

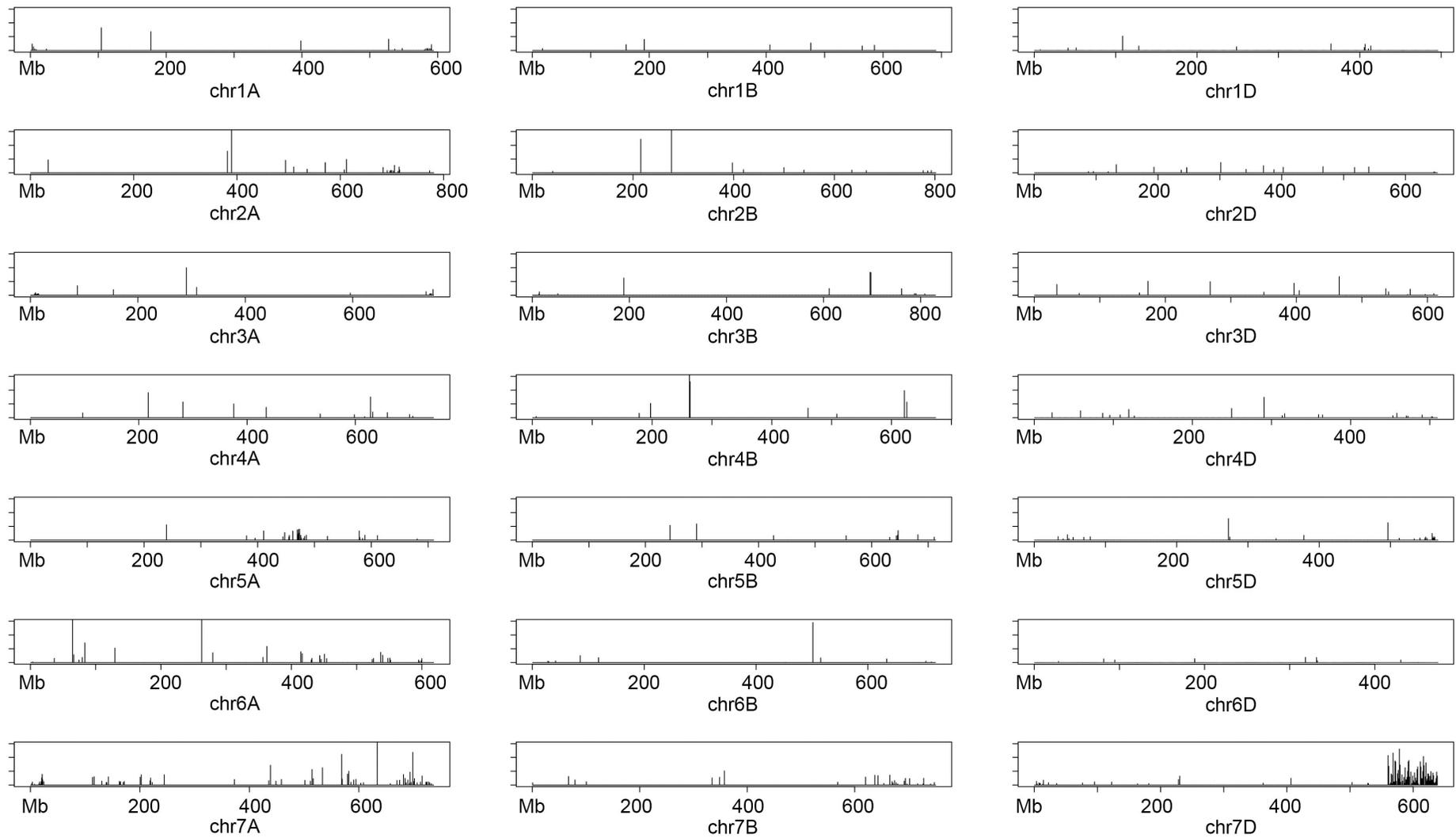


The wheat stem rust resistance gene *Sr43* encodes an unusual protein kinase

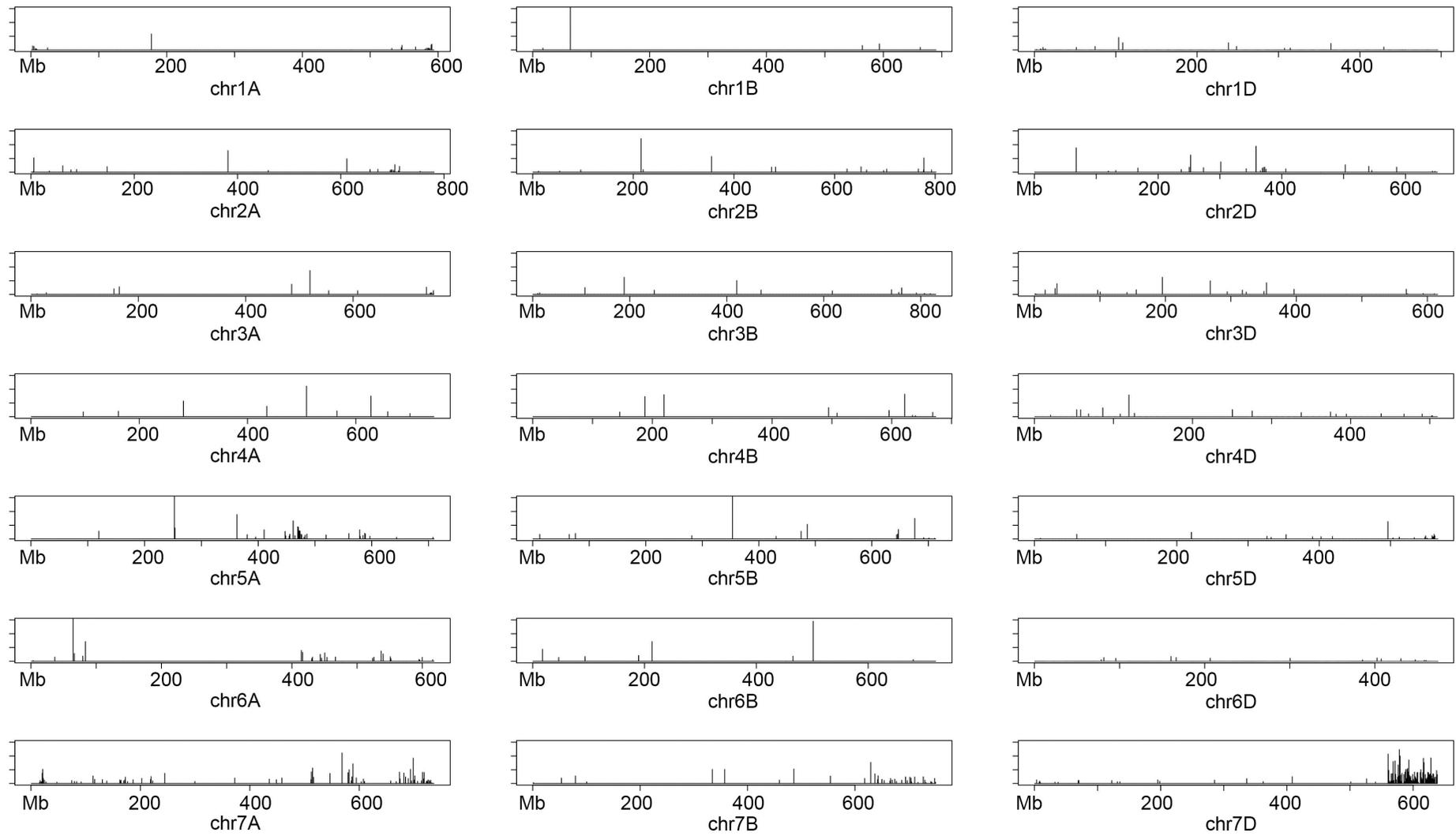
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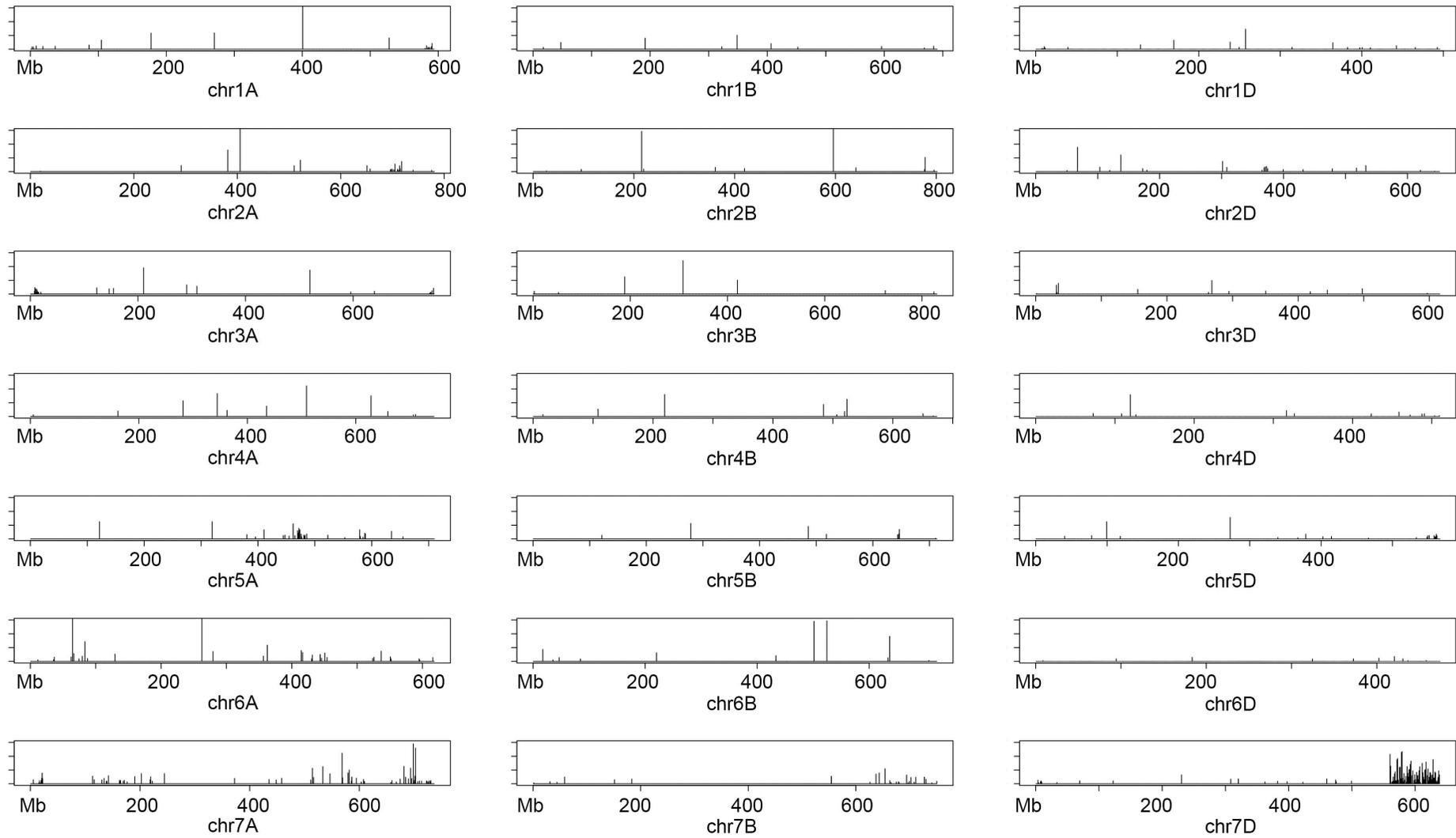
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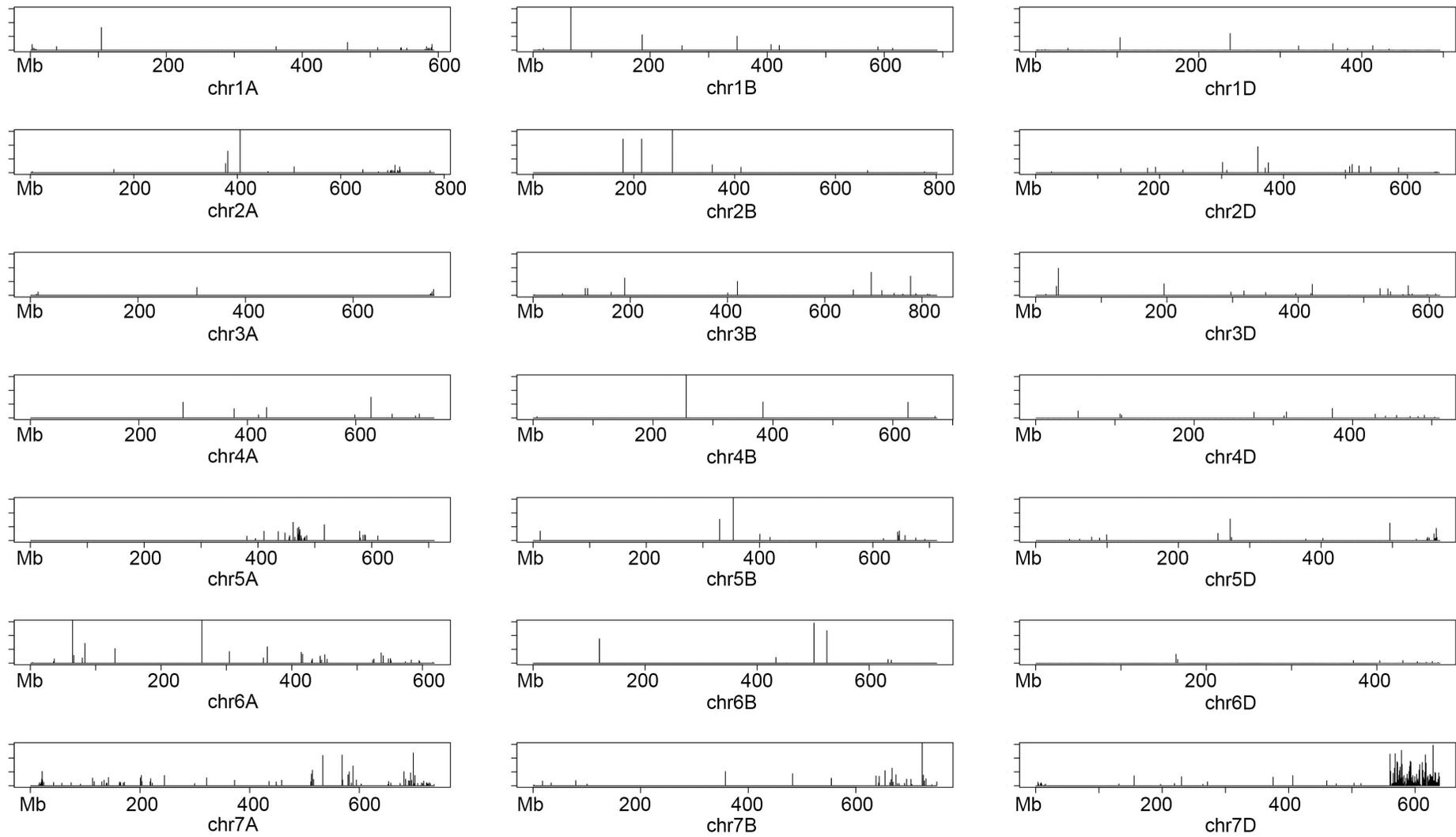
Supplementary Fig. 1: Genotyping-by-sequencing (GBS) plot of the *Sr43* wheat-*Th. elongatum* introgression wild type line. The SNP frequency was plotted in 10 Mb bins along the length of each chromosome relative to wheat cv. Chinese Spring.



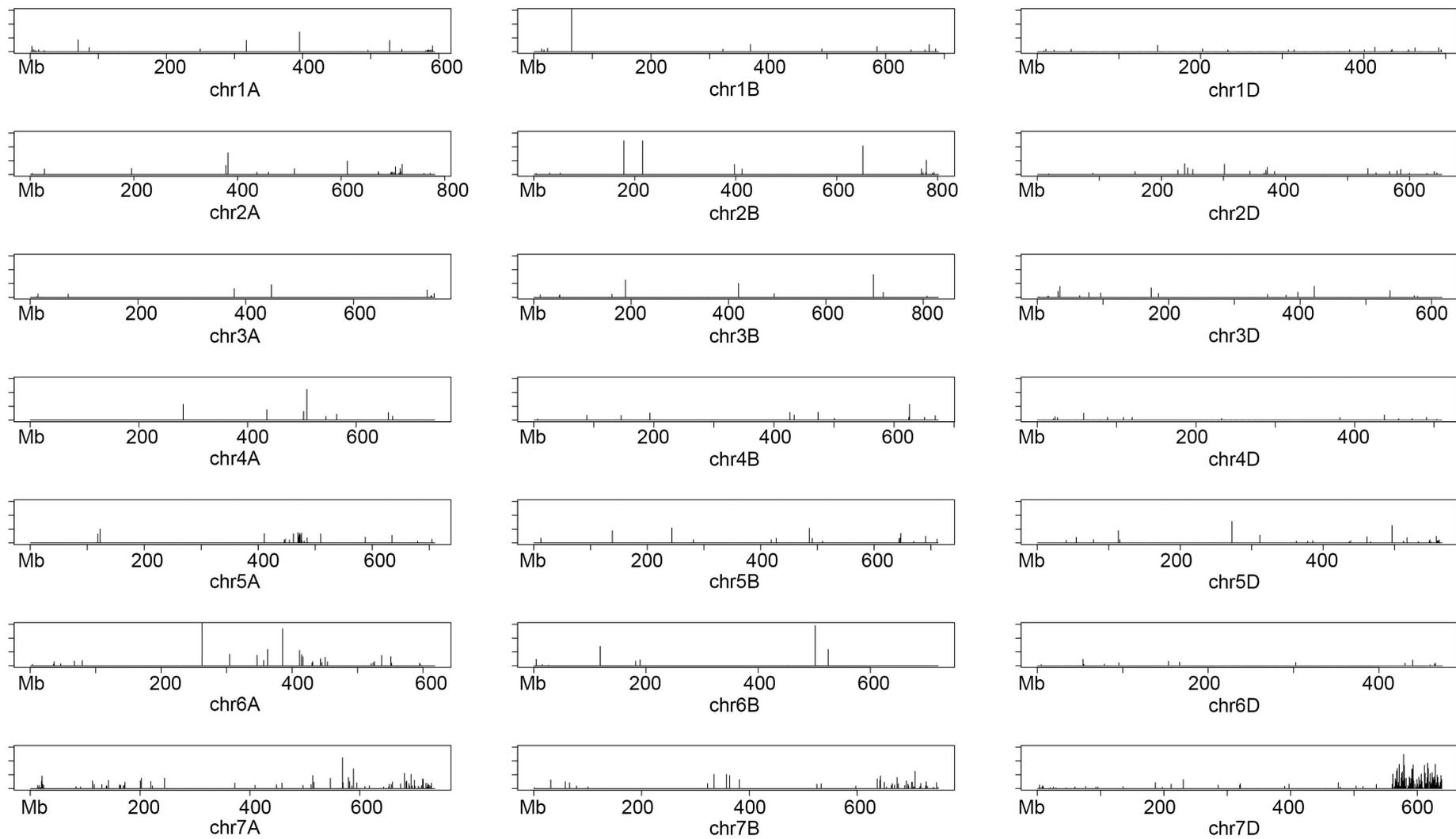
Supplementary Fig. 2: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 31b.



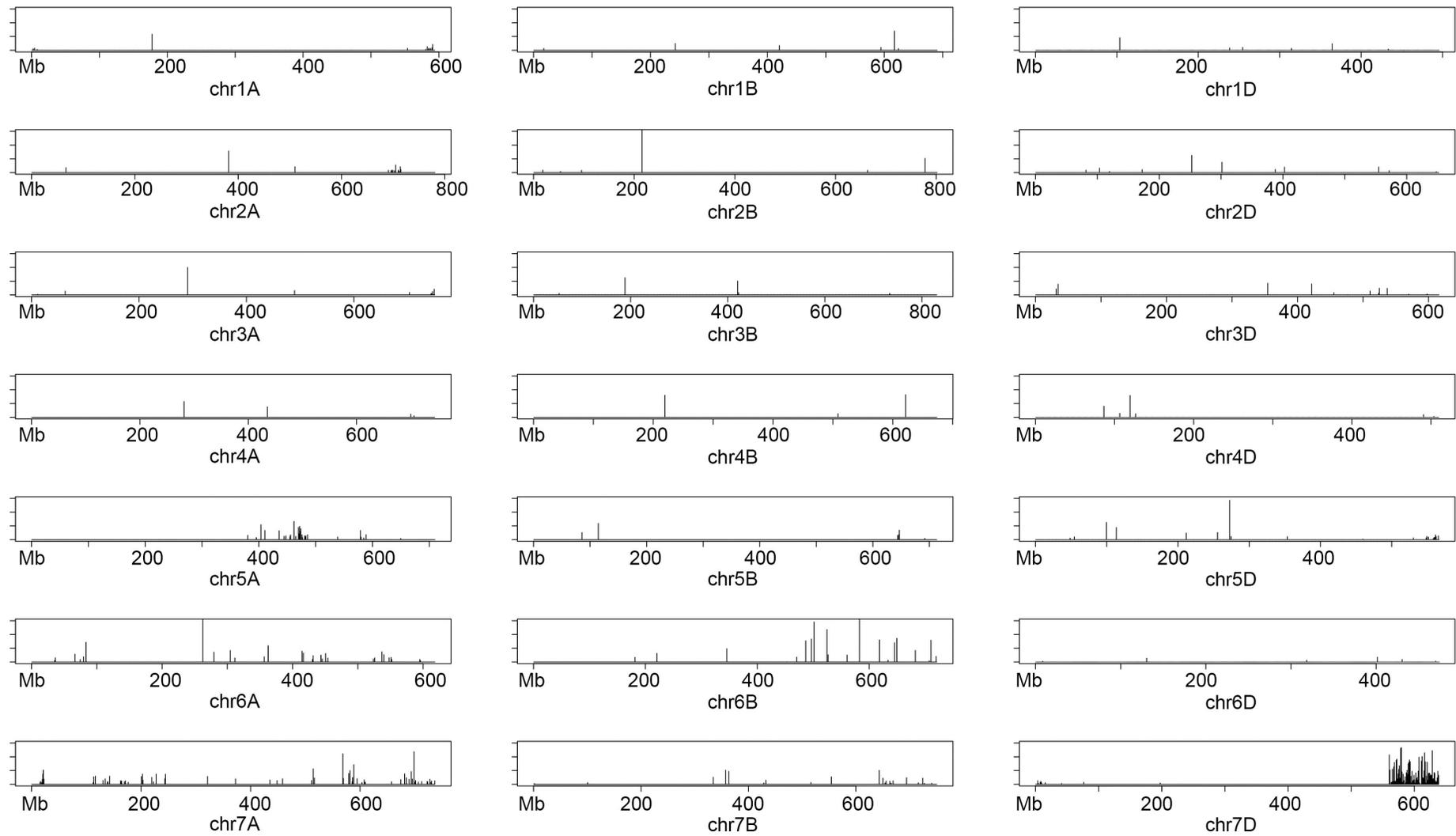
Supplementary Fig. 3: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 187g.



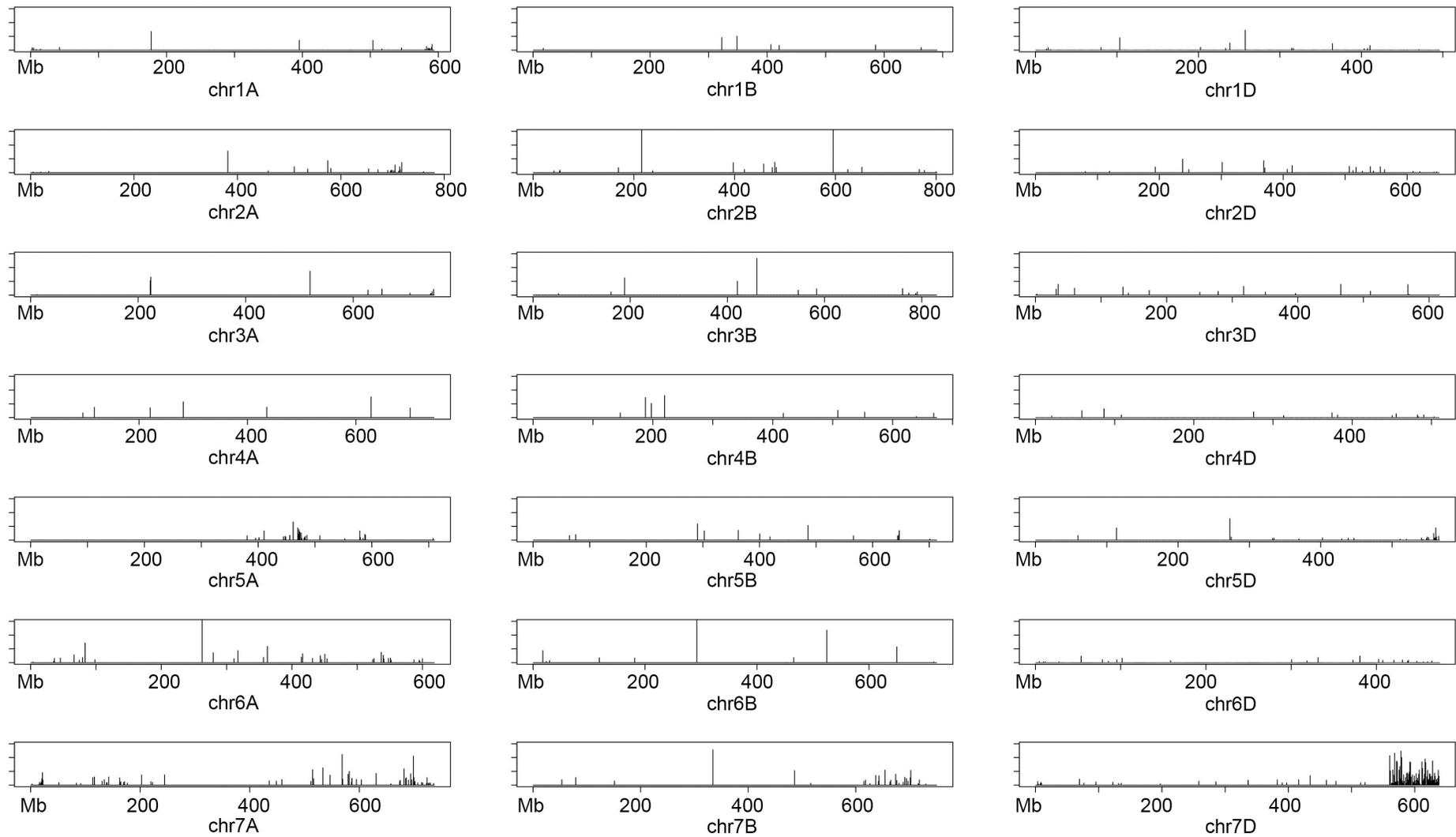
Supplementary Fig. 4: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 276b.



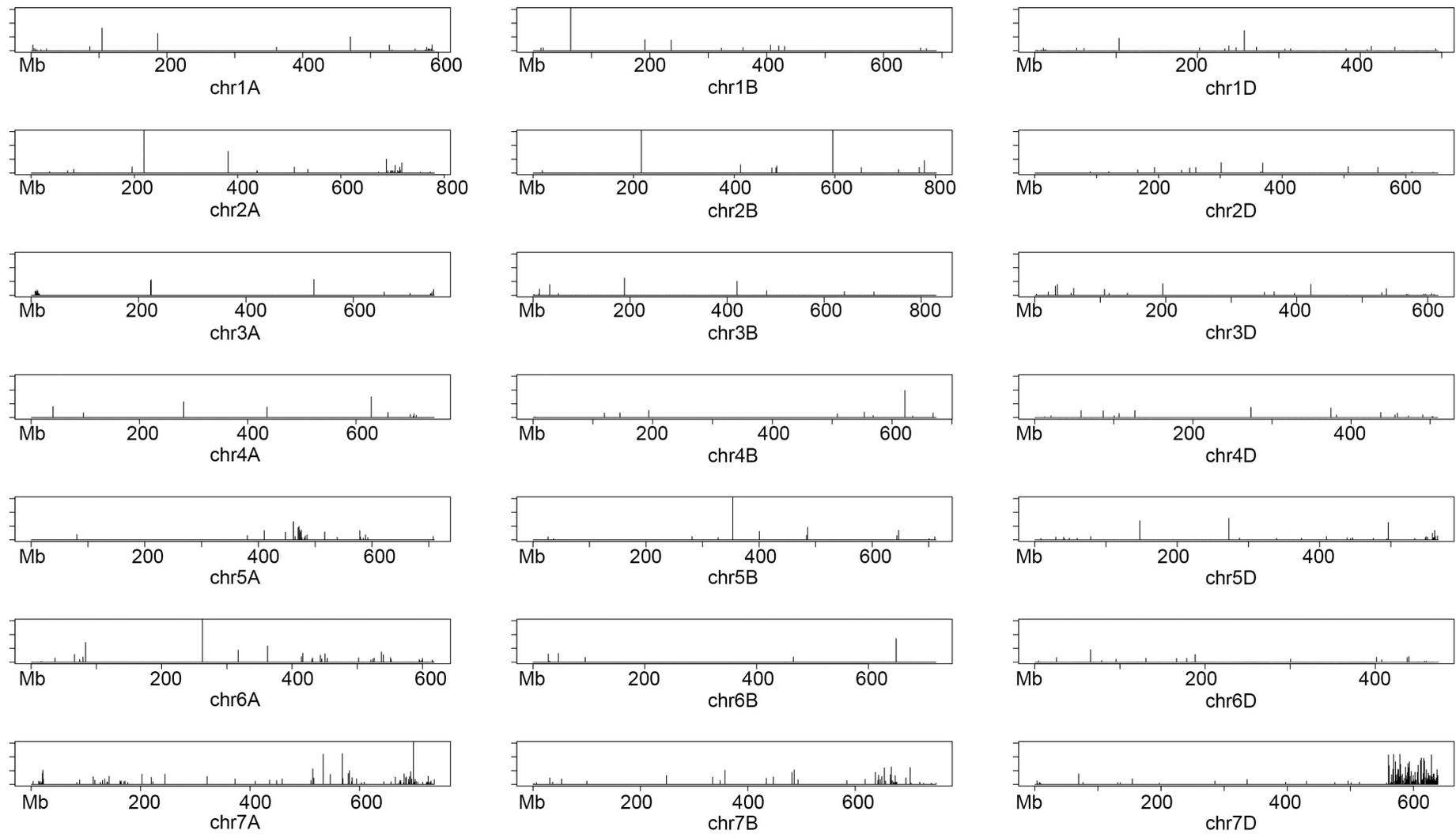
Supplementary Fig. 5: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 450b.



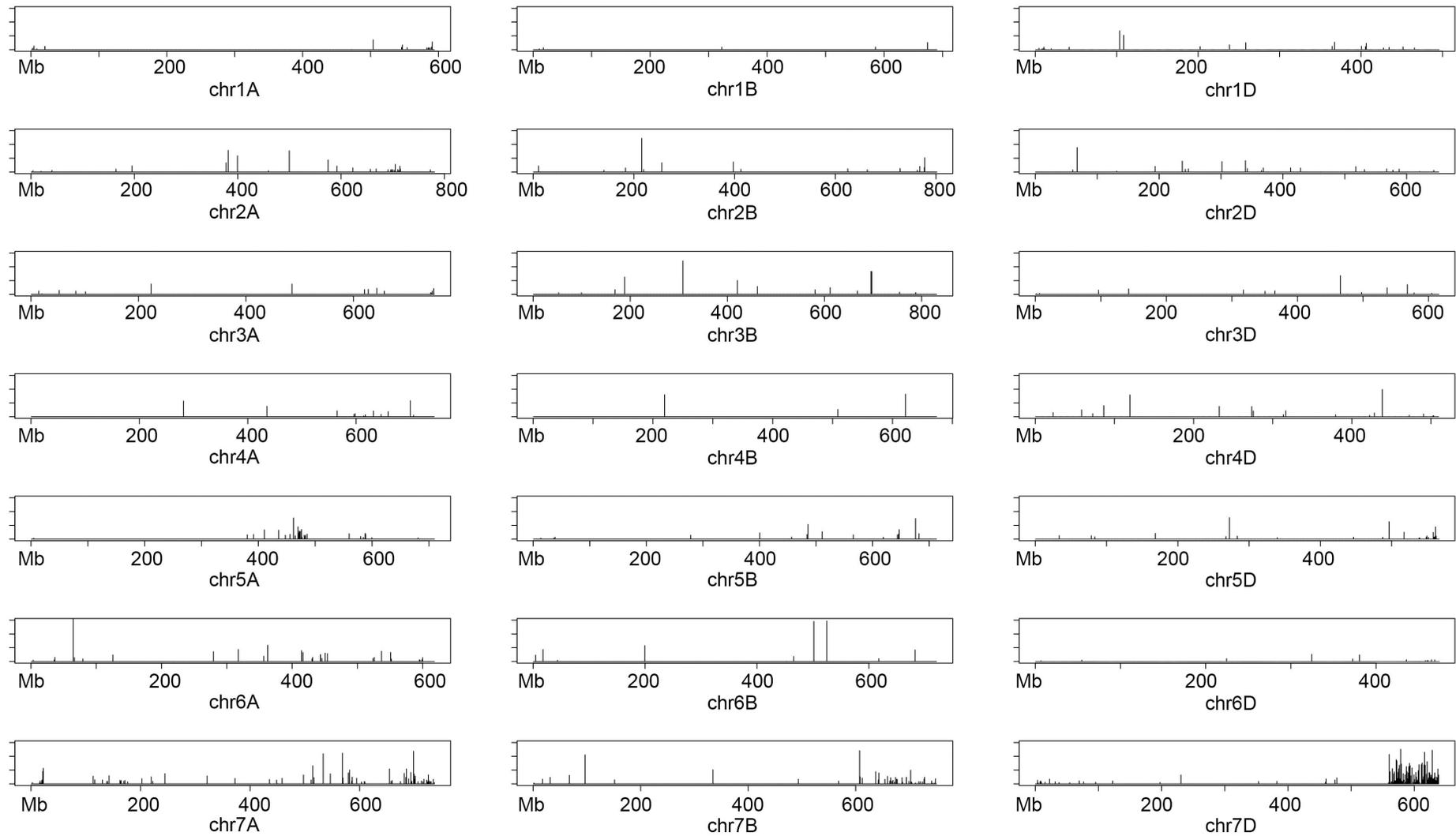
Supplementary Fig. 6: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 706f.



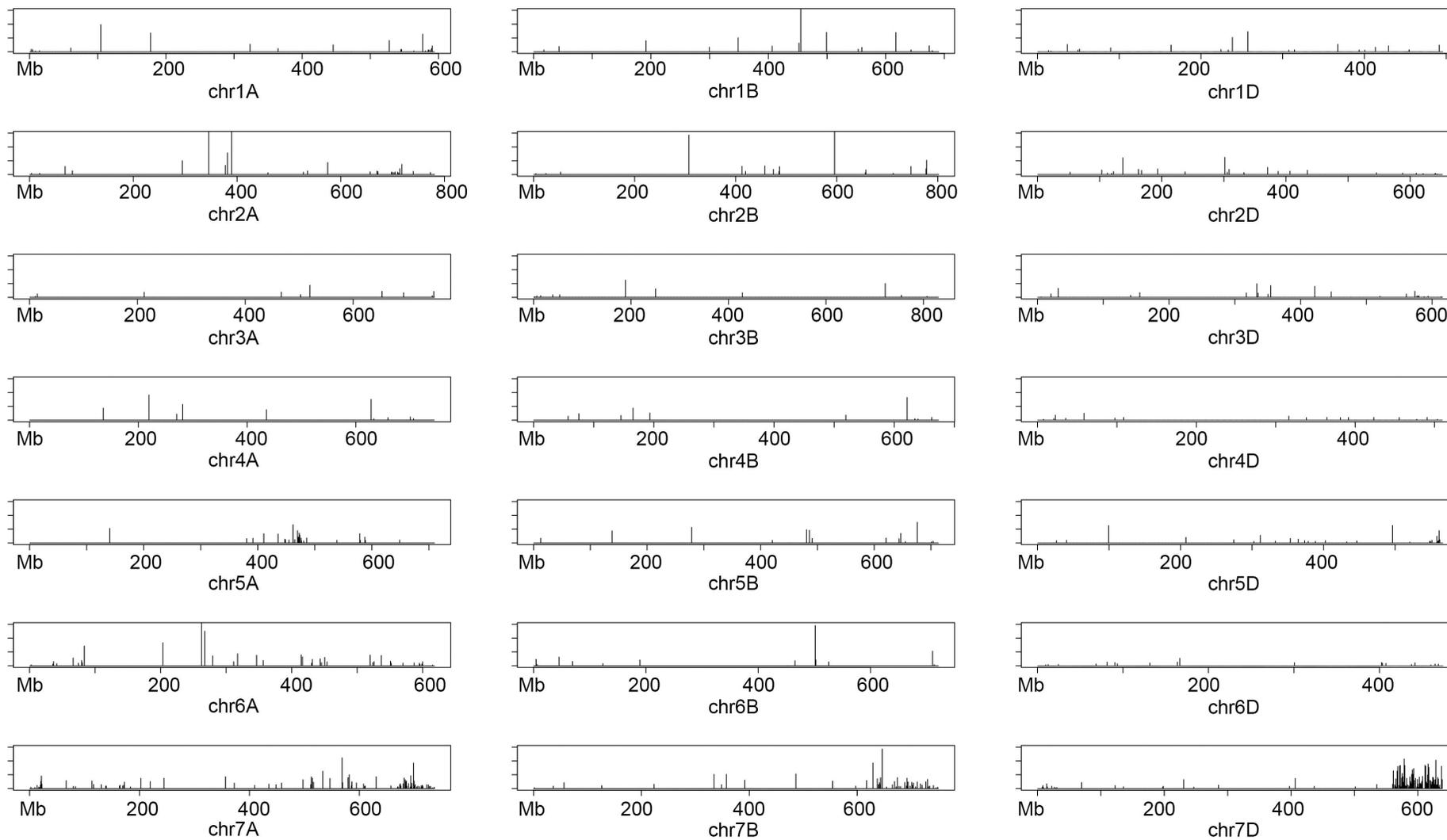
Supplementary Fig. 7: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 1013a.



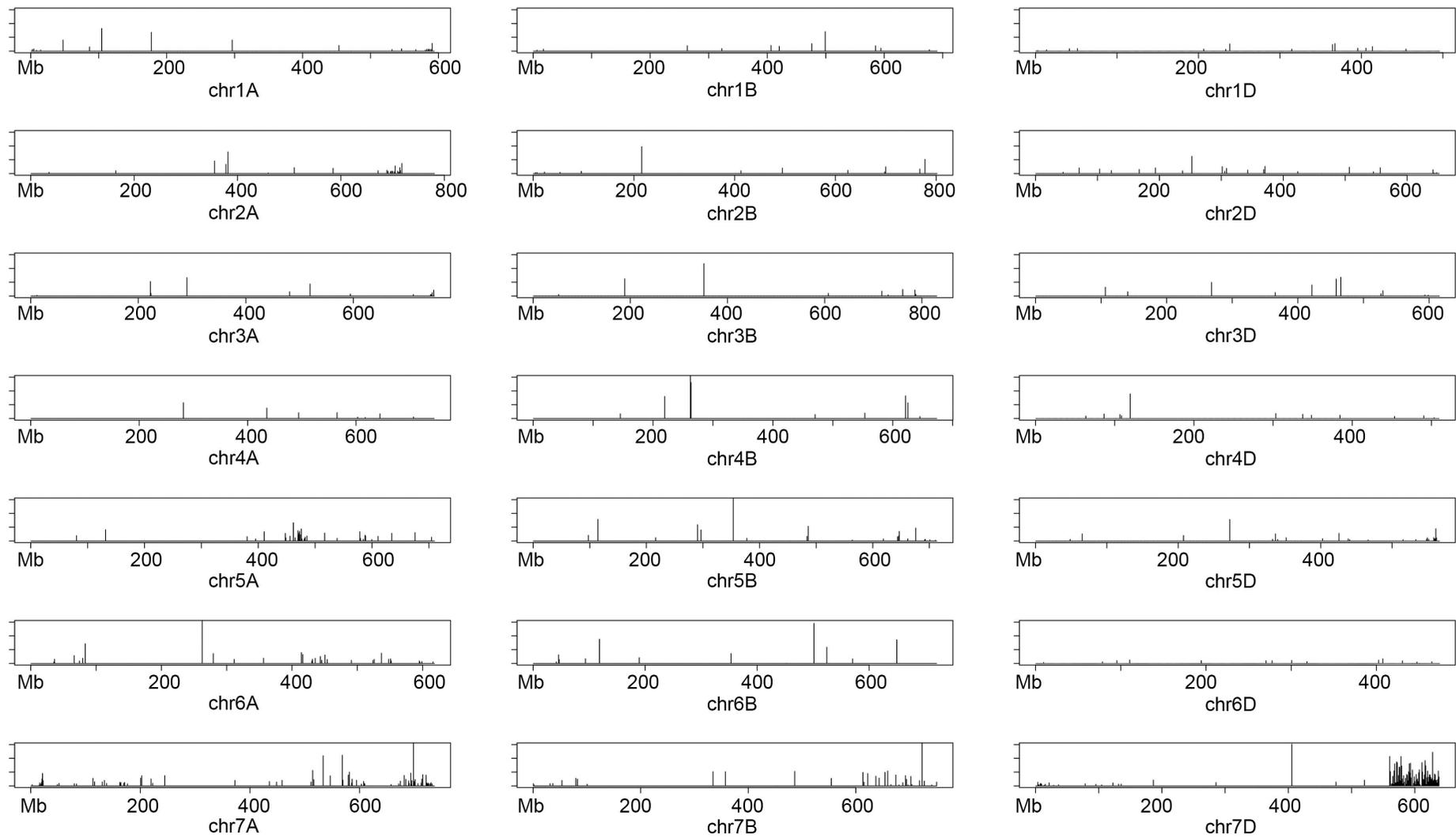
Supplementary Fig. 8: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 1102h.



Supplementary Fig. 9: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 1298g.



Supplementary Fig. 10: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 1513d.

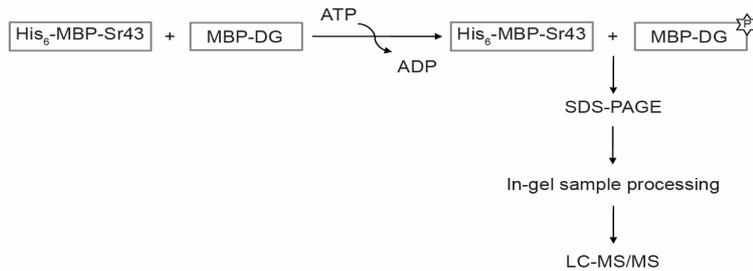


Supplementary Fig. 11: GBS plot of the *Sr43* wheat-*Th. elongatum* introgression EMS mutant 1671a.

Sr43	1	SYHLLGEITNGFSE-----NRKLTGAYGSVYKGEHKDGEKIAVKMLHD	44
		::: ..:: . .: . . :::::	
STKc_IRAK	1	SFYELKNVTNMFDERPISVGGNKMGEFGVYKGYVNNTTVAVKKLAA	49
Sr43	45	TLGLDNE----QFEKEYFNLADLQHKNIIVRLVGYCHETRECVPYNGRMV	90
		:::.. ::: ..: ..: .: . .: .: .: .: .: .: .: .	
STKc_IRAK	50	MVDITTEELKQQFDQEIKVMAKCQHENLVELLGFSSDGDLLC-----	91
Sr43	91	FADITKRALCFEYMRNGG-LDK--CLSDETSGHDWCTRYSIKIGICEGLK	137
		.:: : ... :.	
STKc_IRAK	92	-----LVYVYMPNGSLLDRLSCL-DGTPPLSWHMRCKIAQGAANGIN	132
Sr43	138	YLHEELESMPYHLDLKPANILLDEKMVPKIADFGLSRFF-RGEQSQITKS	186
		: .:.. .: .:: .: .: .: .: .	
STKc_IRAK	133	FLH---ENHHIHRDIKSANILLDEAFTAKISDFGLARASEKFAQTVMTSR	179
Sr43	187	AIGTHGYVPPEYIDASVLSIKFDIFSLGVVVIKIMTGPTGYFRSAE----	232
		:: . .: .: .: .: .: .: .: .: .: .: .: .	
STKc_IRAK	180	IVGTTAYMAPEALRGEI-TPKSDIYSFGVLLLEIITGLPAVDEHREPQLL	228
Sr43	233	MSAQFIELVLANWRKRLPATSVYLLESY-----SEQVKRCTAIAV	273
		:.. .: .: .: .: .: .: .: .: .: .: .: .: .: .	
STKc_IRAK	229	LDIKEEIE-----DEEKTIEDYIDKKMNDADSTSVEAMYSVAS	266
Sr43	274	SCVEADRSKRPSIGEIFVNKLNETETM	299
		. .: .: .: .: .: .: .: .: .: .: .: .: .: .: .	
STKc_IRAK	267	QCLHEKKNKRPDIKKVQQLQEMTAS	292

Supplementary Fig. 12: Amino acid alignment between Sr43 and STKc IRAK. The identity is 30.4%, and the e-value is 2.06e-60.

a



b



MASCOT Search Results

Protein View: Maltose_binding_protein_Recombinant_Substrate

sp|P00015|Maltose_binding_protein_Recombinant_Substrate

Database: SR43
Score: 57069
Monoisotopic mass (M_r): 42456
Calculated pI: 5.08

Sequence similarity is available as [an NCBI BLAST search of Maltose_binding_protein_Recombinant_Substrate against nr.](#)

Search parameters

MS data file: \\datawaha\core\labs\bioscience\bc1p\ProtUsers\HHirt\Nag\2022\20221114_RFS1002207580.
Enzyme: Trypsin/P: cuts C-term side of KR.
Fixed modifications: [Carbamidomethyl \(C\)](#)
Variable modifications: [Oxidation \(M\)](#), [Phospho \(ST\)](#), [Phospho \(Y\)](#)

Protein sequence coverage: 91%

Matched peptides shown in **bold red**.

```

1  MKIEEGKLV I WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ
51  VAATGDGPDI IFWAHDRFGG YAQSGLLAEI TPKAFQDKL YPFTWDAVR Y
101 NGKLIAYPIA VEALS LIY NK DLLPNPPKTW EEIPALDKEL KAKGKSALMF
151 NLQEPYFTWP LIAADGGYAF KYENKDYDIK DVGVDNAGAK AGLTFLVDLI
201 KMKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK VNYGVTVLPT
251 FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL
301 GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN
351 AASGRQTVDE ALKDAQTNSS SNNNNNNNN NLGIEGR

```

c



MASCOT Search Results

Protein View: Maltose_binding_protein_Recombinant_Substrate

sp|P00015|Maltose_binding_protein_Recombinant_Substrate

Database: SR43
Score: 54530
Monoisotopic mass (M_r): 42456
Calculated pI: 5.08

Sequence similarity is available as [an NCBI BLAST search of Maltose_binding_protein_Recombinant_Substrate against nr.](#)

Search parameters

MS data file: \\datawaha\core\labs\bioscience\bc1p\ProtUsers\HHirt\Nag\2022\20221114_RFS1002207580.
Enzyme: Trypsin/P: cuts C-term side of KR.
Fixed modifications: [Carbamidomethyl \(C\)](#)
Variable modifications: [Oxidation \(M\)](#), [Phospho \(ST\)](#), [Phospho \(Y\)](#)

Protein sequence coverage: 97%

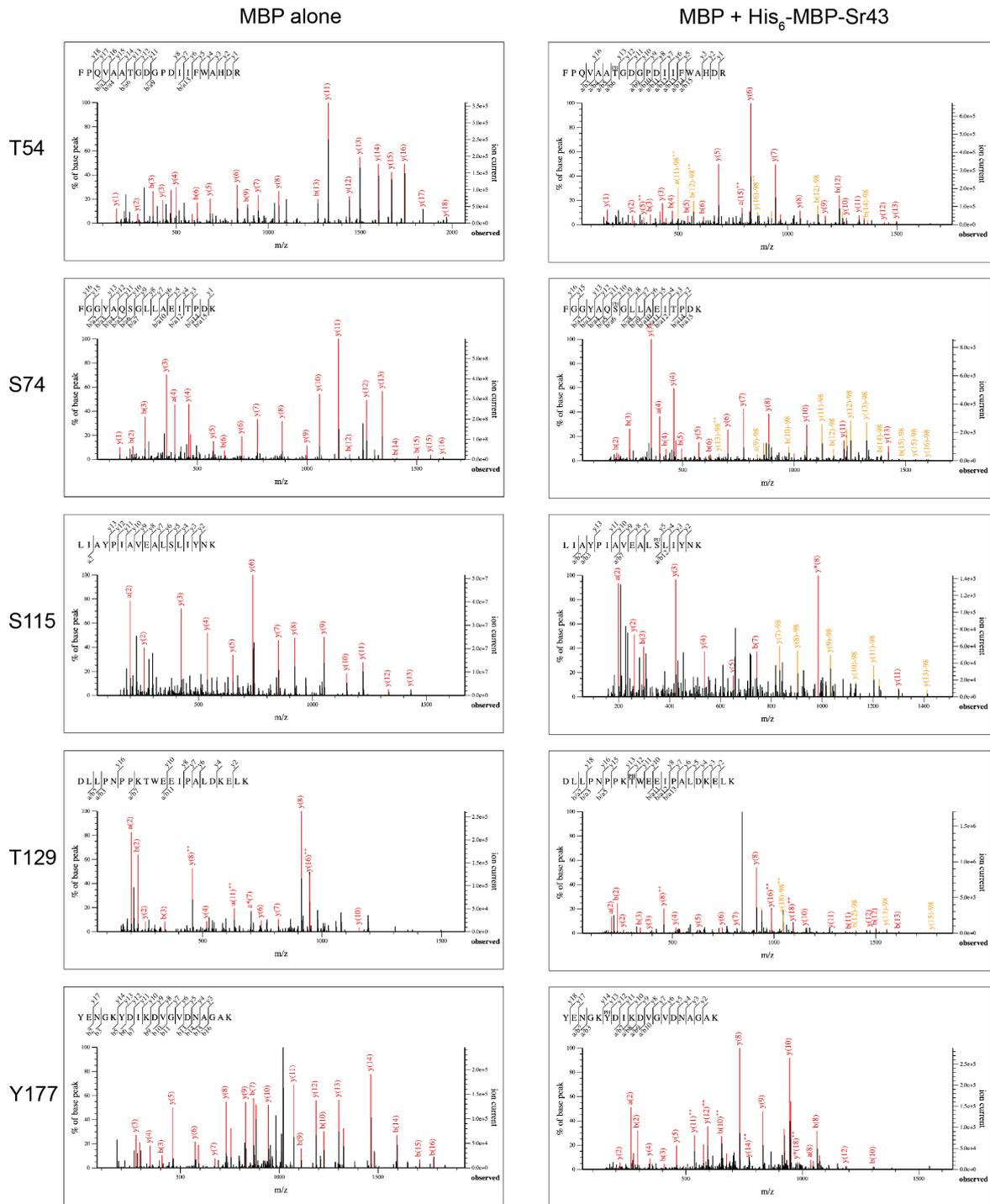
Matched peptides shown in **bold red**.

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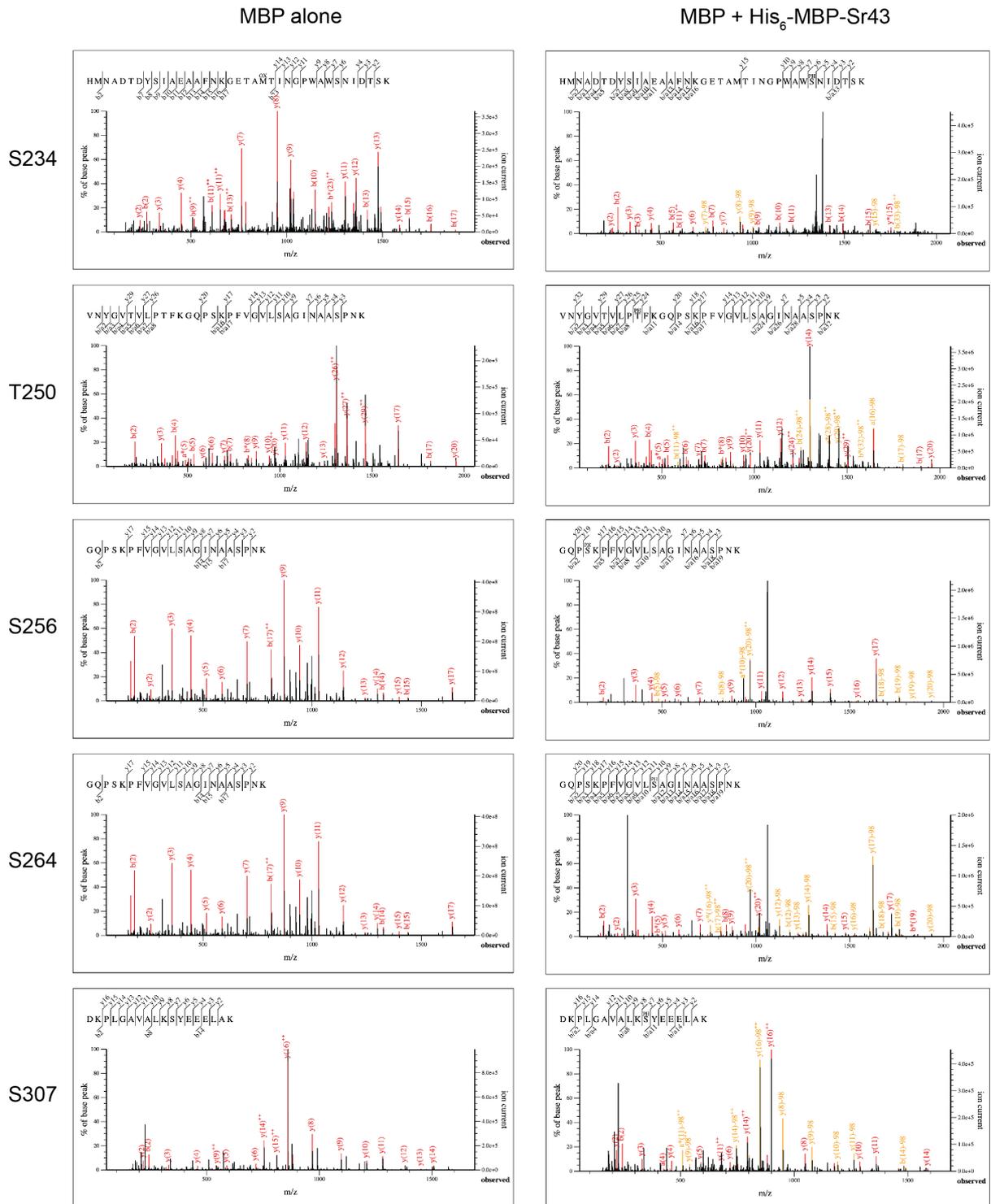
1  MKIEEGKLV I WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ
51  VAATGDGPDI IFWAHDRFGG YAQSGLLAEI TPKAFQDKL YPFTWDAVR Y
101 NGKLIAYPIA VEALS LIY NK DLLPNPPKTW EEIPALDKEL KAKGKSALMF
151 NLQEPYFTWP LIAADGGYAF KYENKDYDIK DVGVDNAGAK AGLTFLVDLI
201 KMKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK VNYGVTVLPT
251 FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL
301 GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN
351 AASGRQTVDE ALKDAQTNSS SNNNNNNNN NLGIEGR

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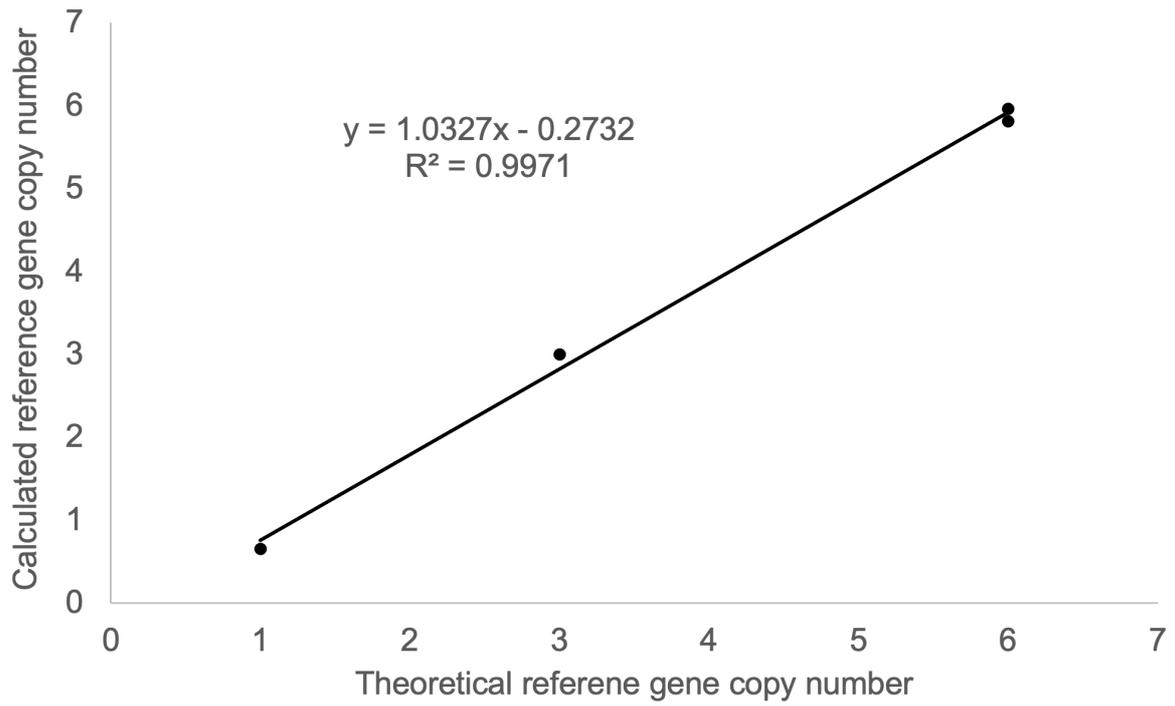
Supplementary Fig. 14. Schematic representation of the phosphorylation reaction and MASCOT search results. **a**, Schematic representation of the phosphorylation reaction and the sample preparation for LC-MS/MS analyses. **b**, Protein sequence coverage of maltose binding protein alone in the absence of His₆-MBP-Sr43. **c**, Protein sequence coverage of maltose binding protein alone in the presence of His₆-MBP-Sr43.



Supplementary Fig. 15. Mass spectra of the peptides that are targeted by His₆-MBP-Sr43 for phosphorylation of residues T54, S74, S115, T129 and Y177. The amino acid numbers are indicated on the left. The spectra on the left panel correspond to the peptides identified from the sample without the kinase added in the reaction. The spectra on the right correspond to the same peptides but phosphorylated on the amino acid.



Supplementary Fig. 16. Mass spectra of the peptides that are targeted by His₆-MBP-Sr43 for phosphorylation of residues S234, T250, S256, S264 and S307. The amino acid numbers are indicated on the left. The spectra on the left panel correspond to the peptides identified from the sample without the kinase added in the reaction. The spectra on the right correspond to the same peptides but phosphorylated on the amino acid.



Supplementary Fig. 17. Regression of calculated reference gene copy number on theoretical reference gene copy number.