Supplementary File: Expression of *MAT1* locus elements in *Teratosphaeria* destructans

RNA-Seq reads derived from both the *MAT1-1* (CMW45661) and *MAT1-2* (CMW44962) isolates of *Teratosphaeria destructans* were used to determine expression of the genes at the *MAT1* locus. The reads of both isolates were mapped to the *MAT1-1* genome of CMW45661 as well as to its *MAT1-1* idiomorph (GenBank accession: MN531144) and the *MAT1-2* genome of CMW44962 and its *MAT1-2* idiomorph (MN531146). Table 6-1 shows the count data for these mappings.

	MAT1-1 reads	MAT1-2 reads	MAT1-1 reads	MAT1-2 reads		
	MAT1-1 Genome (i)		MAT1-1 idiomorph (iii)			
^c Genes in and around the MAT1-1 idiomorph						
APC5	2770	1248	5033	2197		
APN2	13	8	1123	808		
MAT1-1-10	668	1	545	13		
MAT1-1-1	302	0	1121	731		
hypothetical protein	6860	6571	7019	6837		
F-box domain-containing protein	1748	1553	2557	3001		
СОХ6а	4604	4912	4656	4991		
	MAT1-2 Genome (ii)		MAT1-2 idiomorph (iv)			
Genes in and around the MAT1-2 idiomorph						
APC5	3512	1103	6300	4131		
APN2	831	708	1065	802		
MAT1-2-1	264 ^d	2190	1239	3039		
MAT1-2-12	0	280	20	326		
hypothetical protein	6898	6650	6956	6790		
F-box domain-containing protein	1718	1568	2138	2195		
СОХба	5261	5689	4669	4983		

Table 7-1 The number^a of RNA-Seq reads of the *MAT1-1* and *MAT1-2 Teratosphaeria destructans* isolates that mapped^b to the *MAT1* idiomorphs and flanking genes

^a Numbers represent the average RNA reads counted in the three replicates of each isolate.

^b Reads were mapped against four templates, described in the main text. These were the two *T*. *destructans* genomes (i and ii) and the *MAT1-1* (iii) and *MAT1-2* (iv) idiomorphs.

^c Genes are listed in the order they occur in the genome. The *MAT1* genes are in bold.

^d All these MATI-1 reads mapped to a portion of the first exon (194 bp) of MATI-2-1, but their sequences correspond to one of the identified MAT fragment sequences and, therefore, do not suggest expression of the MATI-2-1 gene in a MATI-1 isolate.

The counts of the RNA-Seq reads that mapped to the two *T. destructans* genomes (Table 7-1), indicated expression of all flanking genes in the *MAT1-2* isolate and all flanking genes, except *APN2*, in the *MAT1-1* isolate. The *MAT1-1-1* and *MAT1-1-10* genes were expressed in the *MAT1-1* isolate and the *MAT1-2-1* and *MAT1-2-12* genes in the *MAT1-2* isolate. However, when only considering the *MAT1* idiomorphs as templates, a considerable number of RNA-

Seq reads of the *MAT1-2* isolate mapped to the *MAT1-1-1* gene and, similarly, many reads of the *MAT1-1* isolate mapped to the *MAT1-2-1* gene. These mappings were less than the number of reads identified in the isolate containing the specific mating type gene, but nonetheless suggested expression of these genes in the "incorrect" isolate. A graphical representation of these mappings is shown in Fig. 7-1.





Figure 7-1 Mapping of RNA-Seq reads to the *Teratosphaeria destructans* (a-b) *MAT1-1* and (c-d) *MAT1-2* idiomorphs. Reads of each isolate were mapped to their own *MAT1* idiomorph (a and c) as well as to the opposite idiomorph (b and d). Green and grey arrows represent genes and exons, respectively. Black bars are mapped reads and the relative number of mapped reads is indicated by the coverage plot at the top of the figure. Grey bars below the coverage plot indicate areas with <30 mapped reads.

When comparing the mapped reads in Fig. 7-1, it is clear that mapping the reads of an isolate of opposite mating type to a *MAT* idiomorph indicates some level of expression in certain parts of the *MAT1-1-1* and *MAT1-2-1* genes. However, the entire gene is not expressed in isolates with the opposite mating type. Upon closer inspection, reads that mapped in these scenarios (Fig. 7-1b and 7-1d) aligned primarily in areas homologous to the *MAT* fragment sequences (Fig. 7-2).



Figure 7-2 RNA-Seq reads of the *Teratosphaeria destructans* (a) MAT1-2 isolate mapped to the MAT1-1-1 gene and (b) MAT1-1 isolate mapped to the MAT1-2-1 gene. Yellow arrows represent regions within the genes (green arrows) that are homologous to the MAT fragment sequences identified in the genome.

Two lines of evidence indicated that the *MAT1-1* RNA reads that mapped to the *MAT1-2-1* gene and the *MAT1-2* reads that mapped to the *MAT1-1-1* gene were derived from *MAT* fragment sequences. Firstly, these reads only mapped when the *MAT1* idiomorph, and not the entire genome, was used as a template (Table 7-1). Secondly, the *MAT* fragment sequences and *MAT1* genes had a significant number of single nucleotide polymorphisms (SNPs) and indels (Table 7-2). The RNA reads that mapped to "incorrect" *MAT1* genes had the same SNPs present in the fragment sequences (Fig. 7-3). These mappings, therefore, represent expression of the *MAT* fragment sequences.

	Length	SNPs	Indels	% Polymorphism			
MAT1-1-1 fragments							
A1	89	7	0	7.9%			
B1	112	13	1	12.5%			
C1	59	5	0	8.5%			
D1	148	11	1	8.1%			
E1	68	4	2	8.8%			
F1	73	7	0	9.6%			
G1	74	11	0	14.9%			
H1	149	17	2	12.8%			
MAT1-2-1 fragments							
A2	76	10	1	14.5%			
B2	105	10	1	10.5%			
C2	62	4	0	6.5%			
D2	69	6	0	8.7%			
E2	149	11	0	7.4%			
F2	36	1	1	5.6%			
G2	69	6	1	10.1%			

 Table 7-2 Polymorphisms identified between the MAT1 genes and their homologous MAT fragment sequences in the CMW44962 genome.



Figure 7-3 An example of a region of *MAT* fragments that mapped to the (a) *MAT1-1-1* and (b) *MAT1-2-1* genes. The yellow arrow represents the largest region on the *MAT1-1-1* or *MAT1-2-1* gene (green arrow) homologous to a *MAT* fragment. Bases highlighted in blue are SNPs that correspond to the sequence of the *MAT* fragments.

To further confirm expression of the *MAT* fragment sequences, the RNA-Seq reads were also mapped to the genomic locations where these fragments occur (template v in the main text). All of the identified *MAT* fragment sequences, except perhaps fragment F2 on contig 147, had a sufficient number of mapped reads to conclude that these sequences are being expressed in both the *MAT1-1* and *MAT1-2* isolates (Table 7-2). The visual mappings of the reads to the genome, however, showed that expression is not limited to the *MAT* fragment sequences, but that there are also regions of expression around these sequences. The two contigs on which the most *MAT* fragments were identified is shown as an example of this (Fig. 7-4).

Table 7-3 The number^a of RNA-Seq reads of the *MAT1-1* and *MAT1-2 Teratosphaeria destructans* isolates that mapped to the regions in the CMW44962 genome in which *MAT* fragment sequences were identified

Gene / MAT fragment ^b	MAT1-1 reads	MAT1-2 reads
Contig 28		
Tdes44962_MAKER_02653	403	1251
C1_Contig28.1(1)	694	604
B1_Contig28.2(1)	532	617
Tdes44962_MAKER_02654	3486	3268
Tdes44962_MAKER_02655	1126	1910
Contig 82		
Tdes44962_MAKER_05754	3108	2607
Tdes44962_MAKER_05755	2660	1687
B2_Contig82.1(2)	1156	463
A2_Contig82.2(2)	334	454
H1_Contig82.3(1)	735	862
A1_Contig82.4(1)	760	558
Tdes44962_MAKER_05756	458	572
Contig 147		
Tdes44962_MAKER_07832	4992	4781
Tdes44962_MAKER_07833	1445	1228
Tdes44962_MAKER_07834*	64	47
F2_Contig147.1(2)	21	20
Tdes44962_MAKER_07835	2170	1289
Tdes44962_MAKER_07836	1616	1587
Tdes44962_MAKER_07837	660	1089
Contig 300		
Tdes44962_MAKER_09673	9883	3587
Tdes44962_MAKER_09674	162	191
Tdes44962_MAKER_09675	228	345
Tdes44962_MAKER_09676*	393	456
E2_Contig300.1(2)	947	1312
D1_Contig300.2(1)	655	750
D2_Contig300.3(2)	195	213
G1 Contig300.4(1)	175	206

C2_Contig300.5(2)	54	91		
G2_Contig300.6(2)	130	198		
F1_Contig300.7(1)	393	580		
E1_Contig300.8(1)	47	66		
Tdes44962_MAKER_09677	896	1309		
Tdes44962_MAKER_09678	7266	9034		
^a Counts represent the average of three replicates				

^b Genes and *MAT* fragments are listed in the order they occur in the genome. *MAT* fragments are highlighted in yellow and asterisks indicate gene predictions with a *MAT* fragment in the coding sequence.

(a) Reads of *MAT1-2* isolate mapped to a region of contig 82 containing *MAT* fragment sequences



(b) Reads of *MAT1-2* isolate mapped to a region of contig 300 containing *MAT* fragment sequences



Figure 7-4 RNA-Seq reads of the *MAT1-2* isolate mapped to the two regions of the CMW44962 genome in which the most *MAT* fragment sequences (yellow arrows) were identified.