

Supplementary Files

Table S1. First PtC screening, to detect the insecticidal activity.

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
A5	10	-	-	4/4 *	Black
	11	1/4	1/4	2/4	Black
	13	-	-	2/4	
	18	-	1/4	1/4	
	24	1/4	1/4	4/4	Dark red
	28	-	-	3/4	Black
	29	1/4	2/4	3/4	
	A6	1	-	2/4	4/4
2		1/4	1/4	2/4	Error
4		1/4	1/4	2/4	
6		-	4/4		Black spots
7		-	1/4	2/4	Black/Black spots
11		1/4	1/4	2/4	Black
12		-	1/4	3/4	Dark red
13		-	2/4	4/4	Dark red
14		-	-	3/4	Black
15		1/4	2/4	3/4	Dark red Brown
16		1/3	3/3		Error
17		2/3	3/3		Dark brown
18		1/3	2/3	3/3	
19		2/3	3/3		Black
20		2/3	3/3		Black
21		1/3	3/3		Stiff
22		-	3/3		Black
23		-	3/3		Black
24		-	3/3		Reddish
25		1/3	2/3	2/3	Black
26		-	-	-	
27		-	3/3		Reddish
28		-	-	-	
29		1/3	1/3	1/3	
30		-	-	-	
31		-	-	-	
32		-	2/3	3/3	Error
33		-	-	-	
34		-	-	-	
35		-	-	-	
36		1/3	2/3	2/3	
37		-	-	2/3	
38		-	-	-	
39		-	-	1/3	
40		1/3	1/3	2/3	Black
41		-	-	-	
42		2/3	3/3		Black
43		2/4	4/4		Black

Table S1. *Cont.*

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
	1	-	3/3		Black
	2	1/3	1/3	3/3	Black
	3	-	3/3		Black
	4	1/3	2/3	2/3	Black, Black spots
	5	-	3/3		Black
	6	2/3	3/3		
	7	-	3/3		Black
	8	-	3/3		Black, Error
	9	-	3/3		Black spots
	10	3/3			Black spots
	11	2/3	2/3	3/3	
	12	-	-	-	
	13	-	-	-	
	14	-	-	-	
	15	-	-	-	
	16	-	-	-	
	17	-	-	-	
	18	2/3	2/3	2/3	
	19	-	-	-	
	20	1/3	1/3	1/3	Dark red
	21	-	-	-	
	22	-	-	1/3	
	23	1/3	1/3	1/3	Dark red
A7	24	1/3	1/3	1/3	Dark red
	25	-	-	-	
	26	1/3	1/3	3/3	Black
	27	-	-	3/3	
	28	1/3	1/3	1/3	
	29	-	-	-	
	30	-	-	-	
	31	-	-	-	
	32	1/3	1/3	1/3	
	33	-	-	-	
	34	-	-	-	
	35	-	1/3	1/3	
	36	-	-	-	
	37	-	-	-	
	38	1/3	1/3	1/3	
	39	1/3	1/3	1/3	Black
	40	2/3	2/3	3/3	black
	41	-	2/3	3/3	Error
	42	-	1/3	2/3	Error
	43	1/3	2/3	2/3	Black
	44	-	1/3	1/3	Black
	45	-	-	-	
	46	1/3	2/3	2/3	Black

Table S1. *Cont.*

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
	1	-	1/3	1/3	Black
	2	1/3	2/3	3/3	Black, black spots
	3	-	-	2/3	Black
	4	3/3			Dark red
	5	1/3	2/3	2/3	Black
	6	-	2/3	2/3	
	7	-	-	3/3	Dark red
	8	2/3	3/3		Error
	9	2/3	3/3		Black
	10	1/3	3/3		Black
	11	1/3	2/3	2/3	Black
	12	-	1/3	1/3	
	13	-	-	-	
	14	-	-	-	
	15	-	-	-	
	16	-	-	-	
	17	-	3/3		Dark red
	18	-	-	-	
	19	-	-	-	
	20	-	-	-	
	21	-	-	-	
	22	-	1/3	1/3	Black
A8	23	-	-	-	
	24	-	1/3	1/3	Black
	25	-	-	-	
	26	-	-	-	
	27	-	-	1/3	
	28	-	-	-	
	29	-	-	1/3	
	30	-	-	-	
	31	-	-	-	
	32	-	3/3		Stiff
	33	1/3	3/3		Black spots
	34	-	-	-	
	35	-	3/3		Dark red
	36	-	3/3		
	37	-	3/3		
	38	1/3	1/3	1/3	Error
	39	1/3	3/3		Dark red
	40	-	3/3		
	41	1/3	3/3		stiff
	42	-	3/3		
	43	-	3/3		
	44	-	3/3		
	45	3/3			Black spots

Table S1. *Cont.*

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
	1	-	2/3		
	2	-	2/3		
	3	2/3	3/3		Stiff
	4	-	3/3		Stiff
	5	-	2/3		
	6	-	3/3		Stiff
	7	-	2/3		Reddish
	8	-	3/3		Stiff
	9	-	-	-	Black
	10	-	3/3		Stiff
	11	-	2/3	3/3	Stiff
	12	-	1/3	1/3	Dark red
	13	-	1/3	2/3	
	14	-	1/3	1/3	
	15	-	-	-	
	16	-	2/3	2/3	
	17	-	-	-	
	18	-	-	-	Stiff
	19	-	1/3	1/3	
A9	20	-	1/3	1/3	
	21	-	-	-	
	22	-	2/3	2/3	Dark red
	23	-	2/3	2/3	
	24	-	-	1/3	Black
	25	-	-	1/3	Dark red
	26	-	2/3	2/3	Dark red
	27	-	1/3	1/3	Dark red
	28	-	1/3	1/3	
	29	1/3	2/3	3/3	Stiff
	30	-	1/3	3/3	Dark red, Black
	31	-	1/3	3/3	Black
	32	-	3/3	3/3	Black
	33	-	1/3	3/3	Black
	34	-	-	3/3	Black
	35	-	3/3	3/3	Dark red
	36	-	-	3/3	Black
	37	-	1/3	3/3	Black
	38	-	-	1/3	Black
	39	1/3	1/3	2/3	Black
	40	2/3	2/3	3/3	Black

Table S1. *Cont.*

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
	1	1/3	1/3	1/3	
	2	1/3	2/3	2/3	
	3	1/3	1/3	1/3	
	4	0/3	0/3	0/3	
	5	0/3	0/3	0/3	
	6	0/3	0/3	1/3	
	7	0/3	0/3	0/3	
	8	0/3	0/3	1/3	
	9	0/3	3/3	3/3	
	10	0/3	3/3	3/3	
	11	0/3	3/3	3/3	stiff
	12	0/3	3/3	3/3	
	13	0/3	3/3	3/3	
	14	0/3	3/3	3/3	Stiff
	15	0/3	3/3	3/3	Stiff
	16	0/3	3/3	3/3	Error
	17	0/3	3/3	3/3	
	18	0/3	3/3	3/3	
	19	0/3	3/3	3/3	
	20	0/3	3/3	3/3	
	21	0/3	3/3	3/3	
	22	0/3	3/3	3/3	
	23	0/3	3/3	3/3	
A10	24	0/3	3/3	3/3	Error
	25	0/3	3/3	3/3	
	26	0/3	3/3	3/3	
	27	0/3	3/3	3/3	Error
	28	0/3	3/3	3/3	
	29	0/3	0/3	0/3	
	30	0/3	0/3	0/3	
	31	0/3	0/3	0/3	
	32	0/3	1/3	1/3	
	33	0/3	0/3	0/3	
	34	0/3	0/3	1/3	
	35	0/3	1/3	1/3	
	36	0/3	0/3	0/3	
	37	0/3	1/3	1/3	Stiff
	38	1/3	1/3	1/3	Stiff
	39	0/3	0/3	0/3	
	40	0/3	0/3	0/3	
	41	0/3	0/3	0/3	
	42	0/3	0/3	0/3	
	43	0/3	0/3	0/3	
	44	1/3	1/3	1/3	
	45	0/3	0/3	0/3	
	46	0/3	0/3	0/3	
	47	0/3	0/3	0/3	

Table S1. *Cont.*

Series B Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
B1	1	0/3	0/3	0/3	
	2	0/3	1/3	1/3	Stiff
	3	0/3	1/3	1/3	Stiff
	4	0/3	1/3	1/3	Stiff
	5	0/3	0/3	0/3	
	6	0/3	0/3	0/3	
	7	0/3	0/3	0/3	
	8	0/3	0/3	0/3	
	9	0/3	0/3	0/3	
	10	0/3	0/3	0/3	
	11	0/3	1/3	0/3	
	12	0/3	0/3	0/3	
	13	0/3	0/3	0/3	
	14	0/3	0/3	0/3	
	15	0/3	1/3	1/3	Error
	16	0/3	1/3	1/3	
	17	0/3	1/3	1/3	
	18	0/3	0/3	1/3	Stiff
	19	0/3	1/3	1/3	Error
	20	0/3	0/3	0/3	
B2	1	0/3	0/3	0/3	
	2	0/3	1/3	1/3	
	3	0/3	1/3	1/3	Error
	4	1/3	1/3	1/3	Stiff
	5	0/3	0/3	0/3	
	6	1/3	1/3	1/3	
	7	1/3	2/3	2/3	
	8	1/3	2/3	2/3	Stiff
	9	1/3	1/3	1/3	Stiff
	10	0/3	1/3	1/3	
	11	0/3	0/3	0/3	
	12	0/3	0/3	0/3	
	13	0/3	0/3	0/3	
	14	0/3	0/3	0/3	
	15	0/3	0/3	0/3	
	16	0/3	0/3	0/3	
	17	1/3	2/3	2/3	
	18	1/3	2/3	2/3	
	19	0/3	0/3	0/3	
	20	0/3	1/3	1/3	
	21	1/3	2/3	2/3	
	22	0/3	0/3	1/3	
	23	0/3	0/3	0/3	
	24	1/3	1/3	1/3	
	25	1/3	1/3	2/3	

Table S1. *Cont.*

Series B Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
B2	26	0/3	0/3	1/3	
	27	0/3	0/3	0/3	
	28	1/3	1/3	1/3	
	29	0/3	0/3	0/3	
	30	0/3	1/3	1/3	
	31	0/3	1/3	1/3	
	32	1/3	1/3	1/3	
	33	0/3	0/3	0/3	
	34	0/3	0/3	0/3	
	35	1/3	1/3	1/3	Stiff
	36	1/3	1/3	1/3	Black
	37	0/3	0/3	0/3	
	38	0/3	0/3	0/3	
	39	0/3	0/3	0/3	
	40	0/3	0/3	0/3	
	41	0/3	0/3	0/3	
	42	0/3	0/3	0/3	
	43	0/3	0/3	0/3	
	44	0/3	0/3	0/3	
	45	0/3	0/3	0/3	
46	0/3	0/3	0/3		
47	0/3	0/3	0/3		

* Death/Total.

Table 2. Second round of PtC screening to detect the insecticidal activity.

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
A5	10	-	-	4/4	Black
	11	1/4	1/4	2/4	error
	13	-	-	2/4	
	18	-	1/4	1/4	
	24	1/4	1/4	4/4	Black
	28	-	-	3/4	Black
	29	1/4	2/4	3/4	
	11	-	2/4	4/4	Gray
A6	13	1/4	1/4	4/4	Error
	20	1/4	1/4	2/4	
	21		4/4		Black
	28	-	1/4	2/4	Black
	29	1/4	1/4	2/4	Black
	39	-	1/4	3/4	Dark red
	45	-	2/4	4/4	Dark red
	48	-	-	3/4	Black

Table S2. *Cont.*

Series A Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 days	3 days	
A7	3	3/3	3/3	3/3	Brown
	5	2/3	2/3	3/3	Error
	11	-	-	-	Dark red
	15	-	-	-	
	16	-	-	-	Dark red
	18	-	-	-	Black
	19	-	-	-	Black
	22	2/3	2/3	2/3	Black
	26	-	-	-	Black
	27	1/3	1/3	1/3	Dark red
	38	-	-	-	Black
	39	-	-	1/3	
A9	2	-	3/3	-	Dark red
	5	-	-	-	
	8	1/3	1/3	1/3	
	16	-	-	-	
	19	-	-	-	
	20	-	2/3	3/3	Error
	21	-	-	-	
	30	-	-	-	
	37	-	-	-	
	41	1/3	2/3	2/3	
A10	30	-	1/3	3/3	Dark red
	31	-	1/3	3/3	Black
	32	-	3/3	3/3	Black
	33	-	1/3	3/3	Black
	34	-	-	3/3	Black
	35	-	3/3	3/3	Dark red
	36	-	-	3/3	Black
	37	-	1/3	3/3	Black
	38	-	-	1/3	Black
	39	1/3	1/3	2/3	Black
	40	2/3	2/3	3/3	Black

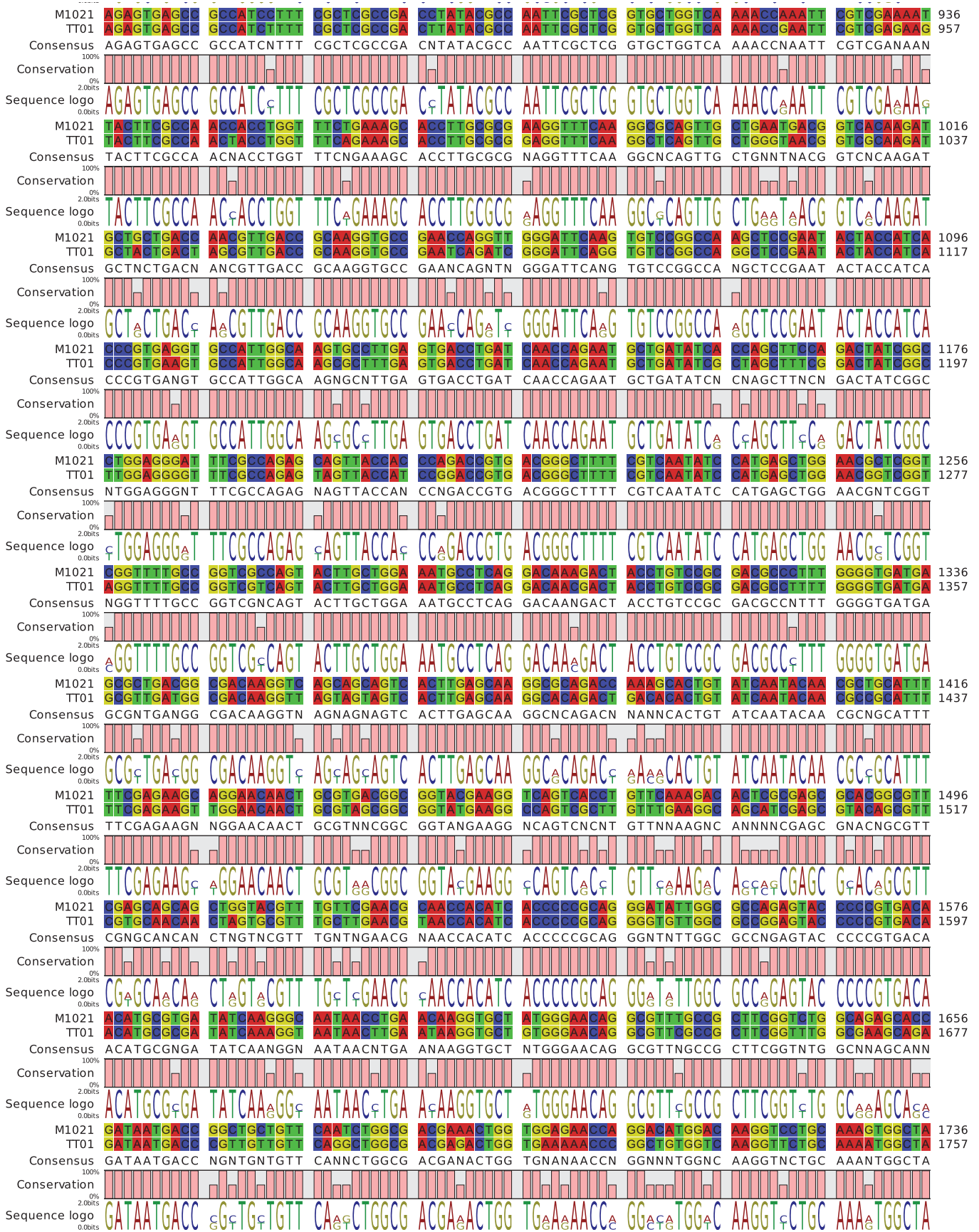
Table S2. Cont.

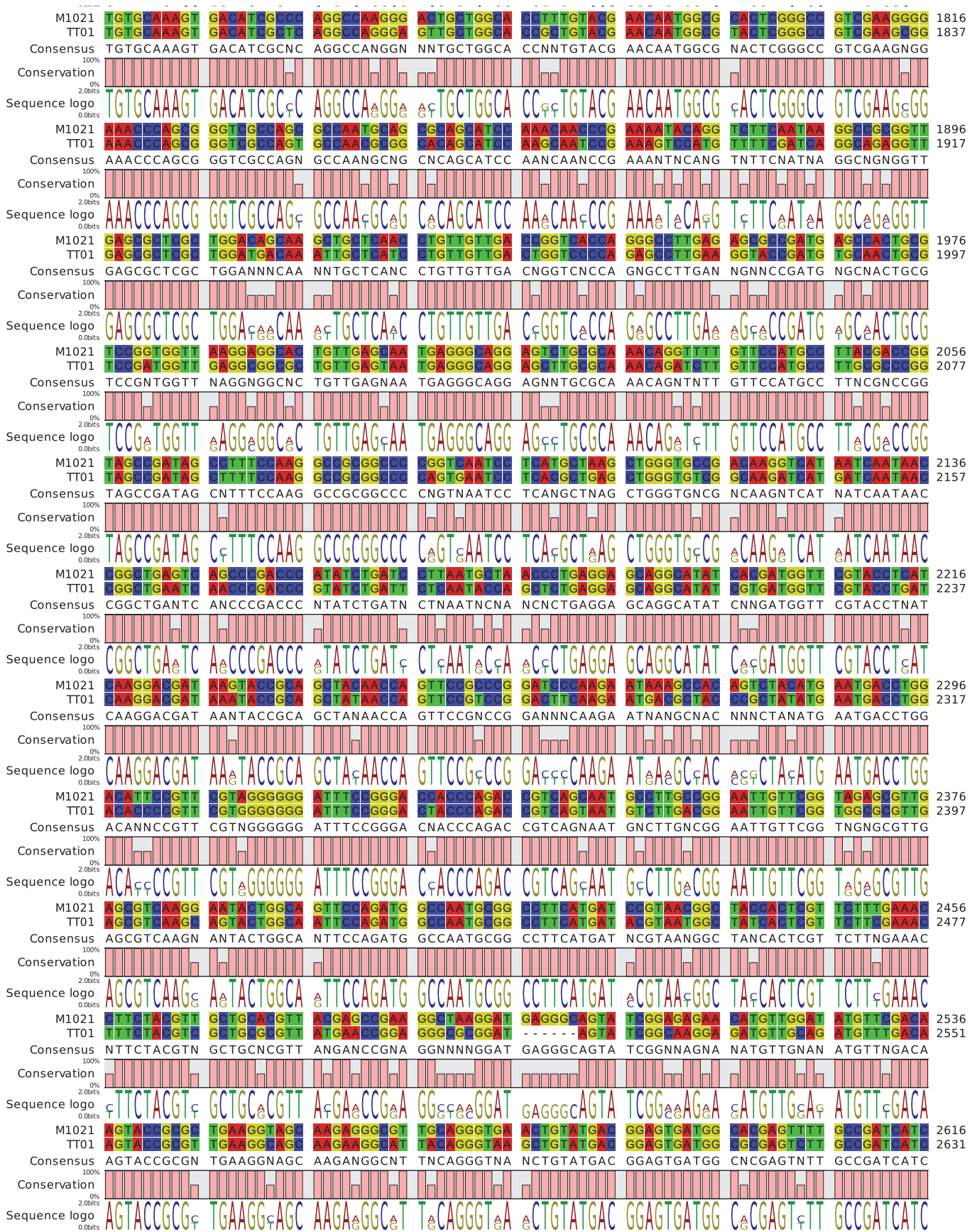
Series B Batch no.	Plate no.	Mortality of Larvae (Time in Days)			Phenotype of Larvae
		1 day	2 day	3 day	
B1	2	1/3	1/3	1/3	
	3	1/3	2/3	2/3	
	5	1/3	1/3	1/3	
	7	-	-	-	
	8	-	-	-	
	10	-	-	1/3	
	12	-	-	-	
	15	-	-	1/3	
	20	-	3/3	3/3	
	21	-	3/3	3/3	
	26	-	3/3	3/3	Stiff
	29	-	3/3	3/3	
	36	-	3/3	3/3	
	38	-	-	1/3	
40	-	1/3	1/3		
41	-	-	-		
B2	1	-	-	2/3	
	2	-	-	-	
	3	-	-	1/3	Black
	9	1/3	1/3	2/3	Dark red
	10	-	-	-	
	29	2/3	3/3		Black
	30	2/3	3/3	3/3	Dark red
	32	1/3	1/3	1/3	
	35	-	-	-	
	38	-	-	-	
42	-	-	-	Black	

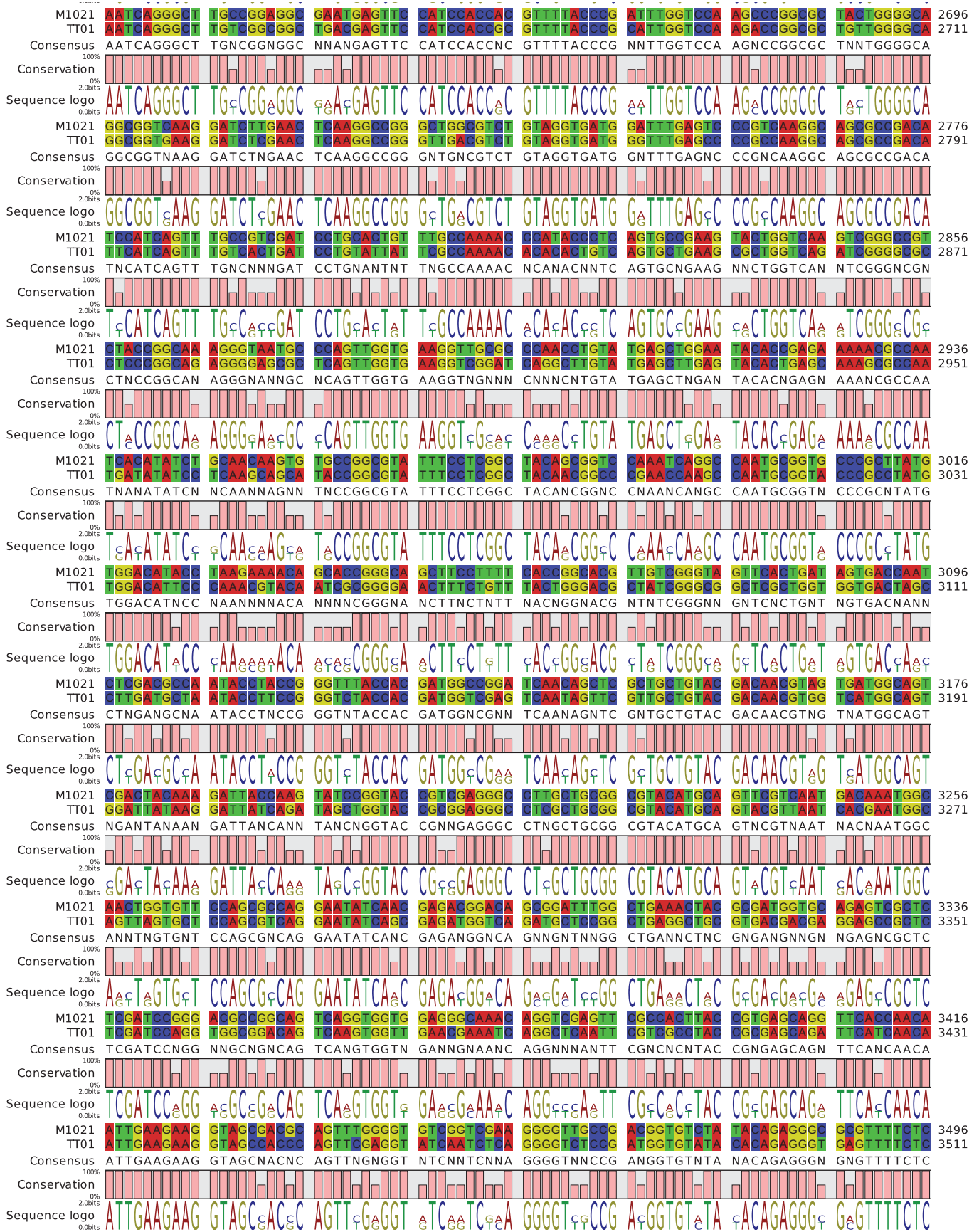
Note: The dash (-) mark represents that the injection cause no mortality in the larvae.

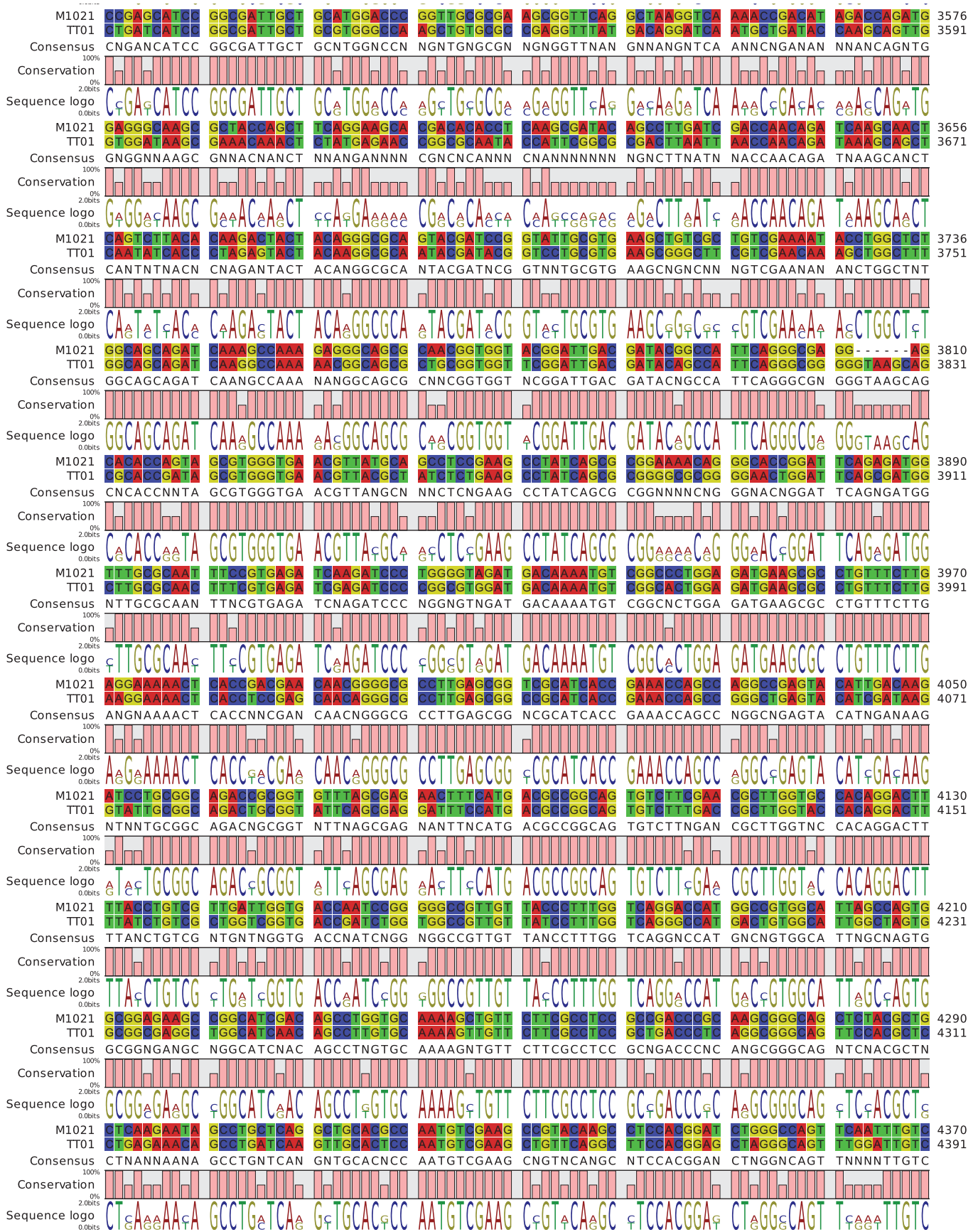
Figure S1. The putative toxin gene sequence from the *Photorhabdus temperata* M1021 was aligned against the *mcf* gene of *Photorhabdus luminescens* TT01 using a CLC genomics work bench version 7.0 alignment tool. The alignment revealed 85% of the sequence similarity and 98% of the query coverage, indicating that the gene isolated and sequenced from *P. temperata* M1021 was the *mcf* gene, encoding “makes caterpillars floppy” (Mcf) toxin.

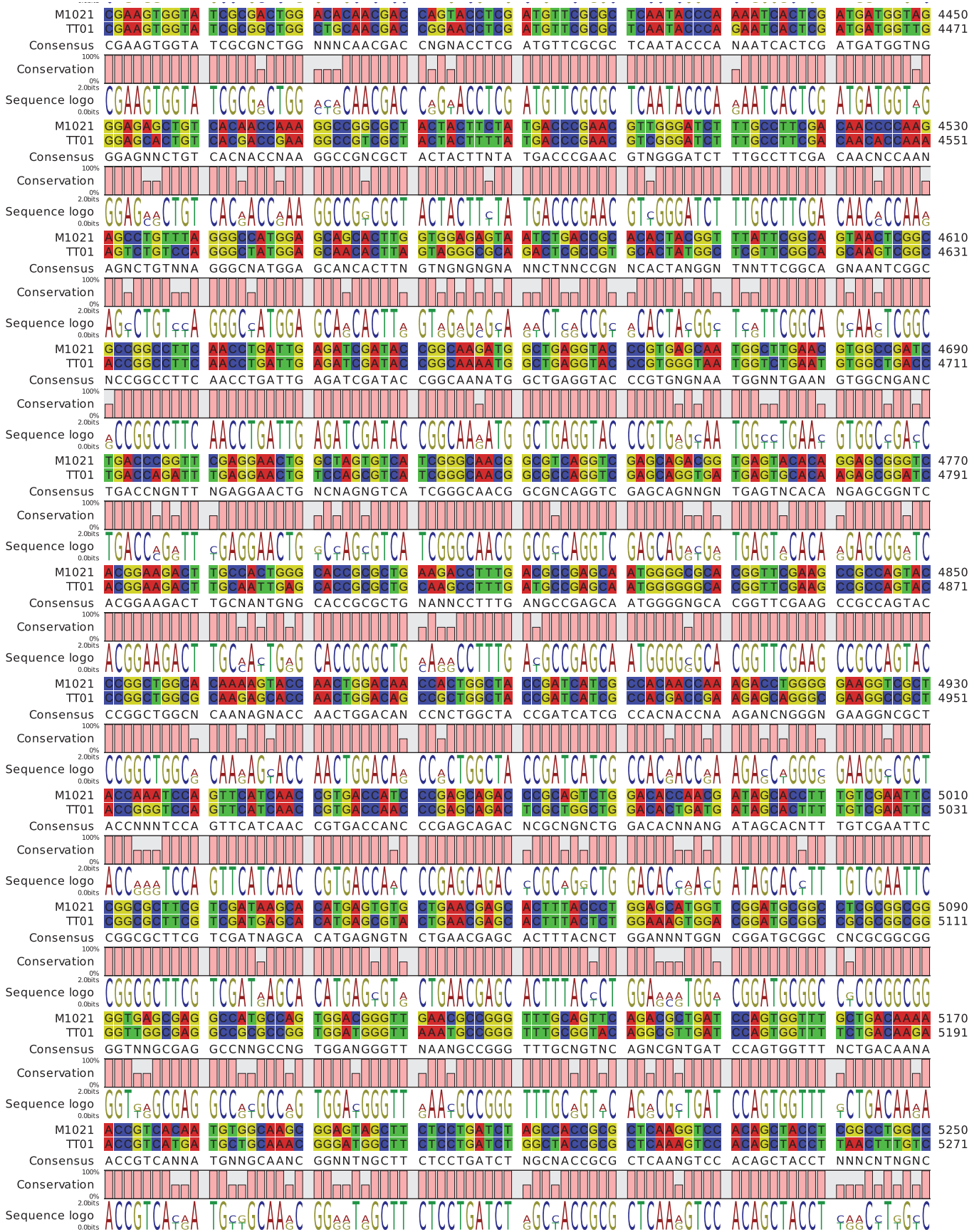


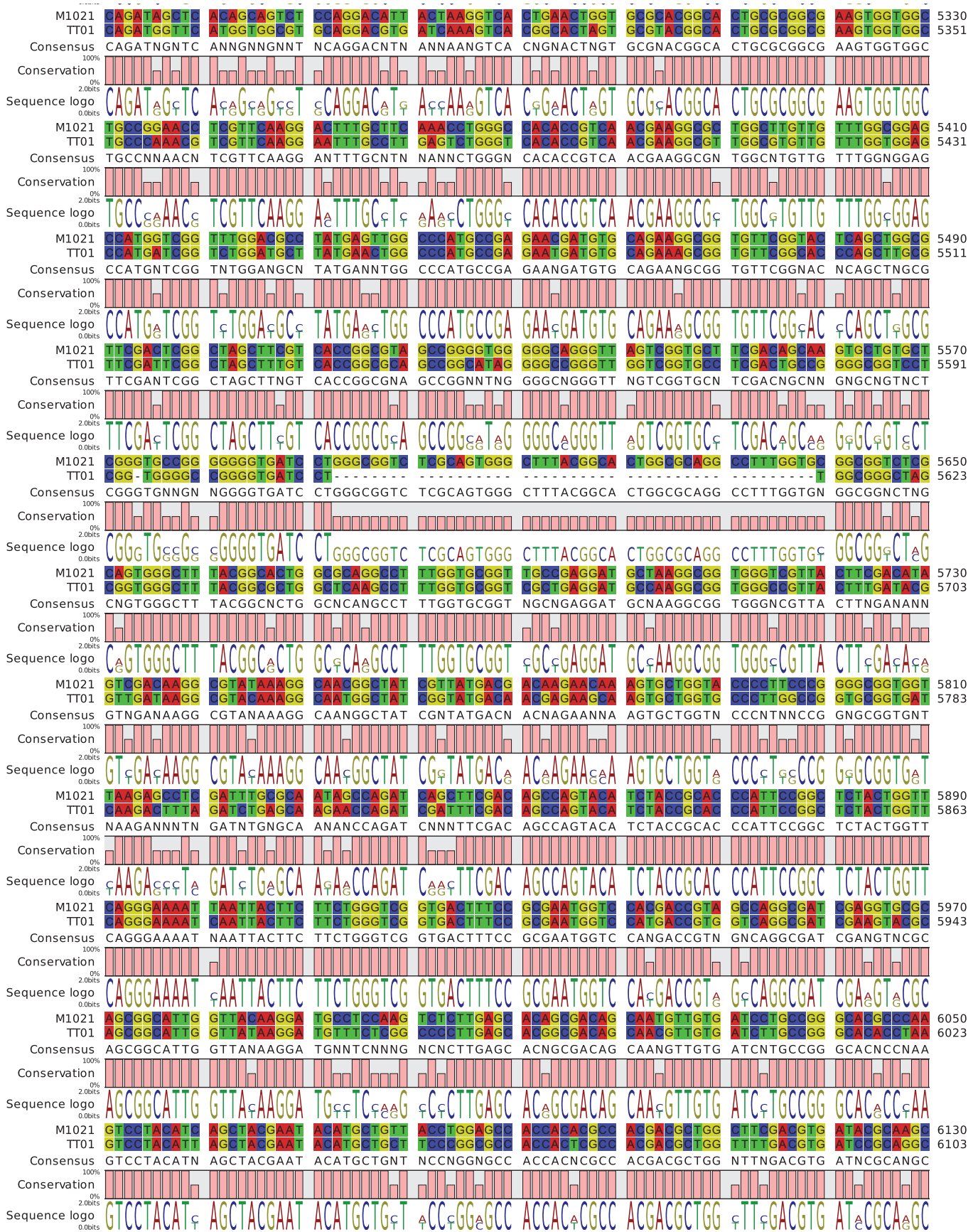


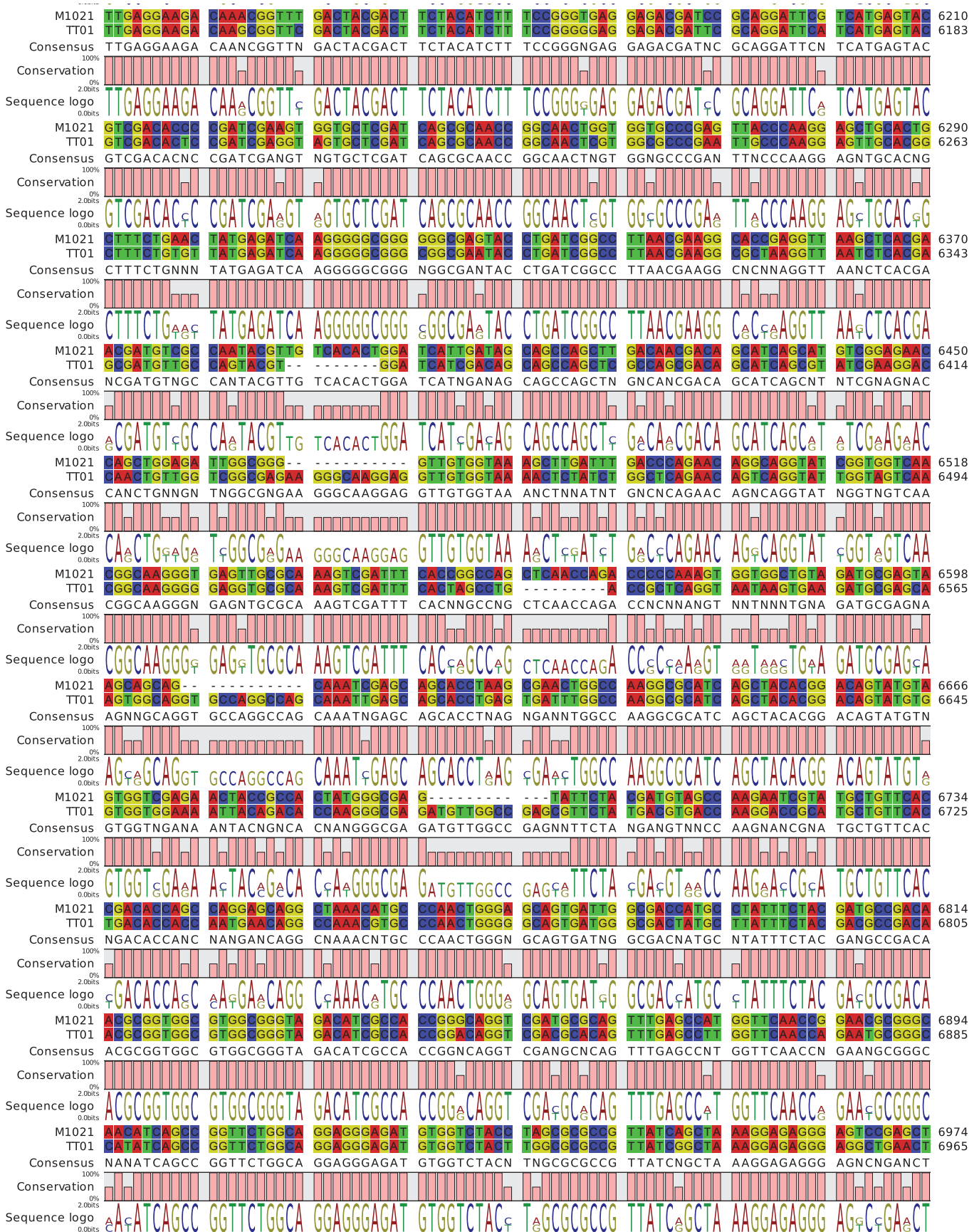


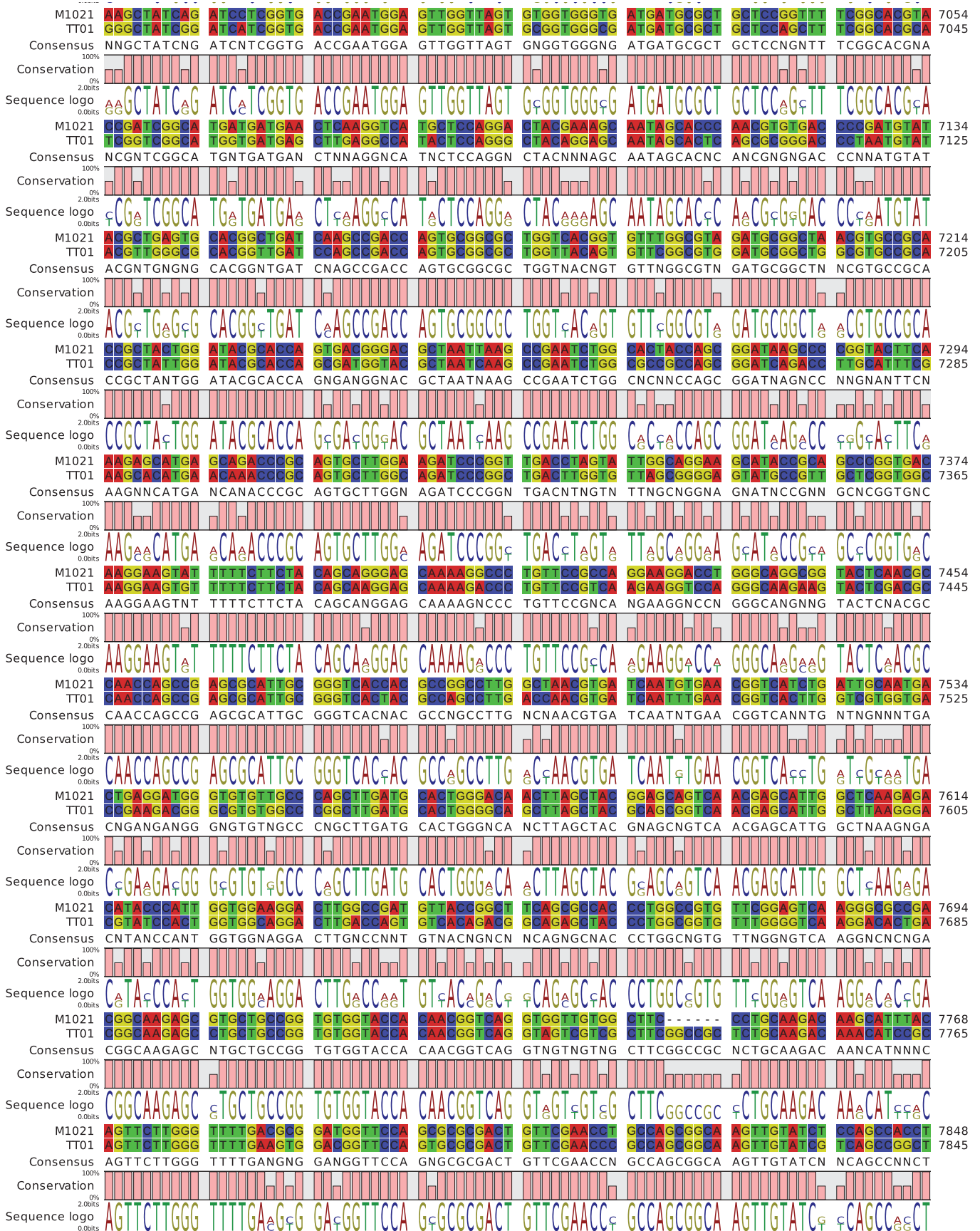


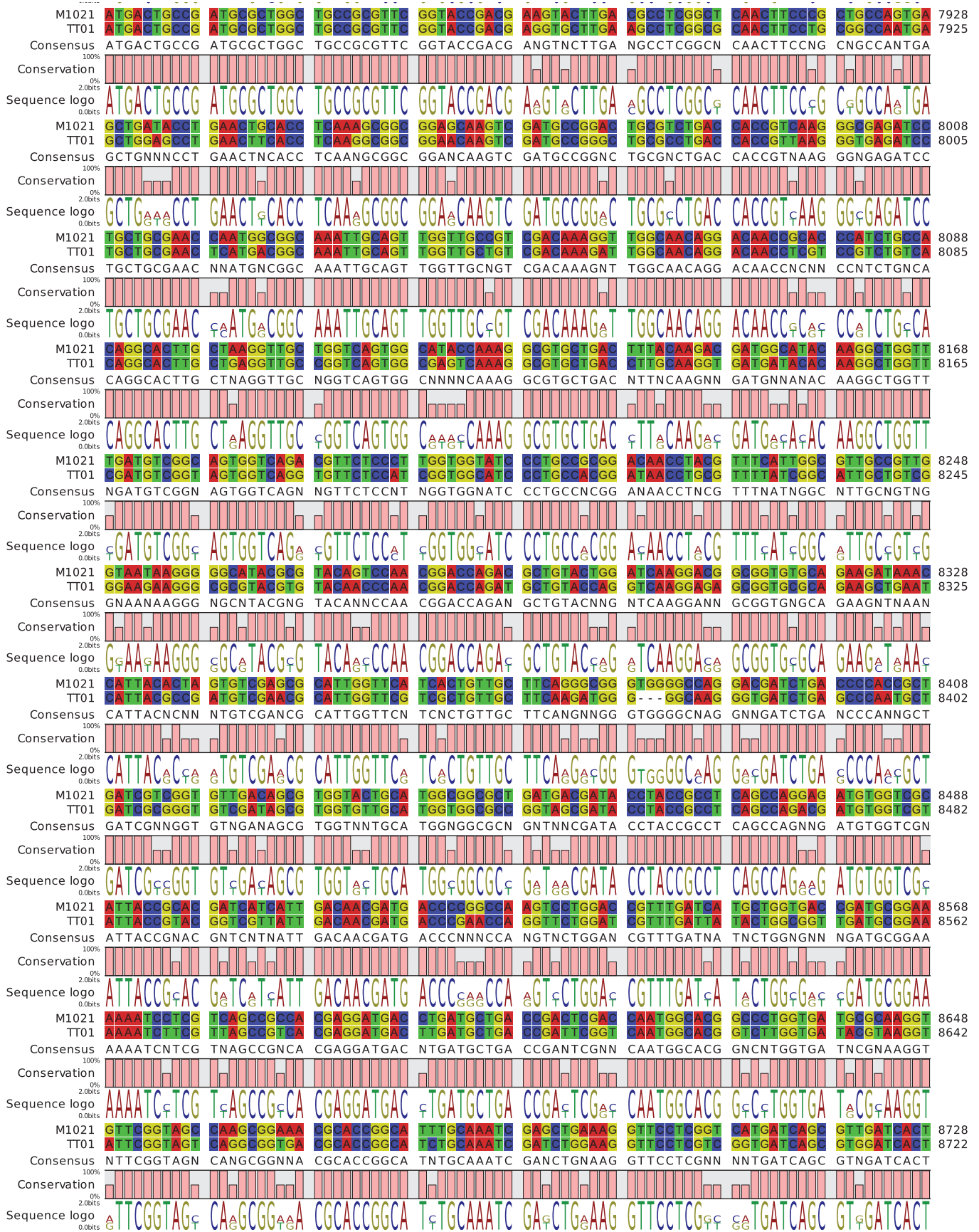












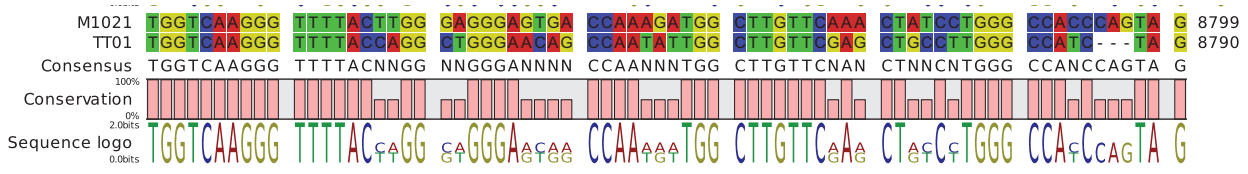
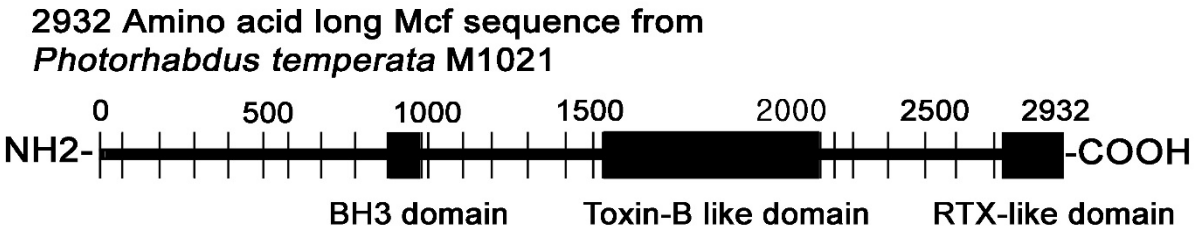


Figure S2. Diagrammatic presentation of domain structure of the Mcf insecticidal toxin from *P. temperata* M1021 and showing the relative location of three potential functional domains in the Mcf protein. **(I)** First, the N-terminus carries a BH3-like consensus motif, suggesting the protein may be pro-apoptotic; **(II)** A centrally located domain with similarity to the translocation domain of *C. difficile* toxin B; **(III)** The C-terminal domain carrying a sequence repeated in the C-terminus of ApxIVA from *Actinobacillus pleuropneumoniae*, an RTX-like toxin.



(I) BH3 domain consensus with Mcf

Mcf - LKAGLASVGDGFESR
 BH3- LXXXLXXVGDDLDLN

(II) Toxin-B domain similarity with Mcf

Toxin B-	861	(2)	.IESISDALCDLKQNELEDSEHIFISFEDISEIDE	GFSIRFIN	KETGESIFVETEKTIFSEYANHIT	926									
Mcf-	1614	(1)	.GARFEVASARLAQEHQLDSRWLPIIATTEDDQGE	(1)	.RYRVQFIN	(1)	.DQPEQTRWLIITSDSTFEVFRFRFD	681							
Toxin B-	927	EEI	SKIKG	(14)	.NLDTTHEVNTLNAAFFIQSLIE	(1)	.NS	(7)	.LSVAMGVQVYAQLFSTGLNITDAAKV	1007					
Mcf-	1682	EEM	AVLNE	(13)	.GVGEAAPVDGLNAGFAVQTLIQ	(9)	.AA	(7)	.LATALKVHSYLNLVQMAHGGVQDIARV	1769					
Toxin B-	1008	VELVSTA	(4)	.IDLLPTL	SEG	L	PIIATIIDGVSLGAAIKELSETSDPLLRQEI	EAKIGIMAV	1069						
Mcf-	1770	TALVRTA	(4)	.VVAAETS	LKD	(9)	.N	EGAGVLFGGAMVGLDAYELAHAENDVQKAVFGTQLAFDSA	1840						
Toxin B-	1070	NLTTATTAXI	(3)	.LGIASG	FSILLVPLAGISAGIPSLVNNELVLRDKATKVVVDYFQHVSLVTEGVF	1136									
Mcf-	1841	SFVTGAAGVG	(6)	.STAGAV	LGGASVILGGLAVGFTALAAQAFGAVAEADAKAVGRYFDIVDKAYKNGY	1910									
Toxin B-	1137	TLLD	(2)	.INMPQDDLVISEIDFNNSIVLKGCEIWR	(15)	.FF	SA	PSITYRE	(2)	.HLSIVDVL	1207				
Mcf-	1911	RYDN	(3)	.VLVPLAGAVIKSLDLRKHQISFDSOYIYR	(13)	.FF	(5)	.PR	MVHDRGO	AIEVRSIG	1984				
Toxin B-	1208	VQKZELDLS	(2)	.DLMVLPNAPNRV	FAWETGWTPLGRSLENDGKLL	DRIRDNY	(3)	.F	(3)	.YFA	1268				
Mcf-	1985	YKDISRPLE	(4)	.NVVILPGTIPKSY	ISYEYMLLPGATTIRHDAGFDVI	RRLEEDK	(2)	.F	(3)	.FYI	2046				
Toxin B-	1269	FIADALITLKPREDTINIRINLDSNTRSFIVPIITTEY	(1)	.REKLSYSFYGSGGTYALSLSQY	(4)	.NI	EL	1338							
Mcf-	2047	FPGEETIRRIHHEYVDTPIEVVDQRNRQLVVPPELPEL	HSEFLRYEIKGAGGEYLIGLNEG	(3)	.KL	TS	2114								
Toxin B-	1339	S	ES	(2)	.WIIDVD	(3)	.RDVTI	ESDKIKKGDIEGI	(7)	.ENKIL	(1)	.SHEIN	(26)	.1416	
Mcf-	2115	(2)	.A	SA	(3)	.WIIDSS	(2)	.ASDSI	(2)	.SKNQLVVGVVVEL	(6)	.QVLVN	(1)	.KGEVR	2169
Toxin B-	1417	EVDLLS	(3)	.KL	(2)	.SGELKILH	(1)	.NSNHQQ	KIDYIGFENSELQKNIPY	(6)	.GKEN	(5)	.STKEG	1482	
Mcf-	2170	EVDVAG	(2)	.TQ	(2)	.SEDAGKQ	(1)	.PGQQIEQ	HLSDLAKAHQLHGQYV	VENY	SHNGR	2223			
Toxin B-	1483	LFSVSELPDVLISKVYM	(1)	.DSKPSFGYYSNNLKDVKVITKDNVNIITGYLLKD	1534										
Mcf-	2224	DVGRAFYDVGKERMLFT	NTTHEQTRHAQLGAVMGDHAIFYDADNAAARVD	2274											

(III) RTX-like domain consensus with Mcf

Mcf-	2556	L A V F G V K G A D K S V L P V W Y H N G Q V V M A S L Q D K H L Q F L G F D A D G S S A R L F E P A S G K L Y L	2613
RTX-	483	L A G I T R K G A D A K S	530
Mcf-	2789	L L G G N G D	2843
RTX-	810	V L L G G A N D I L Y G S D G T N L F D G G V G N D K I Y G G K D I Y R M S K E Y G F H	866
Mcf-	2844	V L D R L I M L V T D A E K I L V S P H E D D L M L T D S T N G T	2876
RTX-	867	T L L S D L S F K V G F	896