

Identifying the functional bases of trait variation in *Brassica napus* using Associative Transcriptomics

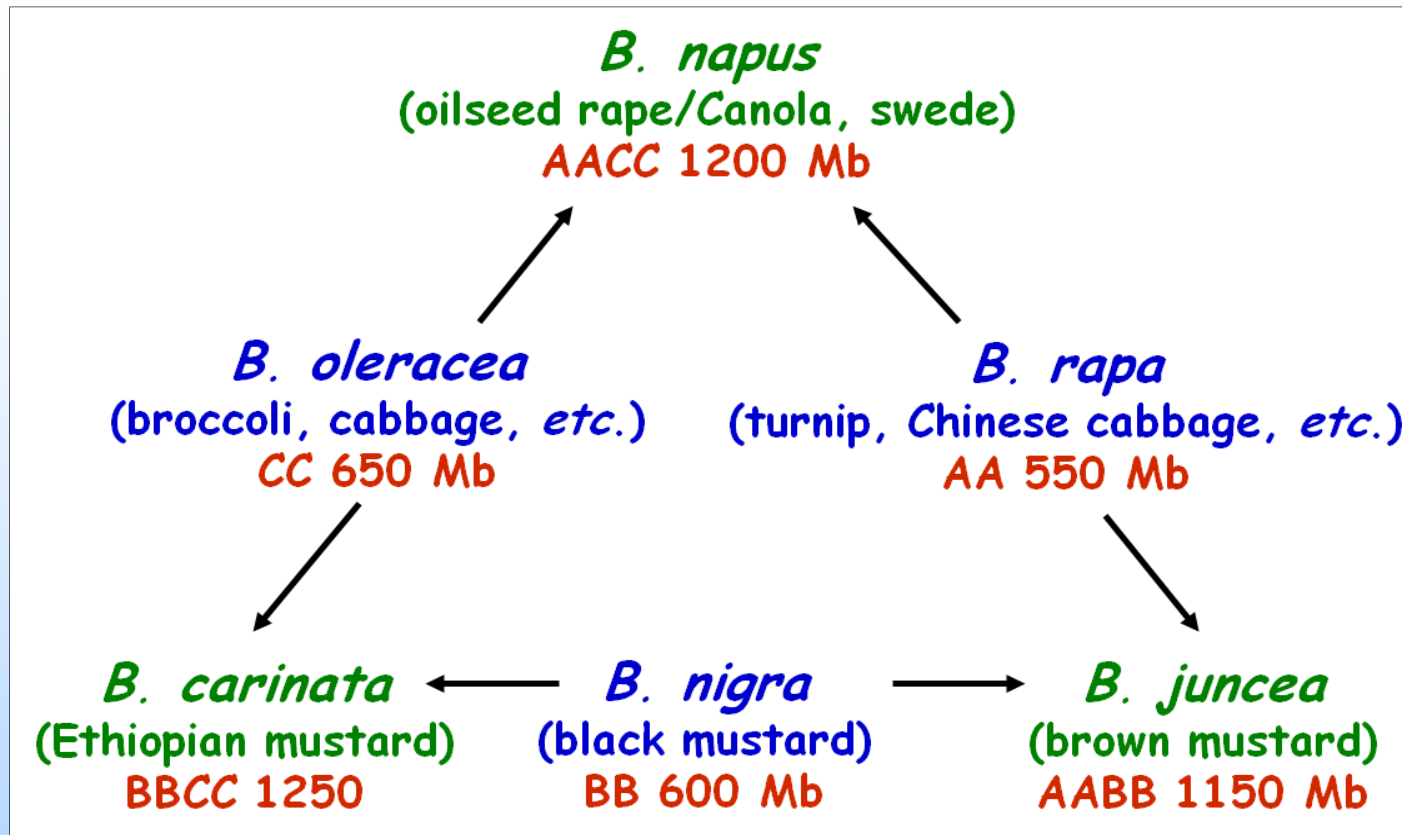
- *Brassica* genome structure and evolution
- Genome framework for association genetics
- Establishing marker-trait associations

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in *Brassica napus* using Associative Transcriptomics

Ian Bancroft

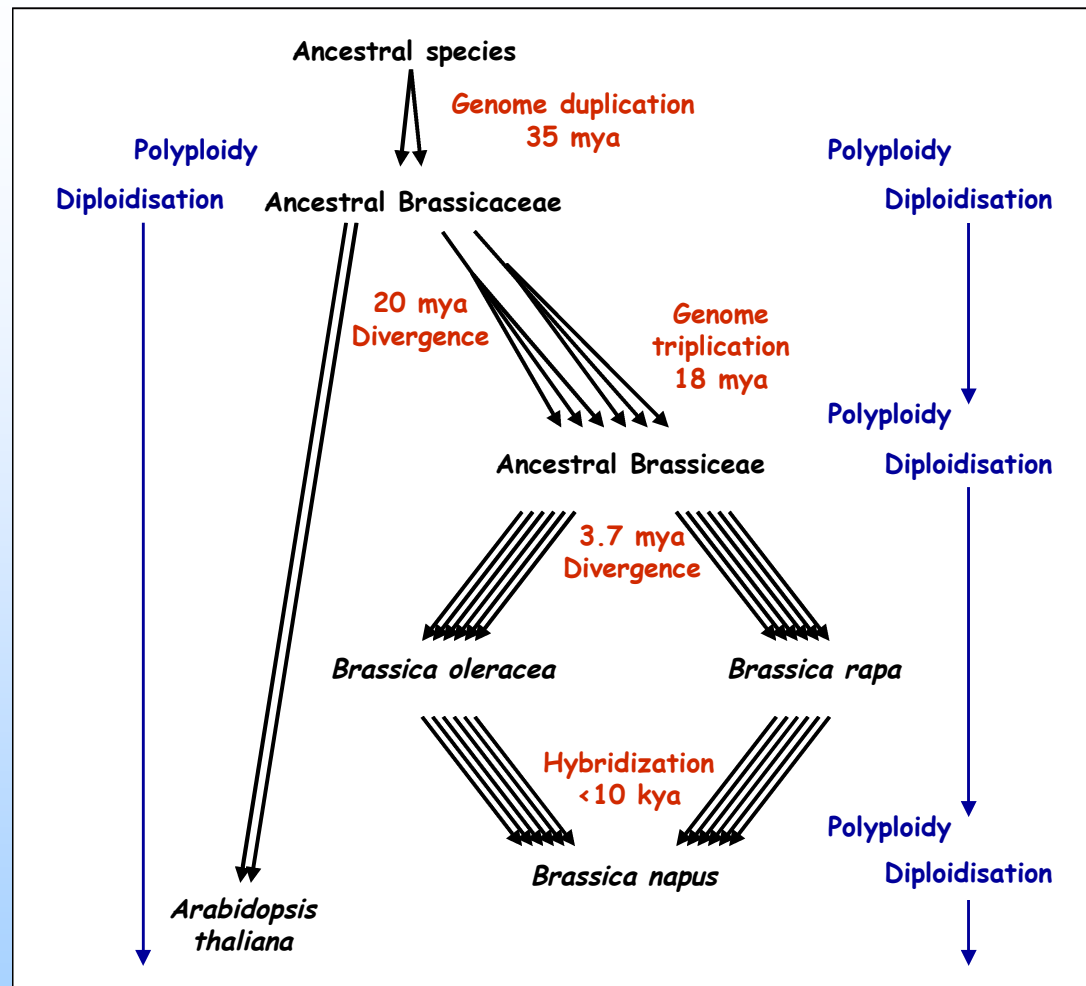
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GENOME RELATIONSHIPS BETWEEN SPECIES - "U's TRIANGLE"



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MULTIPLE ROUNDS OF POLYPLOIDY/DIPLOIDIZATION



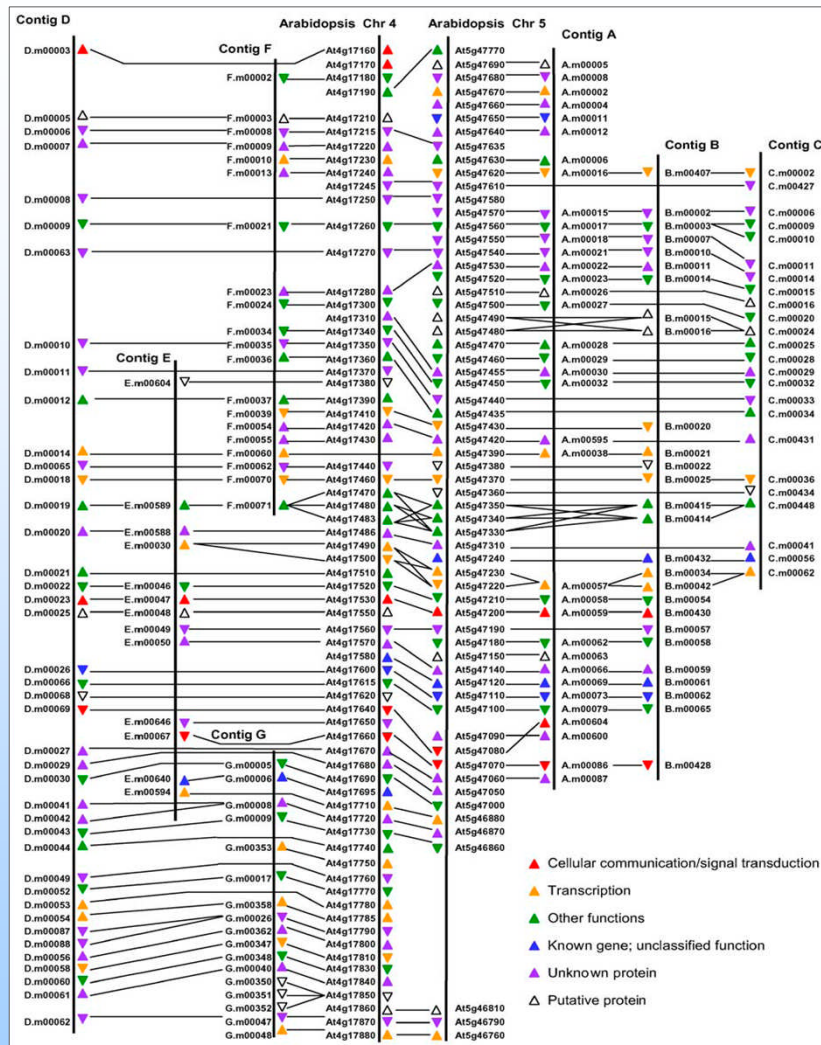
- Model system for the impacts of polyploidy on the structure of plant genomes
- Molecular genetic analyses, particularly comparative, will be problematic

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GENOME MICROSTRUCTURE IN "DIPLOID" BRASSICA SPECIES

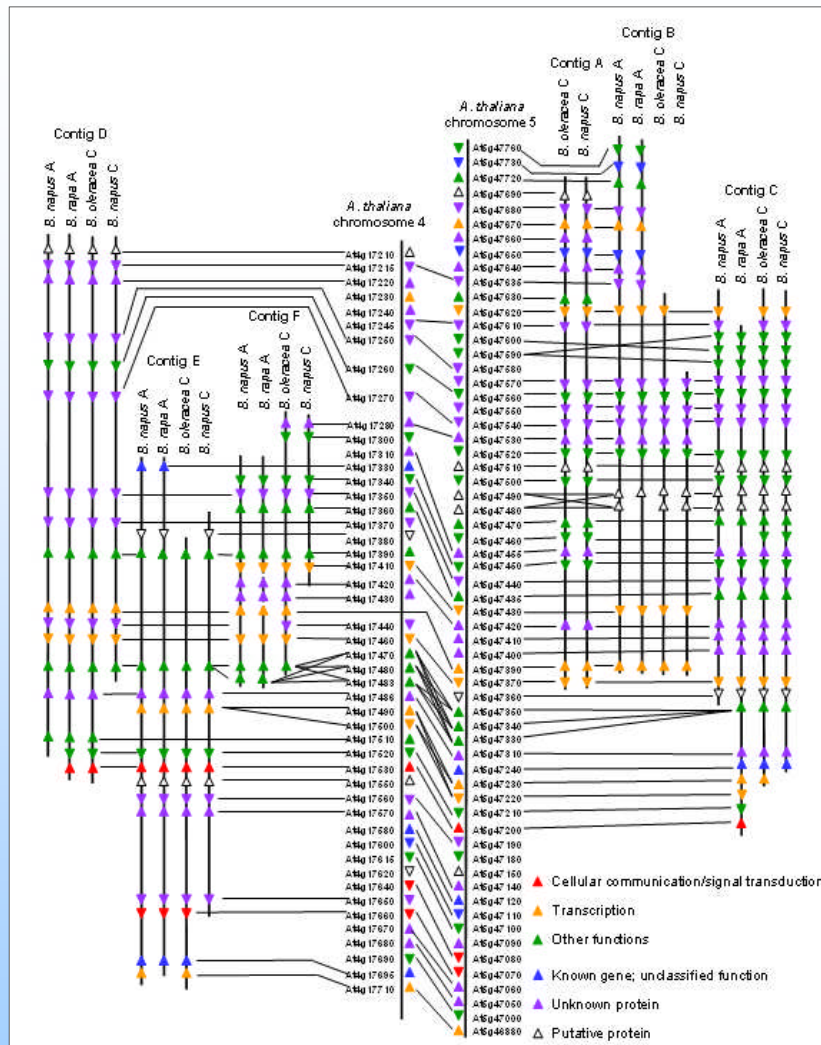


Example (*Brassica oleracea*)

- Triplicated genome - paralogues
- Extensive collinearity between protein-coding genes in *Brassica* and *Arabidopsis*
- Extensive interspersed gene loss
- Evidence for transduplication of genes/gene fragments

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COMPARATIVE ANALYSIS BETWEEN *BRASSICA* SPECIES



Comparison of genome microstructure across orthologous genome segments in *B. oleracea*, *B. rapa* and both genomes of *B. napus*

- High collinearity between protein-coding genes in A and C genomes, as represented in diploids and *B. napus*, but some breakdown
- Considerably more interspersions of transposon-related sequences in gene space of C genome

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POLYMORPHISM TYPES IN A POLYPLOID

	Genomic sequences	Called bases
Diploid <i>B. rapa</i>		
Chiifu locus	A G C T A C C T	A G C T A C C T
Kenshin locus	A G C T A T C T	A G C T A T C T
		simple SNP
Allotetraploid <i>B. napus</i>		
Tapidor locus	A G C T A C C T	A G Y T A C C T
Tapidor homoeologue	A G T T A C C T	A G Y T A C C T
Ningyou 7 locus	A G C T A T C T	A G Y T A Y C T
Ningyou 7 homoeologue	A G T T A C C T	A G Y T A Y C T
	inter-homoeologue polymorphism	hemi-SNP

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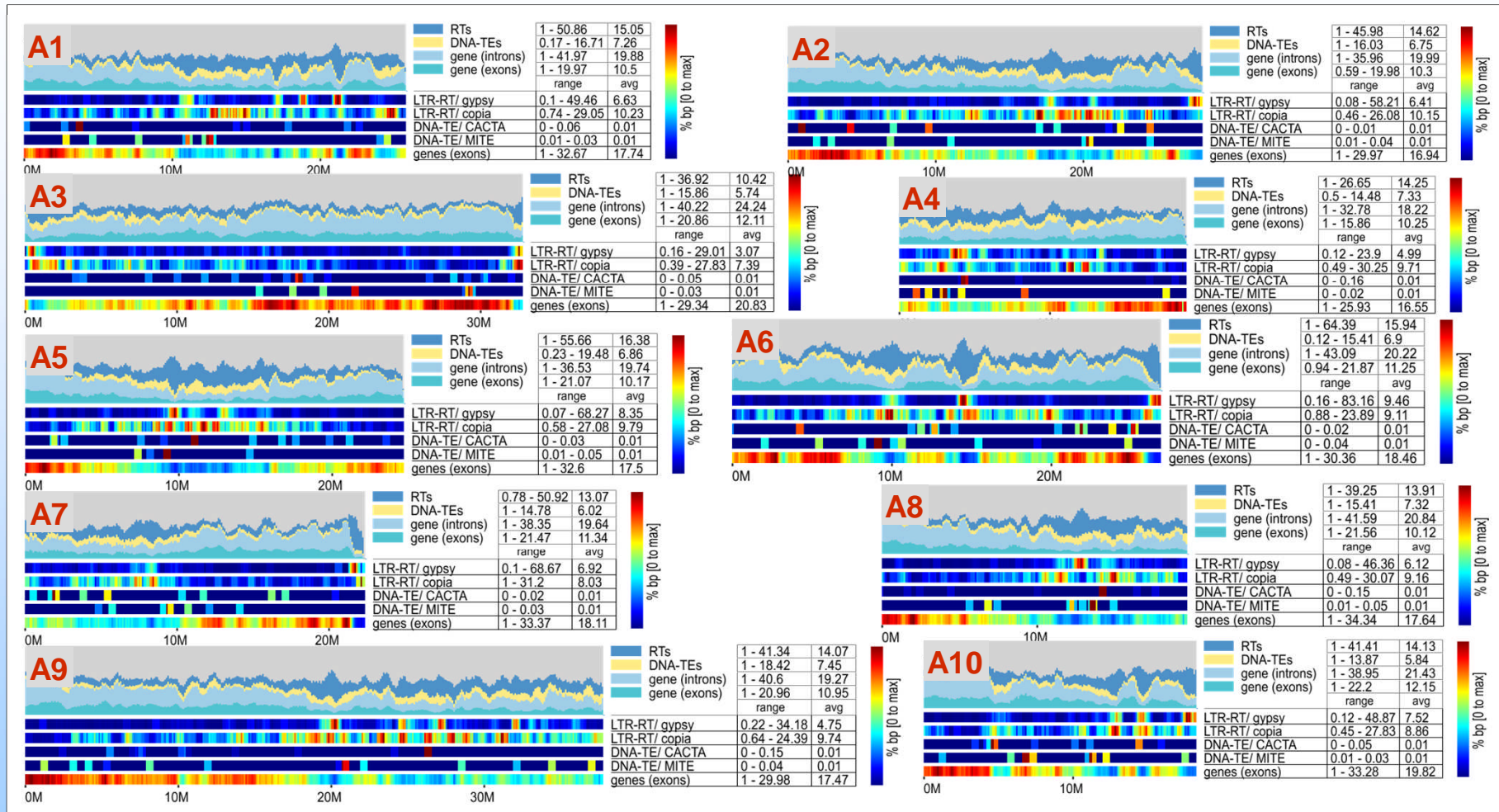
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GENOME SEQUENCING

Brassica rapa:

284 Mb gene space sequenced

41,174 gene models



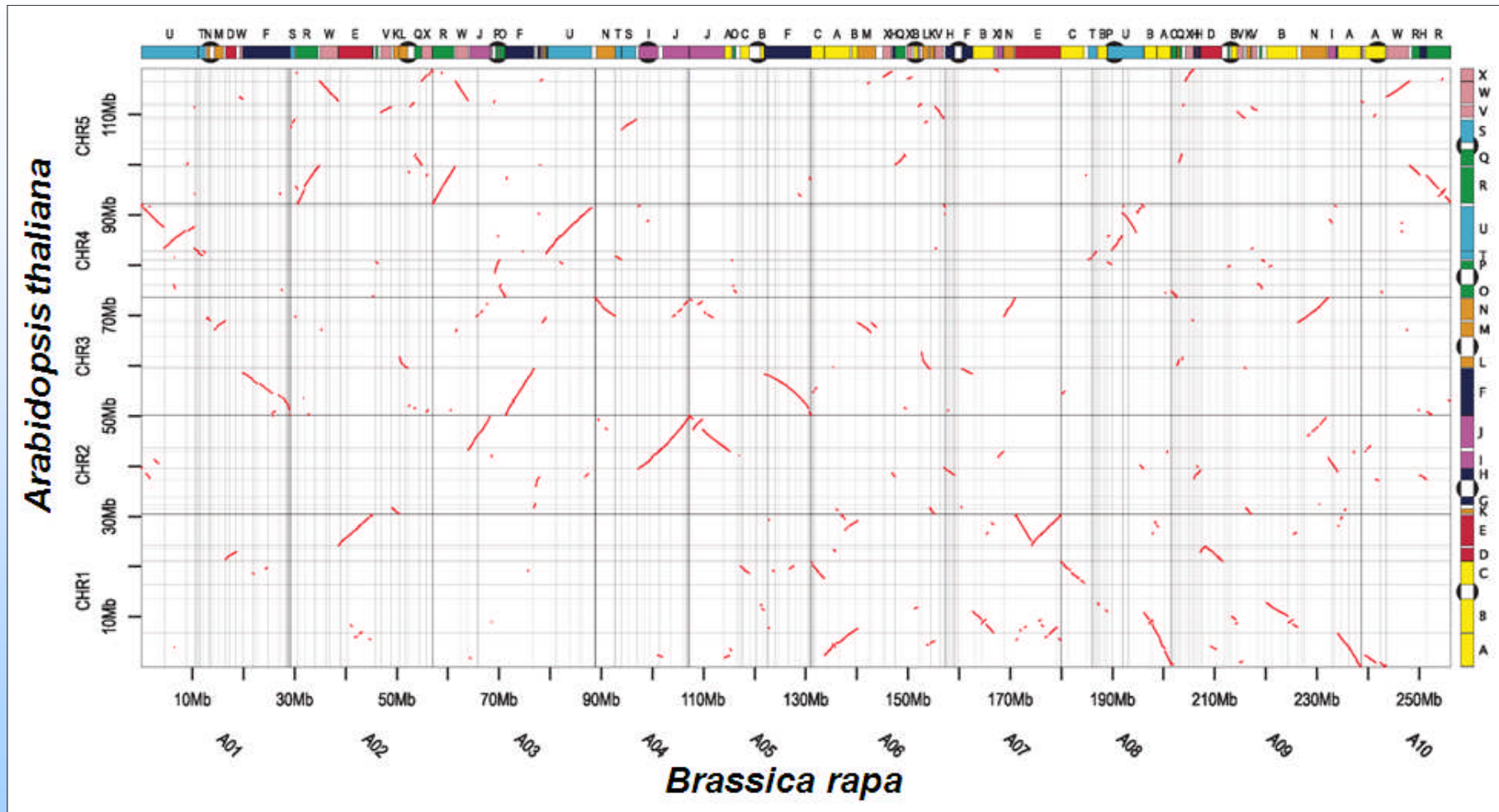
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GENOME - SCALE COMPARATIVE ANALYSIS

Brassica rapa genome sequences aligned to *A. thaliana* and related to AK



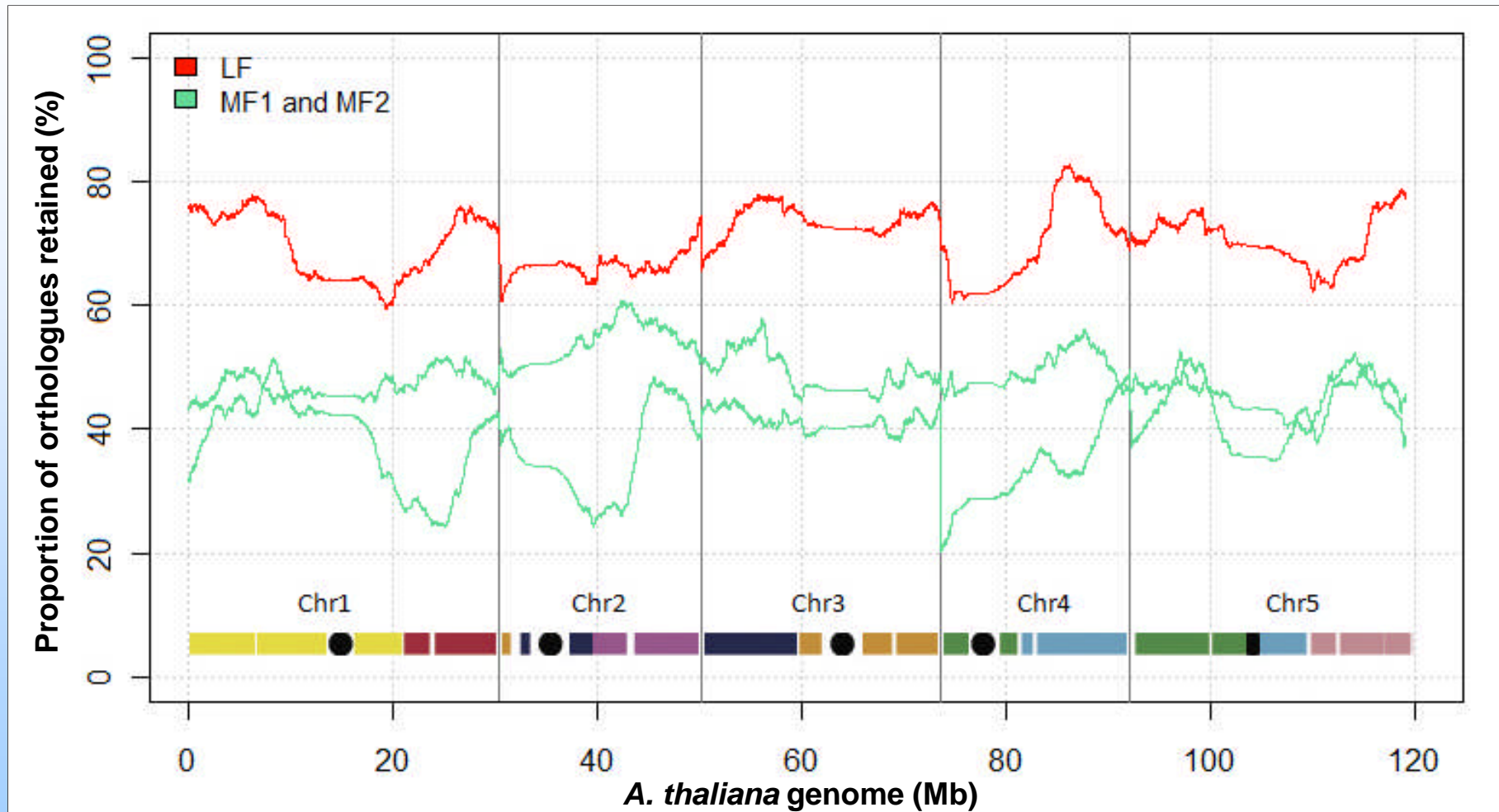
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GENOME - SCALE COMPARATIVE ANALYSIS

Differential gene loss from *Brassica rapa* paralogous genome segments



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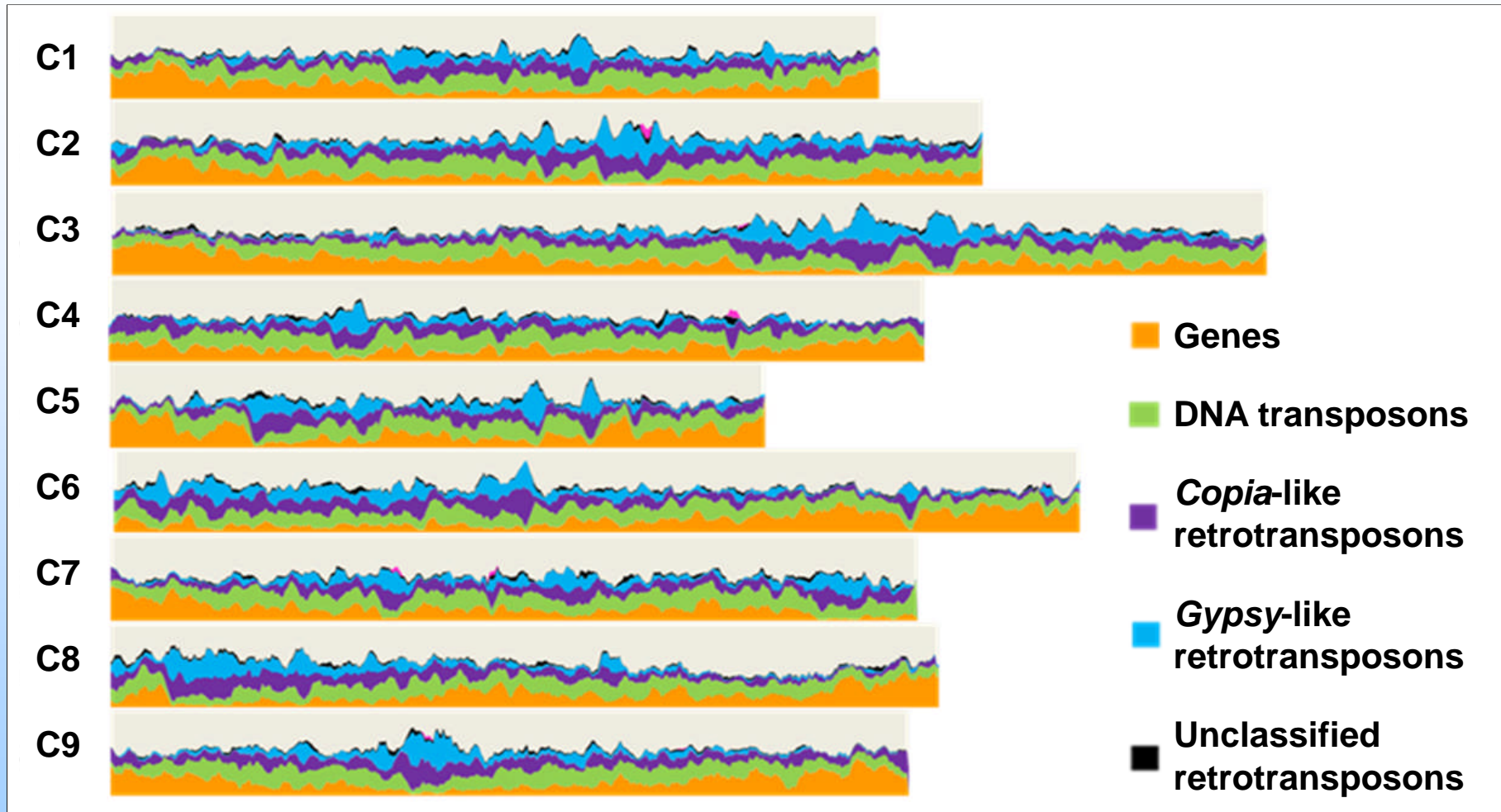
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GENOME SEQUENCING

Brassica oleracea:

540 Mb gene space sequenced

44,940 gene models



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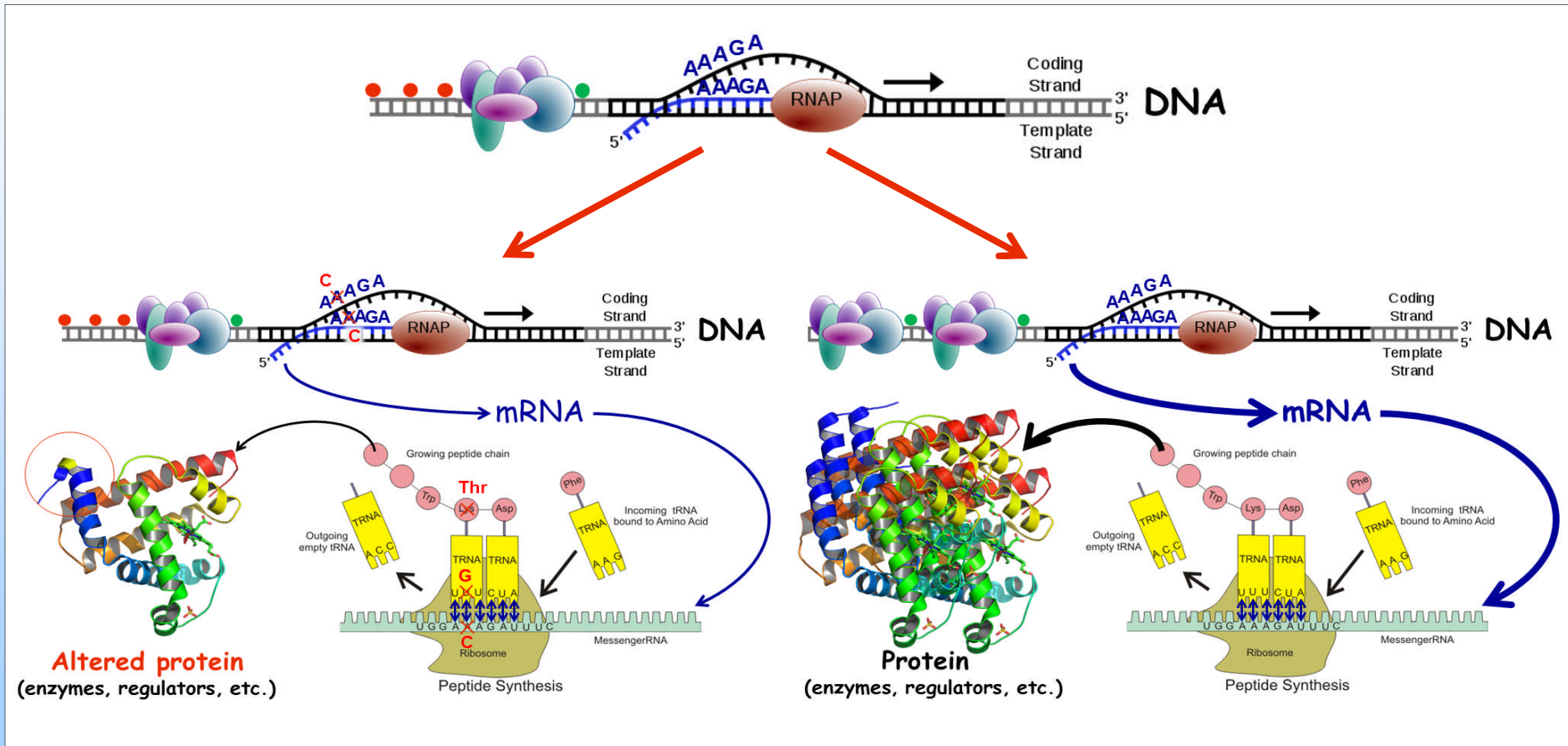
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GENETIC VARIATION

- Genetic variation controls productivity and quality of crops
- Both gene sequence variation and gene expression variation are important

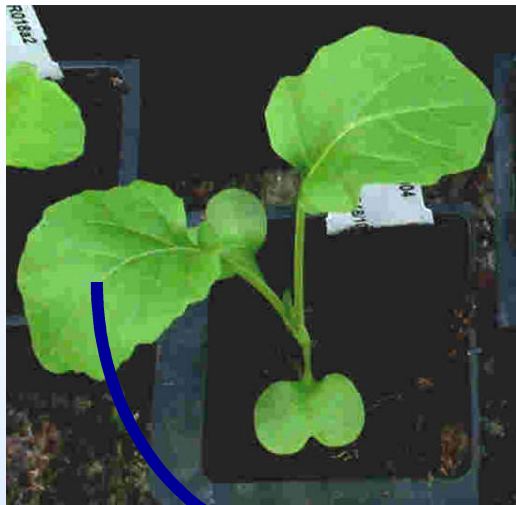


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USE "FUNCTIONAL GENOTYPES" FOR MAPPING



mRNA

3,000,000,000 bases of sequence per plant (~ one complete human genome)

Unigene reference sequence

<pre> TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TcTTcaagGgtctctcttggaaatctat GTGAAAAC TCAAAACACTACTCTCC TGCGA ctctctgaaAcaacctATgTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA ctCtTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA ctctgggacacCtATgTcCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTC caatgggacacctATGtCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TcTTcaatg caactgggacacctATgTccCGTGAAAAC TCAAAACACTACTCTCC TGCGA TcTtcaatgt ctcttgggacacctATGtCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TcTTcaatgg CTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA CTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA CTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA CTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA gtggacacctATGtCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA ggaTgTcccGtGAAAAC TCAAAACACTACTCTCC TGCGA GTGAAAAC TCAAAACACTACTCTCC TGCGA CAAAACACTACTCTCC TGCGA </pre> <p>Tapidor</p> <p>C Y (=C/T)</p>	<pre> TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTcaAgTg ctCtctggACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA tcTTcaaggg ctTggACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAaggTCT ATCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TcTTCAaggTctTTgtttt GTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA ccttcaaggGtCtCcttGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA ggctctcttGgaCaocTatGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA GgGtCtctGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTcaAGgGTCtCTTGgaaactct TCTTCAAGGGTCTCTTGGACACCTATGTCCCGTGAAAAC TCAAAACACTACTCTCC TGCGA TCTTCAAGGGTcTcTtggacacctttt ccttcaagggtctcttggacaccttTATGTCCCGTG </pre> <p>Ningyou 7</p> <p>Y (=C/T) C</p>
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SNP DETECTION USING ILLUMINA SEQUENCING

- Established reference sequence based on the 94k unigenes (EST assemblies) used for the community *Brassica* microarray
- Conducted Illumina sequencing runs on two cultivars (Tapidor & Ningyou 7)
- Used components of M.A.Q. for sequence alignment and SNP calling
- Bioinformatics processing of data for the detection of putative SNPs

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INTEGRATED SNP DISCOVERY AND ASSAY

Transcriptome SNPs distributed throughout gene space

Can be converted to DNA-based assays for marker-assisted selection

NOT dependent upon having to first discover polymorphisms before analysis of sequence variation

Adapted bioinformatics pipeline to simultaneously identify polymorphic loci (positions within *Brassica unigene* reference sequences), assess suitability for use as markers across panels of lines and produce scoring strings

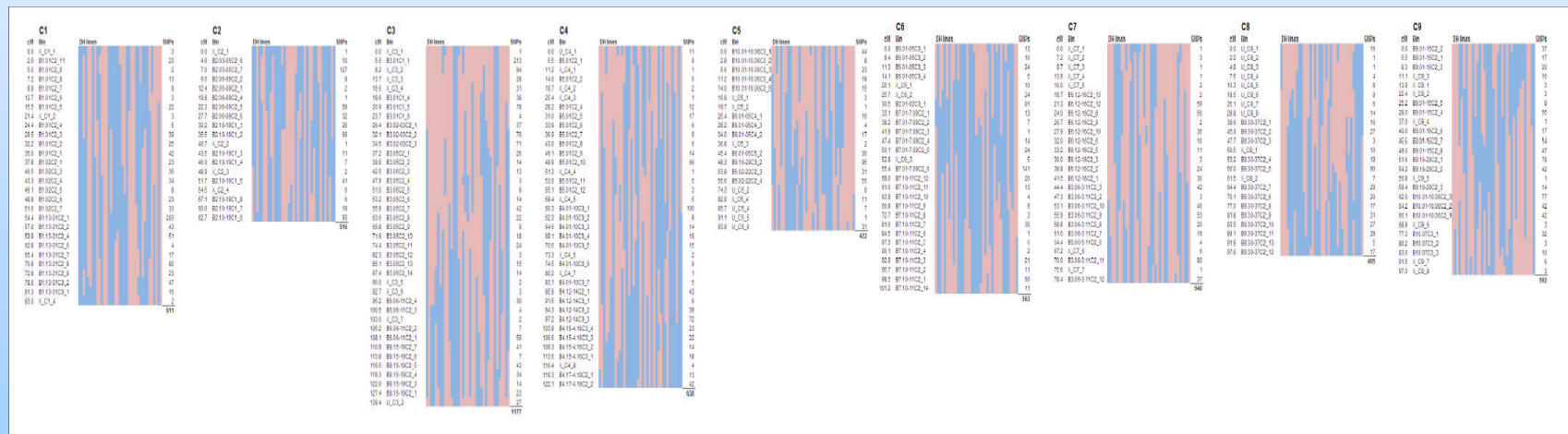
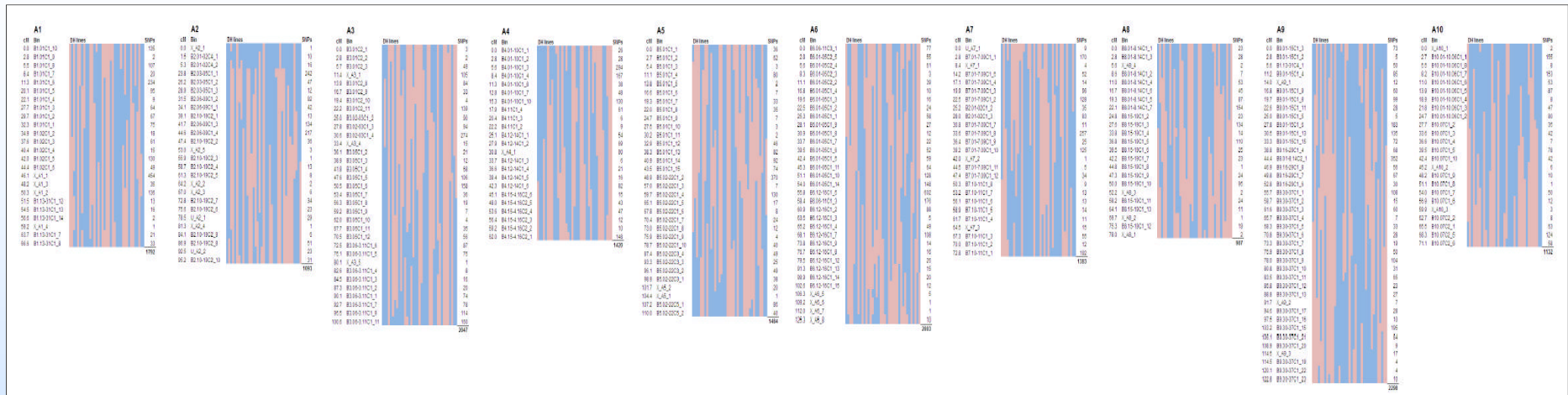
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LINKAGE MAP CONSTRUCTION USING mRNA-seq

TNDH linkage map with 21,323 SNP markers in 527 recombination bins



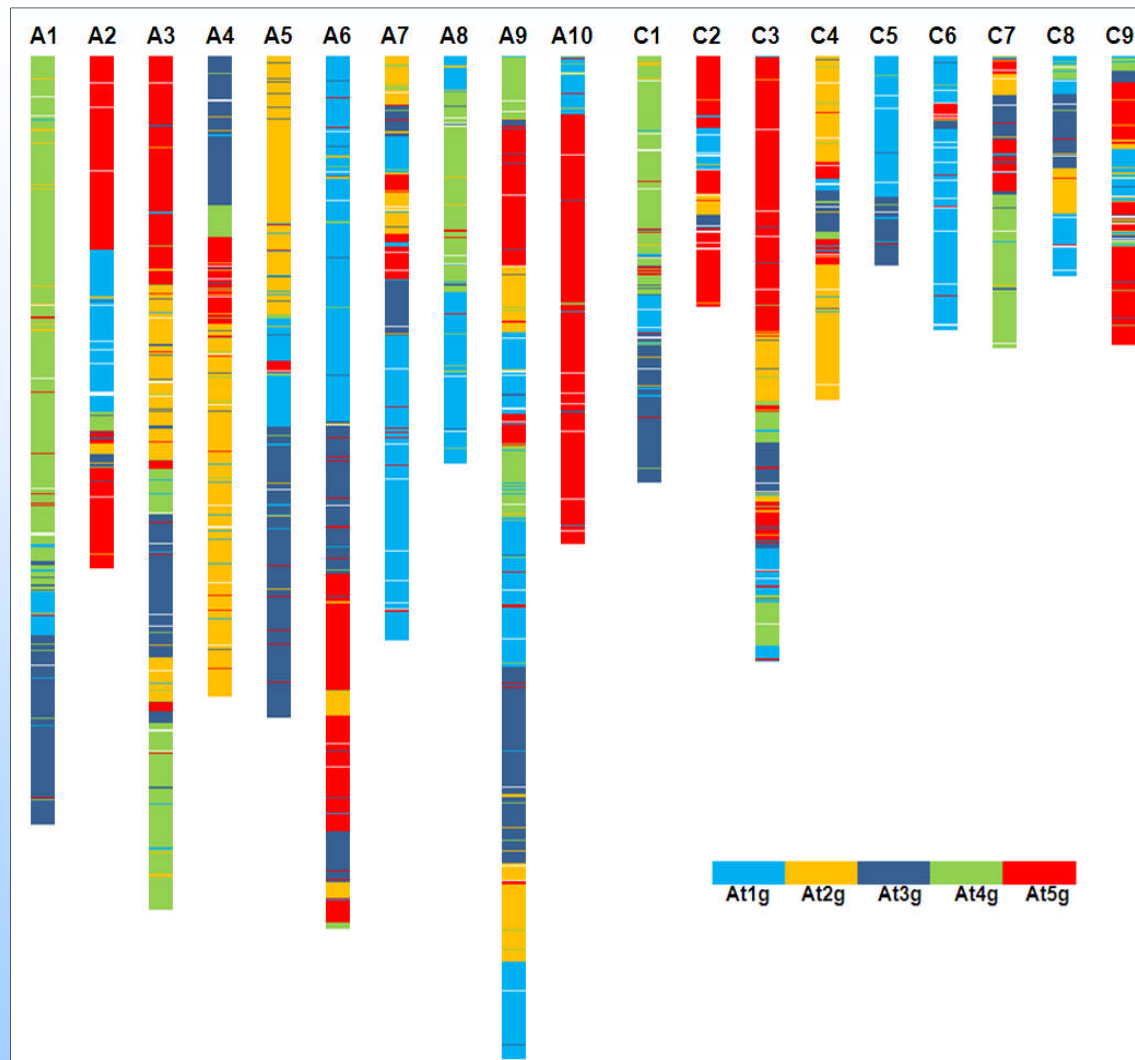
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GENOME ANALYSIS USING mRNA-seq

Analysis of
collinearity with the
genome of
Arabidopsis thaliana
via 9,169 anchoring
unigenes



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UNMAPPED UNIGENES ORDERED BY MAPPED SEQUENCE SCAFFOLDS

1	unigene	A Chr	Start	B. rapa scaffolds	Start	AGI
45240	EV141692	A8	9151176	A_Scaffold000097	796364	AT4G34850.1
45241	JCVI_15733	A8	9151537	A_Scaffold000097	795749	AT4G34850.1
45242	CX192070	A8	9153474	A_Scaffold000097	794210	AT4G34840.1
45243	JCVI_5729	A8	9176822	A_Scaffold000097	759664	.
45244	JCVI_9918	A8	9177527	A_Scaffold000097	759714	.
45245	EV075943	A8	9182978	A_Scaffold000103	470339	AT1G3570.1
45246	DY023723	A8	9197232	A_Scaffold000097	740289	.
45247	JCVI_1057	A8	9212467	A_Scaffold000097	725113	AT4G34720.1
45248	CV546751	A8	9219387	A_Scaffold000097	718170	.
45249	JCVI_10034	A8	9219799	A_Scaffold000097	716806	AT4G34710.1
45250	JCVI_12180	A8	9221365	A_Scaffold000097	716121	AT4G34710.1
45251	EX120610	A8	9222032	A_Scaffold000097	715694	.
45252	EE470793	A8	9227903	A_Scaffold000097	709576	AT4G34700.1
45253	EX066713	A8	9230322	A_Scaffold000097	707204	AT4G34680.1
45254	JCVI_8753	A8	9230420	A_Scaffold000097	706938	AT4G34680.1
45255	BG543396	A8	9231064	A_Scaffold000097	706628	AT4G34680.1
45256	JCVI_4009	A8	9235433	A_Scaffold000097	702318	AT4G34670.1
45257	CV432227	A8	9235439	A_Scaffold000097	702318	AT4G34670.1
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45259	JCVI_16353	A8	9238913	A_Scaffold000097	698854	AT4G34660.1
45260	EE542458	A8	9239928	A_Scaffold000097	697689	AT4G34660.1
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45263	EE536108	A8	9243526	A_Scaffold000097	694157	AT4G34640.1
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45265	JCVI_18916	A8	9254489	A_Scaffold000097	683046	AT4G34590.1
45266	ES907398	A8	9261123	A_Scaffold000097	676452	AT4G34590.1
45267	JCVI_25534	A8	9271398	A_Scaffold000099	755577	.
45268	JCVI_92337	A8	9271400	A_Scaffold000099	755576	.
45269	JCVI_22797	A8	9281778	A_Scaffold000097	654550	AT4G34520.1
45270	JCVI_36670	A8	9285016	A_Scaffold000097	652016	AT4G34510.1
45271	EV163038	A8	9285689	A_Scaffold000097	651611	AT4G34510.1
45272	CX271024	A8	9305343	A_Scaffold000097	631967	.
45273	JCVI_15279	A8	9318289	A_Scaffold000097	619381	AT4G34490.1
45274	JCVI_21808	A8	9322039	A_Scaffold000097	615712	AT4G34460.1
45275	JCVI_18088	A8	9322765	A_Scaffold000097	614719	AT4G34460.1
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45283	ES912981	A8	9345298	A_Scaffold000097	592291	AT4G34430.3
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45285	EX091671	A8	9348079	A_Scaffold000097	589535	AT4G34430.3
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45288	EX127275	A8	9359192	A_Scaffold000097	578204	.
45289	EV085789	A8	9360121	A_Scaffold000097	577295	.
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45292	EX079894	A8	9367763	A_Scaffold000097	569795	AT4G34390.1
45293	EV106761	A8	9367791	A_Scaffold000097	569777	AT4G34390.1
45294	EX058924	A8	9370511	A_Scaffold000097	566973	AT4G34390.1
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45296	ES912902	A8	9377352	A_Scaffold000097	560074	AT1G35920.1

Use BLAST to identify *B. rapa* and *B. oleracea* genome sequence scaffolds corresponding to linkage-mapped unigenes, assemble into pseudomolecules representing the *B. napus* genome.

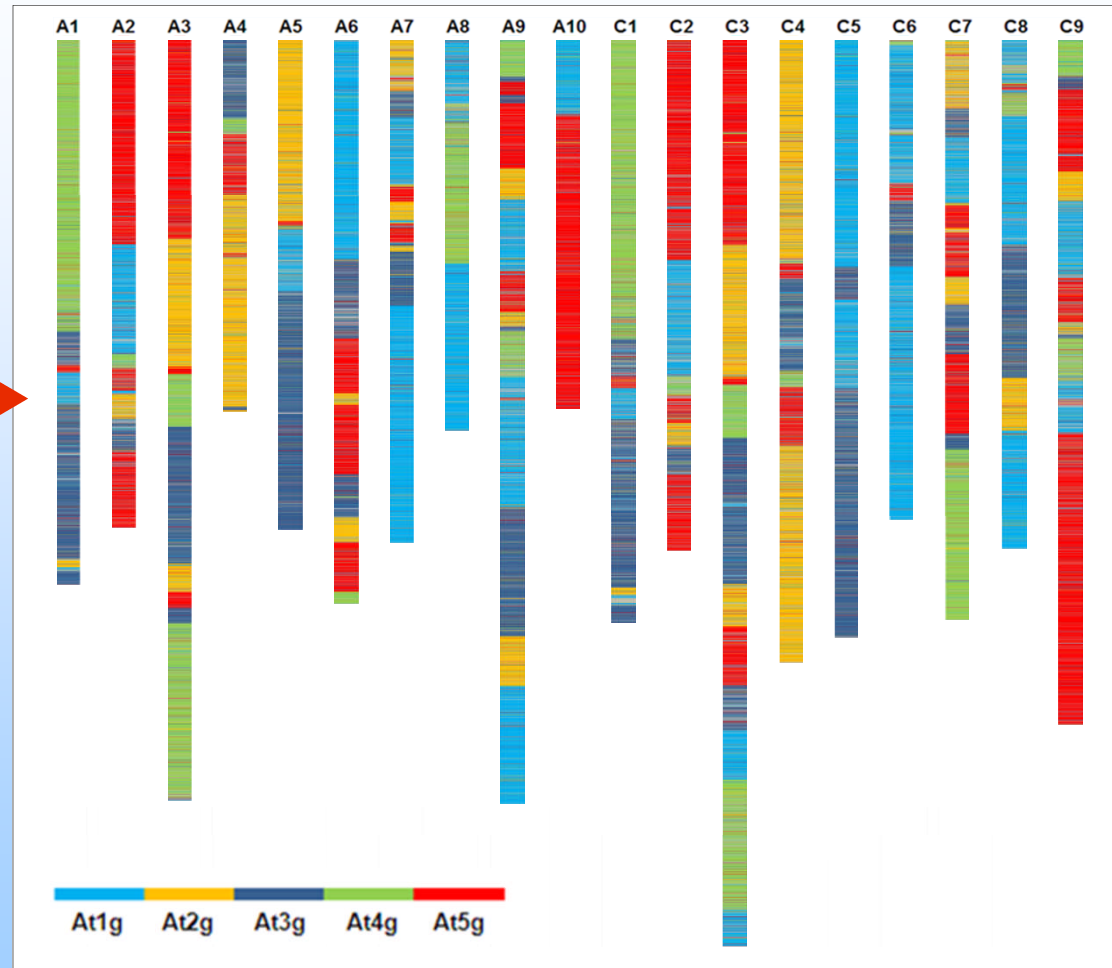
Use BLAST to identify best match between sequence of every unigene and the pseudomolecules representing the *B. napus* genome.

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45243	JCVI_5729	A8	9176822	A_Scaffold000097	759664	.
45244	JCVI_3918	A8	9177527	A_Scaffold000097	759714	.
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45247	JCVI_1057	A8	9212467	A_Scaffold000097	725113	AT4G34720.1
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45256	JCVI_4009	A8	9235433	A_Scaffold000097	702318	AT4G34670.1
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45259	JCVI_16353	A8	9238913	A_Scaffold000097	698854	AT4G34660.1
45260	EE542458	A8	9239928	A_Scaffold000097	697689	AT4G34660.1
45261	JCVI_17975	A8	9240040	A_Scaffold000097	697678	AT4G34660.1
45262	JCVI_7890	A8	9240469	A_Scaffold000097	697187	AT4G34640.1
45263	EE536108	A8	9243526	A_Scaffold000097	694157	AT4G34640.1
45264	JCVI_12520	A8	9243596	A_Scaffold000097	694040	AT4G34640.1
45265	JCVI_18916	A8	9254489	A_Scaffold000097	683046	AT4G34590.1
45266	ES907398	A8	9261123	A_Scaffold000097	676452	AT4G34580.1
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45275	JCVI_18088	A8	9322765	A_Scaffold000097	614719	AT4G34460.1
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45282	JCVI_34283	A8	9344371	A_Scaffold000097	593251	AT4G34430.1
45283	ES912981	A8	9345298	A_Scaffold000097	592291	AT4G34430.3
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45286	JCVI_23541	A8	9356323	A_Scaffold000097	581044	AT4G34410.1
45287	JCVI_19991	A8	9356523	A_Scaffold000097	580865	AT4G34410.1
45288	EX127275	A8	9359192	A_Scaffold000097	578204	.
45289	EV085789	A8	9360121	A_Scaffold000097	577295	.
45290	EV106406	A8	9367444	A_Scaffold000097	569822	AT4G34390.1
45291	EV106450	A8	9367446	A_Scaffold000097	569785	AT4G34390.1
45292	EX079894	A8	9367763	A_Scaffold000097	569795	AT4G34390.1
45293	EV106761	A8	9367791	A_Scaffold000097	569777	AT4G34390.1
45294	EX058924	A8	9370511	A_Scaffold000097	566973	AT4G34390.1
45295	ES984770	A8	9373170	A_Scaffold000097	564202	AT4G34380.1
45296	ES912902	A8	9377352	A_Scaffold000097	560074	AT1G35920.1

61,613 unigenes anchored to A genome
64,292 unigenes anchored to C genome



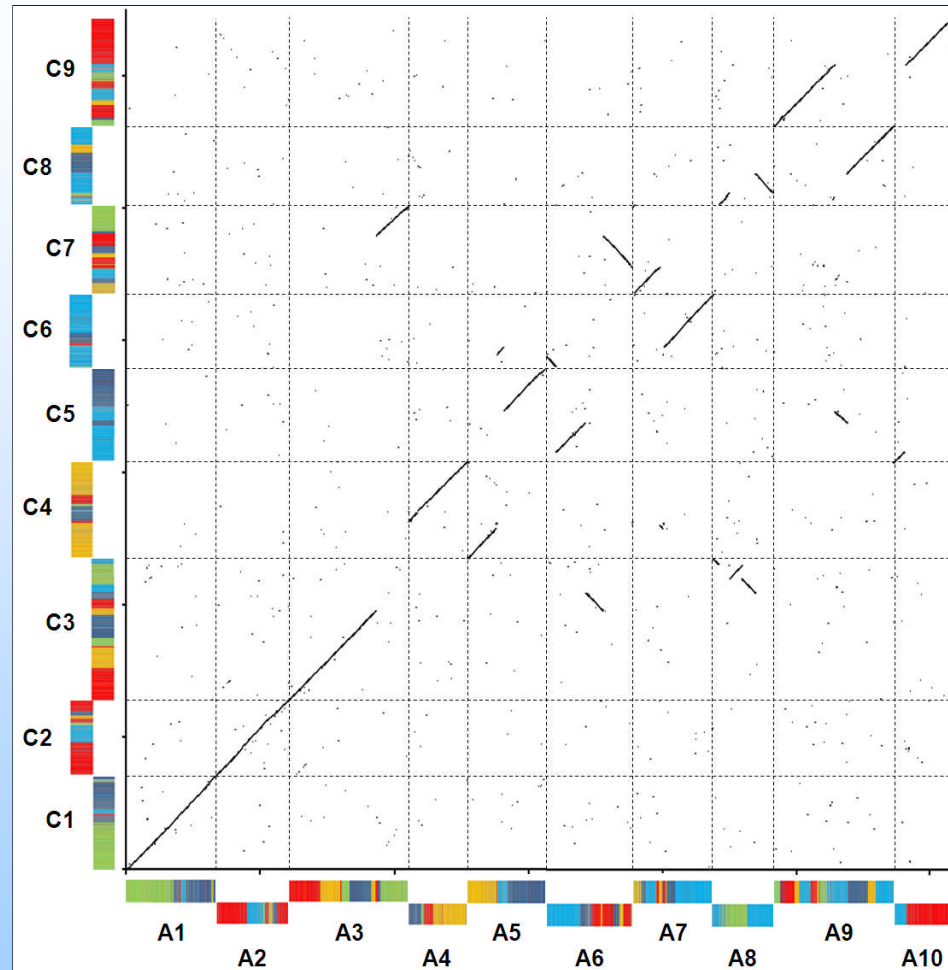
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BEST UNIGENE SEQUENCE MATCHES TO A AND C GENOMES

Rearrangements between A and C genomes (as represented in *B. napus*)

48,442 unigenes
collinear between
genomes

(BLAST-mapped 1E-30 to
both genomes, filtered to
exclude those with
inconsistent cross-genome
matches for both flanking
unigenes)



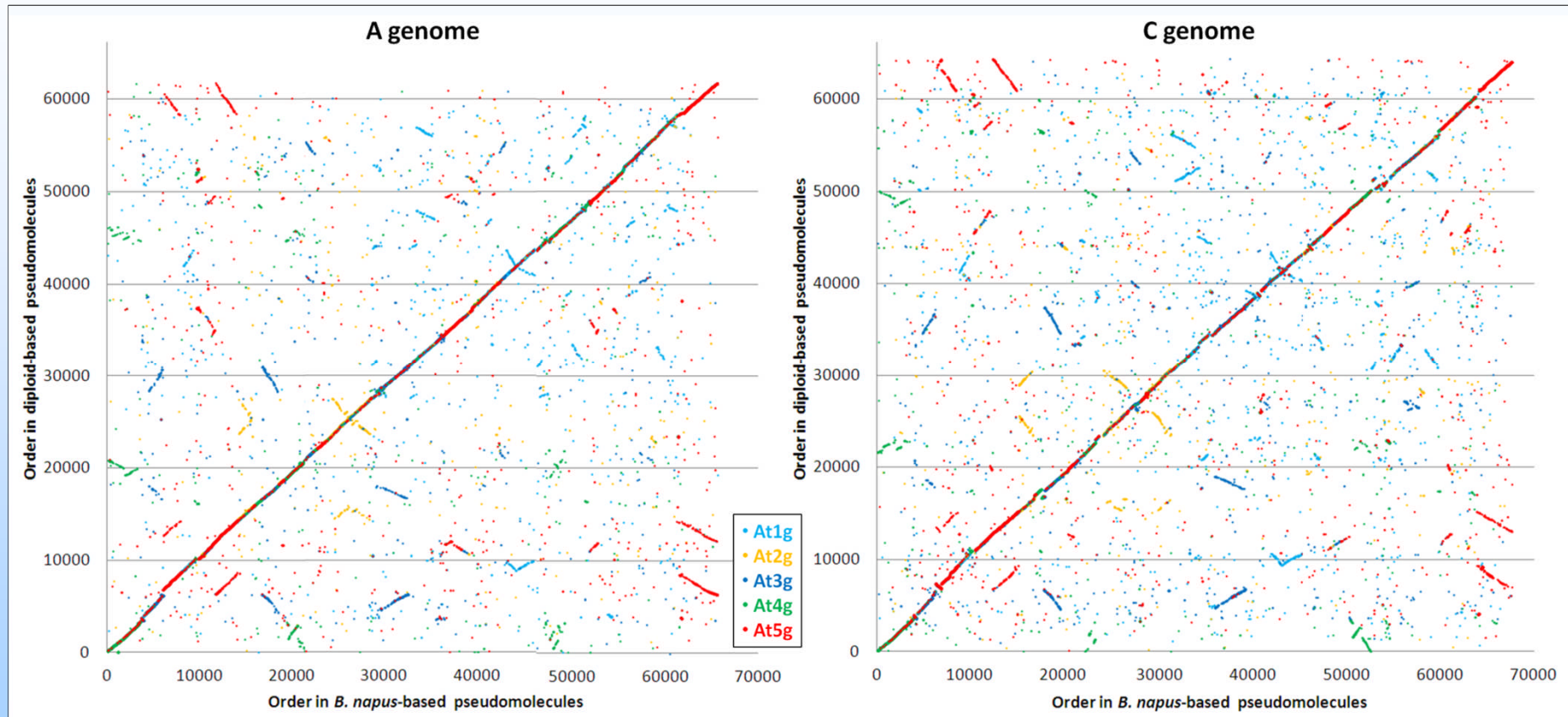
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BEST UNIGENE SEQUENCE MATCHES TO RAPESEED GENOME

Good (but not perfect) collinearity between diploid-based and *B. napus*-based pseudomolecules representing the *B. napus* genome



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- *Brassica* genome structure and evolution
- Genome framework for association genetics
- Establishing marker-trait associations

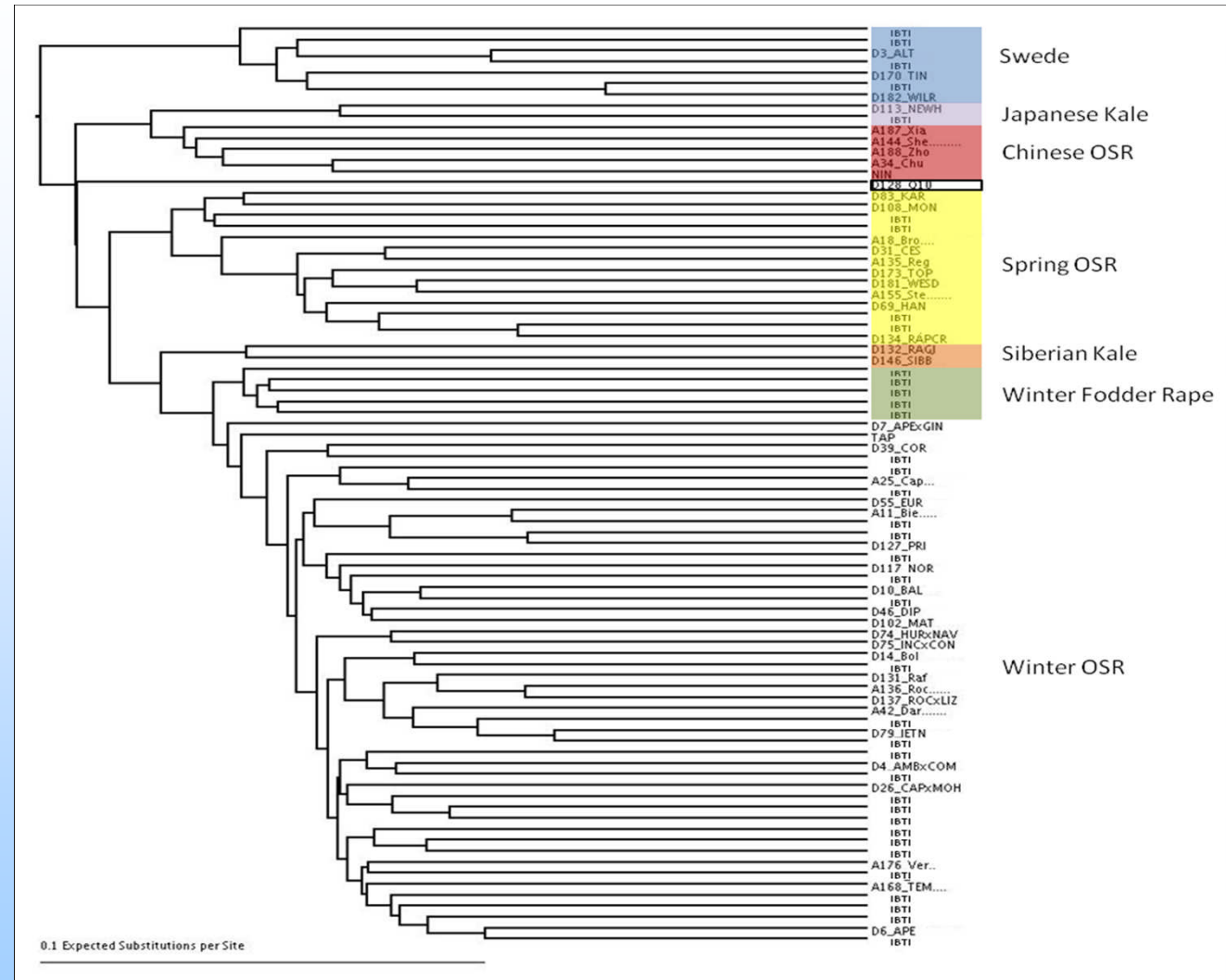
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RELATEDNESS ANALYSIS

Tree construction
across 83 *B. napus*
accessions with
101,644
transcriptome
SNPs.



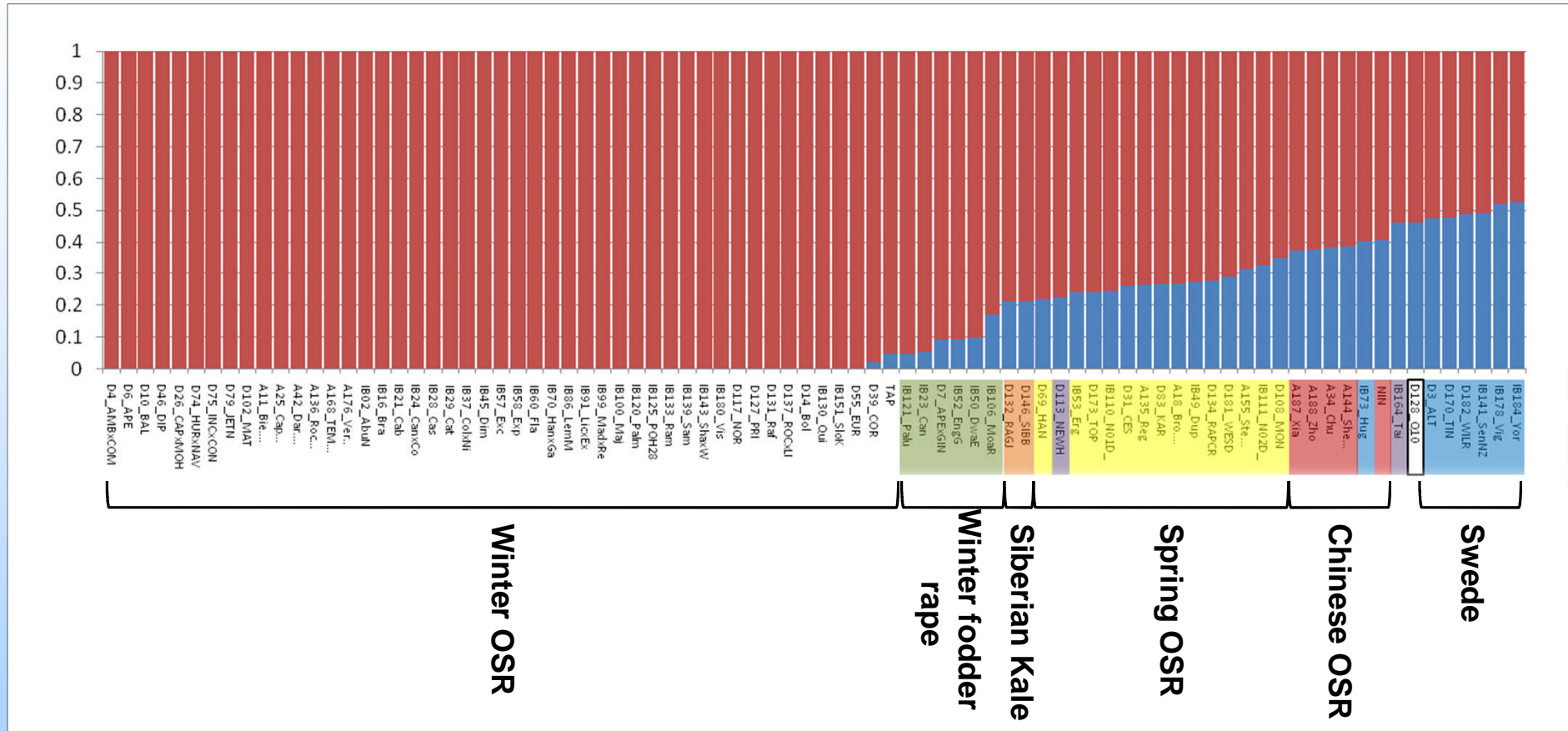
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POPULATION STRUCTURE ANALYSIS

STRUCTURE analysis across 83 *B. napus* accessions



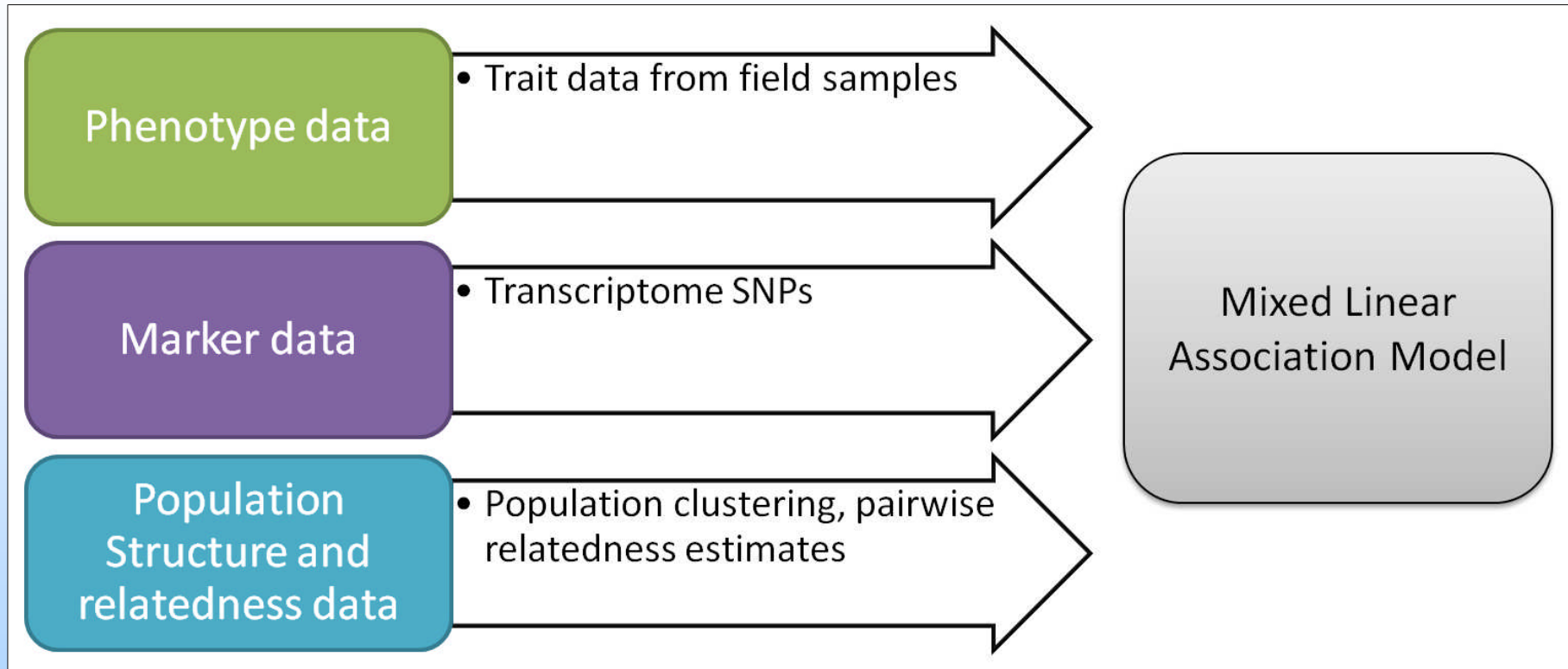
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ASSOCIATION GENETICS

Linkage Disequilibrium analysis



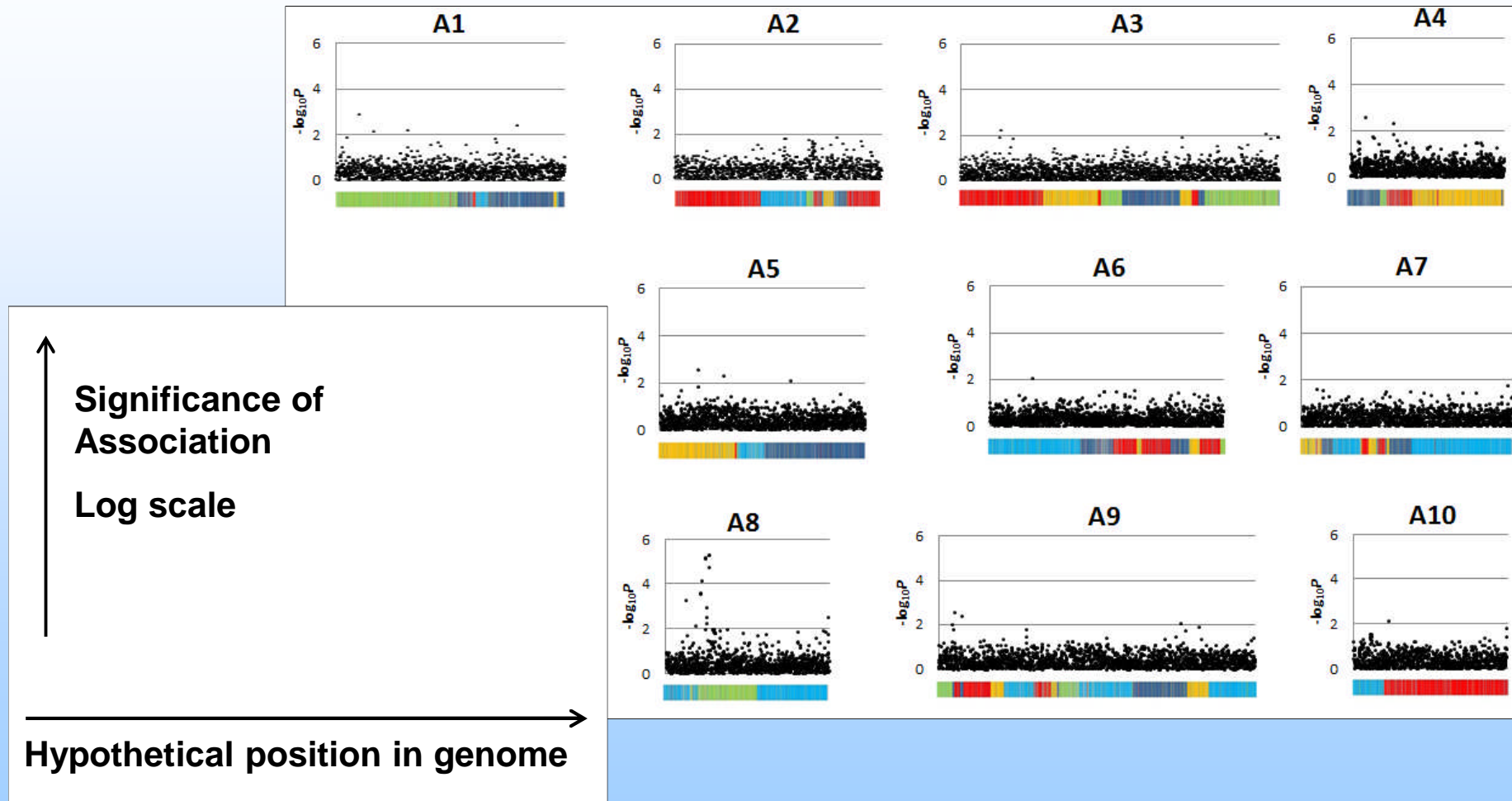
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VISUALISING MARKER-TRAIT ASSOCIATIONS

Genome-Wide Association Scans: "Manhattan plots"



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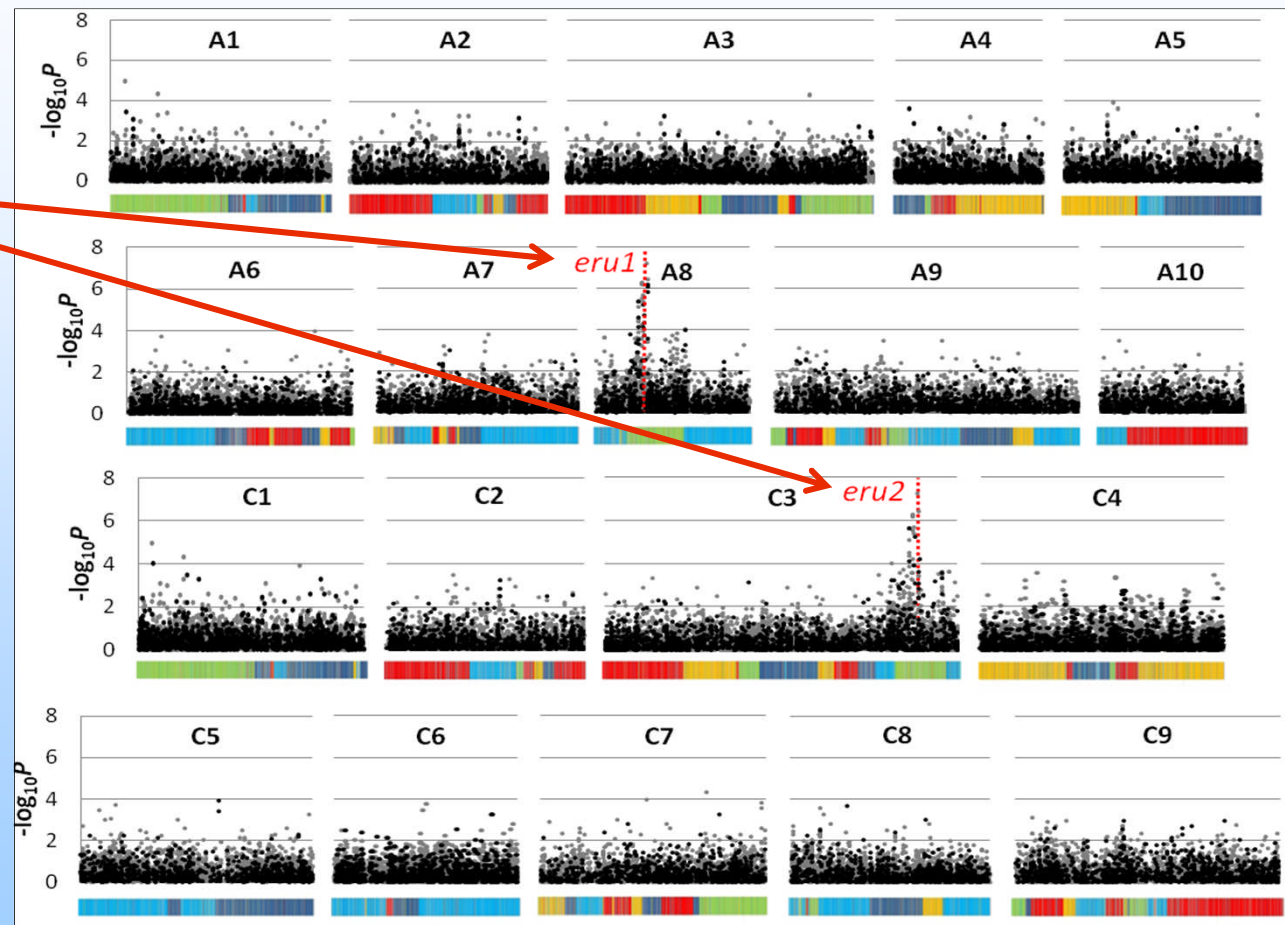
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EXAMPLE OF ASSOCIATION GENETICS USING TRANSCRIPTOME SNPs

Validation experiment: Linkage Disequilibrium analysis for **erucic acid** content of seed oil (53 *B. napus* accessions)

Clusters of highly significant associations coincide precisely with the positions of the genes known to control the trait



Identifying the functional bases of trait variation in *Brassica napus* using Associative Transcriptomics

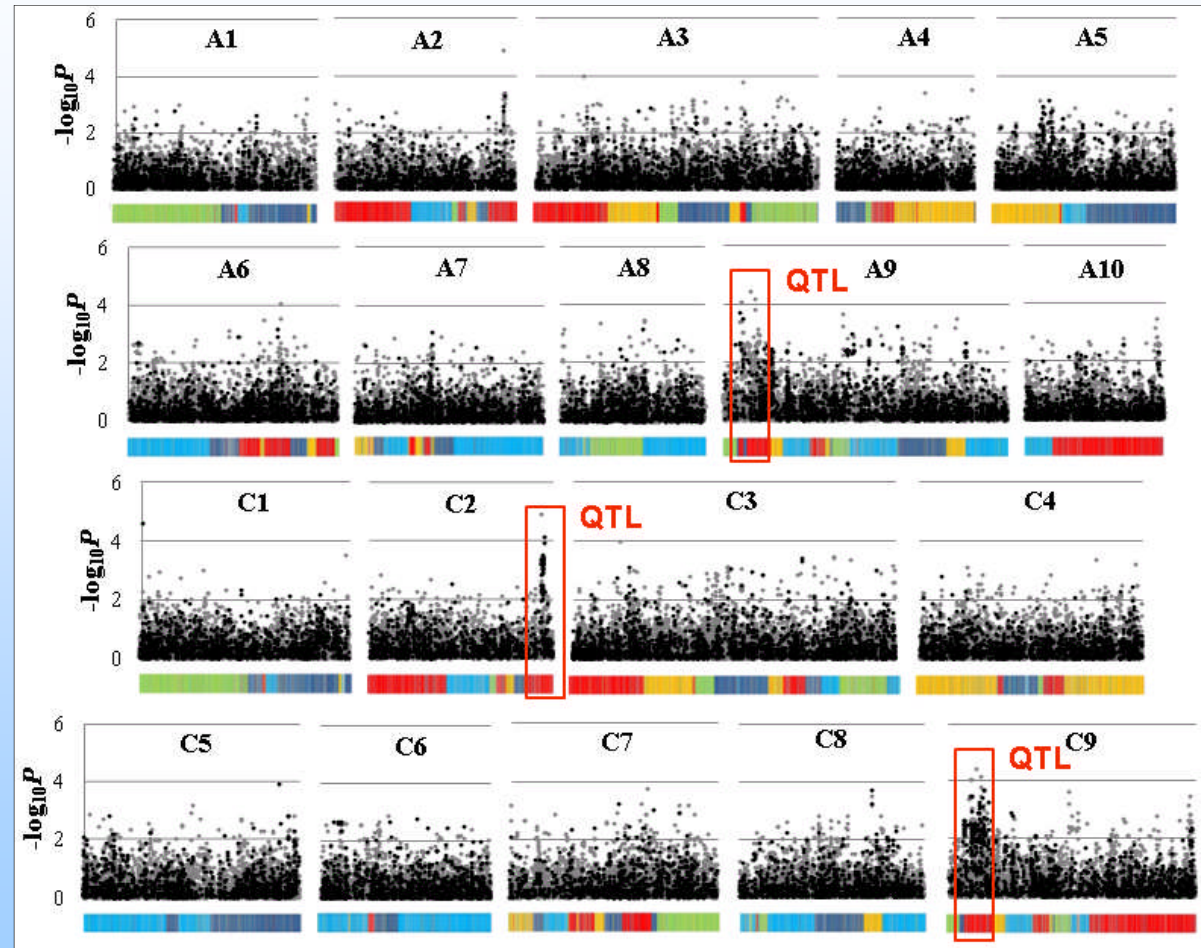
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ASSOCIATION GENETICS USING TRANSCRIPTOME SNPs: GLUCOSINOLATES

Trait of unknown basis: Linkage Disequilibrium analysis for **glucosinolate** content of seeds (53 *B. napus* accessions)

Genome-wide association scan for SNP markers in 125,905 hypothetically ordered unigenes



Identifying the functional bases of trait variation in *Brassica napus* using Associative Transcriptomics

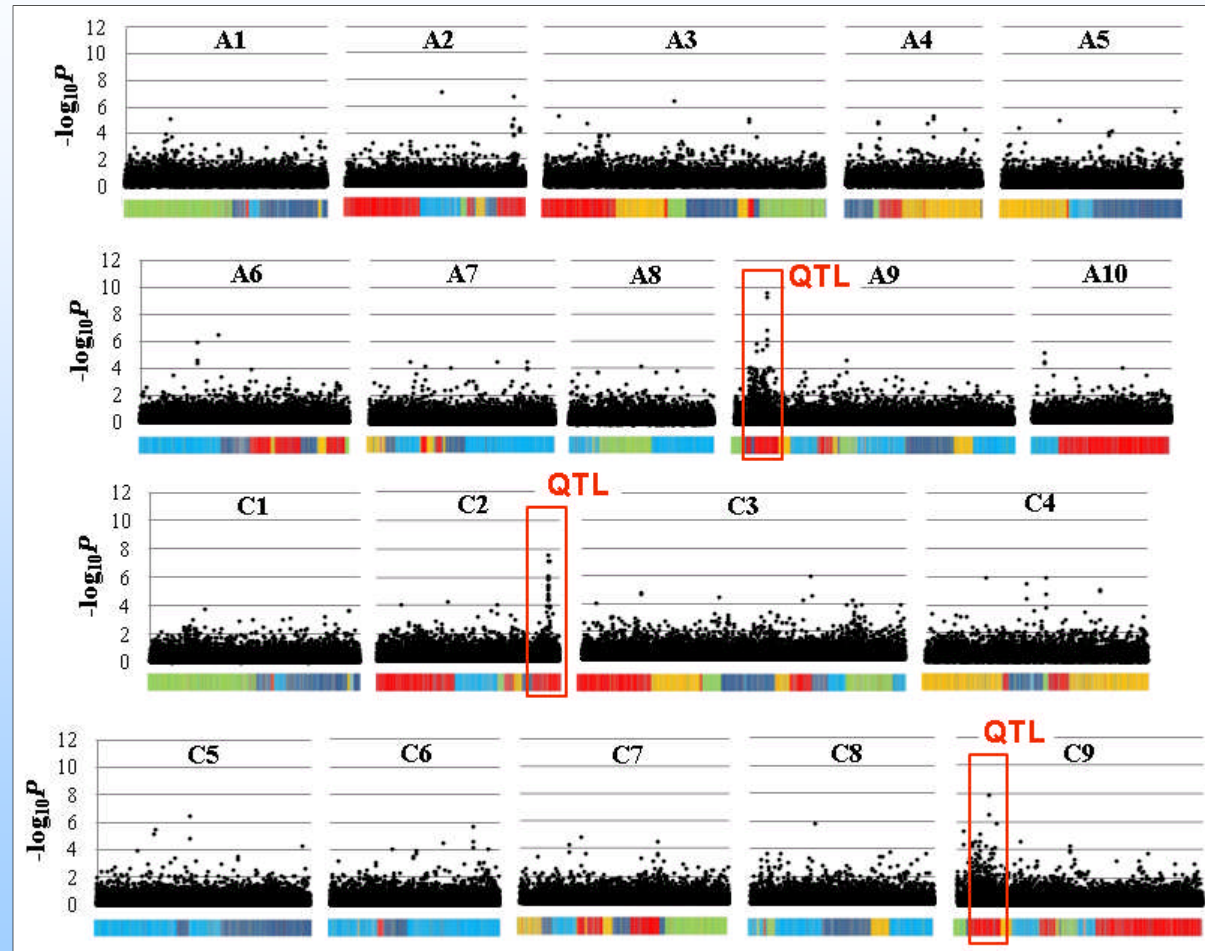
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CORRELATE GENE EXPRESSION VARIATION WITH TRAIT VARIATION

Trait of unknown basis: Regression analysis for **glucosinolate** content of seeds (53 *B. napus* accessions)

Genome-wide regression for expression variation in 125,905 hypothetically ordered unigenes



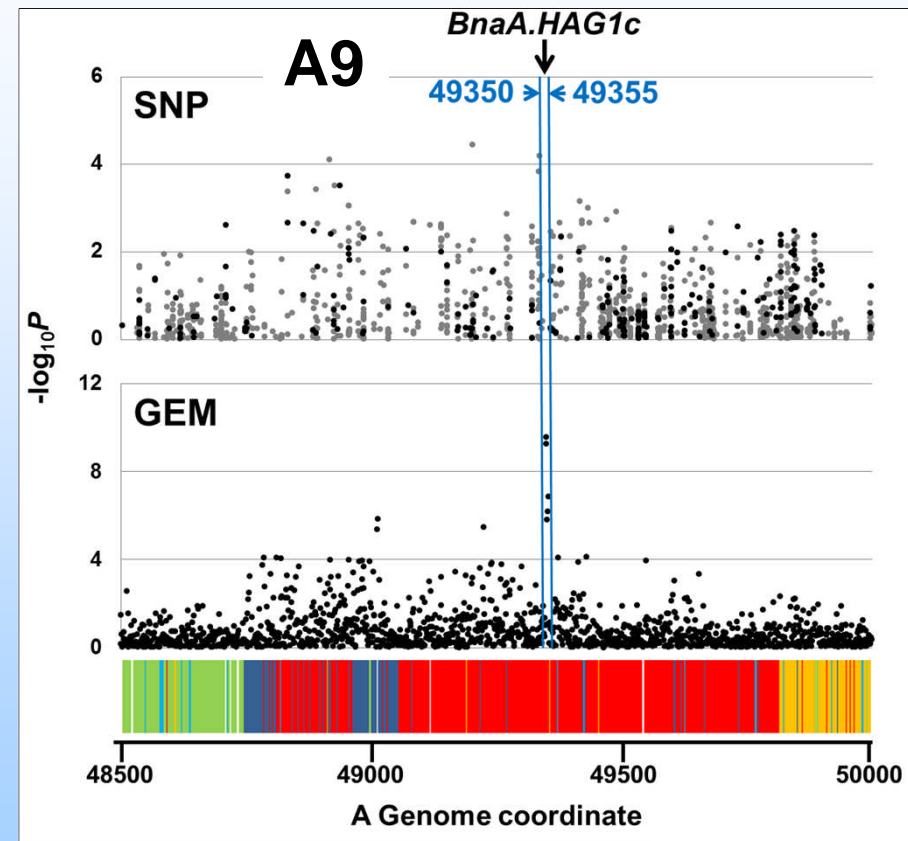
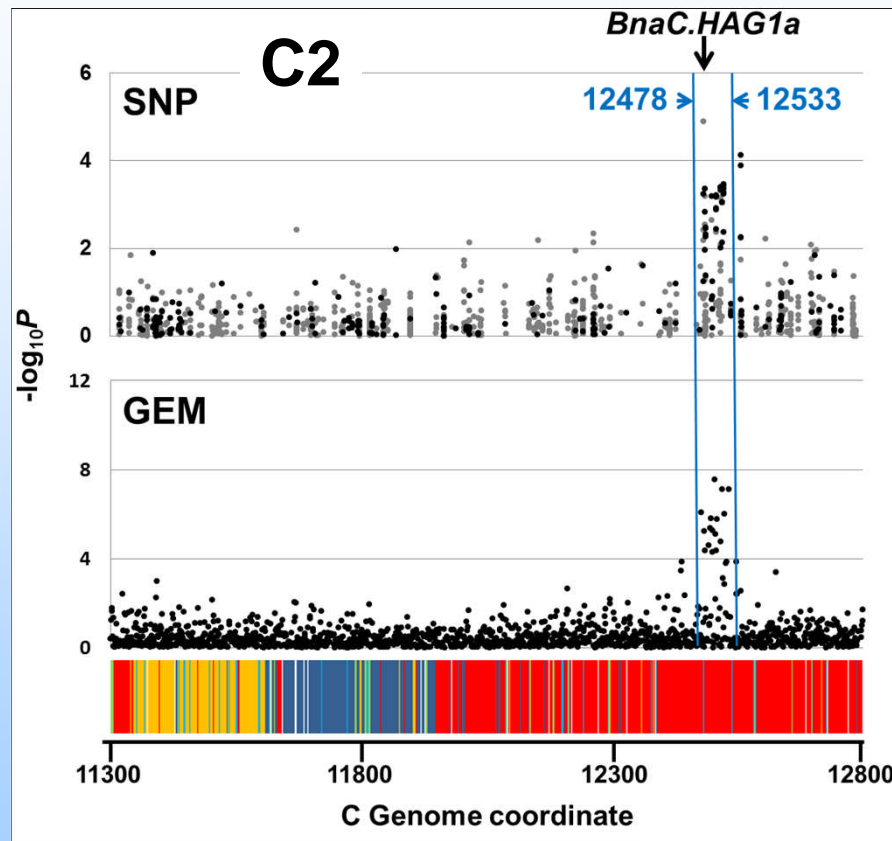
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IDENTIFICATION OF CANDIDATE GENES

- Association analyses provide molecular markers to support breeding
- Associative Transcriptomics often identifies causative gene variants



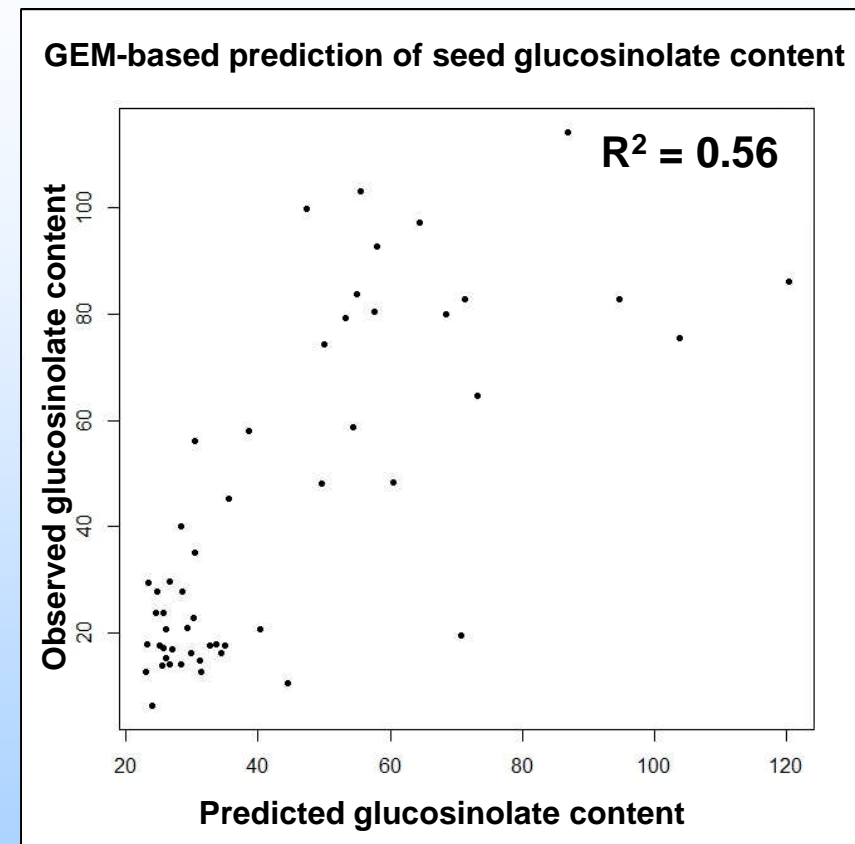
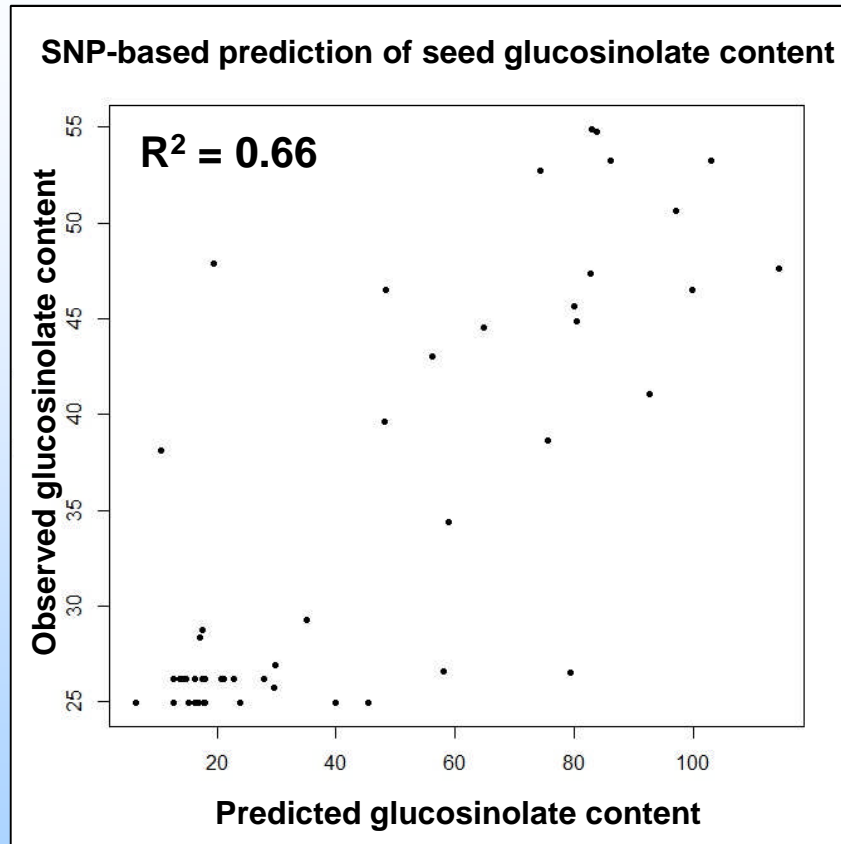
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TRAIT PREDICTION

Markers associated with variation are predictive of quantitative trait values



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