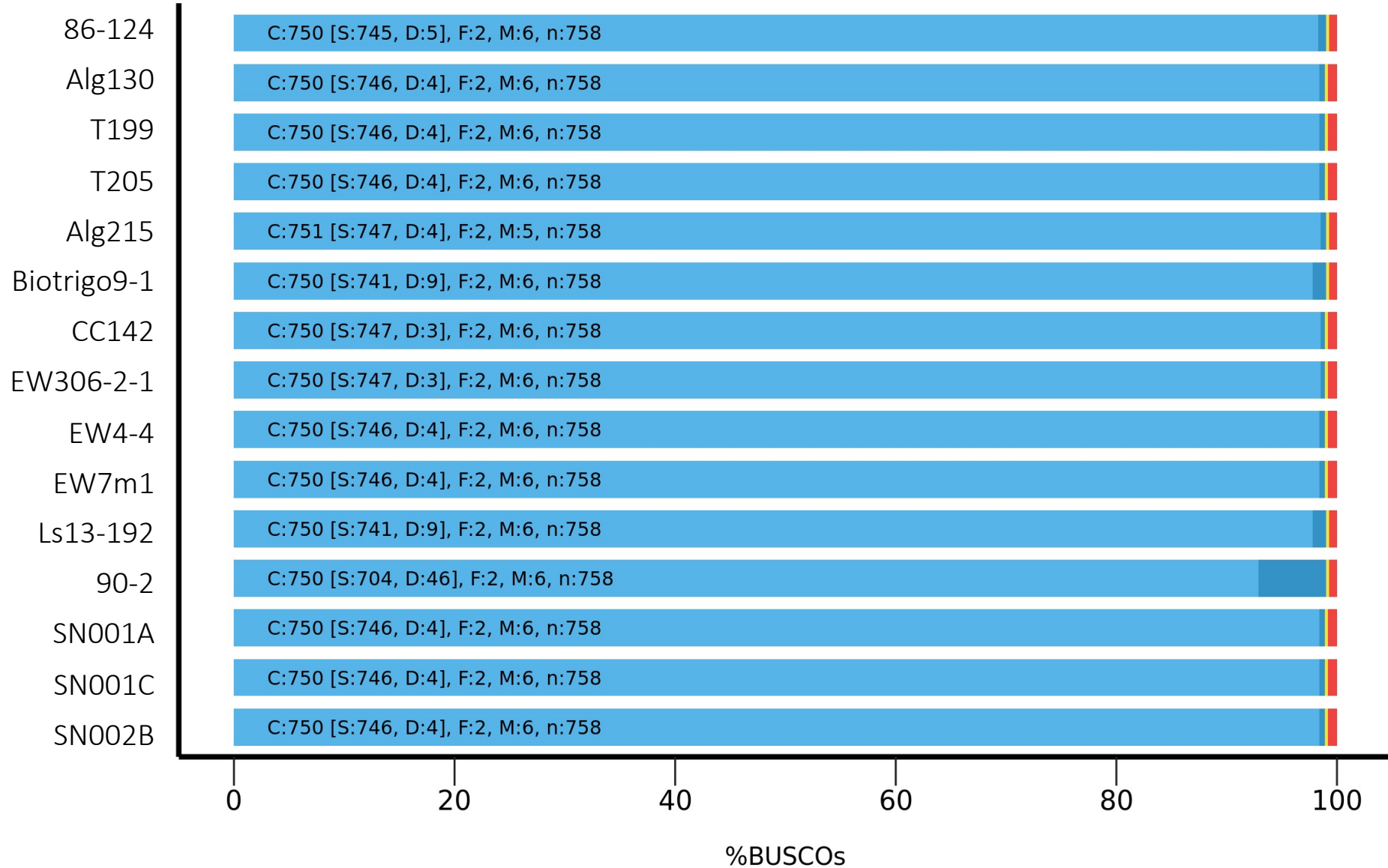
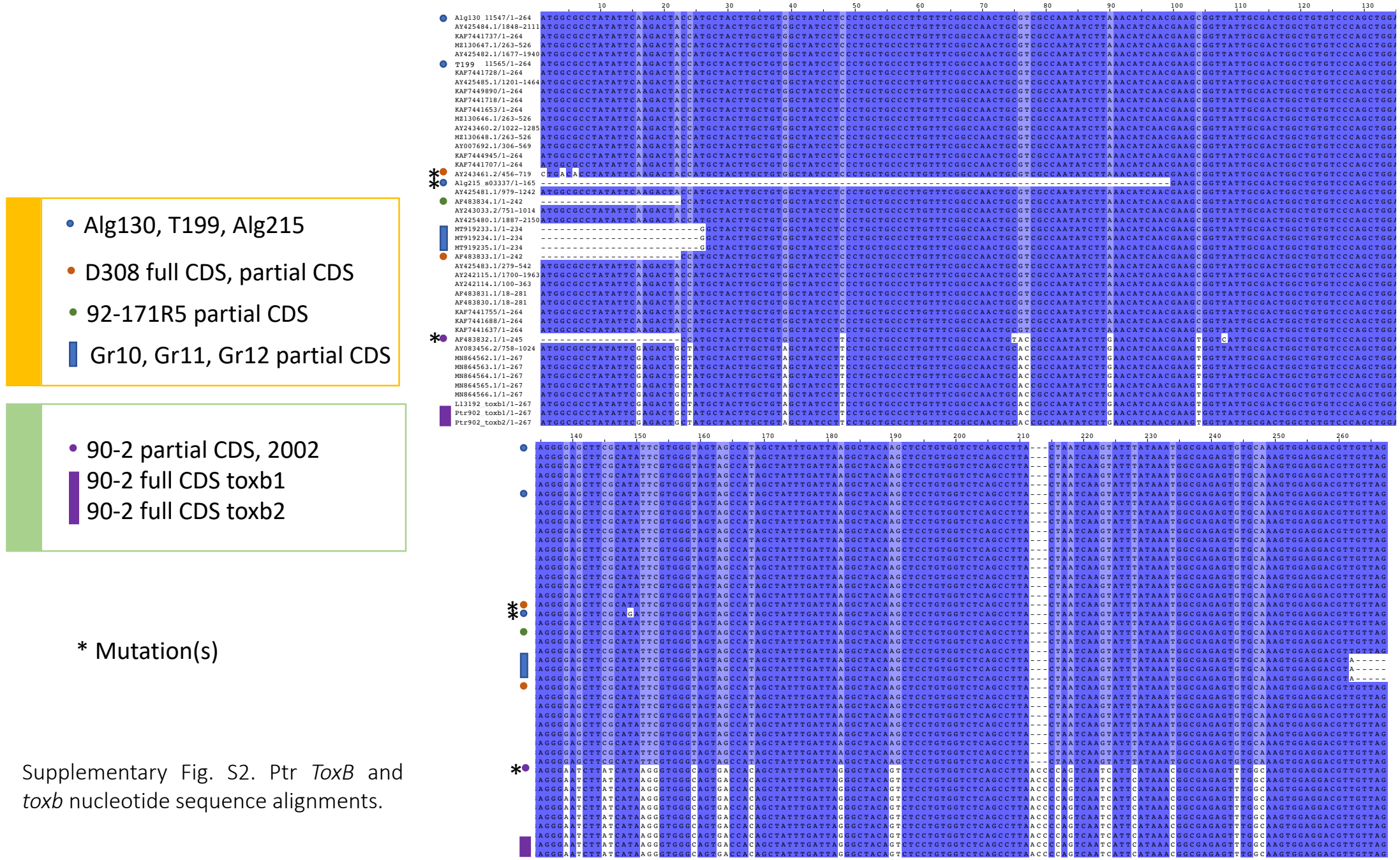


BUSCO Assessment Results



Supplementary Fig. S1. BUSCO quantitative assessment of the completeness of genome assemblies in terms of expected gene content.



- Alg130, T199, Alg215
- D308 full CDS, partial CDS
- 92-171R5 partial CDS
- Gr10, Gr11, Gr12 partial CDS

- 90-2 partial CDS, 2002
- 90-2 full CDS toxb1
- 90-2 full CDS toxb2

* Mutation(s)

Supplementary Fig. S2. Ptr *ToxB* and *toxb* nucleotide sequence alignments.

ToxB

toxb

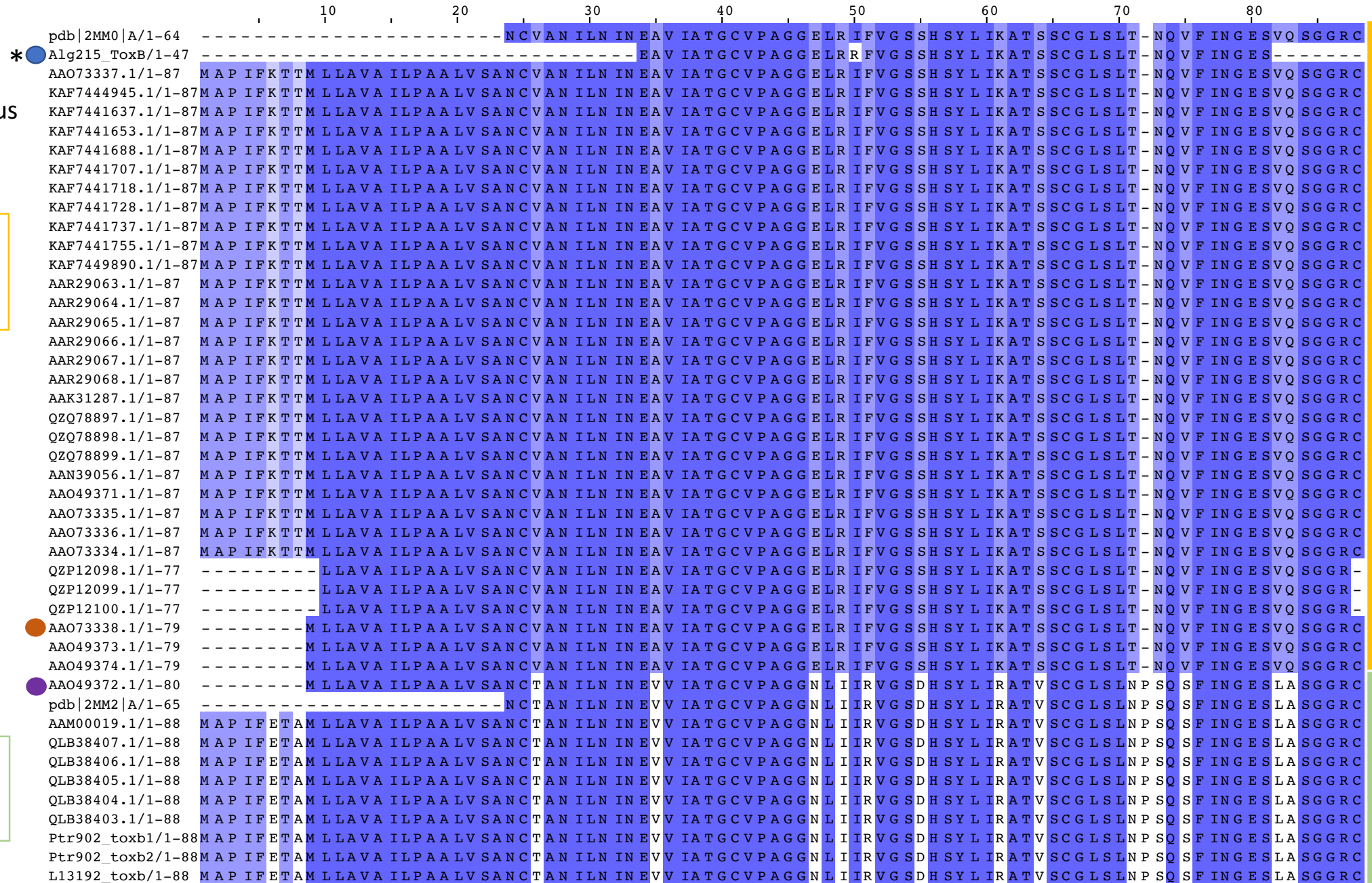
ToxB

toxb

*Non-synonymous Mutation

- Alg215
- D308 full CDS

- 90-2 partial CDS 2002



Supplementary Fig. S3. Ptr ToxB and toxB protein sequence alignments.

A

```

ToxB CDS/1-261 nt      1 ATGGCGCCTATATTTCAAGACTACCATGCTACTTGCTGTGGCTATCCTCCCTGCTGCCCTTGTTTCGG 67
Alg215_scaffold_03337/1-300  -----
68 CCAACTGCGTCGCCAATATCTTAAACATCAAC GAAGCGGTTATTGCGACTGGCTGTGTCCCAGCTGG 134
1 ----- GAAGCGGTTATTGCGACTGGCTGTGTCCCAGCTGG 35
          SNP T > G
135 AGGGGAGCTTCGCATATATTCGTGGGTAGTAGCCATAGCTATTTGATTAAGGCTACAAGCTCCTGTGGT 201
36 AGGGGAGCTTCGCAGATATTCGTGGGTAGTAGCCATAGCTATTTGATTAAGGCTACAAGCTCCTGTGGT 102

202 CTCAGCCTTACTAATCAAGTATTTATAAATGGCGAGAGTGTGCAAAGTGGAGGACGTTGT ----- 261
103 CTCAGCCTTACTAATCAAGTATTTATAAATGGCGAGAGTGTGCAAAGTGGAGGACGTTGT TAGTAAA 169

-----
170 CAGAGTTTAGGCGCTACAAGATTACTACATAGTAAAGTAGCCCTACATTAGGTATAGGGGTTTTTTTA 236

-----
237 TCTGGC ATAGCACAGTTTTCTCTTAATTCAACCTATTGTACCCTTAGTTAAACGACACGTA CT 300

```

B

```

                                Cleavage site
                                ▼
          Signal peptide 1-22 aa
          ┌──────────────────────────┴──────────────────────────┐
ToxB CDS/1-87 aa      1 M A P I F K T T M L L A V A I L P A A L V S A N C V A N I L N I N E A V I A T G C V P A G G E L R I F V G S S H S Y L I K A T S S C G 67
Alg215_scaffold_03337/1-87 1 ----- E A V I A T G C V P A G G E L R R F V G S S H S Y L I K A T S S C G 34

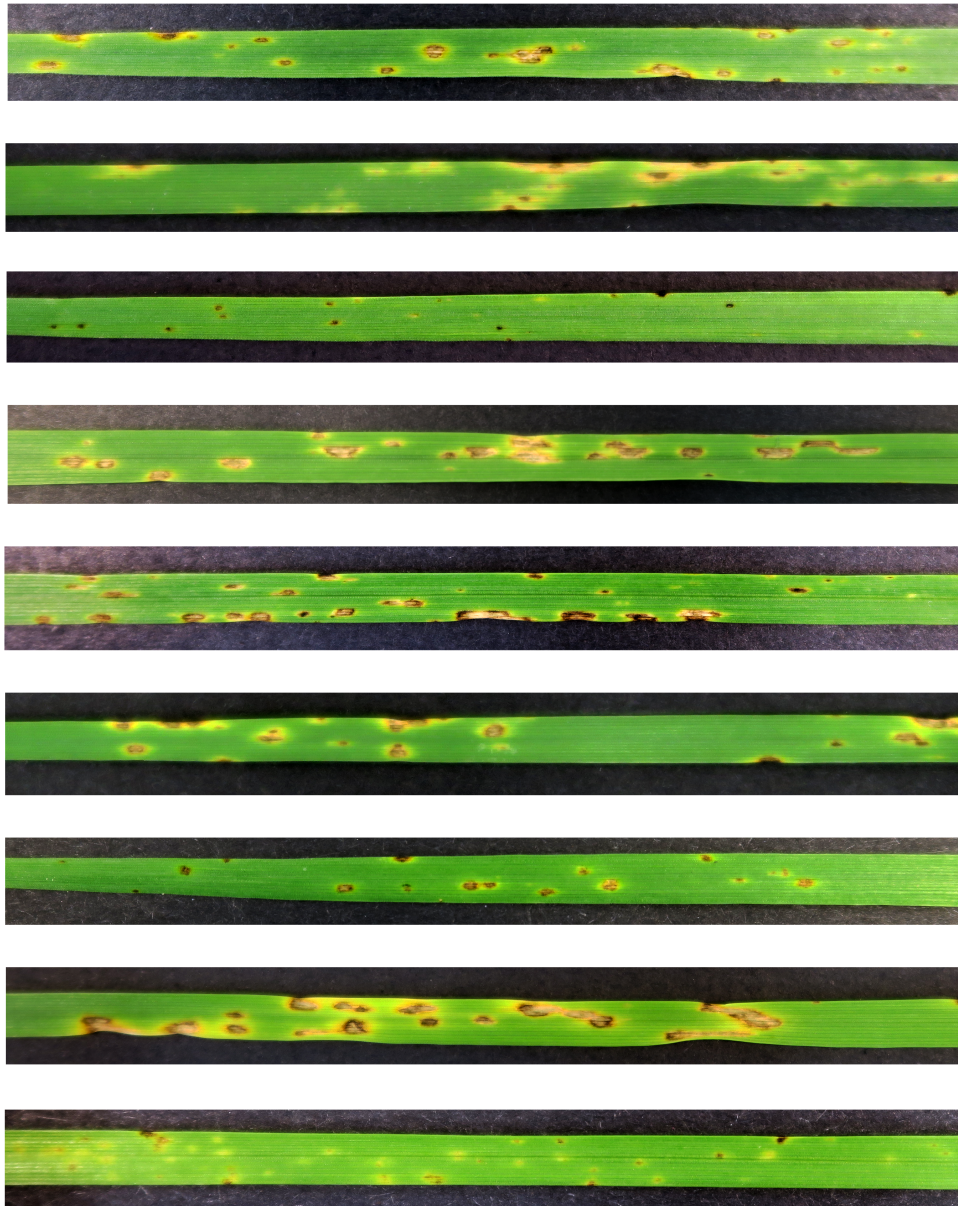
68 L S L T N Q V F I N G E S V Q S G G R C ----- 87
35 L S L T N Q V F I N G E S V Q S G G R C T E F R R Y K I T T S S P T L G I G V F Y L A H S F P L N S T Y C T L S T T R T 94

                                Nonsynonymous change
                                I > R

```

Supplementary Fig. S4. Ptr Alg215 isolate partial *ToxB* sequence alignments show Alg215 *ToxB* is truncated at the 5' end of the sequence. A) The first 162 nucleotide bases of Alg215 scaffold 03337 sequence aligned to the *ToxB* coding sequence (CDS) (99-261 bp). A *ToxB* single nucleotide polymorphism (SNP; at the 149 bp position) shows a thiamine nucleotide change to guanine (T > G). B) The Alg215 *ToxB* region protein translated (1-94 aa) aligned to the *ToxB* protein sequence (1-87 bp). A nonsynonymous amino acid residue change (I>R) at *ToxB* residue position 50 is beyond the *ToxB* signal peptide cleavage site between amino acid positions 23 and 24.

Wheat line Glenlea (ToxA-sensitive)



Alg130

T199

T205

Alg215

CC142

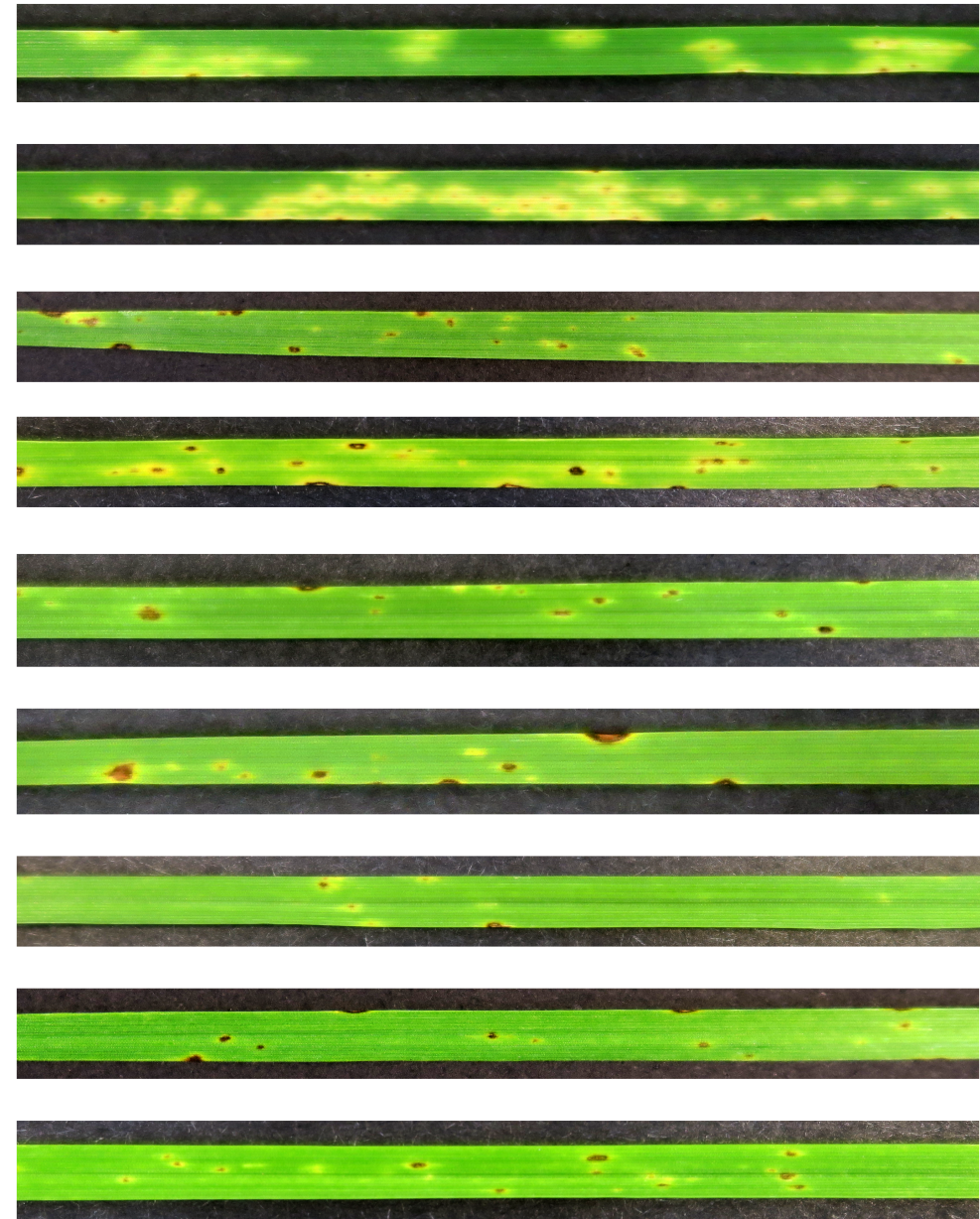
EW4-4

EW7m1

EW306-2-1

SN001A

Wheat line 6B662 (ToxB-sensitive)



Alg130

T199

T205

Alg215

CC142

EW4-4

EW7m1

EW306-2-1

SN001A

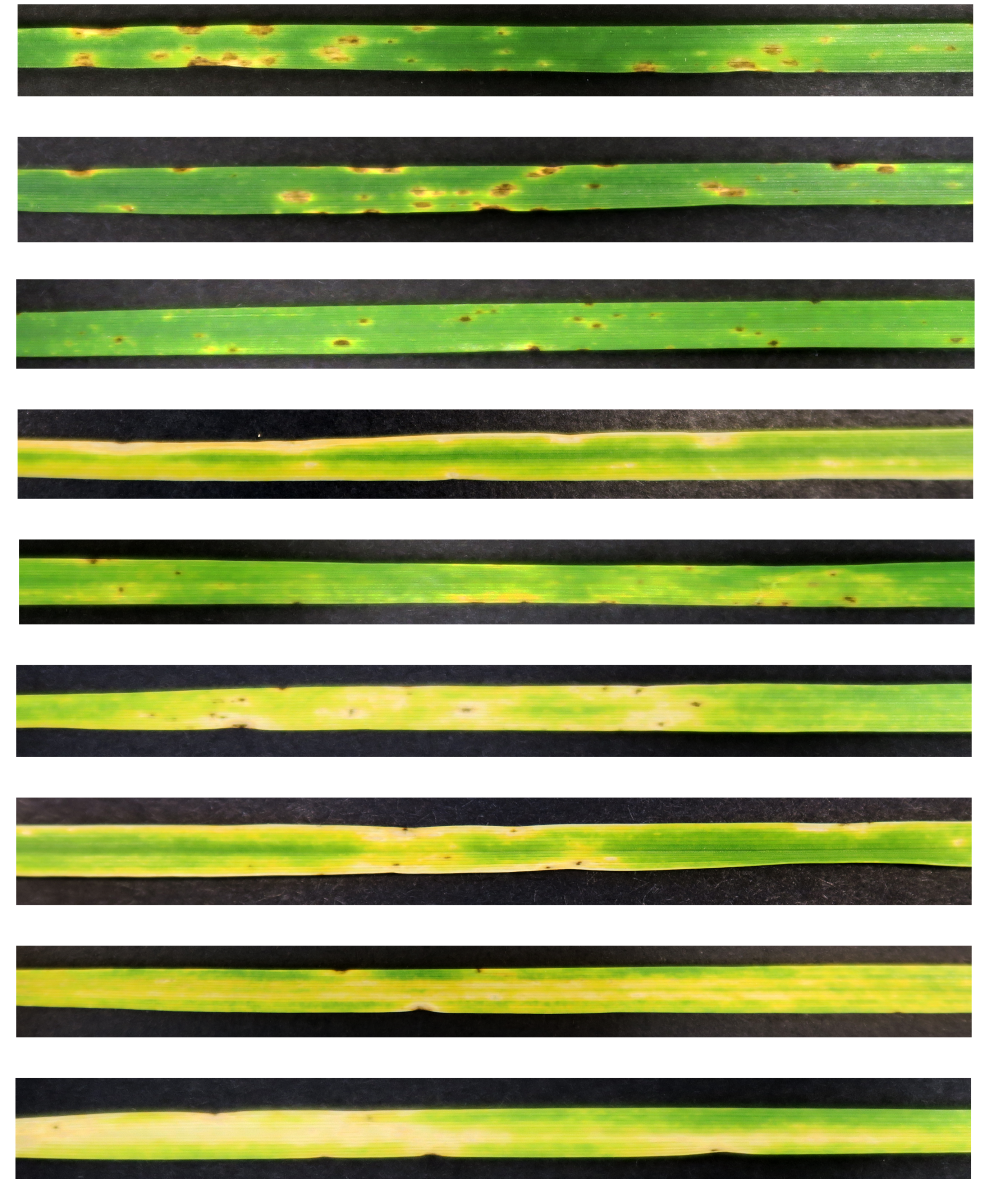
Supplementary Fig. S5. Ptr plant leaf infection assays to identify isolate ToxA production by the development of necrosis symptoms on the differential wheat cultivar Glenlea (left hand side) and ToxB production by chlorosis symptoms on the differential wheat line 6B662 (sensitive) (right hand side).

Wheat line Auburn (insensitive all 3 effectors)



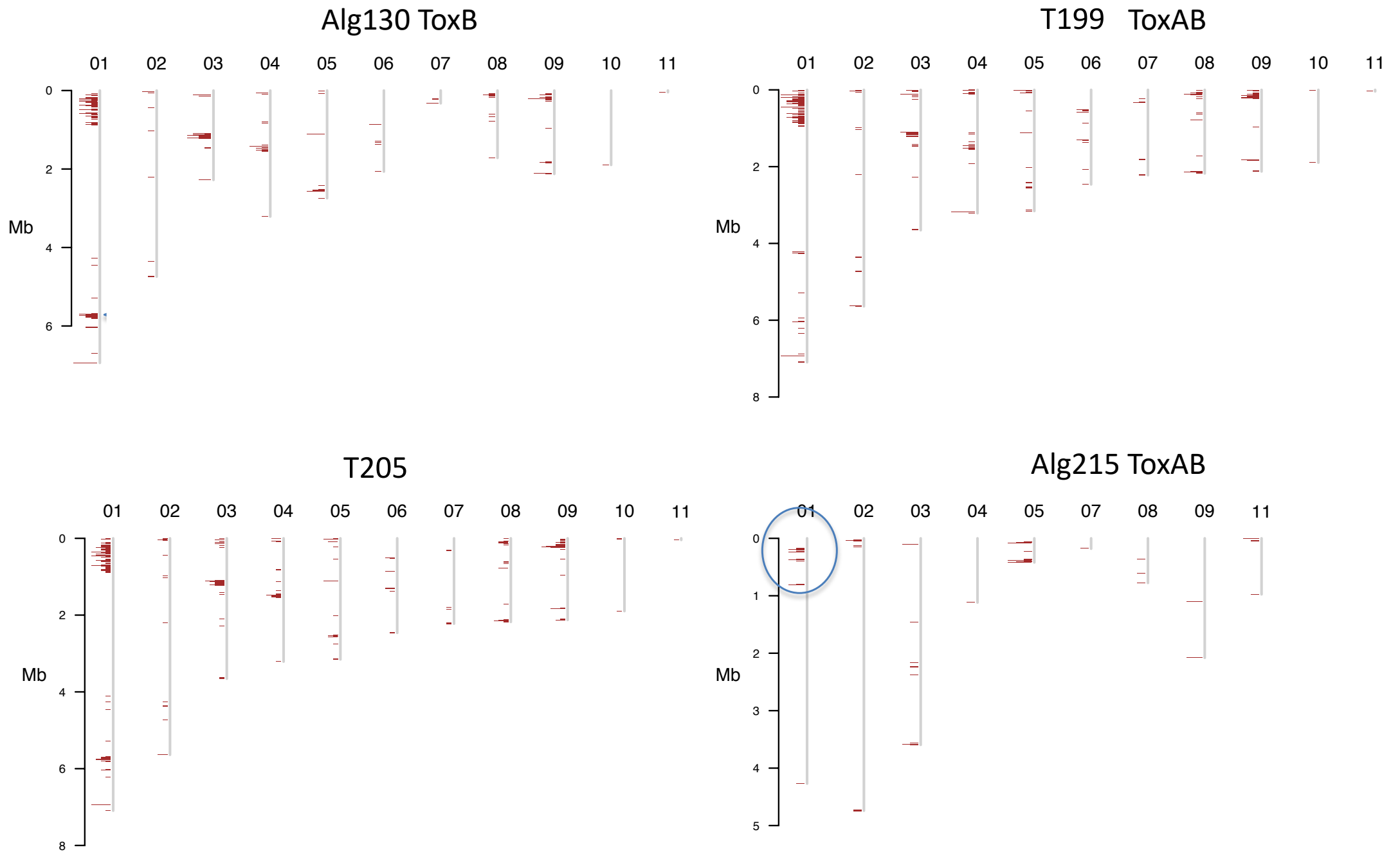
Alg130
T199
T205
Alg215
CC142
EW4-4
EW7m1
EW306-2-1
SN001A

Wheat line 6B365 (ToxC-sensitive)

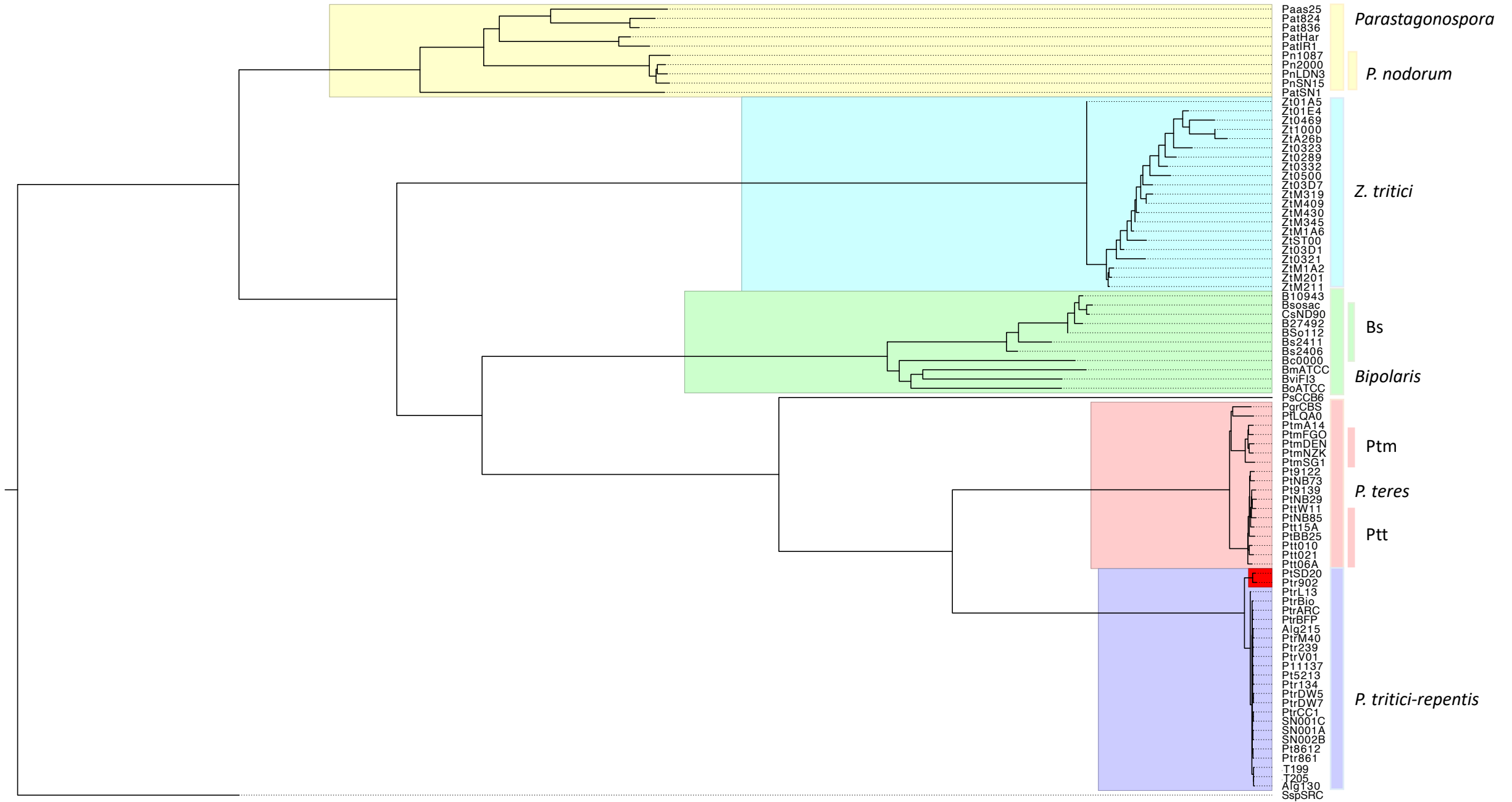


Alg130
T199
T205
Alg215
CC142
EW4-4
EW7m1
EW306-2-1
SN001A

Supplementary Fig. S6. Ptr plant infection assays to confirm no symptoms on response to the differential wheat line Auburn (insensitive) (left hand side) and ToxC production by chlorosis symptoms on the differential wheat line 6B365 (right hand side).

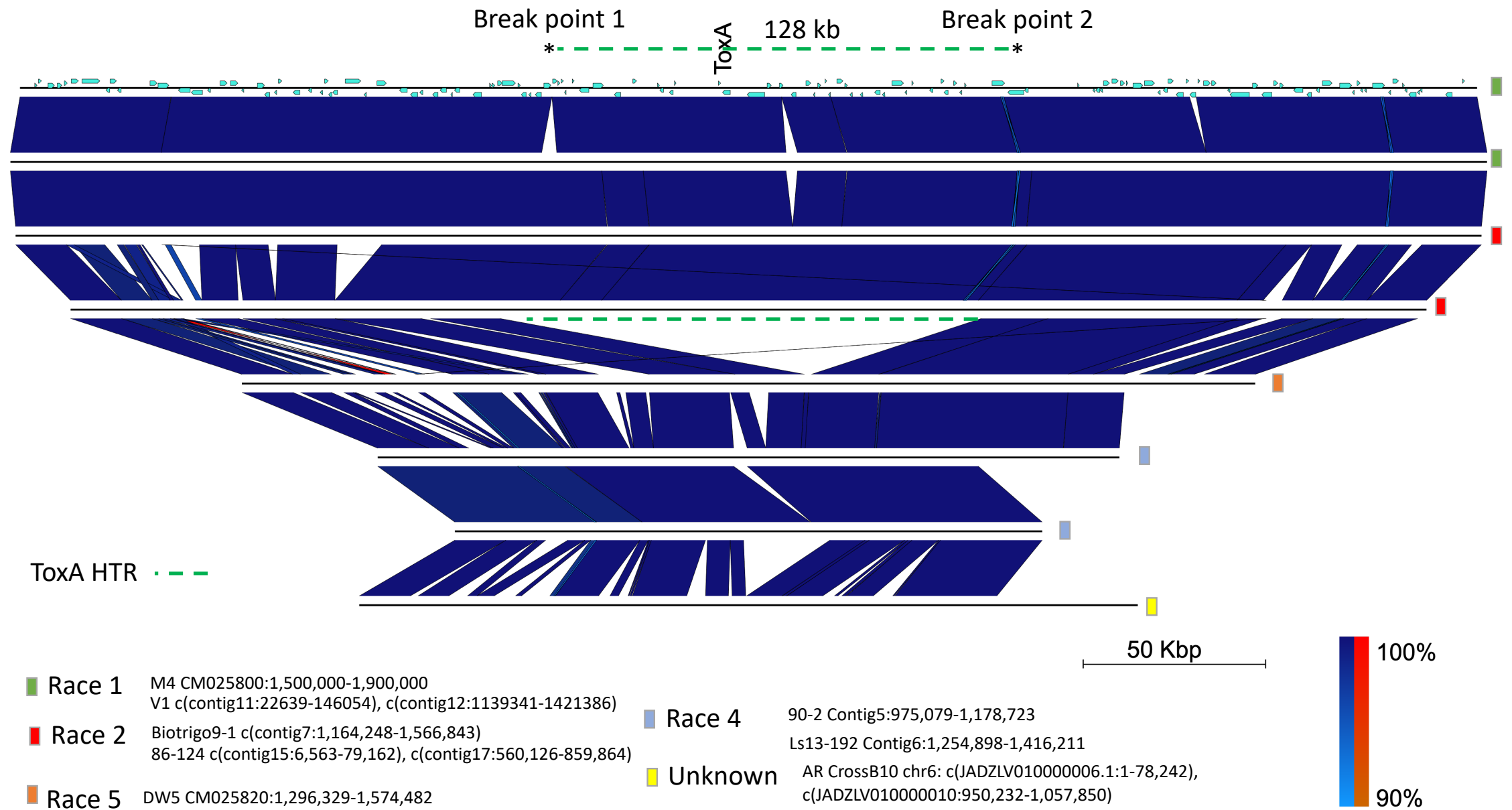


Supplementary Fig. S7. Closer examination a large 1 Mb distal region on isolate M4 contig 1 and many smaller regions were absent in isolates Alg130, T199 and T205 but present in Alg215.



0.02

Supplementary Fig. S8. Phylogenetic analysis of publicly available ascomycete genomes downloaded from NCBI.

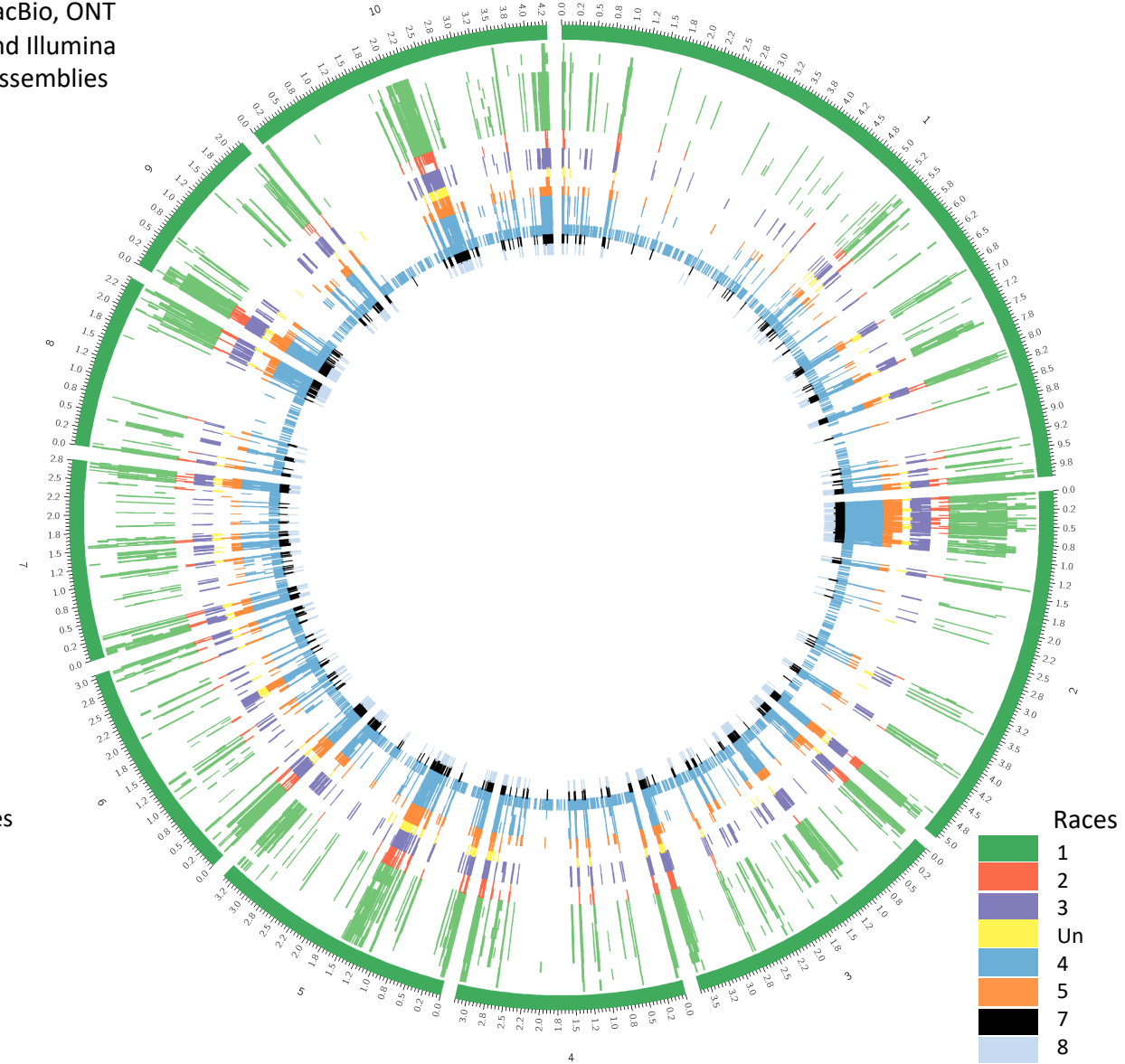


Supplementary Fig. S9. Ptr isolate M4 ToxA horizontal transfer and flanking genomic region (400 kb) alignments for race 1, 2, 4, 5 and unknown races. Break points are displayed for the large 128 kb insertion in the M4 isolate. The ToxA horizontal transfer region is absent in all non-ToxA producing races (race 4, 5 and unknown) and present in ToxA producing races (race 1 and 2).

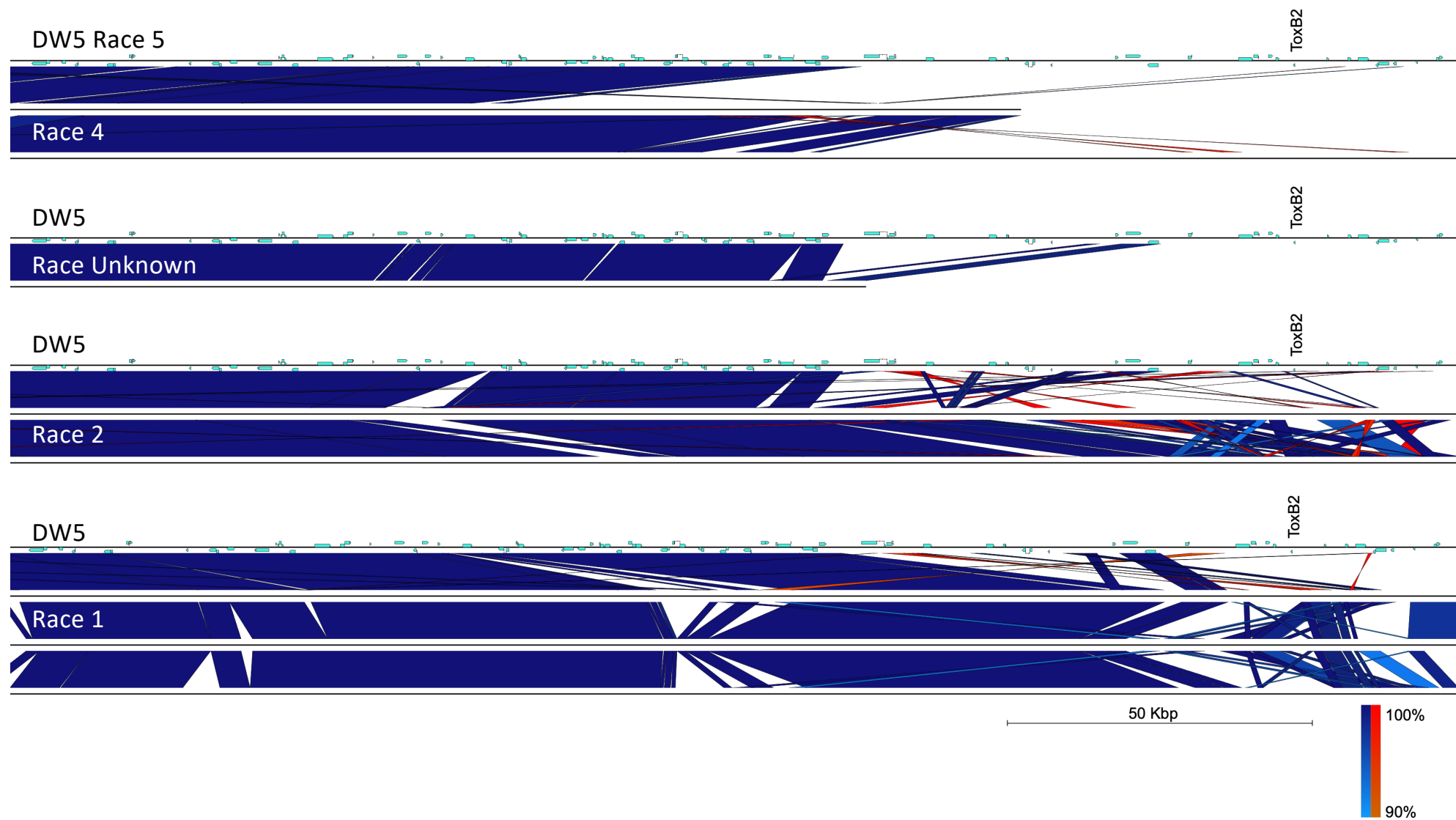
PacBio and
ONT
assemblies



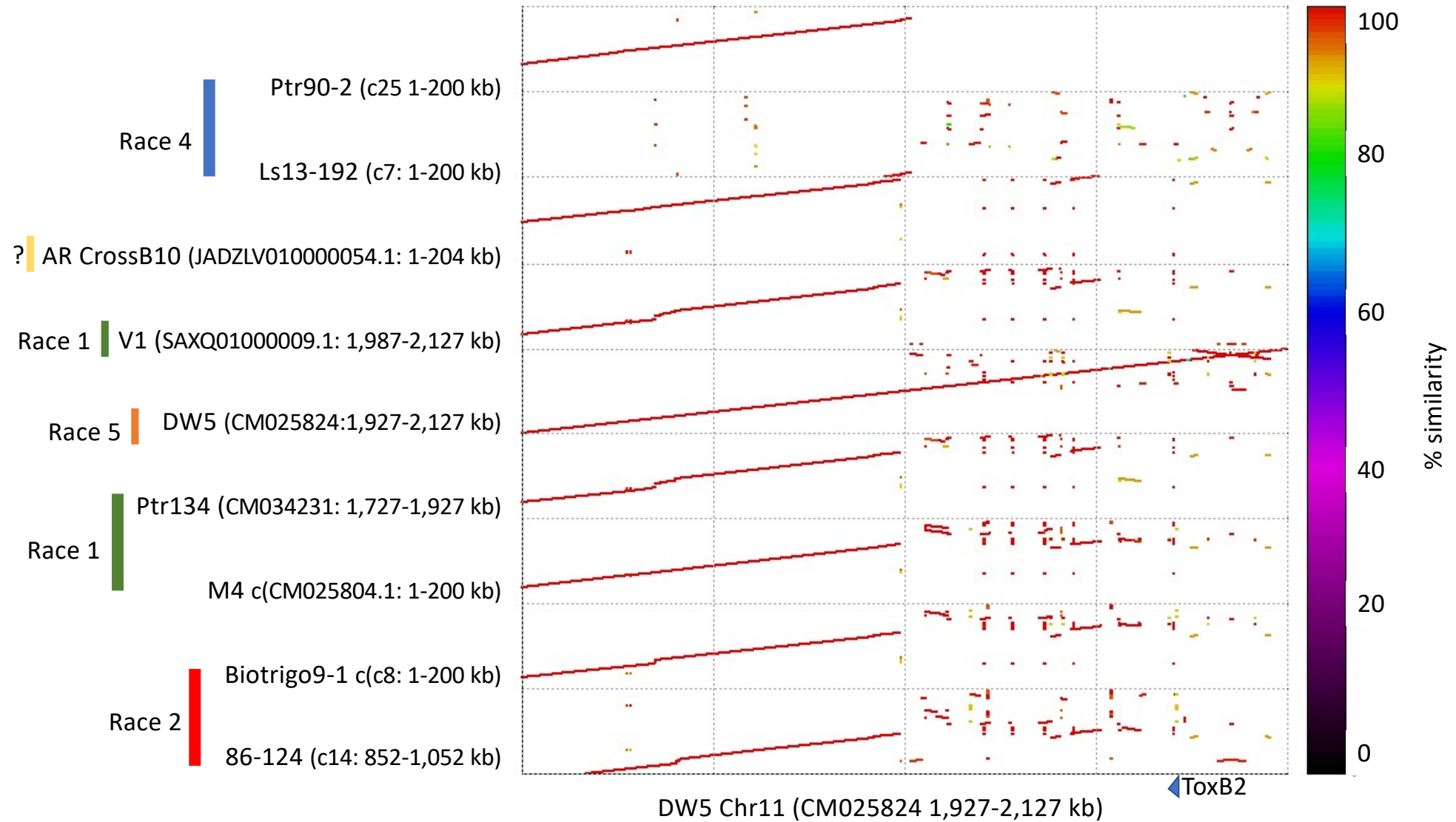
PacBio, ONT
and Illumina
assemblies



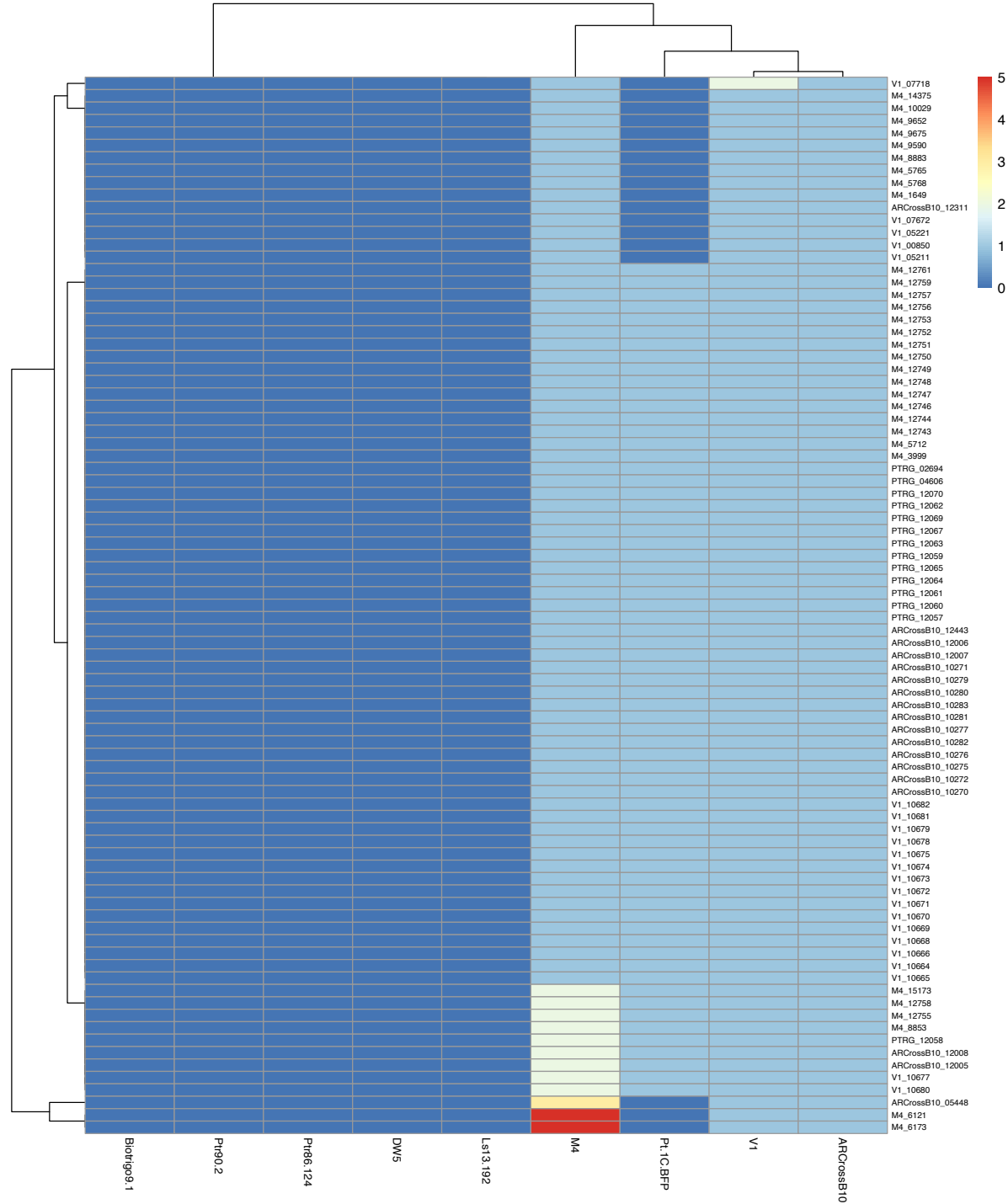
Supplementary Fig. S10. Circular plots show 10 kb regions of absence for *Ptr* isolates as compared to M4, coloured by race. The left plot shows assembled isolate genomes sequenced from long-read technologies, PacBio and Oxford Nanopore Technology. The right plot displays all the genomes.



Supplementary Fig. S11. DW5 chromosome 11 *ToxB2* and flanking genomic region alignments for race 1, 2, 4, 5 and unknown races. **Slide 1**, EasyFig Blastn alignments for 400 kb region. Top to bottom DW5 aligned to race 4 (90-2 and Ls13-192), race unknown (AR CrossB10), race 2 (86-124 and Biotrigo9-1) and race 1 (V1, M4 and Ptr134).

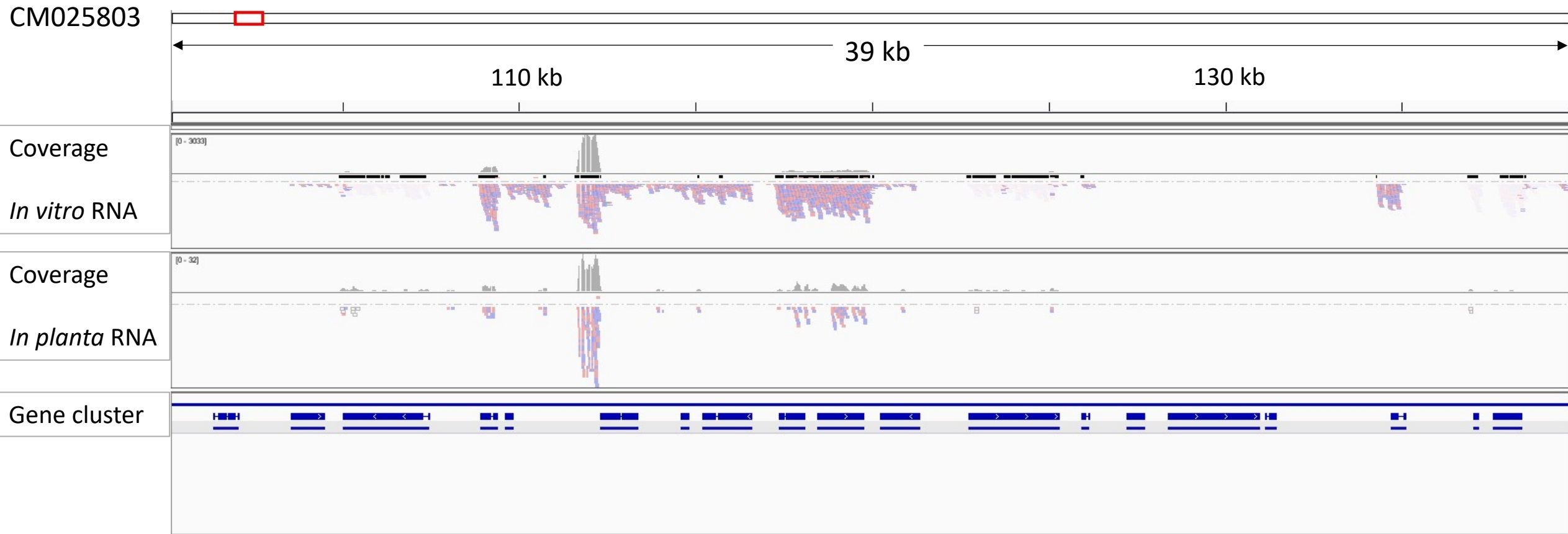


Supplementary Fig. S11. DW5 chromosome 11 *ToxB2* and flanking genomic region alignments for race 1, 2, 4, 5 and unknown races. **Slide 2**, NUCmer sequence dot plot for 200 kb region.

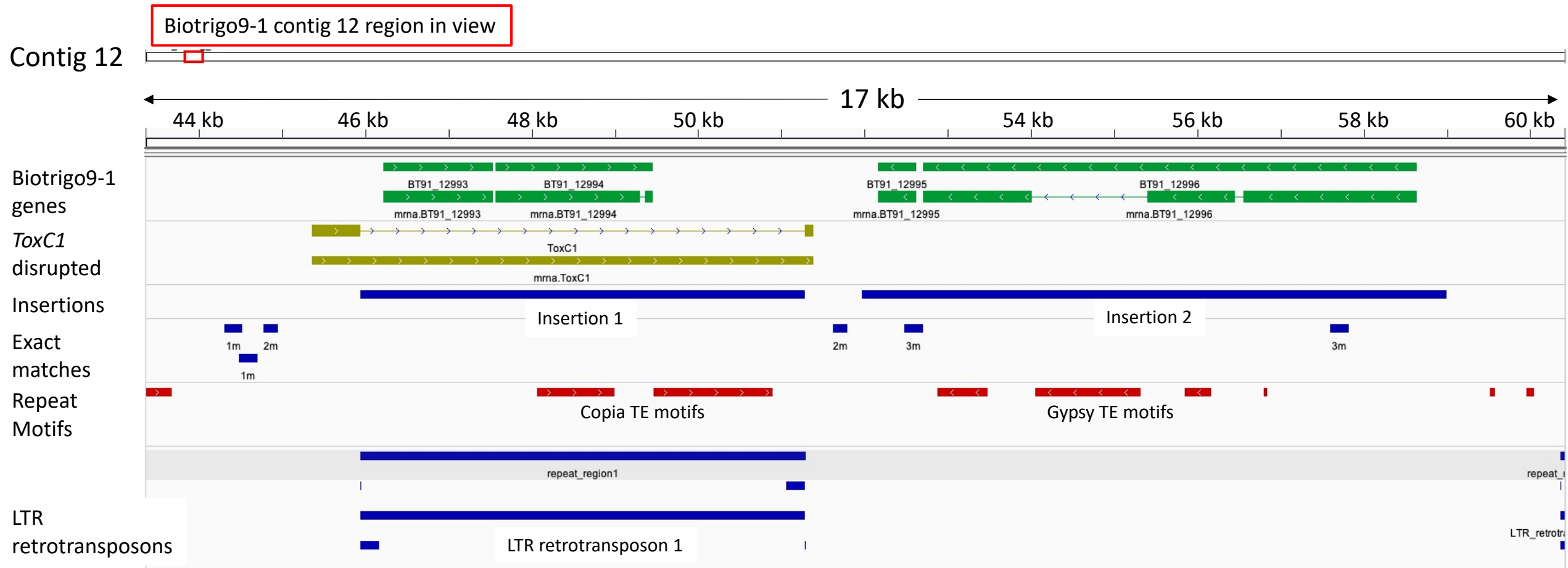


Supplementary Fig. S12. Ptr loci found only in PacBio sequenced ToxC-producing isolates. Loci are absent in non-ToxC producing Ptr isolates Ls13-192 (race 4), 86-124 and Biotrigo9-1 (race 2), DW5 (race 5) and are present in ToxC producing isolates AR CrossB10 (AR, race unknown), V1 and M4 (race 1) isolates. M4 isolate had multiple gene copies, while some genes were absent in Pt-1C-BFP (race 1).

Ptr M4 chr 9 region in view

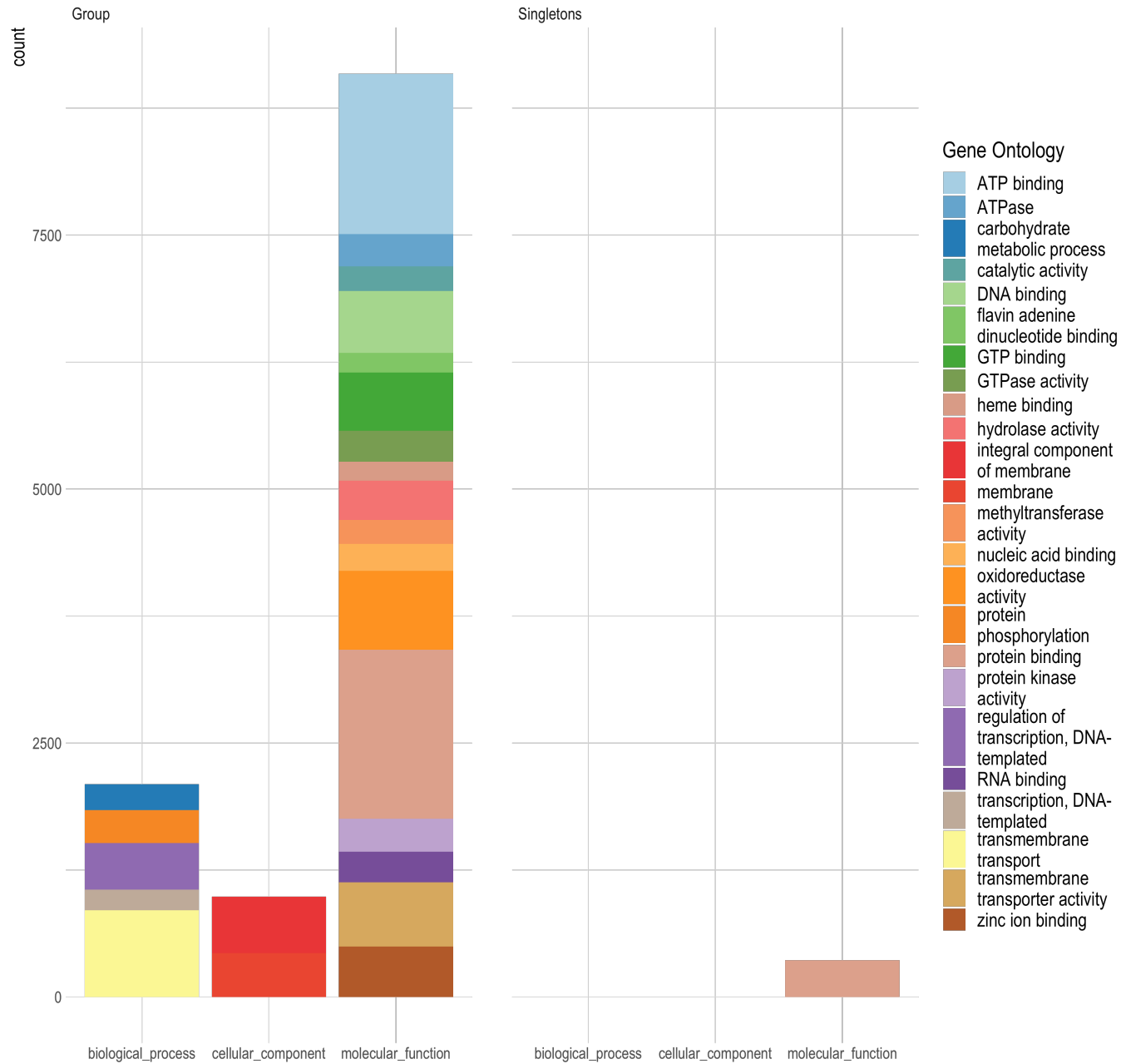


Supplementary Fig. S13. RNA expression for *Pyrenophora tritici-repentis* isolate M4 for the M4 ToxC producing isolate specific gene cluster 64 kb region (red). The read coverage and alignments show RNA expression *in vitro* (top) and *in planta* (below) on chromosome 9 (CM025803.1).

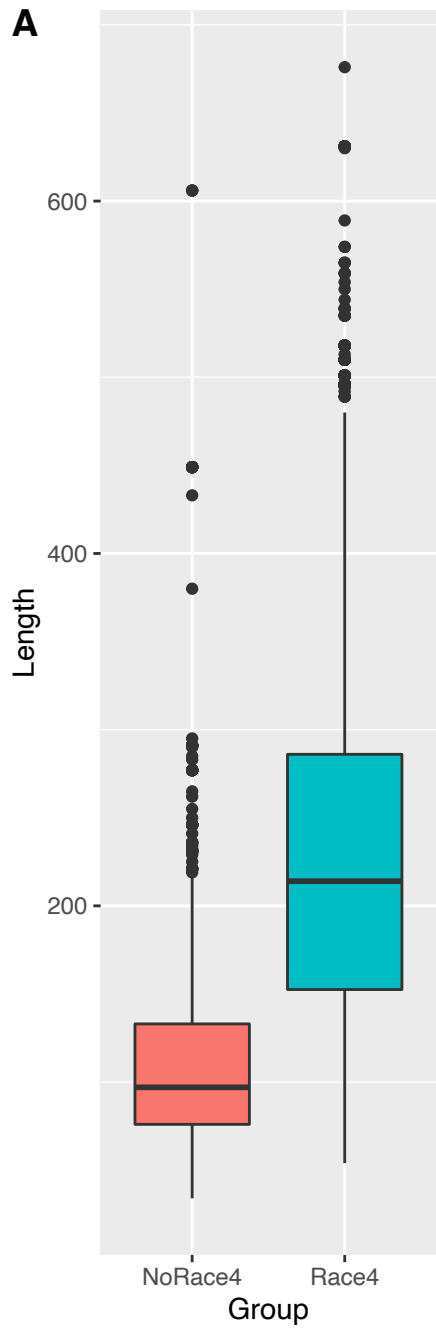


Supplementary Fig. S14. Ptr isolate Biotrigo9-1 *ToxC1* genomic region on contig 12 (43,362-60,409 bp) shows *ToxC1* is disrupted by a large single insertion (5,348 bp in size) positioned from 45,946 to 51,292 bp that carries a nested long terminal repeat (LTR) retrotransposon and the motifs for the Copia retrotransposon transposable element (TE).

Orthogroup Gene Ontology

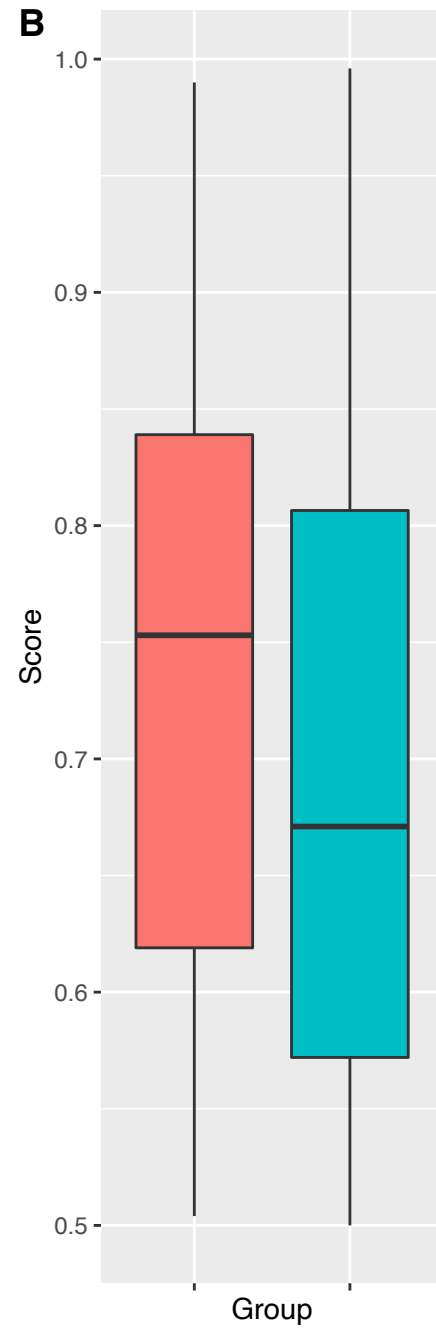


Supplementary Fig. S15. The number of Ptr orthologous protein groups (OG) and singletons assigned a Gene Ontology (GO) term. The stack bar charts for GO biological processes, cellular components and molecular functions show terms with counts $\geq 1\%$ of the total number of OGs and singletons.



Group

- NoRace4
- Race4



Group

- NoRace4
- Race4

Supplementary Fig. S16. Ptr pangenome predicted effectors specific to race 4 and specific to known effector producing isolates (non-race 4). A) Boxplot of protein lengths. B) Boxplot of effector probability scores.