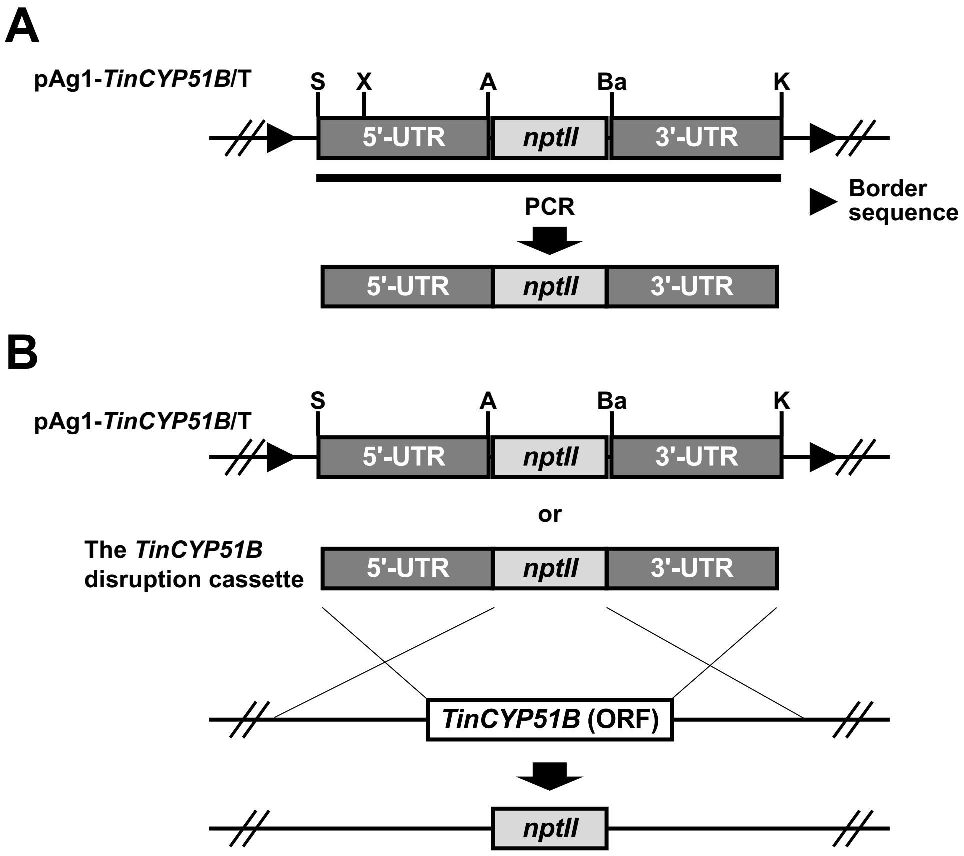

FIG. S1 Disruption of the *TinCYP51B* gene of *T. indotineae* strains with low azole susceptibility by gene replacement strategy. (A) Schematic representation of a part of the *TinCYP51B*-targeting vector pAg1-*TinCYP51B*/T. Plasmid DNA of pAg1-*TinCYP51B*/T was used as a template for PCR, to amplify the sequence indicated by the thick line. The *nptII* cassette is composed of *PtrpC*, *nptII*, and *TcgrA*. Border sequences are the specific regions that delineate the DNA to be transferred during *Agrobacterium tumefaciens*-mediated transformation. Restriction enzyme site: A, ApaI; Ba, BamHI; E, EcoRI; K, KpnI; P, PstI; S, SpeI. (B) Schematic representation of the *TinCYP51B* locus before and after homologous recombination.

TABLE S1 Primers used in this study

Primer name	Sequence (5'-3')	Chain	Purpose
P1	ATGGCCGTGTTCACAGTGG	Sense	Amplification of *TinCYP51A*
P2	TCAATGTGACTGAGGACGTCG	Anti-sense	Amplification of *TinCYP51A*
P3	ATGGGCCTCTTAGCCGAC	Sense	Amplification of *TinCYP51B*
P4	TCATTTCTGGGAAGGCTGC	Anti-sense	Amplification of *TinCYP51B*
P5	TGTTGGTGATGAGGCACAGT	Sense	qRT-PCR (*TinACTIN*)
P6	CCATGTCATCCCAGTTTGTG	Anti-sense	qRT-PCR (*TinACTIN*)
P7	GCGTGACCCAGCCAACA	Sense	qRT-PCR (*TinGAPDH*)
P8	TTGGCACCTCCCTTCAAGT	Anti-sense	qRT-PCR (*TinGAPDH*)
P9	CCTAATGCCCTTCCTGGATT	Sense	qRT-PCR (*TinMDR1*)
P10	AAATTGCCAGCTCGTTCTGT	Anti-sense	qRT-PCR (*TinMDR1*)
P11	TGACTCTGAATCCGAAAAGG	Sense	qRT-PCR (*TinMDR2*)
P12	GTCGGTGAGCAACAGCAATA	Anti-sense	qRT-PCR (*TinMDR2*)
P13	TCACTGGTGAAATGCTCGTTGACG	Sense	qRT-PCR (*TinMDR3*)
P14	GGTTGACGGAGCAAGGCACTG	Anti-sense	qRT-PCR (*TinMDR3*)
P15	GAGACGTAGATATTTCGAGTGCC	Sense	qRT-PCR (*TinCYP51A*)
P16	TTGAGCGTACCTCTTCCCCCTG	Anti-sense	qRT-PCR (*TinCYP51A*)
P17	AAGTTCGTCAAGTTCGGCCT	Sense	qRT-PCR (*TinCYP51B*)
P18	GAGAGGCGGTGTAGATGGTG	Anti-sense	qRT-PCR (*TinCYP51B*)
P19	GCTGATGCCAATGCCATGGAG	Sense	qRT-PCR (*TinMFS2*)
P20	TCGGGTTTCGAGGATCGCTTGG	Anti-sense	qRT-PCR (*TinMFS2*)
P21	AAA*GGATCC*CCACTAAGACTCCTTCAAGCTCCCCA	Sense	Amplification of *Ptef1α* for pAg1-*TinCYP51B*/OE
P22	GGCTAAGAGGCCCATGGTGACGGTGTATTTTTGTG	Anti-sense	Amplification of *Ptef1α* for pAg1-*TinCyp51B*/OE
P23	GCAGCCTTCCCAGAAATGACAGCAGAAGAATCTCT	Sense	Amplification of *TcgrA* for pAg1-*TinCYP51B*/OE
P24	AAAA*GGTACC*AGATGATTCATGA	Anti-sense	Amplification of *TcgrA* for pAg1-*TinCYP51B*/OE
P25	AAATACACCGTCACCATGGGCCTCTTAGCCGACAT	Sense	*TinCYP51B* cDNA

P26	AGAGATTCTTCTGCTGTCATTTCTGGGAAGGCTGC	Anti-sense	*TinCYP51B* cDNA
P27	TTT*CTCGAG*CCTCACCGATGGGAGAACAACGTCGG	Sense	Amplification of the *TinCYP51B* cDNA fragment for pSilent1-*TinCYP51B*; Amplification of the *TinCYP51B*-specific hybridization probe
P28	TTT*AAGCTT*TGGCGGCACAACATCTTTGGTGCCTT	Anti-sense	Amplification of the *TinCYP51B* cDNA fragment for pSilent1-*TinCYP51B*; Amplification of the *TinCYP51B*-specific hybridization probe
P29	TTT*AGATCT*TGGCGGCACAACATCTTTGGTGCCTT	Sense	Amplification of the anti-sense *TinCYP51B* cDNA fragment for pSilent1-*TinCYP51B*
P30	TTT*GGTACC*CCTCACCGATGGGAGAACAACGTCGG	Anti-sense	Amplification of the anti-sense *TinCYP51B* cDNA fragment for pSilent1-*TinCYP51B*
P31	TTT*ACTAGT*CCACTAAGACTCCTTCAAGCT	Sense	Amplification of *Ptef1α* for pSilent1-*TinCYP51B*
P32	TTCTACAGATGGTAACTgGTCGAGAGCAGAGAGTA	Anti-sense	Inactivation of the internal SpeI site by overlap extension PCR
P33	TACTCTCTGCTCTCGACcAGTTACCATCTGTAGAA	Sense	Inactivation of the internal SpeI site by overlap extension PCR
P34	TTT*CTCGAG*GGTGACGGTGTATTTTTGTGTGGTT	Anti-sense	Amplification of *Ptef1α* for pSilent1-*TinCYP51B*
P35	CATG*ACTAGT*GCGGTATGCCTTTACCCATTGCATA	Sense	Amplification of the 5' UTR of *TinCYP51B* gene
P36	AAA*GGGCCC*ATGGTGATCGTCTTATCCTCCTGCT	Anti-sense	Amplification of the 5' UTR of *TinCYP51B* gene; Amplification of the *TmeCYP51B*-specific hybridization probe
P37	CCAG*GGATCC*TAATCACGACTGCGGATTGAATCAT	Sense	Amplification of the 3' UTR of *TinCYP51B* gene
P38	TTATCAGCCATGCGTACcGTACCTTCATCAATCTC	Anti-sense	Inactivation of the internal KpnI site by overlap extension PCR
P39	GAGATTGATGAAGGTACgGTACGCATGGCTGATAA	Sense	Inactivation of the internal KpnI site by overlap extension PCR
P40	CCCTTACCGGTTTACCTtGGCCCTTGGTGCCGTGG	Anti-sense	Inactivation of the internal ApaI site by overlap extension PCR
P41	CCACGGCACCAAGGGCCaAGGTAAACCGGTAAGGG	Sense	Inactivation of the internal ApaI site by overlap extension PCR
P42	CCCA*GGTACC*ATATGTCGACTGCACCGGAATATTG	Anti-sense	Amplification of the 3' UTR of *TinCYP51B* gene
P43	GTGT*ACTagt*ACCGTGGTCATCGTGTAGATGATAT	Sense	Amplification of the 5' UTR of *TinMDR3* gene
P44	TATTGTGAGCCATAGGGaACCGATTTACTCTGAAA	Anti-sense	Inactivation of the internal KpnI site by overlap extension PCR
P45	TTTCAGAGTAAATCGGTtCCCTATGGCTCACAATA	Sense	Inactivation of the internal KpnI site by overlap extension PCR

P46	CAGCGTATGTTCAGGGCCtAATCATATGCCACAGA	Anti-sense	Inactivation of the internal ApaI site by overlap extension PCR
P47	TCTGTGGCATATGATTaGGCCCTGAACATACGCTG	Sense	Inactivation of the internal ApaI site by overlap extension PCR; Amplification of the *TinMDR3*-specific hybridization probe
P48	TTT*GGGCCC*CTGCTGATCTCTGTGGACGATAGCAC	Anti-sense	Amplification of the 5' UTR of *TinMDR3* gene; Amplification of the *TinMDR3*-specific hybridization probe
P49	TTCC*GGATCC*CATGAGCCATTACACATGGGTTACA	Sense	Amplification of the 3' UTR of *TinMDR3* gene
P50	TACCATGGACATGGTACtTGGTGCAACCAGGACGA	Anti-sense	Inactivation of the internal KpnI site by overlap extension PCR
P51	TCGTCCTGGTTGCACCAaGTACCATGTCCATGGTA	Sense	Inactivation of the internal KpnI site by overlap extension PCR
P52	TGA*GGTACC*TACTACTCTGTGAAATCAGTTAACTT	Anti-sense	Amplification of the 3' UTR of *TinMDR3* gene
P53	GGC*ACTAGT*TATGGCTAACCGTTGTCGTAGCATGA	Sense	Amplification of the TmeCYP51B1 and TmeCYP51B2 fragments
P54	AAA*GGGCCC*TCATTTCTGGGAAGGCTGCTTTCGCT	Anti-sense	Amplification of the TmeCYP51B1 and TmeCYP51B2 fragments
P55	AGACAGATTACGaATACGGCTTGGTT	Sense	Generation of a point mutation in *TmeCYP51B*
P56	AACCAAGCCGTATtCGTAATCTGTCT	Anti-sense	Generation of a point mutation in *TmeCYP51B*
P57	TTGACGGACCGTTGATAGCTTAACCTGCCATGACT	Sense	Amplification of the *TmeCYP51B*-specific hybridization probe
P58	TTT*CTCGAG*ATGGGCCTCTTAGCCGACATTGTCTC	Sense	Amplification of the *TinCYP51B*-specific hybridization probe
P59	TTT*AAGCTT*CTTGCAGTCGTCAAAGAACTTGTACG	Anti-sense	Amplification of the *TinCYP51B*-specific hybridization probe
P60	TTC*AGATCT*AGAAGATGATATTGAAGG	Sense	Amplification of the *nptII*/*TinCYP51B* cDNA cassette
P61	CGATTAAGTTGGGTAACGCCAG	Anti-sense	Amplification of the *nptII*/*TinCYP51B* cDNA cassette
P62	ATGAAAAAGCCTGAACTCAC	Sense	Amplification of the *hph* gene
P63	GTTTCCACTATCGGCGAGTA	Anti-sense	Amplification of the *hph* gene
T7	TAATACGACTCACTATAGGG	Sense	Amplification of *TinCYP51B* RNAi cassette
M13rv	CAGGAAACAGCTATGAC	Anti-sense	Amplification of *TinCYP51B* RNAi cassette

Restriction sites are italicized and underlined, and nucleotide substituitions are shown in lower case letters.

TABLE S2 Overall genetic features of six *T. indotineae* strains

Species and isolate no.[a]	BioProject number	GenBank accession number (wgs)	Total Number of scaffolds	N50 (bp)	Longest sequence length (bp)	Total sequence length (bp)	Depth of coverage (×)	GC content (%)
T. indotineae								
TIMM20114 (UKJ1676/17; IFM 67092)	PRJNA780377	JAJVHL000000000	3	8,011,651	10,184,091	22,261,634	40	49
TIMM20115 (UKJ1700/17II; IFM 67093)	PRJNA780377	JAJVHI000000000	6	4,361,119	6,825,914	22,331,438	38	49
TIMM20116 (UKJ1708/17; IFM 67094)	PRJNA780377	JAJVHK000000000	8	4,066,087	7,369,201	22,383,831	46	49
TIMM20117 (200087/18; IFM 67095)	PRJNA780377	JAJVHH000000000	8	3,672,843	6,160,204	22,303,869	32	49
TIMM20118 (UKJ1687/17; IFM 67096)	PRJNA780377	JAJVHJ000000000	6	4,204,240	4,528,007	22,316,814	43	49
TIMM20119 (200123/18; IFM 67097)	PRJNA780377	JAJVHG000000000	8	3,646,268	4,540,326	22,297,103	32	49

[a] All the *T. indotineae* isolates were collected in India and preserved in the culture collection of Teikyo University Institute of Medical Mycology (TIMM) and Medical Mycology Research Center, Chiba University (IFM), through the National Bio-Resource Project, Japan (http://www.nbrp.jp/).

TABLE S3 Conserved sequences HR1 and HR2 that could allow amplification of *CYP51B* in selected dermatophyte species of *Trichophyton* (T.), *Nannizia* (N.) and *Microsporum* (M).

Species	HR1 sequence[a]	HR2 sequence[b]
T. indotineae	**5’-AACCGAAAAGCAGC-3’**	**5’-AAGCGAAAGCAGC-3’**
T. tonsurans	5’AACCGAAAAGCAGC-3’	5’-AAACGAAAGCAGC-3’
T. equinum	5’-AACTGAAAAGCAGC-3’	5’-AAGCGAAAGCAGC-3’
T. interdigitale	5’-AACCAAAAAGCAGC-3’	5’-AAGCGAAAGCAGC-3’
T. rubrum	5’-AACCAAAAAGCAAC-3’	5’-AAACGAAAGCAGG-3’
T.violaceum	5’-AACCAAAAAGCAAC-3’	5’-AAACGAAAGCAGG-3’
T. soudanense	5’-AACCAAAAAGCAAC-3’	5’-AAACGAAAGCAGG-3’
T. verrucosum	5’-AACCGAAAAGCAAA-3’	5’-AAGCGAAAGCAGC-3’
T. benhamiae	5’-AAACGAAAAGCAAA-3’	5’-AAACGAAAGCAGC-3’
N. gypsea	5’-AACCGAAAAGCAGC-3’	5’-AAACGAAAGCAGC-3’
M. canis [c]	-	5’-AAACGAAAGCAGC-3’

[a] The HR1 sequence is centered at about position -640 in relation to the initiator codon of the *CYP51B* gene.

[b] The HR2 sequence is localized within the CYP51B coding region, -27 to -14 nucleotides in relation to the last nucleotide of the stop codon of the *CYP51B* gene.

[c] The sequence in *M. canis* differs too much to identify an HR1-related sequence.

CYP51B locus in TIMM20114

The original 3’ of the *CYP51* gene (in TIMM20118_CYP51B1) is marked in red (corresponding to H109_04528 in *Trichophyton interdigitale* strain MR816).

CYP51B promoter is in violet
CYP51B ORF is in green

The two nearly identical sequences 5′-AACCGAAAAGCAGC-3′ (HR1, for homologous recombination site 1) and 5′-AAGCGAAAGCAGC-3′ (HR2) at the borders of the CYP51B origin block are highlighted.

At 5’.: CAMK protein kinase (corresponding to H109_04528 in *Trichophyton interdigitale* strain MR816)
At 3’: Protein FYV4, mitochondrial (corresponding to H109_04526 in *Trichophyton interdigitale* strain MR816)

The *TinCyp51B* gene in the genome of TIMM20114 (5' to 3')

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CATGCAGAGTGCGGTATGCCTTTACCCATTGCATACTCTCTGCCGTCTAGTGATCAATTGCTAA
CCATCTGGGTACTGCAGATTTCAGGATCAAGCTCCCCACGATATCCAACCGCCATTGCCTGATA
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CATCCTGCAAGAATCCAGATTCATCTTCAACTACCCGCGGAGTCGGAACACAAGCAAGTTCCGA
CAGCAGTATAAGCTCTTTGATATGCTGGGTAGGGGACACTTTGCCTCGGTTTATCTATGCGCTG
AACGTTCTACGGGGATCAAGTATGCCGTCAAACATTTCGAGAAACGCCCGGGCGCAGTTCAGAG
GCTAGACCGTGAAGCACTACAGCAAGAGATTTCAATGCTGATGAGTGTCAACCATCCGAACATG
CTGTGCCTAAAGGATACCTTTGACGAGAGCGATGGTGTCTTCCTGATTCTTGAGCTTGCGCCAG
AGGGCGAACTGTTCAACTGGATCATTAGGCATCAGAAACTCAGCGAGGAGGAGACTCGAAAAGT
ATTCATTCAGCTTTTTGAAGGTTTGAGATACCTGGTATGTCGTTCGTTTTCTGGTTGATCAGAC
TCATGGCAGTGTATATGGCTAACCGTTGTCGTAGCATGAACGAAACATCATTCACCGTGACATA
AAGCCAGAGAACATTCTGGTTGTCGACAAAGATCTTACCGTAAAATTAGCAGACTTCGGCCTTG
CAAAGATTGTCGGCGAACACTCCTTTACCACCACGCTGTAAGTTCACCTACCTTCTGCCGTTCA
AATCTGGGGTCAATCTTACTAAGAGATCATAAAATCTAGATGTGGAACGCCTGGCTGTAAGTTT
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TGTCCAAAGAACGCCGGCCAACTATGGTCCGCAGGCTGTTGAGTGACATACATGAAGTCACGGT
CAAGCAGGTGGTGAAGACCAAGGAAGACGACCCTGCTTCCCAGGACATAACTATATACCAGAAT
CATCCTGTGCCTCGAGCCCAGGAGCCCAAACCGGCTGGCAACCGGAACTCAAAGGAGTTCATGG
AACTCGGTGGAAGTGGAGACCAGGTGTTATTCGATGAGCAGTCTGACGACTCGCTGGAGCTTGA
GAGTGAGGCACGGAAGCCAGTTGTCCACCAAGACGACGCAGACAAGACTCCACGAGAAAGAAAG
AGGTAGCAGAAGCCCGATCCGTCTTGGGATGGTATTCTGCTAGCTATACCGTGCTATTCCGGAC
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TCGCTGCCTTGGAGACGACGGCGATTCTCGTGTTTAGGATTTCATTTTTGAGTGATATAACTAT
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GGGTACAATAGCGTCCACTGGGCAATGTAATTACAGTCTCTTCTCACTCACCACCGGACAATAA
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AAGCCGCGATGGCCTGATGATGCGCCCACCGTCAGGTCTCGCTCGCTCAGGCAGCAGAGAATCC
AGCGGAGGAGATGACGCCCTGCACGAACACCGTCCAGGGACCGTCTCCGCCCCGTCGCGAGCTC
GAATTGTAATCATCTCGACGGCCGTTGGCCTGCTCGCCGCAAGGGAGAAAAAGGGGGGCAGAAG
GGCAGCGGTAATTTTGGTATTGGAGATGCAGCTGTAAACCAATAAAAAACGACGTAAAATACCA
GGAACAATGCATTATCTTACAGTACCGTACGCCCCTTGCATAAAATGCGAGTTCGATCTCAAGA
TGCAACTTTTTTTTTCCCTTGCTTCGTCCGCAGCAGCTTCAAGGCATTCATCACAAACAACGAA
CAAGTCTTGAGCGGAAGCAGGAGGATAAGACGATCACC**ATG**GGCCTCTTAGCCGACATTGTCTC
TCGTTTCTGCGAGAACTGCTCGACCCTGTCCACCGCCGCGCTCGTCGCAAGTGCCGTATCGGCT
TTTATCGTCCTCTCCATTGTTATCAACGTCCTGCAGCAGCTCCTGTTCAAGGACCCTACAAAGC
CTCCGGTGGTCTTCCACTGGGTTCCGGTCATTGGAAGCACGATCTCCTATGGAATTGACCCGTA
CAAGTTCTTTGACGACTGCAAGGAGAAGGTTAGTTGGTGCAATTCAACTTAGCTATATACCAGG
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GTGGTGTATGATTGCCCAAACTCCAAGCTCATGGAGCAGAAGAAGGTGTGTACAGCAATTTATT
CCCGTCTACATCTCCTGGGCCTCCATTGCTAATTTAATCGTCTACCAGTTCGTCAAGTTCGGCC
TCACCTCTGAAGCTCTCCGATCCTATGTCACCCTGATCACCAAGGAAGTTGAGCAGTTCTTCGA
GTCCTCCCCCGTCTTCAAGGGCGACTCCGGAGTTTTCAACGTCAGCAAGGTCATGGCTGAAATC
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TGTCGTCCGTGTACAAGAATGGAACGCCAATTCCAGATATCGAAGTCGCCCACATGATGATTGC
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CGATTTTACTAACTTTGCATTAATAGTCCCTCTTCTCGAAGCCCCTTGGCGACCCAATGGTCTC
GTGGGAGAAGCGAAAGCAGCCTTCCCAGAAA**TGA**TAATCACGACTGCGGATTGAATCATGTTAA
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CATGCTTTTGAAGATGGGGGATGTAGATAATAGTATTATTCTAATACAGCCATTTATTACAATA
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TACATTGGAAATAGTACATCTTCCCGTAGCTGGGATAATCTAGCATCTAAACAGCGATATCCAA
GCCAGTAATGTCAGGAAGTAACCAGCCAGCAACGTCGTTAATCCCGTGCCCCCTTTCAACTCCG
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CACCTCCTGGGCCGTAGTCCTGCTGTCTGAATTTCTCTCTCCACCGCAGTATGTATCGTCTGTC
CCTTGTCATTTCTATGCCCAATTCTCGAAGCTCAGCCGAGTCCGCTGTAAACAATTGCTTCCAT

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TTTGATAACAAACAAAGCTGTATTGTTGATTGAATACTAGGAGAGCTGCTCAGAGCAGTGTCTC
ACTCTTGTGCCACTCTATCCTTTGTCTCGCGAAATTGGTGGAAGCCAATTTCGCTTGACATTAT
GGCAGTGTCGCCGCTTCCCTCTCAATTCCCTCCCATCACTACTACCGGTGCTCCAAAGTCGTTA
GCGATGAGAAATATTGCTTCTCAAGAGCCTCGGACGGCGCCTAGTGGTGATCCTGCAGTTCCTA
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CAAAGGTGGGCGGGCAAATACACCCCAACCATTGGGACCCCATAATGCTAACATTTGGTTATTC
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CTACTCTTCGGCGCCAGGCAATATTCCGGTGCAGTCGACATATGGTCTATGGG
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CYP51B locus in TIMM20118

The original 3’ of the *CYP51* gene (in TIMM20118_CYP51B1) is marked in red (corresponding to H109_04528 in *Trichophyton interdigitale* strain MR816).

CYP51B promoters are in violet
CYP51B alleles are in green

The two nearly identical sequences 5′-AACCGAAAAGCAGC-3′ (HR1, for homologous recombination site 1) and 5′-AAGCGAAAGCAGC-3′ (HR2) at the borders of the CYP51B origin block are highlighted.

At 5’.: CAMK protein kinase (corresponding to H109_04528 in *Trichophyton interdigitale* strain MR816).

At 3’: Protein FYV4, mitochondrial (corresponding to H109_04526 in *Trichophyton interdigitale* strain MR816).

The 7 *TinCyp51B* gene tandem repeats in the genome of TIMM20118 (5' to 3')

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CATGCAGAGTGCGGTATGCCTTTACCCATTGCATACTCTCTGCCGTCTAGTGATCAATTGCTAA
CCATCTGGGTACTGCAGATTTCAGGATCAAGCTCCCCACGATATCCAACCGCCATTGCCTGATA
TTCCACGAAAACAGGGGAGGAGACTTTGTTGCAATTGTGGAAGATCTCTCTATCAATGGCACTT
TCATCAACGACGCCATCATCGGCAAGAACAAACGCCGAGAGCTCGAAAACGGCGACGAGATTAC
CATCCTGCAAGAATCCAGATTCATCTTCAACTACCCGCGGAGTCGGAACACAAGCAAGTTCCGA
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GCTAGACCGTGAAGCACTACAGCAAGAGATTTCAATGCTGATGAGTGTCAACCATCCGAACATG
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Protein alignments:
Green: Typical cytochrome P450 monooxygenases ER anchoring TM
Blue: Inhibitor binding sites (from A. fumigatus)
Red: Heme-binding cysteine
Gray: New C-terminus of the additional copies.

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