.

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 20 µl |
| Forward Primer | 0.5 µM | 1 µl |
| Reverse Primer | 0.5 µM | 1 µl |
| Phusion DNA Polymerase Mastermix | 0.02 $U.\mu l^{-1}$ | 10 µl |
| Template DNA | 10 ng | 1 µl |

| Primer | Sequence |
|---|---|
| HygF | AACGCTGGTGAAAGTAAAAGATGCTGAA |
| HygR | ACGCCTGGTTGCTACGCCTGAATAAGT |

| | | | |
|---|---|---|---|
| Initial denaturation | 98 °C | 01:00 | |
| Denaturation | 98 °C | 00:10 | X 30 |
| Annealing | 62 °C | 01:00 | |
| Extension | 72 °C | 03:00 | |
| Final Extension | 72 °C | 10:00 | |

Table B.7: PCR protocol used to combine the 5' region the dDNA with the hygromycin resistance cassette

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 25 µl |
| Forward Primer | 0.5 µM | 1 µl |
| Reverse Primer | 0.5 µM | 1 µl |
| LongAmp Taq 2 X Mastermix | 1 X | 12.5 µl |
| Template DNA 1 | 5 ng | 1 µl |
| Template DNA 2 | 5 ng | 1 µl |

| Primer | Sequence |
|---|---|
| dDNA_F | TGGCGTAATGGACATTGA |
| HygR | ACGCCTGGTTGCTACGCCTGAATAAGT |

| | | | |
|---|---|---|---|
| Initial denaturation | 94 °C | 00:30 | |
| Denaturation | 94 °C | 00:30 | X 30 |
| Annealing | 50 °C | 00:30 | |
| Extension | 65 °C | 02:00 | |
| Final Extension | 65 °C | 10:00 | |

Table B.8: PCR protocol used to combine the 3' region the dDNA with the hygromycin resistance cassette

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 20 μl |
| Forward Primer | 0.5 μM | 1 μl |
| Reverse Primer | 0.5 μM | 1 μl |
| Phusion DNA Polymerase Mastermix | 0.02 $U.\mu l^{-1}$ | 10 μl |
| Template DNA 1 | 5 ng | 1 μl |
| Template DNA 2 | 5 ng | 1 μl |

| Primer | Sequence |
|---|---|
| HygF | AACGCTGGTGAAAGTAAAAGATGCTGAA |
| dDNA_R | GAAATCCCCATAAAGCCT |

| | | | |
|---|---|---|---|
| Initial denaturation | 98 °C | 01:00 | |
| Denaturation | 98 °C | 00:30 | X 30 |
| Annealing | 50 °C | 00:30 | |
| Extension | 72 °C | 02:00 | |
| Final Extension | 72 °C | 10:00 | |

Table B.9: PCR protocol used to assemble the full-length dDNA

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 25 μl |
| LongAmp Taq 2 X Mastermix | 1 X | 12.5 μl |
| DMSO | - | 1 μl |
| Template DNA 1 | 5 ng | 1 μl |
| Template DNA 2 | 5 ng | 1 μl |

| | | | |
|---|---|---|---|
| Initial denaturation | 94 °C | 01:00 | |
| Denaturation | 94 °C | 01:00 | X 30 |
| Annealing | 50 °C | 01:00 | |
| Extension | 65 °C | 02:30 | |
| Final Extension | 65 °C | 10:00 | |

Table B.10: Degradation of the germling/mycelia solution with lysing enzymes from *Trichoderma harzianum*

| Reaction | Enzyme Concentration | Degradation Time |
|---|---|---|
| A | 1.250 μg.ml$^{-1}$ | 3 hours |
| B | 1.875 μg.ml$^{-1}$ | 3 hours |
| C | 2.500 μg.ml$^{-1}$ | 2.5 hours |
| D | 3.750 μg.ml$^{-1}$ | 2.5 hours |
| E | 4.375 μg.ml$^{-1}$ | 2 hours |
| F | 5.000 μg.ml$^{-1}$ | 2 hours |

Table B.11: PCR protocol used to confirm integration of the dDNA into the genome of *H. omanensis.*

| Reagent | Final Concentration | Volume |
|---|---|---|
| dd$H_2O$ | - | Up to 25 μl |
| Forward Primer | 0.5 μM | 1 μl |
| Reverse Primer | 0.5 μM | 1 μl |
| LongAmp Taq 2 X Mastermix | 1 X | 12.5 μl |
| Template DNA | 10 ng | 1 μl |

| Primer | Sequence |
|---|---|
| AF | GTCAGCCCTAAACCTTGAAAT |
| BR | ATTTTTGGTTAAGTTGGGCGG |
| ygF | GATGTAGGAGGGCGTGGATATGTCCT |
| HyR | GTATTGACCGATTCCTTGCGGTCCGAA |

| | | | |
|---|---|---|---|
| Initial denaturation | 94 °C | 05:00 | |
| Denaturation | 94 °C | 00:30 | X 30 |
| Annealing | 55 °C | 00:30 | |
| Extension | 65 °C | [a] | |
| Final Extension | 65 °C | 10:00 | |

[a] AF + BR: 04:00, AF + HyR: 02:00, ygF + BR: 03:00

Table B.12: DIG-labeled probe synthesis PCR protocol

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | 17.13 µl |
| Buffer, with $MgCl_2$ | 1X | 5X |
| DIG mix | - | 2.5 µl |
| Forward Primer | 0.3 µM | 0.75 µl |
| Reverse Primer | 0.3 µM | 0.75 µl |
| High fidelity enzyme | - | 0.37 µl |
| Template DNA | 10 ng | 1 µl |

| Primer | Sequence |
|---|---|
| ygF | GATGTAGGAGGGCGTGGATATGTCCT |
| hyR | GTATTGACCGATTCCTTGCGGTCCGAA |

| | | | |
|---|---|---|---|
| Initial denaturation | 94 °C | 05:00 | |
| Denaturation | 94 °C | 00:30 | X 40 |
| Annealing | 66 °C | 00:30 | |
| Extension | 72 °C | 00:30 | |
| Final Extension | 72 °C | 10:00 | |

Table B.13: Mating tests performed

| | Combination | Partner 1 | Partner 2 |
|---|---|---|---|
| A x B | WT MAT1 x WT MAT1 | CMW 44436 | CMW 44437 |
| A x C | WT MAT1 x WT MAT2 | CMW 44436 | CMW 44439 |
| A x D | WT MAT1 x WT MAT2 | CMW 44436 | CMW 44442 |
| A x E | WT MAT1 x Δ MAT2 | CMW 44436 | CMW 54810 |
| A x F | WT MAT1 x Δ MAT2 | CMW 44436 | CMW 54811 |
| B x C | WT MAT1 x WT MAT2 | CMW 44437 | CMW 44439 |
| B x D | WT MAT1 x WT MAT2 | CMW 44437 | CMW 44442 |
| B x E | WT MAT1 x Δ MAT2 | CMW 44437 | CMW 54810 |
| B x F | WT MAT1 x Δ MAT2 | CMW 44437 | CMW 54811 |
| C x D | WT MAT2 x WT MAT2 | CMW 44439 | CMW 44442 |
| C x E | WT MAT2 x Δ MAT2 | CMW 44439 | CMW 54810 |
| C x F | WT MAT2 x Δ MAT2 | CMW 44439 | CMW 54811 |
| D x E | WT MAT2 x Δ MAT2 | CMW 44442 | CMW 54810 |
| D x F | WT MAT2 x Δ MAT2 | CMW 44442 | CMW 54811 |
| E x F | Δ MAT2 x Δ MAT2 | CMW 54810 | CMW 54811 |

Table B.14: RT-PCRs of *MAT1-2-1*, the two pheromones and the two pheromone receptors

| Reagent | Final Concentration | Volume |
|---|---|---|
| $ddH_2O$ | - | Up to 25 µl |
| 10 X PCR Buffer | 1 X | 2.5 µl |
| $MgCl_2$ | 0.5 mM | 0.5 µl |
| dNTPS | 200 µM each | 0.5 µl |
| Forward Primer | 0.5 µM | 0.5 µl |
| Reverse Primer | 0.5 µM | 0.5 µl |
| FastStart Taq DNA Polymerase | 1 U | 0.2 µl |
| Template DNA | 10 ng | 1 µl |

| Primer | Sequence | |
|---|---|---|
| B121F | ATTGCTGGCTGATTTCACG | *MAT1-2-1* |
| BM121R | TAGTCTGGGTGGGTGTTC | |
| OαF2 | TTCTCTACCATCCTGGCT | α pheromone |
| OαR2 | AGTTTTCCAAGAAGTGGC | |
| OaF | CAAGAACACCACCACCTCCA | a pheromone |
| OaR | AACACCGCGCATGACAGT | |
| Oste2F | TGACGCCGATGGAGATTT | α pheromone receptor |
| Oste2R | CATTGTCTTGTTGGTTGCTG | |
| Oste3F | CTTATCAAATCTCGCTGCCT | a pheromone receptor |
| Oste3R | ATGACGAGACGACGACGA | |

| | | | |
|---|---|---|---|
| Initial denaturation | 95 °C | 04:00 | |
| Denaturation | 95 °C | 00:30 | |
| Annealing | [a] | 00:30 | X 15 [b] |
| Extension | 72 °C | 01:00 | |
| Denaturation | 95 °C | 00:30 | |
| Annealing | [a] | 00:30 + 5s/cycle | X 25 [b] |
| Extension | 72 °C | 01:00 | |
| Final Extension | 72 °C | 10:00 | |

[a] B121F & BM121R: 56 °C, OαF2 & OαR2: 60°C, OaF & OaR: 62°C, Oste2F & Oste2R: 56 °C, Oste3F & Oste3R; 56°C.

[b] These PCR conditions include two separate denaturation, annealing and extension cycles with different annealing conditions. Thus, each amplification cycle is repeated a total of 40 times.

Table B.15: The identified potential protospacer regions of the sgRNA molecules and their target positions in the *H. omanensis MAT1-2-7* gene

| Name | Strand | Position | Protospacer and PAM Sequences [a] | Reason for exclusion |
|---|---|---|---|---|
| sgRNA_1 | - | 126 – 148 | TCAATACGCAAGGATGGATGAGG | Folding |
| sgRNA_2 | - | 136 – 158 | GCTGATGGTATCAATACGCAAGG | Chosen |
| sgRNA_3 | - | 158 – 180 | GATGACGTCGAGCAAAGAGGCGG | Folding |
| sgRNA_4 | - | 161 – 183 | GGGGATGACGTCGAGCAAAGAGG | Position |
| sgRNA_5 | - | 183 – 205 | AGTTCTGGAGATATCGATTTGGG | Folding |
| sgRNA_6 | - | 198 – 220 | GTGGCTGTTGGAAGCAGTTCTGG | Folding |
| sgRNA_7 | - | 210 – 232 | TTGTAAAGGGCGGTGGCTGTTGG | Specificity |
| sgRNA_8 | - | 237 – 259 | GTTTCTTGAACAGAAGGGGGAGG | Folding |
| sgRNA_9 | + | 271 – 293 | AAAGGCTTTATGGGGATTTCCGG | Position |
| sgRNA_10 | - | 290 – 312 | GTATCGGTACATGTATCGACCGG | Folding |
| sgRNA_11 | - | 335 – 357 | ATCTTCTGGGAGATCAAGCATGG | Position |
| sgRNA_12 | + | 342 – 364 | TGATCTCCCAGAAGATGCAGTGG | Folding |
| sgRNA_13 | + | 353 – 375 | AAGATGCAGTGGCATTGCATGGG | Folding |

[a] The sequence of the protospacer is indicated in black text, the PAM sequence is indicated in red text and the scaffold sequence is not indicated. All sequences are written in a 5' to 3' orientation.

Table B.16: Growth measurements at 60 hours post-inoculation

| | | Repeats | | | | |
|---|---|---|---|---|---|---|
| | Measurement | 1 | 2 | 3 | 4 | 5 |
| MAT1-1 (CMW44436) | 1 | 55 | 55 | 56 | 57 | 58 |
| | 2 | 56 | 56 | 55 | 57 | 57 |
| | Average | 55.5 | 55.5 | 55.5 | 57 | 57.5 |
| MAT1-1 (CMW44437) | 1 | 58 | 57 | 59 | 57 | 57 |
| | 2 | 57 | 59 | 58 | 58 | 57 |
| | Average | 57.5 | 58 | 58.5 | 57.5 | 57 |
| MAT1-2 (CMW44439) | 1 | 58 | 56 | 58 | 55 | 57 |
| | 2 | 57 | 56 | 57 | 58 | 58 |
| | Average | 57.5 | 56 | 57.5 | 56.5 | 57.5 |
| MAT1-2 (CMW 44442) | 1 | 54 | 51 | 50 | 52 | 53 |
| | 2 | 54 | 52 | 49 | 52 | 51 |
| | Average | 54 | 51.5 | 49.5 | 52 | 52 |
| ***Δ****MAT127-H1* (CMW 54810) | 1 | 38 | 40 | 41 | 40 | 41 |
| | 2 | 41 | 39 | 41 | 41 | 39 |
| | Average | 39.5 | 39.5 | 41 | 40.5 | 40 |
| ***Δ****MAT127-H4* (CMW 54811) | 1 | 39 | 40 | 41 | 41 | 40 |
| | 2 | 40 | 39 | 39 | 40 | 39 |
| | Average | 39.5 | 39.5 | 40 | 40.5 | 39.5 |

Table B.17: The mutant isolates both grew significantly slower than the wild type isolates of both mating types. The p-values indicated below are the results from a two-tailed, independent t-test.

| Isolate | Average growth in 60 hours (mm) | *p-value* | |
|---|---|---|---|
| | | ***Δ****MAT127-H1* | ***Δ****MAT127-H4* |
| MAT1 (CMW 44436) | 56.2 | 1.3 E-09 | 5.8 E-10 |
| MAT1 (CMW 44437) | 57.7 | 6.1 E-11 | 1.3 E-11 |
| MAT2 (CMW 44439) | 57.0 | 1.9 E-10 | 5.5 E-11 |
| MAT2 (CMW 44442) | 51.8 | 3.7 E-07 | 2.21 E-7 |
| ***Δ****MAT127-H1* (CMW 54810) | 40.1 | - | 0.42 |
| ***Δ****MAT127-H4* (CMW 54811) | 39.8 | 0.42 | - |