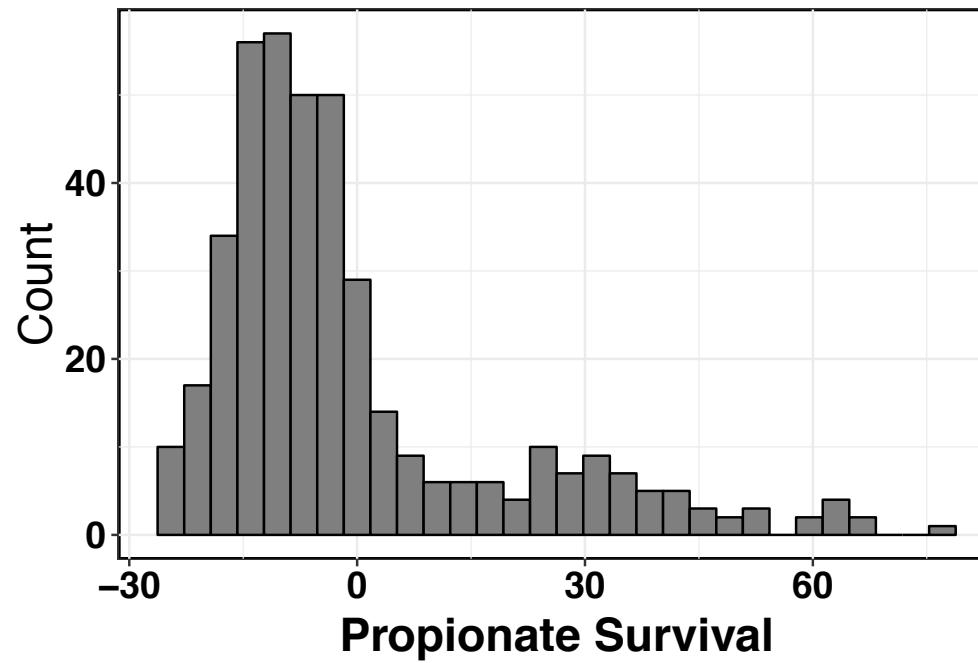


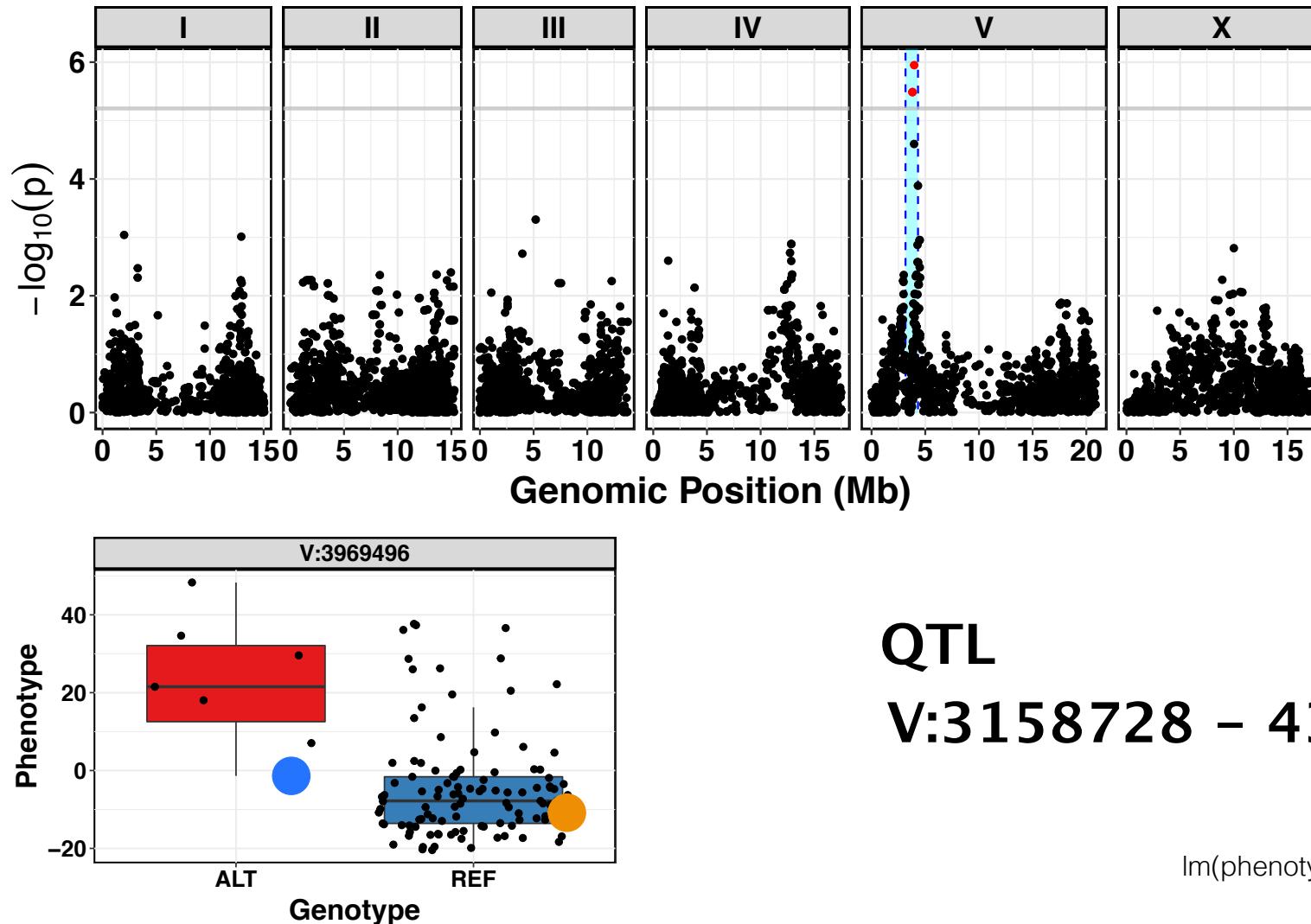
Propionate QTL summary

Distribution of processed propionate survival phenotypes



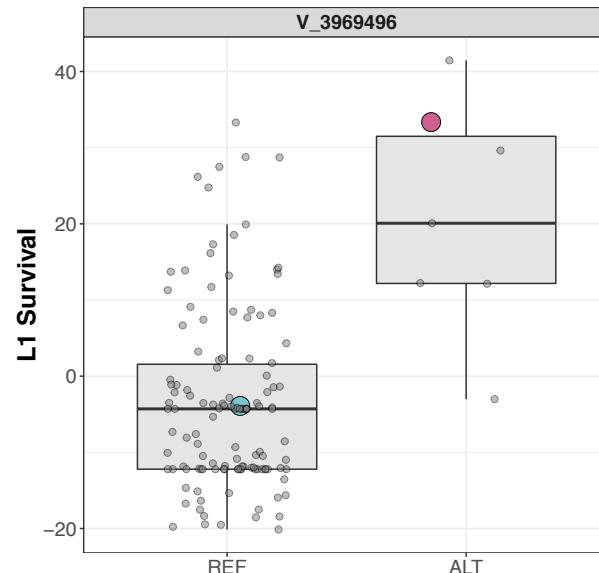
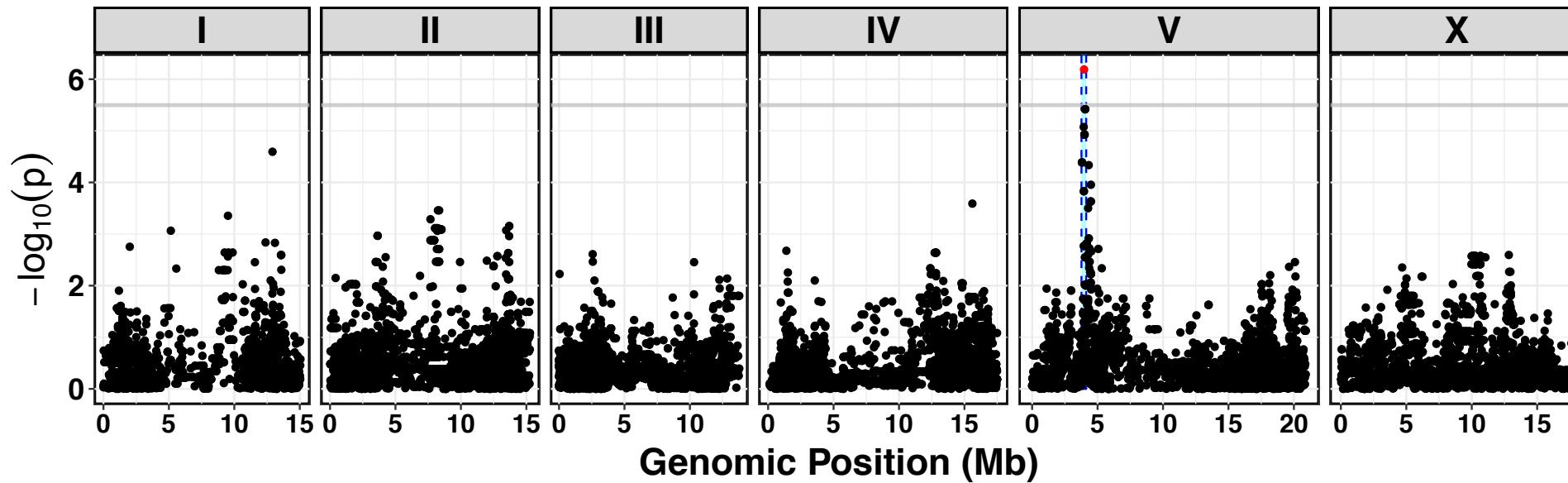
lm(phenotype ~ set) and removal of outliers

Original QTL on the left arm of chromosome V



QTL on the left arm of chromosome V (updated marker set)

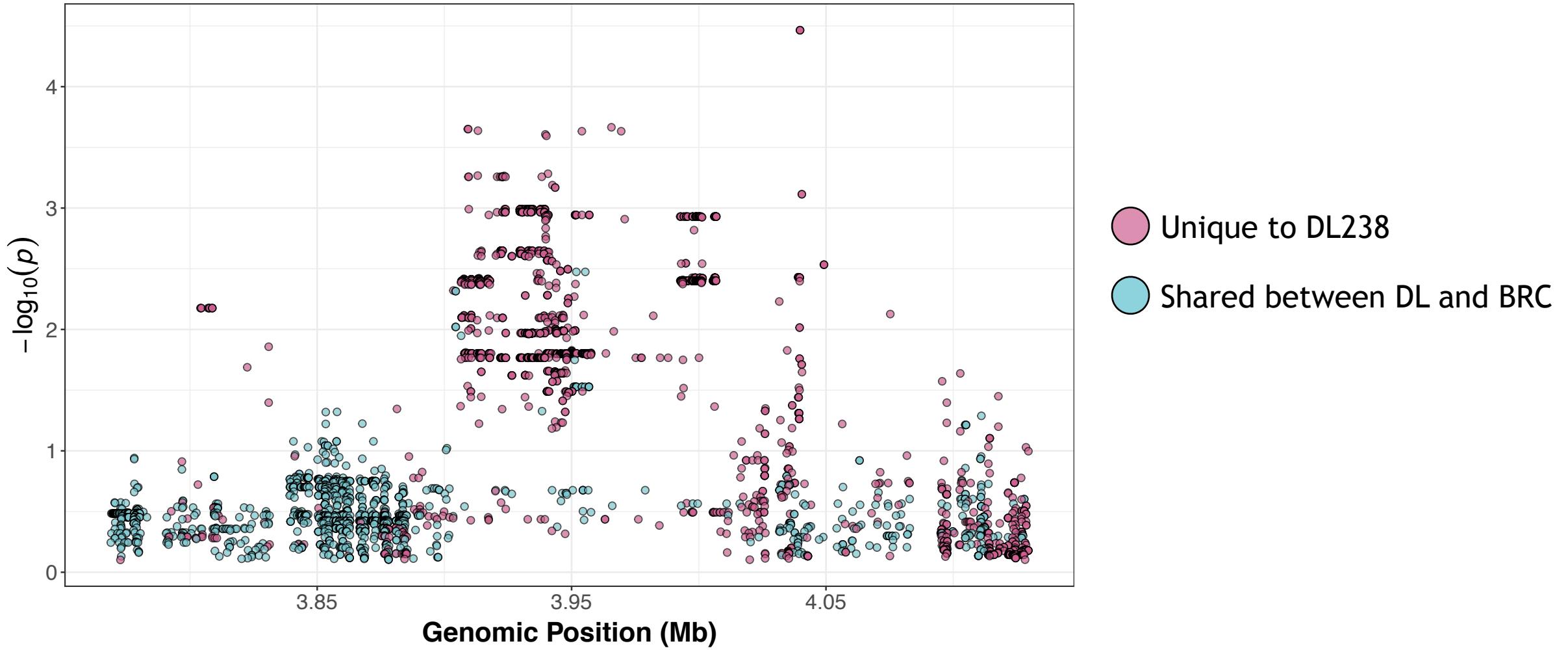
L1 Survival



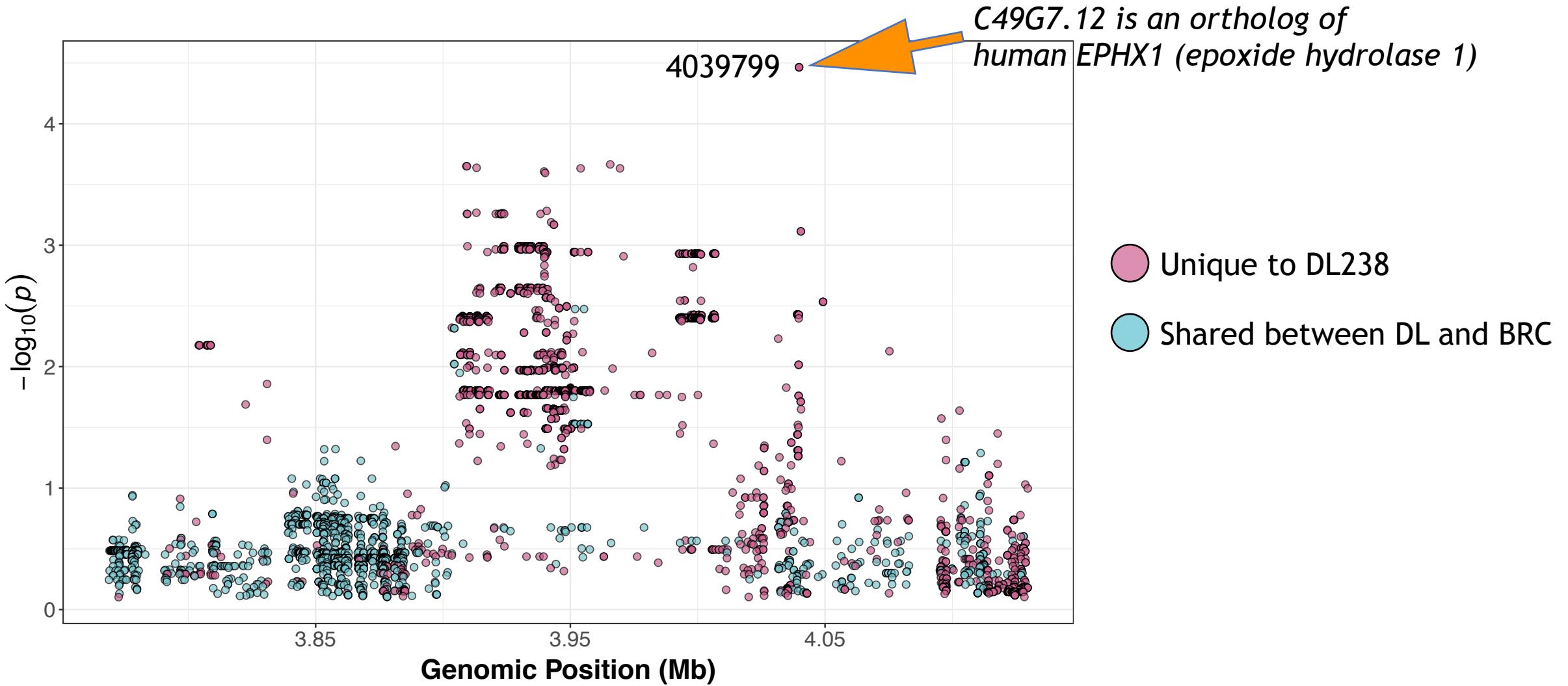
QTL
V: 3768868 – 4129900

lm(phenotype ~ set) and removal of outliers

Fine mapping of updated QTL confidence interval



Fine mapping of updated QTL confidence interval

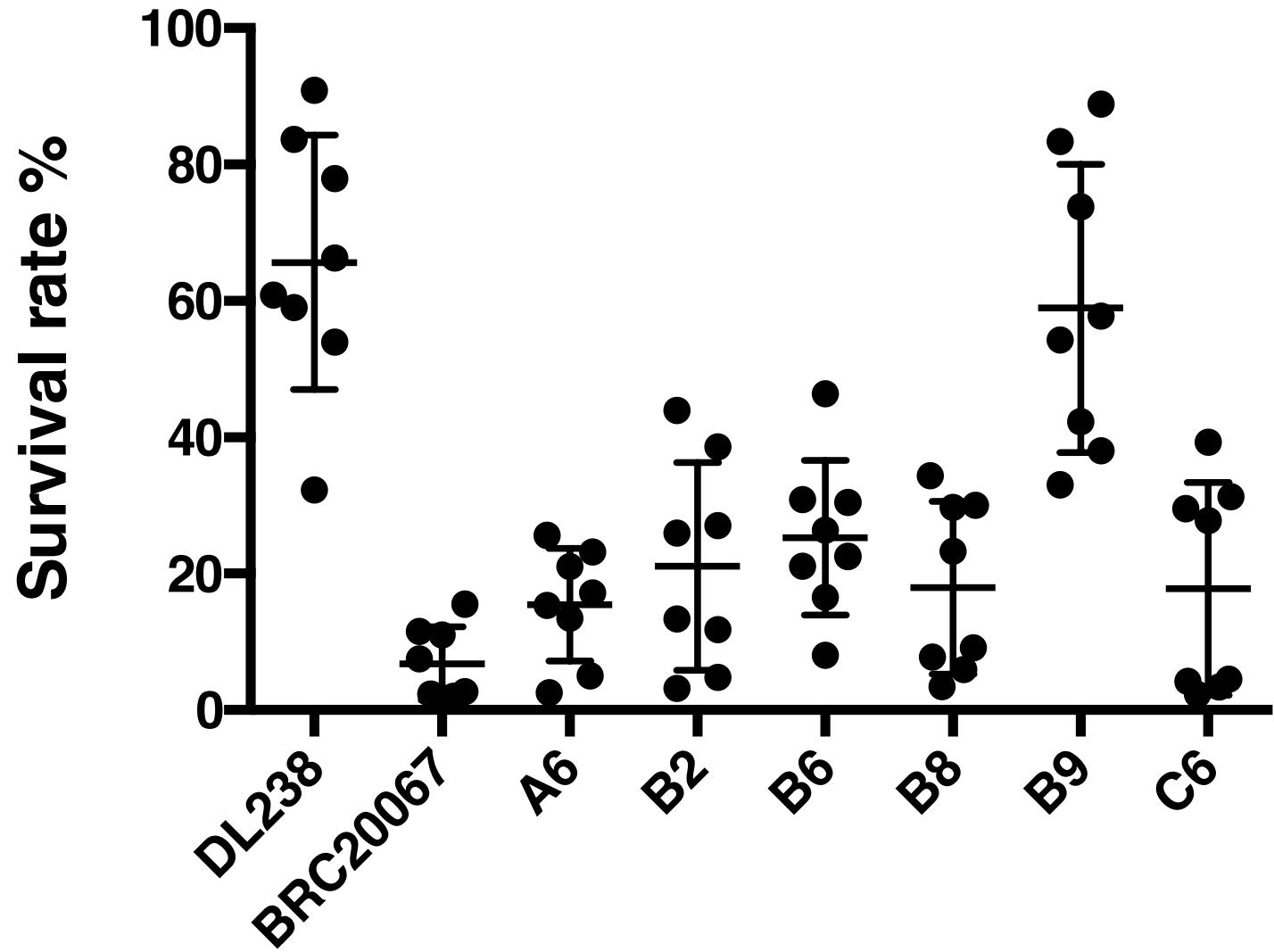


CHROM	POS	aa_change	gene_id	gene_name	log10p
V	4039787	p.Tyr261Phe	WBGene00077593	C49G7.12	4.464938
V	4039799	p.Pro265Gln	WBGene00077593	C49G7.12	4.464938
V	4039800	p.Pro265Pro	WBGene00077593	C49G7.12	4.464938
V	3965658		WBGene00015043	cyp-34A8	3.665353
V	3909185		WBGene00005952	srx-61	3.650420
V	3909384		WBGene00005952	srx-61	3.650420
V	3913185		WBGene00005959	srx-68	3.637398
V	3954044	p.Phe2Leu	WBGene00006274	str-247	3.632746
V	3969496		WBGene00015044	cyp-34A9	3.632746
V	3939690		WBGene00019472	cyp-35B1	3.606947
V	3940099	p.Ala155Thr	WBGene00019472	cyp-35B1	3.594843
V	3940722	p.Ala362Ala	WBGene00019472	cyp-35B1	3.282753
V	3913098		WBGene00005959	srx-68	3.267520
V	3923520		WBGene00005960	srx-69	3.264983
V	3909400		WBGene00005952	srx-61	3.257626
V	3909565		WBGene00005952	srx-61	3.257626
V	3920719	p.Ile267Ile	WBGene00005957	srx-66	3.257626
V	3921940	p.Ala2Ala	WBGene00005956	srx-65	3.257626
V	3922653	p.Ala206Ala	WBGene00005956	srx-65	3.257626
V	3922782	p.Leu249Leu	WBGene00005956	srx-65	3.257626
V	3922877	p.Gly265Gly	WBGene00005956	srx-65	3.257626
V	3922889	p.Leu269Leu	WBGene00005956	srx-65	3.257626
V	3922940	p.Val286Val	WBGene00005956	srx-65	3.257626
V	3924032		WBGene00005960	srx-69	3.257626

Other correlated genes within QTL

NIL Genotyping

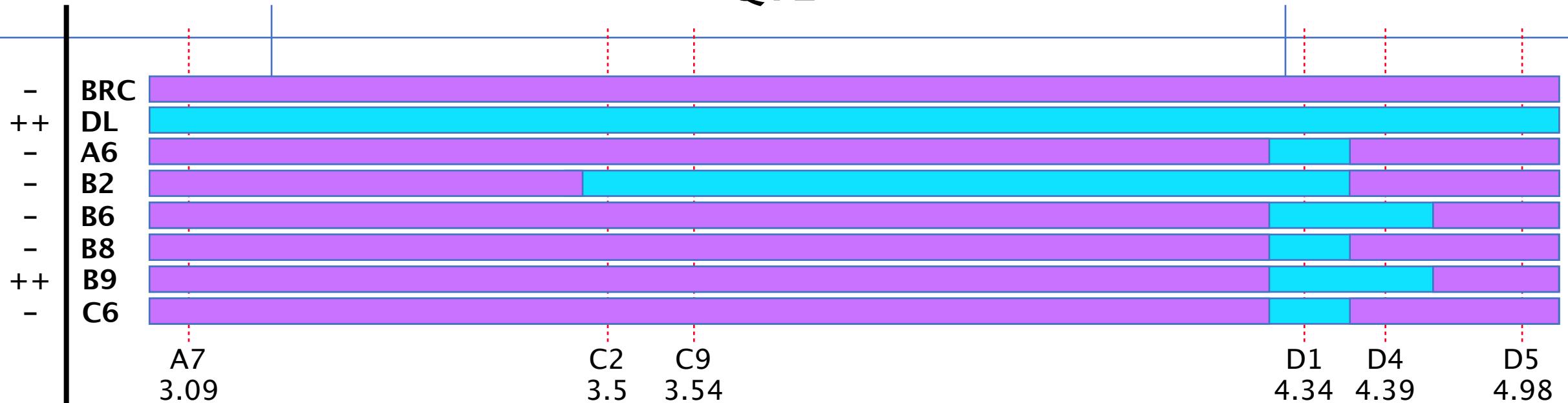
NIL L1 survival



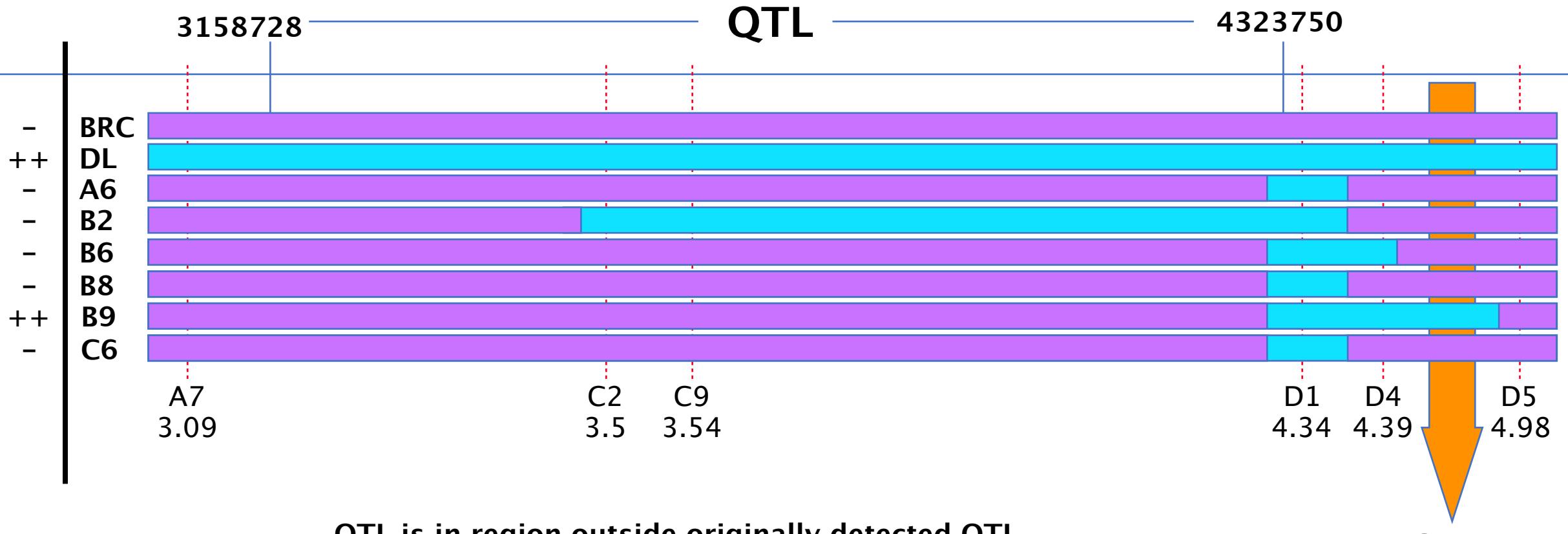
Making sense of NIL phenotypes

6 times introgression	Genomic location	A6	B2	B6	B8	B9	C6	BRC20067	DL238
Leftout primer_A7	V:3096975-3097641	+	+	+	+	+	+	+	-
Left primer_C2	V:3504515-3505301	+	-	+	+	+	+	+	-
Left primer_C9	V:3542692-3543404	+	-	+	+	+	+	+	-
Right primer_D1	V:4344262-4344867	-	-	-	-	-	+	-	
Right primer_D4	V:4393127-4393882	+	+	-	+	-	+	+	-
Rightout primer_D5	V:4989989-4990709	+	+	+	+	+	+	+	-

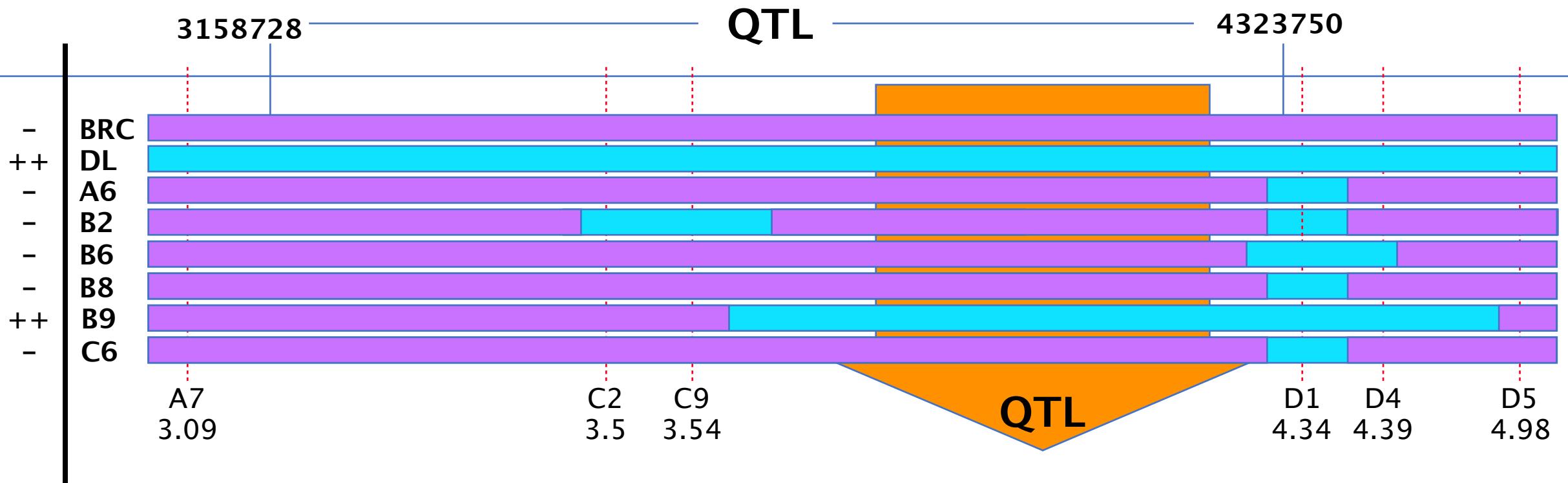
3158728 **QTL** 4323750



Making sense of NIL phenotypes : Scenario A



Making sense of NIL phenotypes : Scenario B

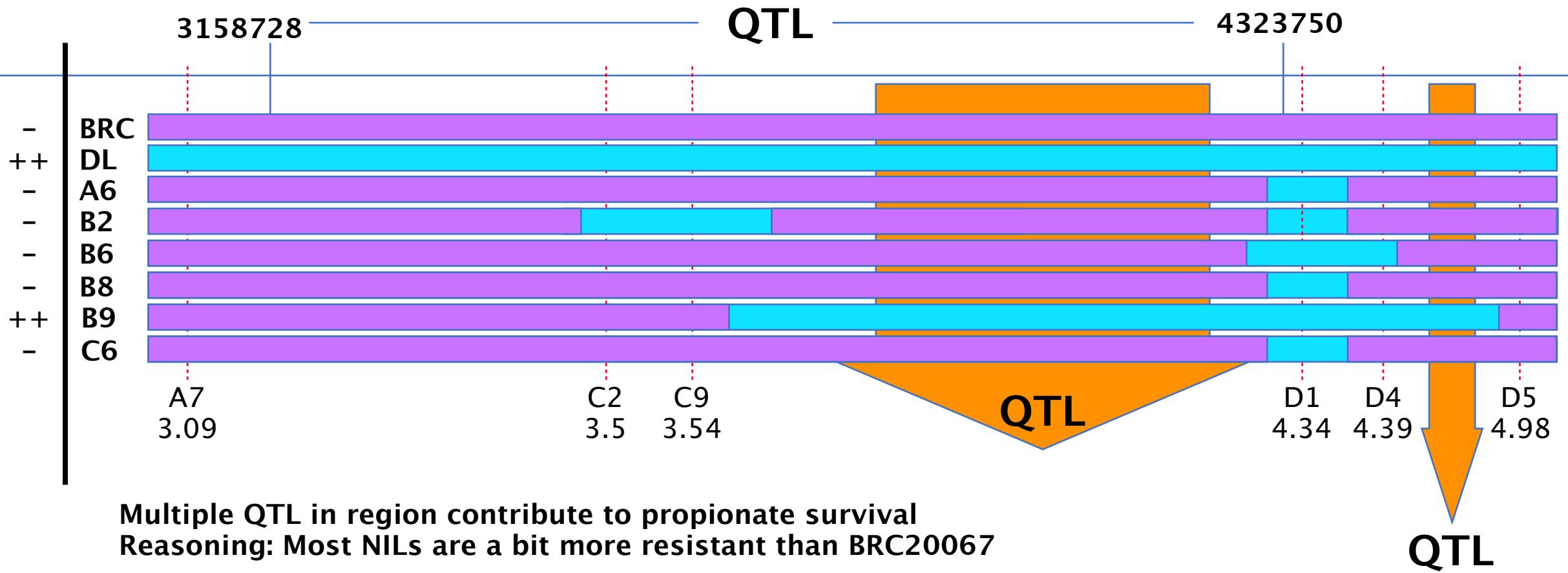


Double recombination occurred in B2 strain between markers C9 and D1.

QTL corresponds to difference in DL238 region between C9 and D1

Test markers between C9 and D1 in strain B2 to confirm genotype is BRC20067

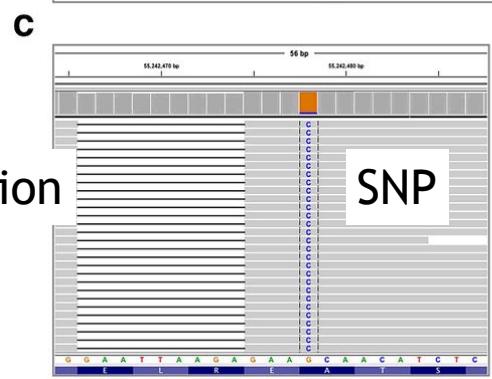
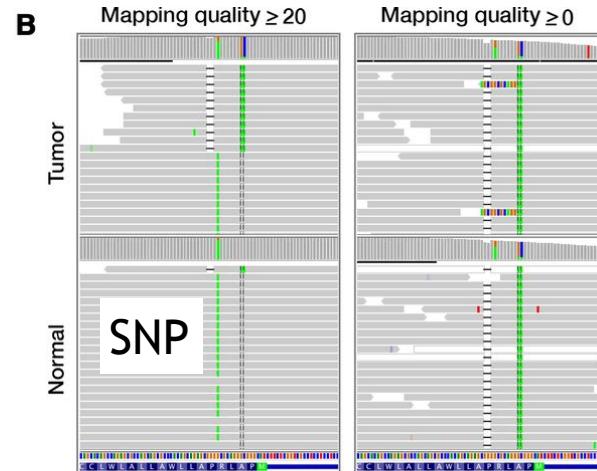
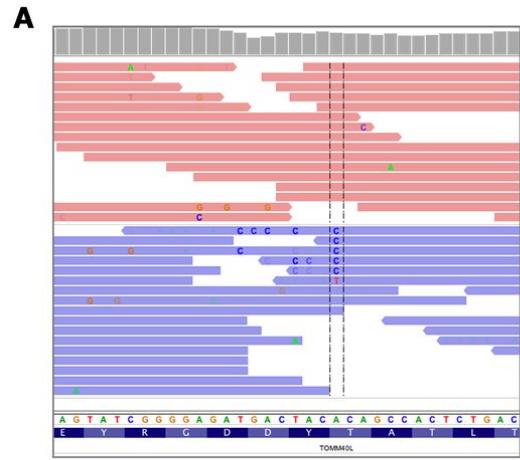
Making sense of NIL phenotypes : Scenario C



Making sense of NIL phenotypes

More likely scenario A because double recombination doesn't happen often

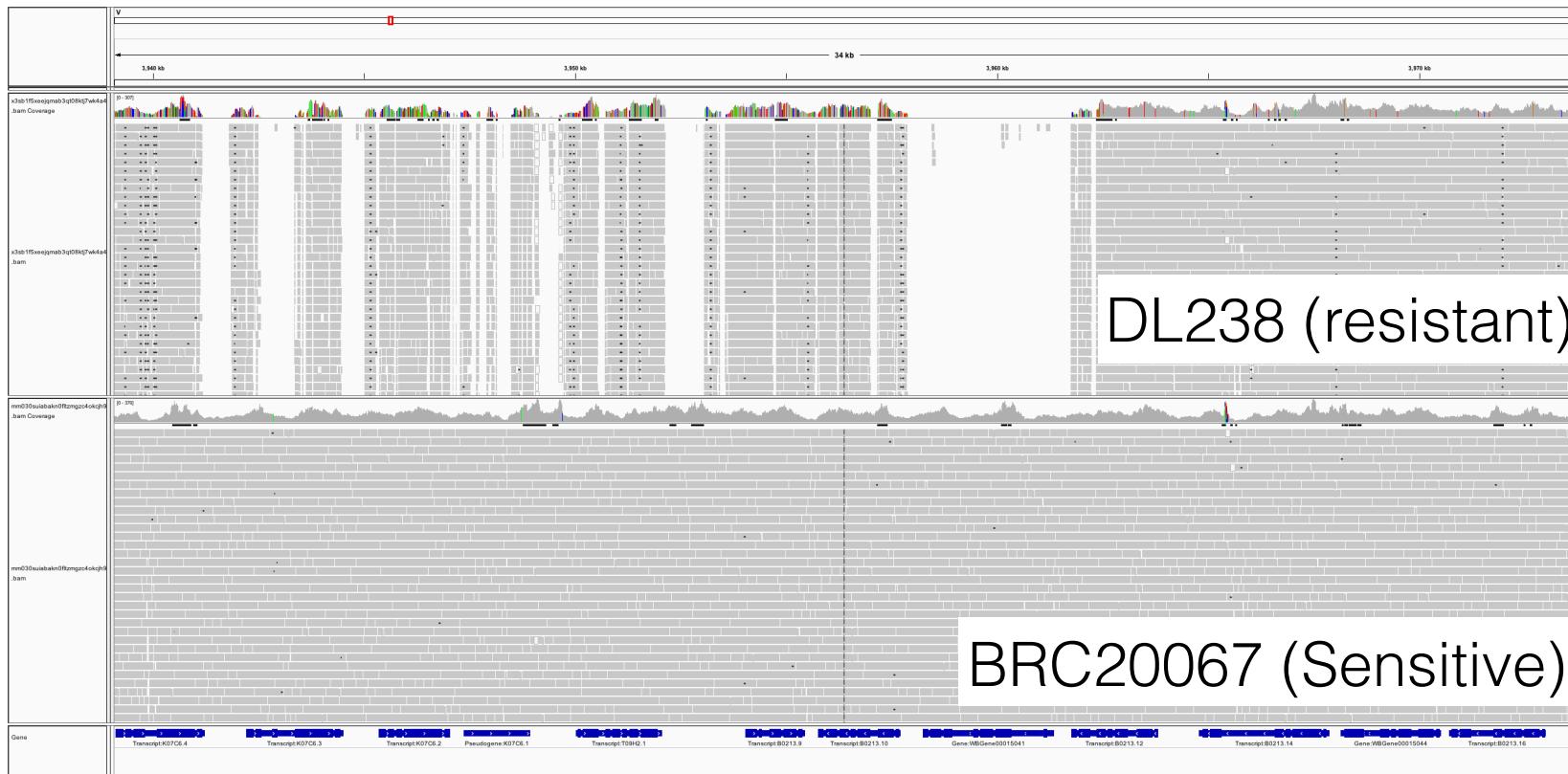
IGV interpretation



Making sense of NIL phenotypes

More likely scenario A because double recombination doesn't happen often

This is a diverged region with lots of SVs, which may facilitate double recombination



Diverged region extends from 3.886Mb to 4.054 MB on ChrV

Making sense of NIL phenotypes

More likely scenario A because double recombination doesn't happen often

This is a diverged region with lots of SVs, which may facilitate double recombination

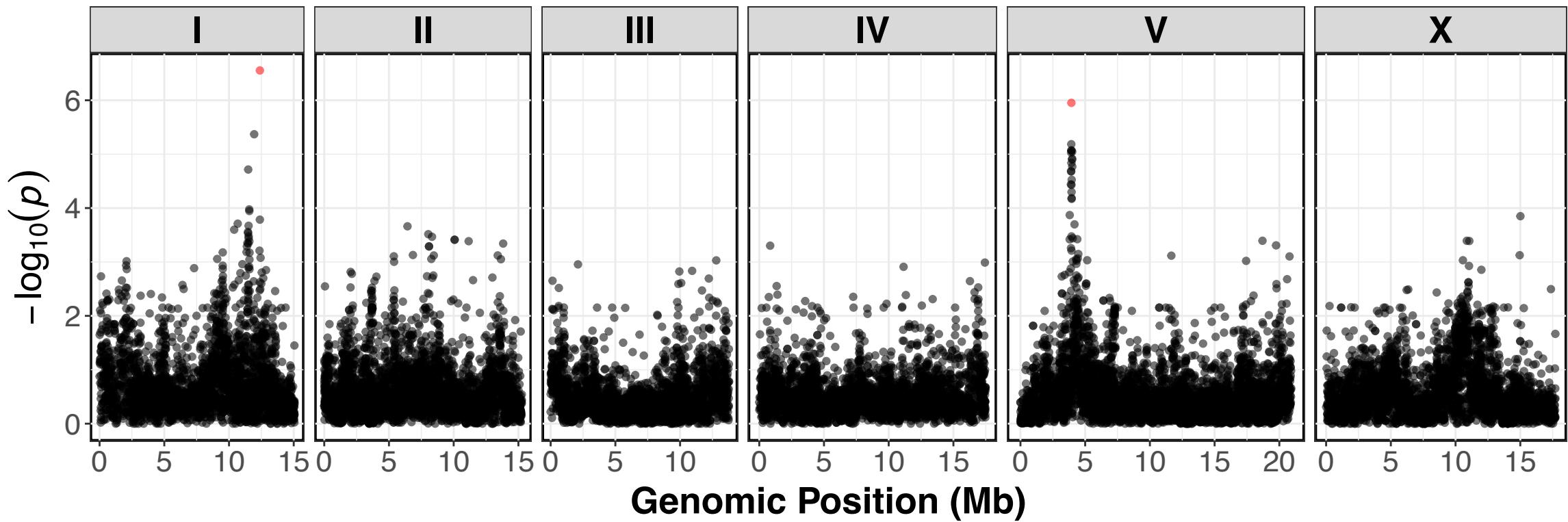
Either way, whole-genome sequencing will resolve genotype–phenotype discrepancies among NILs and parental lines

Burden testing - multiple allele for one gene

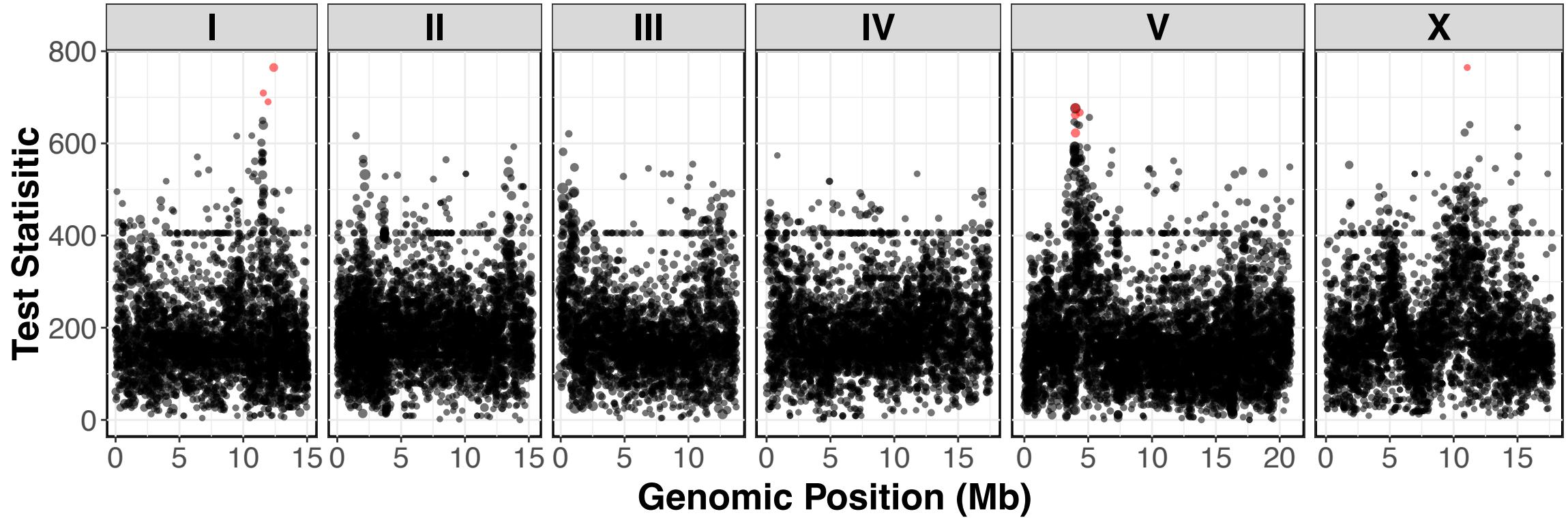
Variable threshold

The BURDEN test is more powerful than SKAT when the proportion of causal variants in a region is large and all causal variants are deleterious/protective. SKAT, however, is superior to the BURDEN test when the number of neutral variants increases and/or both deleterious and protective variants coexist in a gene

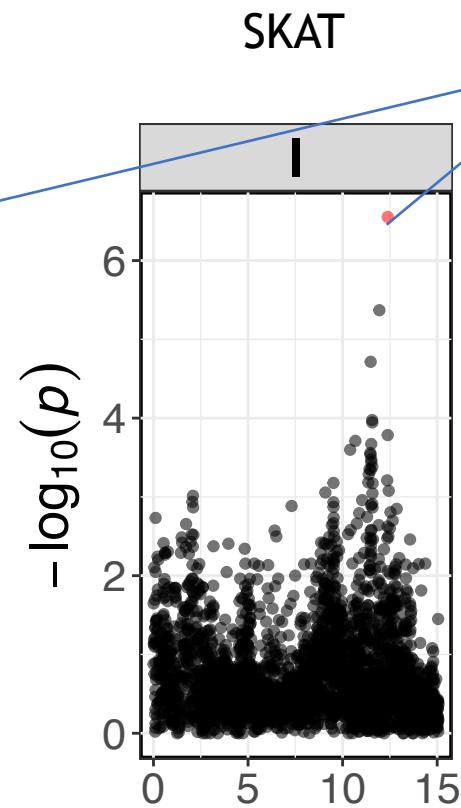
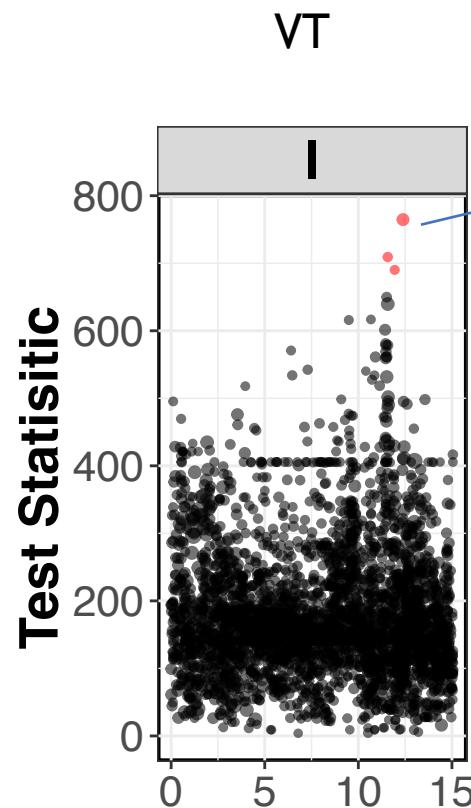
SKAT results



Variable threshold burden test results

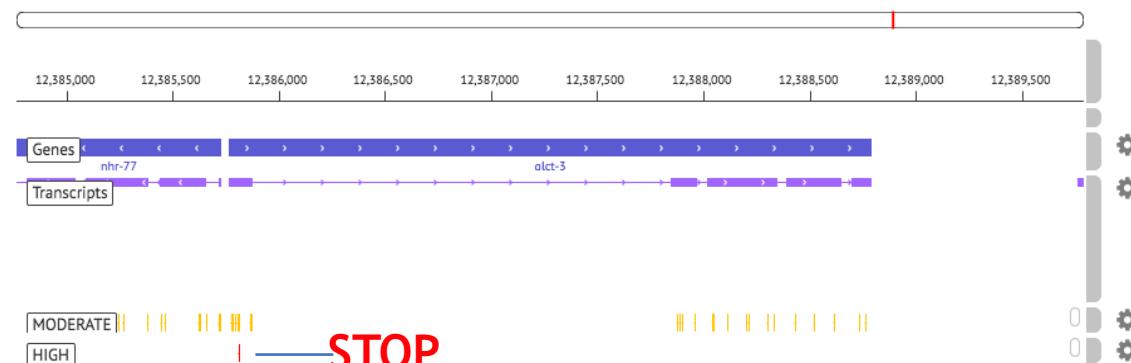


Both tests found same gene on chromosome I



glct-3

Contains an early stop-gained mutation



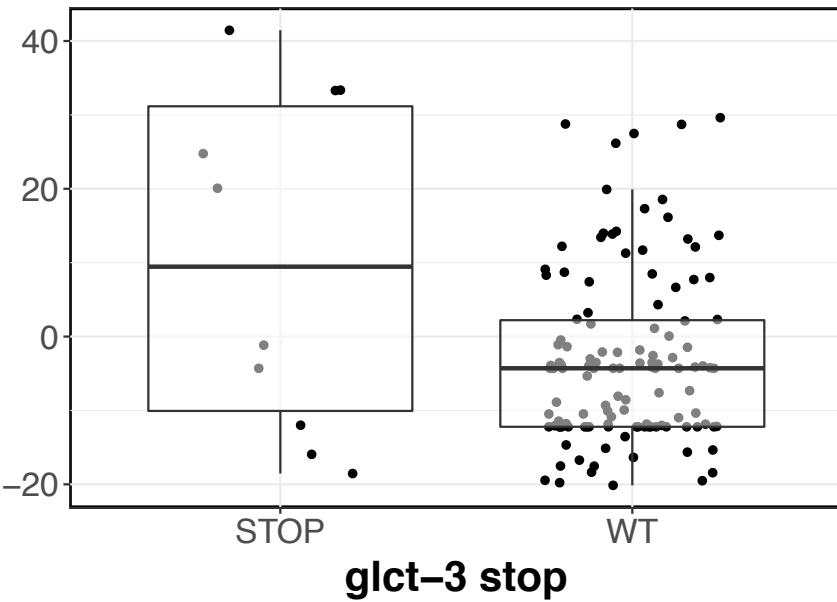
glct-3 is an ortholog of human B3GAT1 (*beta-1,3-glucuronyltransferase 1*), B3GAT3 (*beta-1,3-glucuronyltransferase 3*) and B3GAT2 (*beta-1,3-glucuronyltransferase 2*); *glct-3* is predicted to have galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase activity, based on protein domain information.

network fun

https://bioinfo.uth.edu/ccmGDB/gene_search_result_top20_network.cgi?page=page&type=quick_search&quick_search=B3GAT1

DL238 contains stop codon in *glct-3*

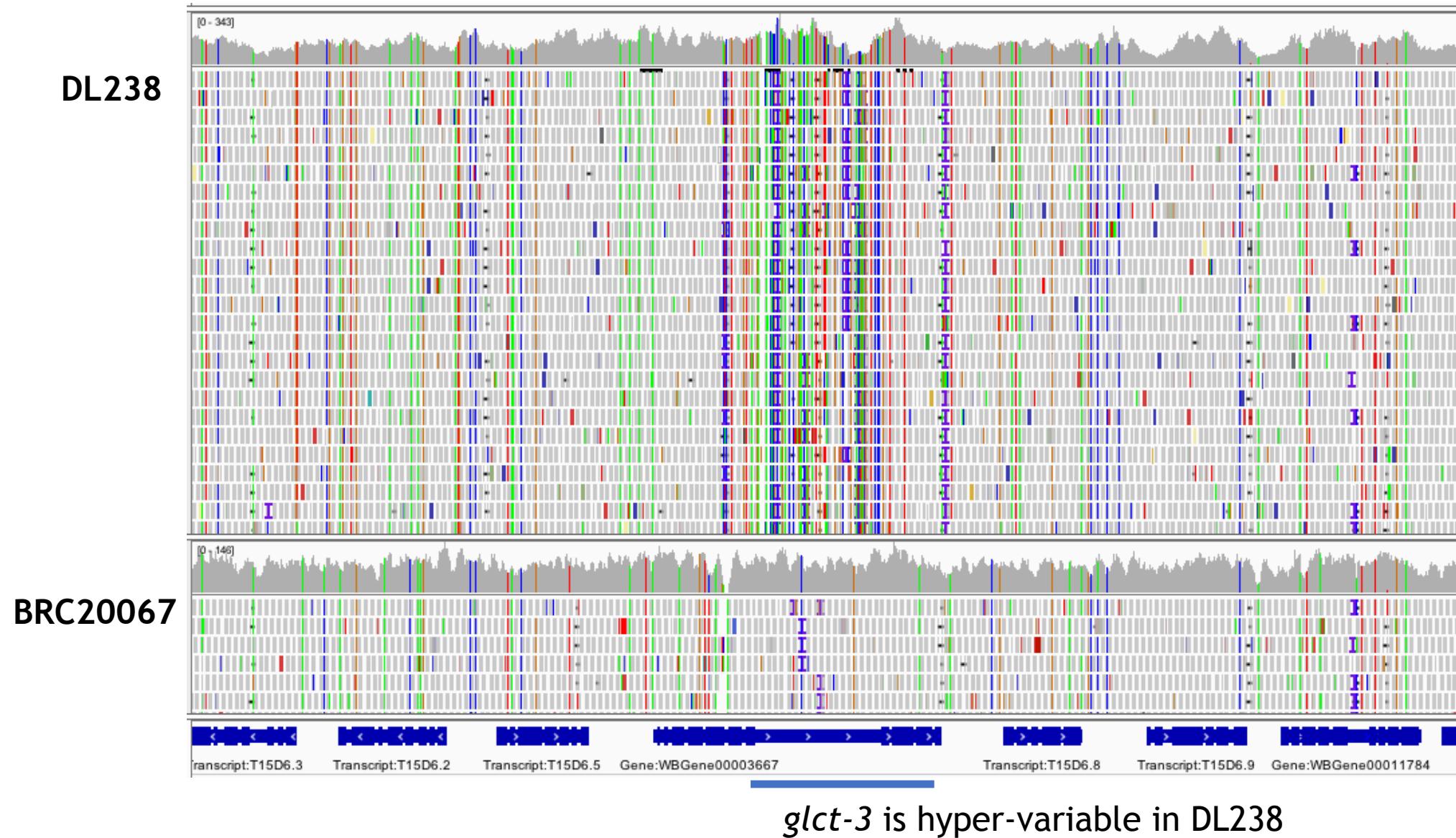
L1 survival



glct-3 stop codon strain genotypes

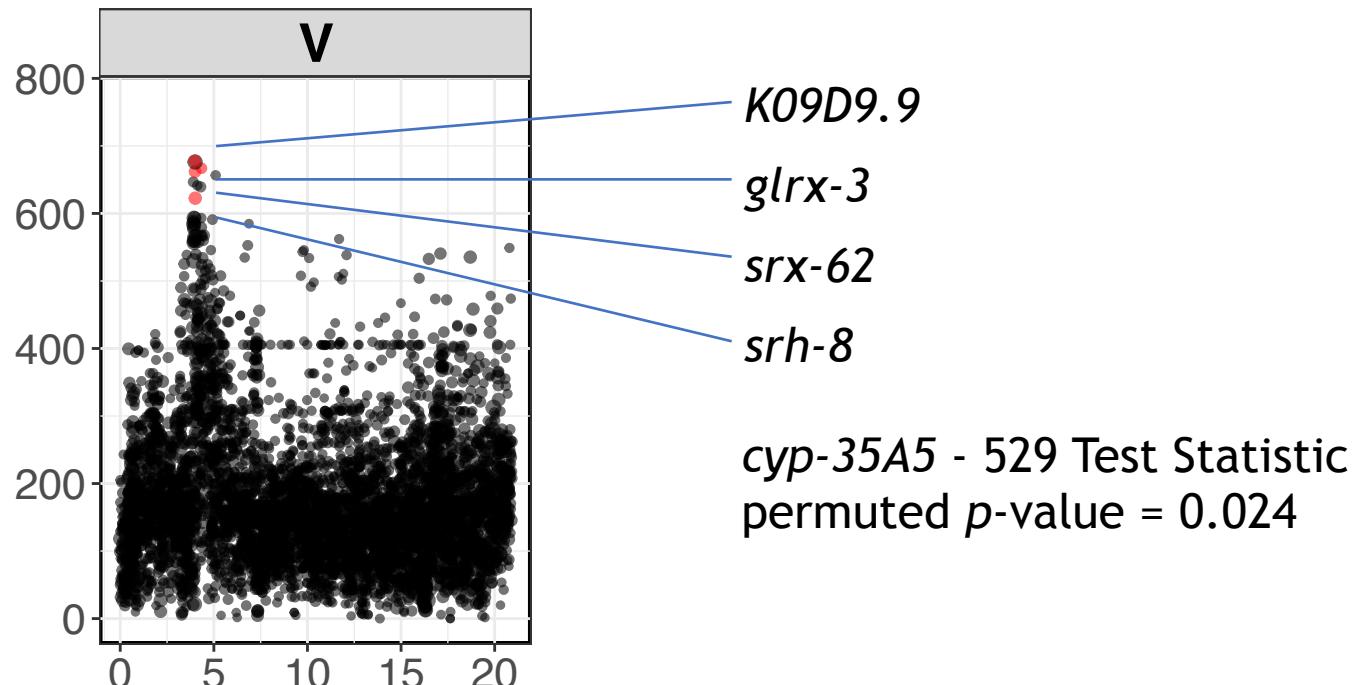
I:12,385,811	G / T	PASS	glct-3	WBGene00011781	T15D6.7	p.Gly16*	protein_coding	stop_gained	HIGH	
Reference										
NIC511 : G/G	NIC3 : G/G	NIC267 : G/G	NIC271 : G/G	NIC268 : G/G	JU346 : G/G	LSJ1 : G/G	NIC272 : G/G	NIC277 : G/G	ED3073 : G/G	JU778 : G/G
JU2619 : G/G	JU406 : G/G	JU2513 : G/G	JU2906 : G/G	JU3132 : G/G	CB4855 : G/G	ED3011 : G/G	JU792 : G/G	NIC526 : G/G	ED3040 : G/G	NIC207 : G/G
MY2573 : G/G	MY795 : G/G	JU2829 : G/G	GXW1 : G/G	NIC527 : G/G	JU2017 : G/G	JU1896 : G/G	NIC265 : G/G	JU1666 : G/G	JU1581 : G/G	MY18 : G/G
CX11262 : G/G	NIC269 : G/G	JU2862 : G/G	NIC513 : G/G	PS2025 : G/G	JU774 : G/G	JU1792 : G/G	ECA252 : G/G	JU2234 : G/G	NIC2 : G/G	JU2578 : G/G
JU2838 : G/G	NIC266 : G/G	EG4724 : G/G	CB4853 : G/G	JU3125 : G/G	NIC256 : G/G	JU2007 : G/G	JU2610 : G/G	NIC523 : G/G	NIC260 : G/G	CX11307 : G/G
NIC166 : G/G	JU1543 : G/G	NIC255 : G/G	JU2811 : G/G	JU2572 : G/G	JU782 : G/G	QX1233 : G/G	JU2587 : G/G	JU1088 : G/G	EG4725 : G/G	JU1172 : G/G
JU2565 : G/G	NIC259 : G/G	NIC195 : G/G	BRC20067 : G/G	CX11271 : G/G	MY1 : G/G	JU2001 : G/G	JU2575 : G/G	JU775 : G/G	ED3012 : G/G	JU2464 : G/G
WN2050 : G/G	JU3134 : G/G	QX1212 : G/G	ED3017 : G/G	JU397 : G/G	JU2106 : G/G	CB4857 : G/G	JU1395 : G/G	NIC1049 : G/G	JU2878 : G/G	NIC1107 : G/G
QX1794 : G/G	MY2530 : G/G	NIC236 : G/G	DL200 : G/G	NIC501 : G/G	MY772 : G/G	XZ1515 : G/G	JU2141 : G/G	JU1793 : G/G	NIC514 : G/G	JU1200 : G/G
JU2534 : G/G	EG4347 : G/G	JU2522 : G/G	JU2257 : G/G	CB4854 : G/G	ED3005 : G/G	ED3077 : G/G	JU258 : G/G	NIC251 : G/G	JU2576 : G/G	MY2147 : G/G
CX11254 : G/G	JU367 : G/G	MY2535 : G/G	JU2841 : G/G	CB4856 : G/G	JU1934 : G/G	JU1808 : G/G	CB4932 : G/G	JU2866 : G/G	NIC1 : G/G	JU1213 : G/G
QW947 : G/G	JU3127 : G/G	JU393 : G/G	AB1 : G/G	NIC522 : G/G	QG557 : G/G	JU2853 : G/G	MY2741 : G/G	JU2570 : G/G	JU3128 : G/G	NIC242 : G/G
BRC20263 : G/G	CX11292 : G/G	JU847 : G/G	JU2131 : G/G	JU2519 : G/G	NIC231 : G/G	JU2466 : G/G	PB303 : G/G	XQ1211 : G/G	WN2002 : G/G	MY920 : G/G
JU2907 : G/G	JU323 : G/G	JU1212 : G/G	KR314 : G/G	NIC276 : G/G	JU2592 : G/G	MY518 : G/G	ECA349 : G/G	NIC529 : G/G	MY2585 : G/G	JU1530 : G/G
NIC275 : G/G	JU2600 : G/G	JU1652 : G/G	JU2016 : G/G	EG4349 : G/G	JU2566 : G/G	CX11314 : G/G	JU3137 : G/G	DL226 : G/G	QG2075 : G/G	PB306 : G/G
JU2825 : G/G	JU3135 : G/G	EG4946 : G/G	CX11285 : G/G	NIC528 : G/G	QG536 : G/G	N2 : G/G	CX11315 : G/G	JU1440 : G/G	JU1568 : G/G	NIC515 : G/G
JU311 : G/G	JU2800 : G/G	LKC34 : G/G	NIC258 : G/G	JU2316 : G/G	NIC262 : G/G	JU1409 : G/G	MY2713 : G/G	ECA348 : G/G	JU1249 : G/G	ED3052 : G/G
ED3048 : G/G	JU3140 : G/G	PX179 : G/G	CX11264 : G/G	JU1400 : G/G	MY2453 : G/G	MY2212 : G/G	QG556 : G/G	JU440 : G/G	JU2250 : G/G	JT11398 : G/G
WN2001 : G/G	JU642 : G/G	MY10 : G/G	JU1242 : G/G	RC301 : G/G	JU310 : G/G	JU1586 : G/G	JU394 : G/G	JU1246 : G/G	JU751 : G/G	ECA369 : G/G
NIC261 : G/G	WN2033 : G/G	MY679 : G/G	MY2693 : G/G	JU3144 : G/G	JU830 : G/G	JU561 : Q/G	XZ1514 : G/G	JU1491 : G/G	NIC199 : G/G	CB4858 : G/G
JU2581 : G/G	CB4852 : G/G	JU2526 : G/G	CB4851 : G/G	JU360 : G/G	MY16 : G/G	JU2593 : G/G	NIC274 : G/G	JU2478 : G/G	JU1580 : G/G	
Alternative										
MY23 : T/T	JU2679 : T/T	ED3049 : T/T	CX11276 : T/T	ECA363 : T/T	QX1793 : T/T	QX1791 : T/T	ECA189 : T/T	ED3046 : T/T	ECA396 : T/T	ECA191 : T/T
DL238 : T/T	ECA36 : T/T	XZ1516 : T/T	QX1792 : T/T	NIC252 : T/T	XZ1513 : T/T	ECA372 : T/T				

Raw reads at *glct-3* locus

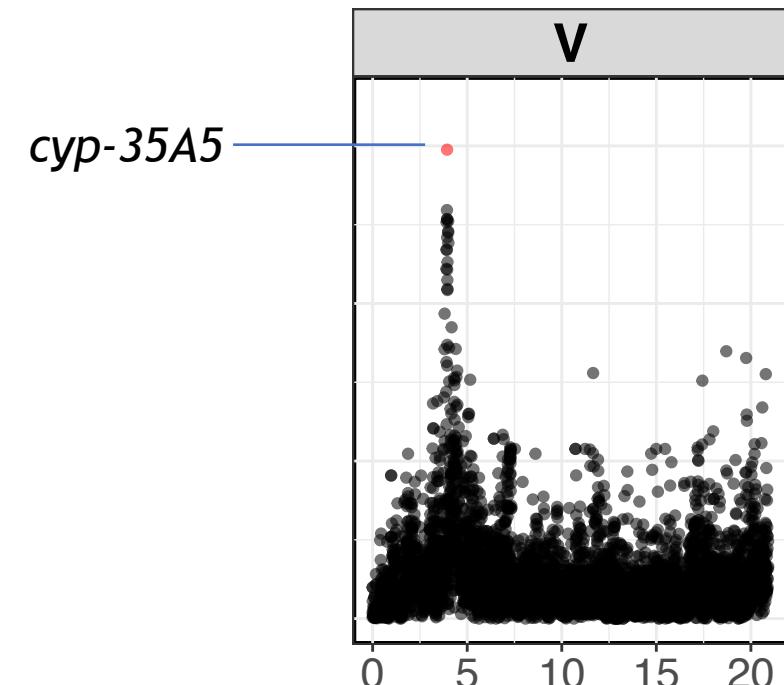


Associated genes on chromosome V

VT

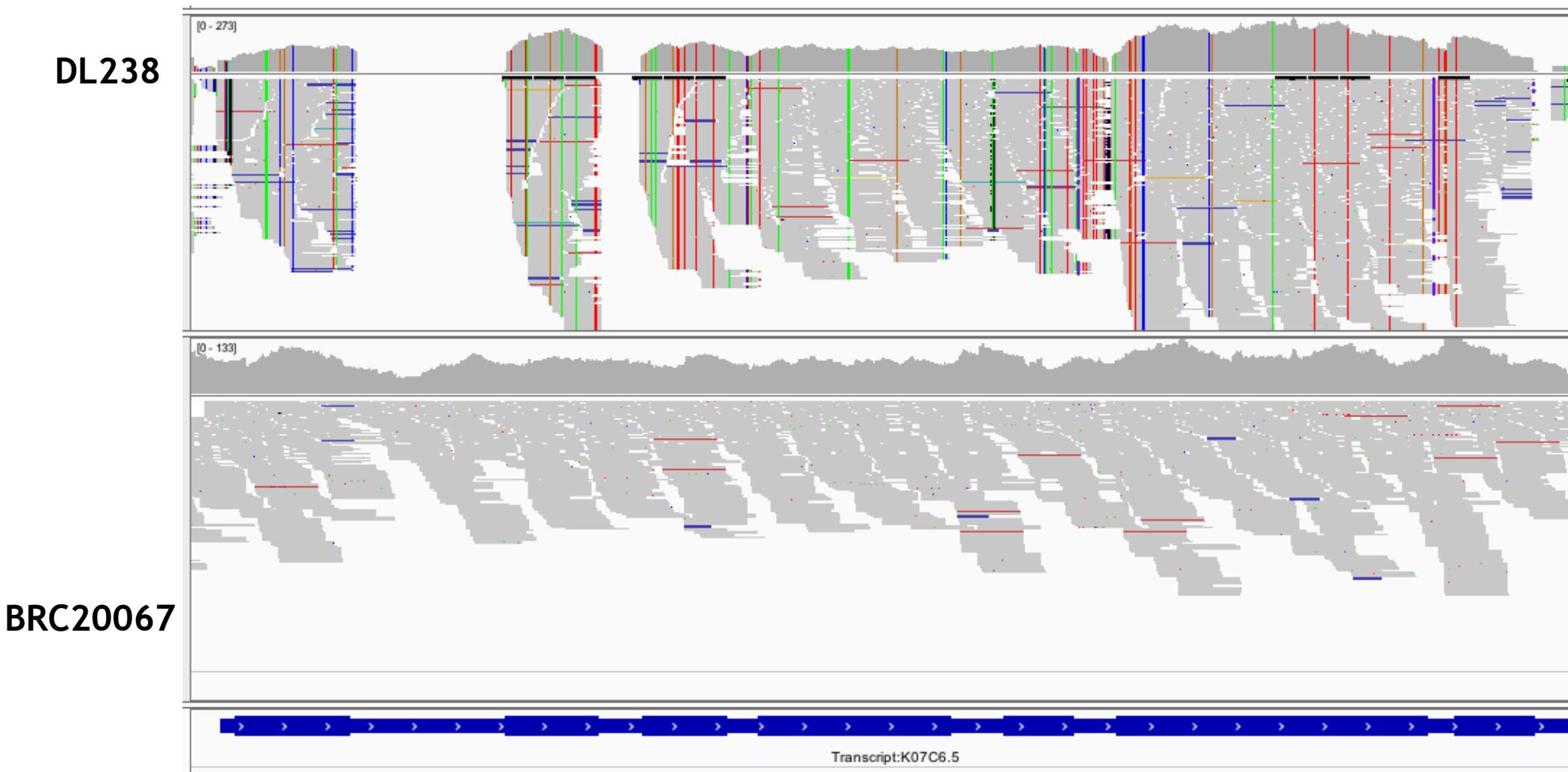


SKAT



All associated genes are between 3936373 - 4341308

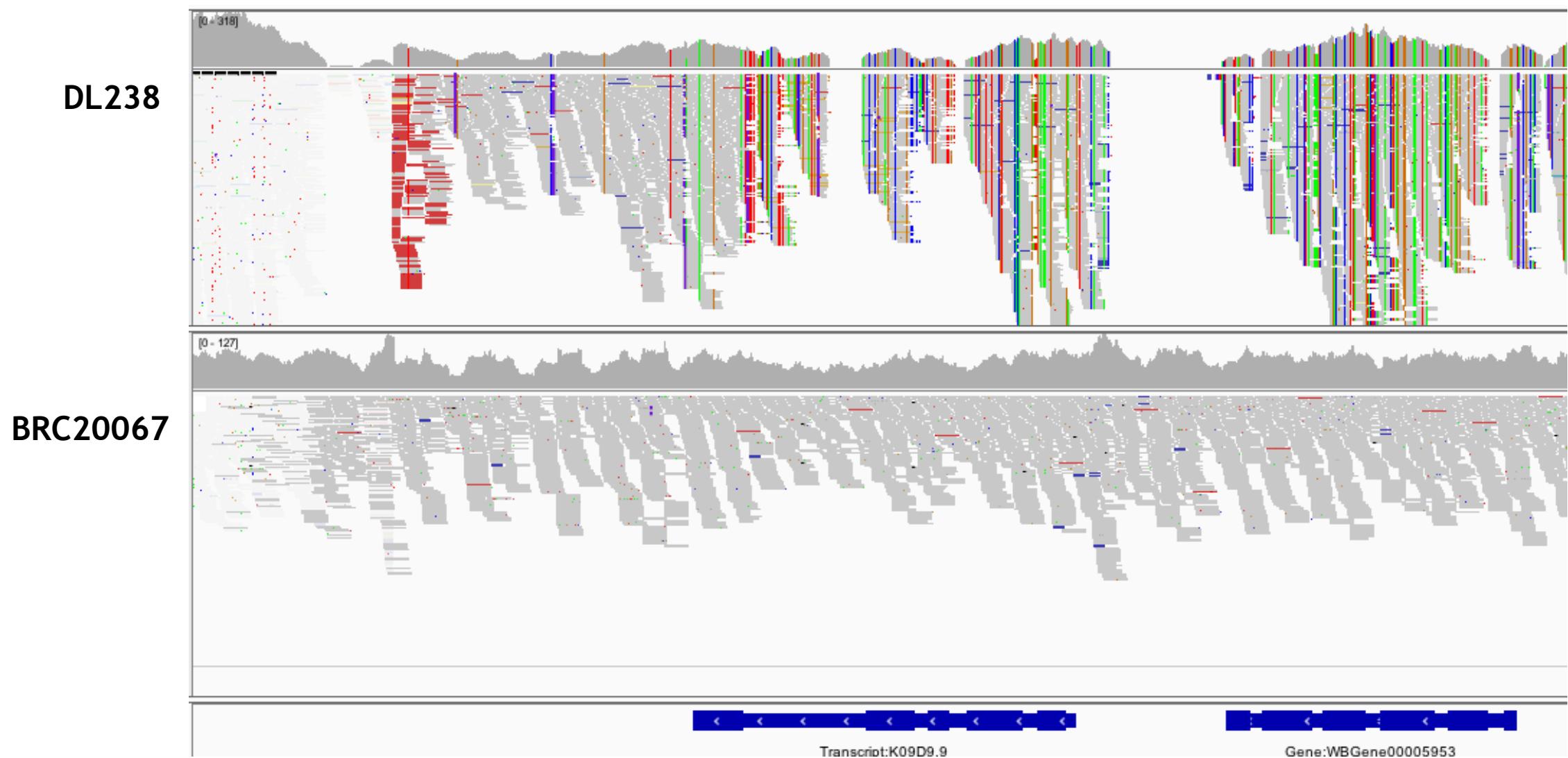
Raw reads at *cyp-35A5* locus



CHROM	POS	aa_change	gene_id	gene_name	log10p
V	4039787	p.Tyr261Phe	WBGene00077593	C49G7.12	4.464938
V	4039799	p.Pro265Gln	WBGene00077593	C49G7.12	4.464938
V	4039800	p.Pro265Pro	WBGene00077593	C49G7.12	4.464938
V	3965658		WBGene00015043	cyp-34A8	3.665353
V	3909185		WBGene00005952	srx-61	3.650420
V	3909384		WBGene00005952	srx-61	3.650420
V	3913185		WBGene00005959	srx-68	3.637398
V	3954044	p.Phe2Leu	WBGene00006274	str-247	3.632746
V	3969496		WBGene00015044	cyp-34A9	3.632746
V	3939690		WBGene00019472	cyp-35B1	3.606947
V	3940099	p.Ala155Thr	WBGene00019472	cyp-35B1	3.594843
V	3940722	p.Ala362Ala	WBGene00019472	cyp-35B1	3.282753
V	3913098		WBGene00005959	srx-68	3.267520
V	3923520		WBGene00005960	srx-69	3.264983
V	3909400		WBGene00005952	srx-61	3.257626
V	3909565		WBGene00005952	srx-61	3.257626
V	3920719	p.Ile267Ile	WBGene00005957	srx-66	3.257626
V	3921940	p.Ala2Ala	WBGene00005956	srx-65	3.257626
V	3922653	p.Ala206Ala	WBGene00005956	srx-65	3.257626
V	3922782	p.Leu249Leu	WBGene00005956	srx-65	3.257626
V	3922877	p.Gly265Gly	WBGene00005956	srx-65	3.257626
V	3922889	p.Leu269Leu	WBGene00005956	srx-65	3.257626
V	3922940	p.Val286Val	WBGene00005956	srx-65	3.257626
V	3924032		WBGene00005960	srx-69	3.257626
V	3938289		WBGene00019473	cyp-35A5	3.257626
V	3942456	p.Thr67Thr	WBGene00019471	cyp-35B2	3.188847
V	3943540	p.Ala209Ala	WBGene00019471	cyp-35B2	3.168931
V	3943547	p.Leu212Val	WBGene00019471	cyp-35B2	3.168931
V	4040504	p.Ile276Ile	WBGene00016788	C49G7.10	3.113776
V	4040509	p.Ile275Val	WBGene00016788	C49G7.10	3.113776

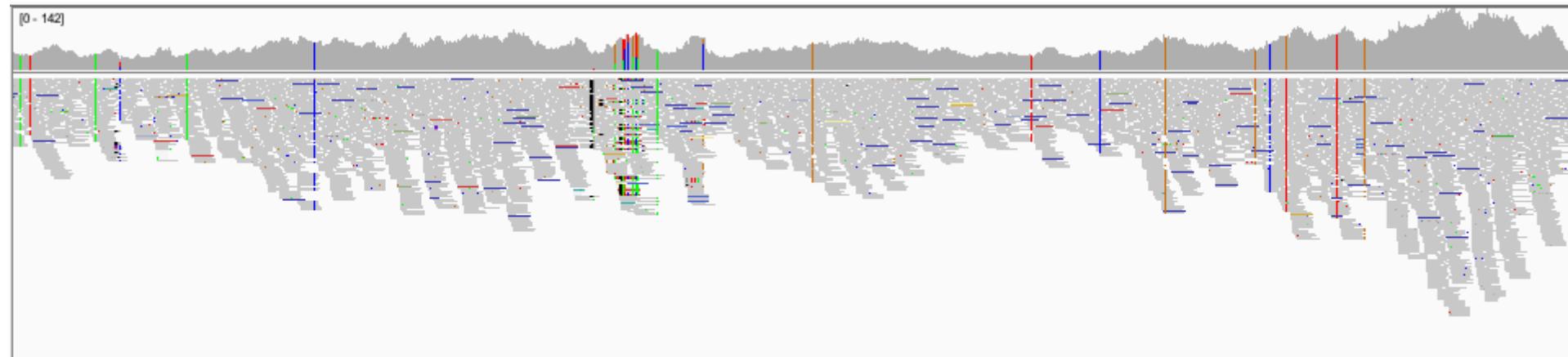
Single marker in *cyp-35A5* locus is also associated with L1 phenotype

Raw reads at *K09D9.9* locus

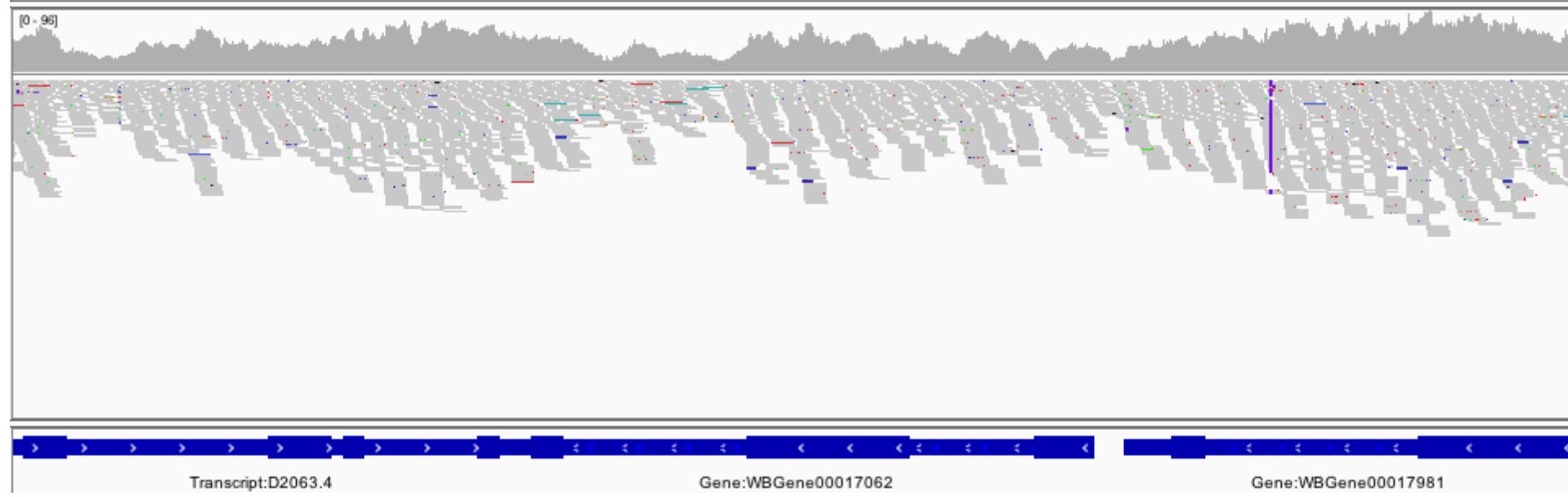


Raw reads at *g/rx-3* locus

DL238



BRCA20067



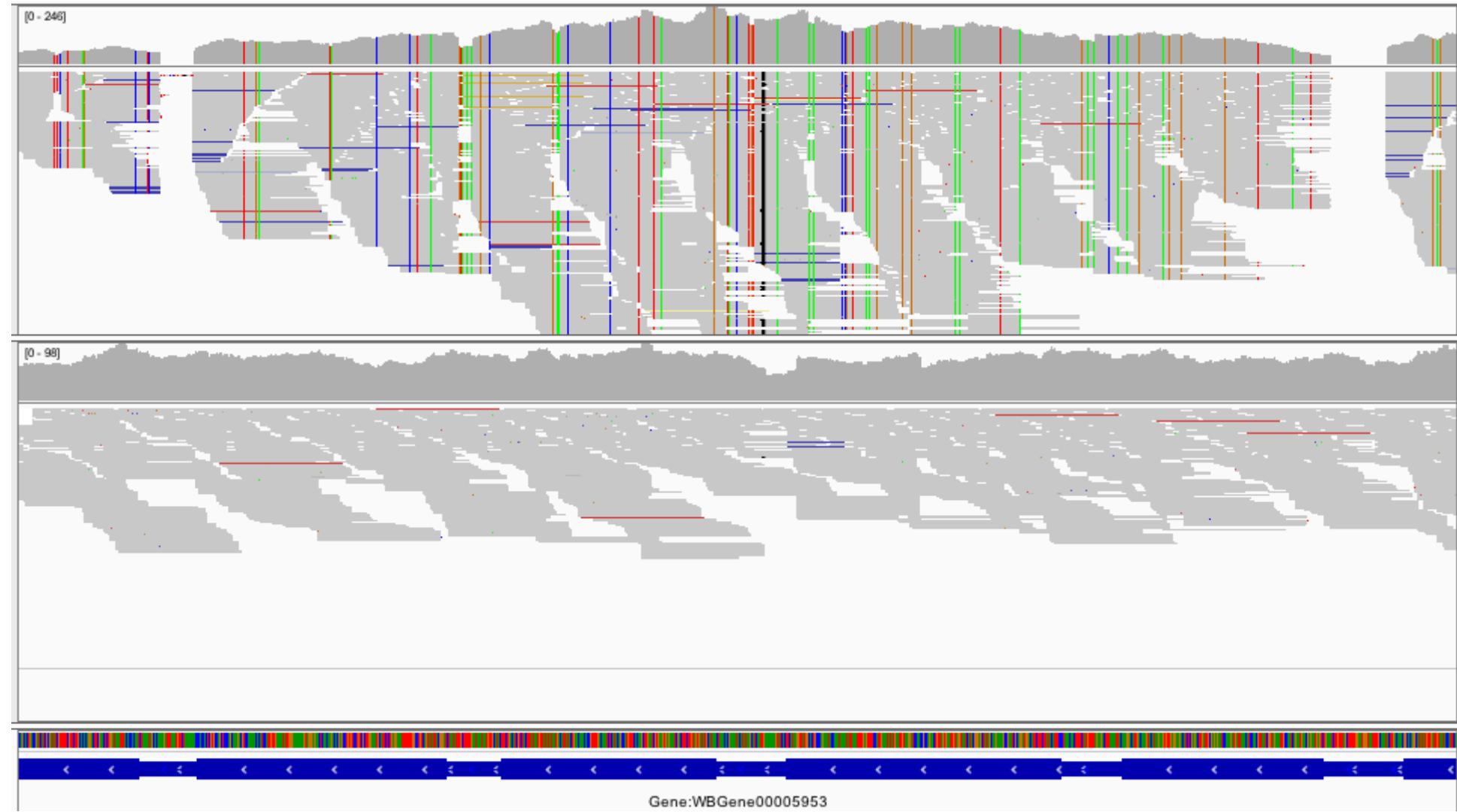
Transcript:D2063.4

Gene:WBGene00017062

Gene:WBGene00017981

Raw reads at *srx-62* locus

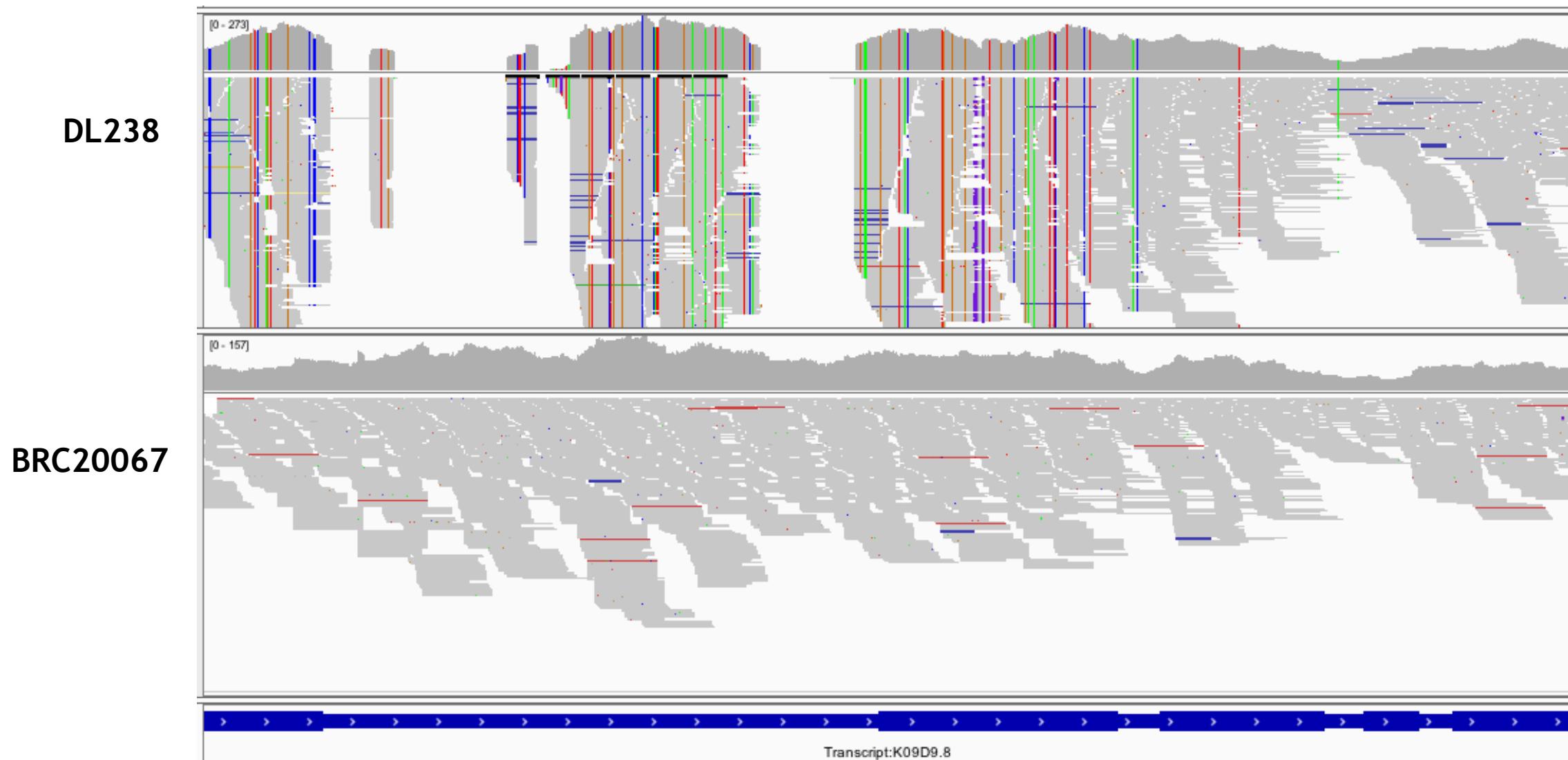
DL238



BRC20067

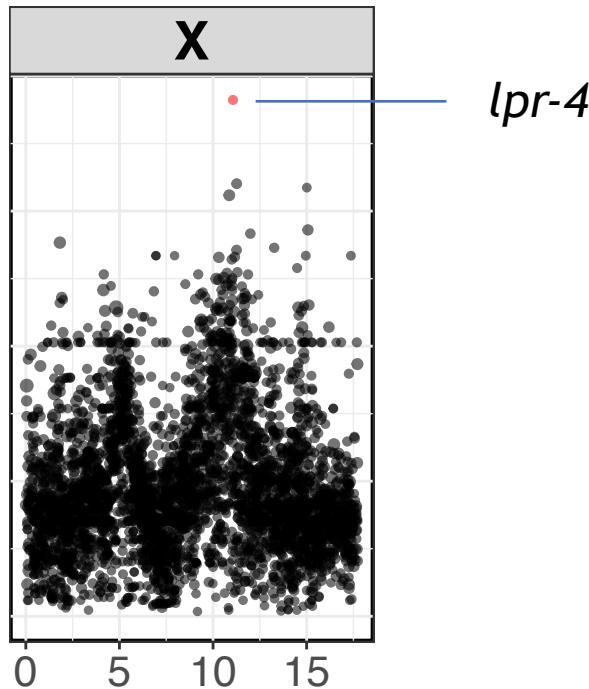
Gene:WBGene00005953

Raw reads at *srh-8* locus



Unique gene found by variable threshold burden test on ChrX

VT



Conclusions

ChrV

Burden/SKAT/single marker association mapping all implicate ChrV QTL in propionate response

NIL phenotypes and genotypes do no match most straightforward expectation from GWA

Makes sense in context of highly diverged genomic region

Whole-genome sequencing will hopefully resolve this issue (Long-read Seq may be needed?)

Potential A few candidates on chromosome V

All associated genes are to the left of marker D1 (4.34 Mb)

Chrl

Burden/SKAT implicate *glct-3* in propionate response. This may be a candidate to test.

Things to do

ChrV

Re-do burden test:

- After filtering non-synonymous variants

- Including large deletions

- Potentially test additional markers in region of interest before we get whole-genome data back

Chrl

Introduce stop-gained SNV into *glct-3* with CRISPR and test in propionate response