Is *KIT* locus polymorphism rs328592739 related to white belt phenotype in Krškopolje pig?

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Coat colour in pigs

- Colour determined by presence/absence of melanocytes in skin and type of melanin being produced.
- Polygenic trait with several major genes (*e.g. MC1R, KIT*).







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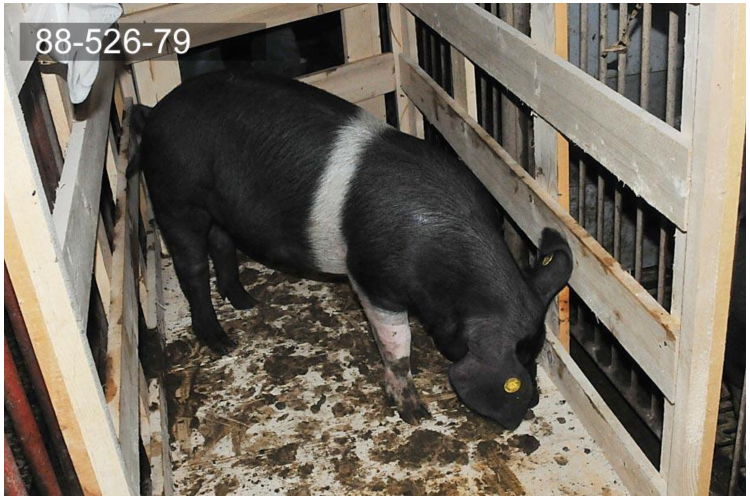
Krškopolje pig – belted phenotype

- In the past Krškopolje pigs (KP) crossbred with different European breeds to improve productivity.
- For preservation purpose crossbred with belted breeds (Wessex and German Saddleback) to reduce inbreeding and preserve the phenotype.
- Diversity of belted phenotypes in KP.



- Belt in pigs was attributed to an allele of the „dominant white“ (*KIT*) locus (Giuffra *et al*., 1999; Fontanesi *et al*., 2010).

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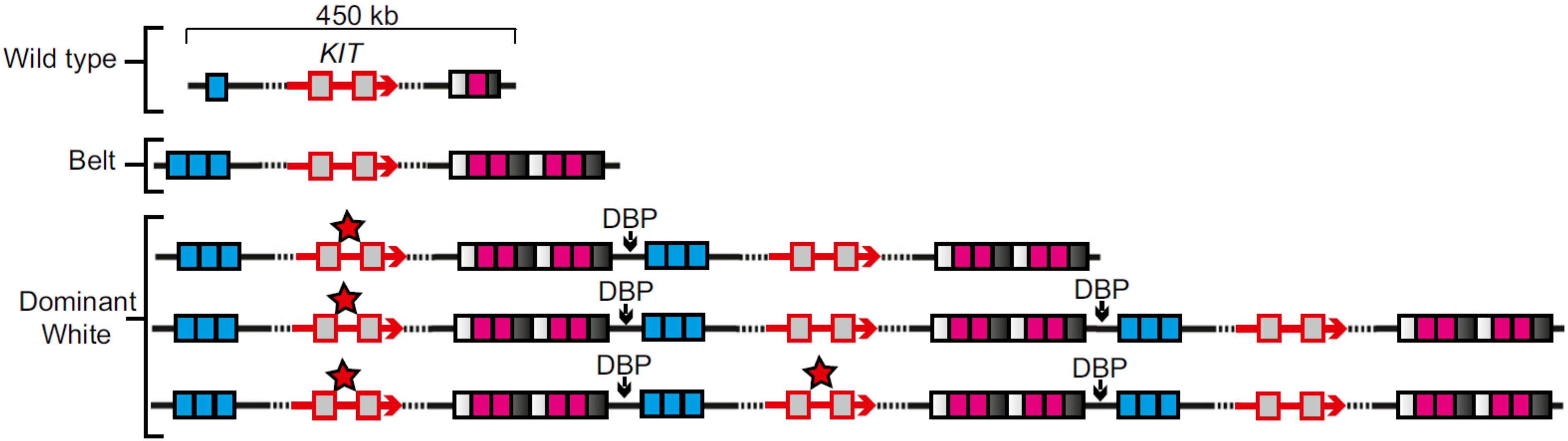
KIT („dominant white“) locus

- *KIT* = KIT proto-oncogene receptor tyrosine kinase (Chr. 8).
- Very compelx, controls development of many cell types, including migration and survival of melanocytes.
- Different KIT alleles (wt, dominant white, patch, belt, roan/dilute).
- Belted phenotype is believed to be caused by a regulatory mutation



Rubin et al., 2012

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Background

Livestock Science 184 (2016) 71–77

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

ELSEVIER

LIVESTOCK SCIENCE

Authentication of "mono-breed" pork products: Identification of a coat colour gene marker in Cinta Senese pigs useful to this purpose

CrossMark

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Is the SNP (rs328592739) associated to white belt phenotype also in Krškopolje pig / distinctive to all belted pig breeds (useful to distinguish meat of belted from non-belted breeds)?

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Materials and methods

DNA extraction from ear tissue or hair sacks of 38 Krškopolje pigs (KP), 9 Swäbisch-Hall (SH), 5 Cinta Senese (CS), and 5 wild boars (WB).

- **PCR/RFLP** based genotyping of the rs328592739 SNP in the KIT gene (ENSSSCG00000008842):

…AACTATACTTGTGACCCACGTATTTCAGAAGCAGTCAGGGTCATCCAAGGTCAT
Intron 17 GGCTCAAAGGACTTTGTGAGATGCCCTCAAGTTCTCACCCCCACCTCGCAGCAGG
AGCAGTATCTACAGGAATATTTTGGAGCTTCATAATGAACATTGCTGACTCCCCT
GTGCTTCCACTGCAGGCTCGGCTACCCGTGAAGTGGATGGCACC[C/T]GAGAGC
Exon 18 ATTTTCAACTGTGTCTACACATTTGAAAGCGATGTCTGGTCCTATGGGATTTTTC
TGTGGGAGCTCTTCTCTTTAGGTAAAATGCACCTTGCCAAAGGCACCTCAGTTAG
ACTCTGGGCATCTTCTTTAAGATGTTCCCATTGT…

CTNAG – DdEI restriction site (230 bp = 180 bp + 50 bp)

- **Sanger sequencing** of Exon 18 for several Krškopolje pigs.

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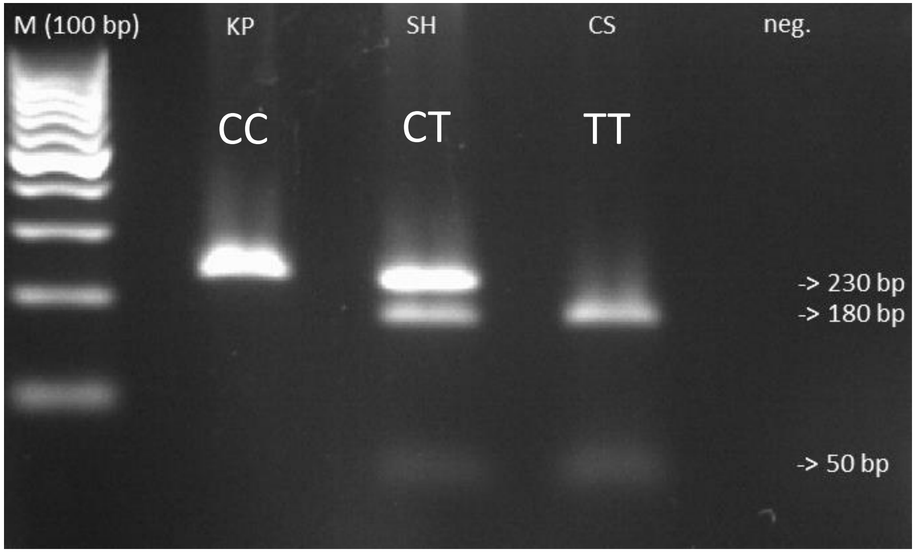
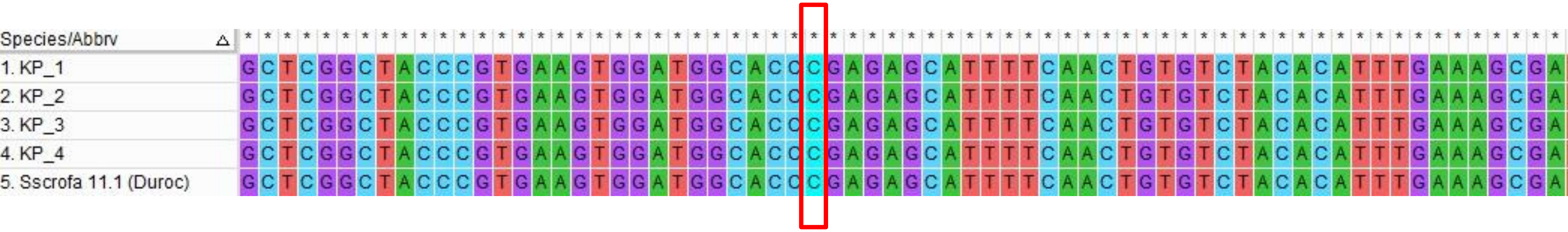
Results



Breed / Gen.	C/C	C/T	T/T
KP	38	0	0
SH	7	2	0
CS	0	0	5
WB	5	0	0

rs328592739 (C/T)



Species/Abbrv △	**
1. KP_1	GCTCGGCTACCCGTGAAGTGGATGGCACCCGAGAGCATTTTCAACTGTGTCTACACATTTGAAAGCGA
2. KP_2	GCTCGGCTACCCGTGAAGTGGATGGCACCCGAGAGCATTTTCAACTGTGTCTACACATTTGAAAGCGA
3. KP_3	GCTCGGCTACCCGTGAAGTGGATGGCACCCGAGAGCATTTTCAACTGTGTCTACACATTTGAAAGCGA
4. KP_4	GCTCGGCTACCCGTGAAGTGGATGGCACCCGAGAGCATTTTCAACTGTGTCTACACATTTGAAAGCGA
5. Sscrofa 11.1 (Duroc)	GCTCGGCTACCCGTGAAGTGGATGGCACCCGAGAGCATTTTCAACTGTGTCTACACATTTGAAAGCGA

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Results

- C allele seems to be fixed in KP – diverse phenotypes
- CC and CT genotypes in SH show no phenotypic differences.

KP

C/C

C/C

C/C

SH

C/C

C/T

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Conclusions

- SNP rs328592739 in exon 18 is **not associated** to the belted phenotype in KP (and SH).

- Exon 18 is **not causative** for the belted phenotype in KP pigs.

- We suggest that the SNP is not (a genetic marker) specific for the belted phenotype across the breeds, but seems to be associated with the belted phenotype in Cinta Senese and Hampshire breeds.

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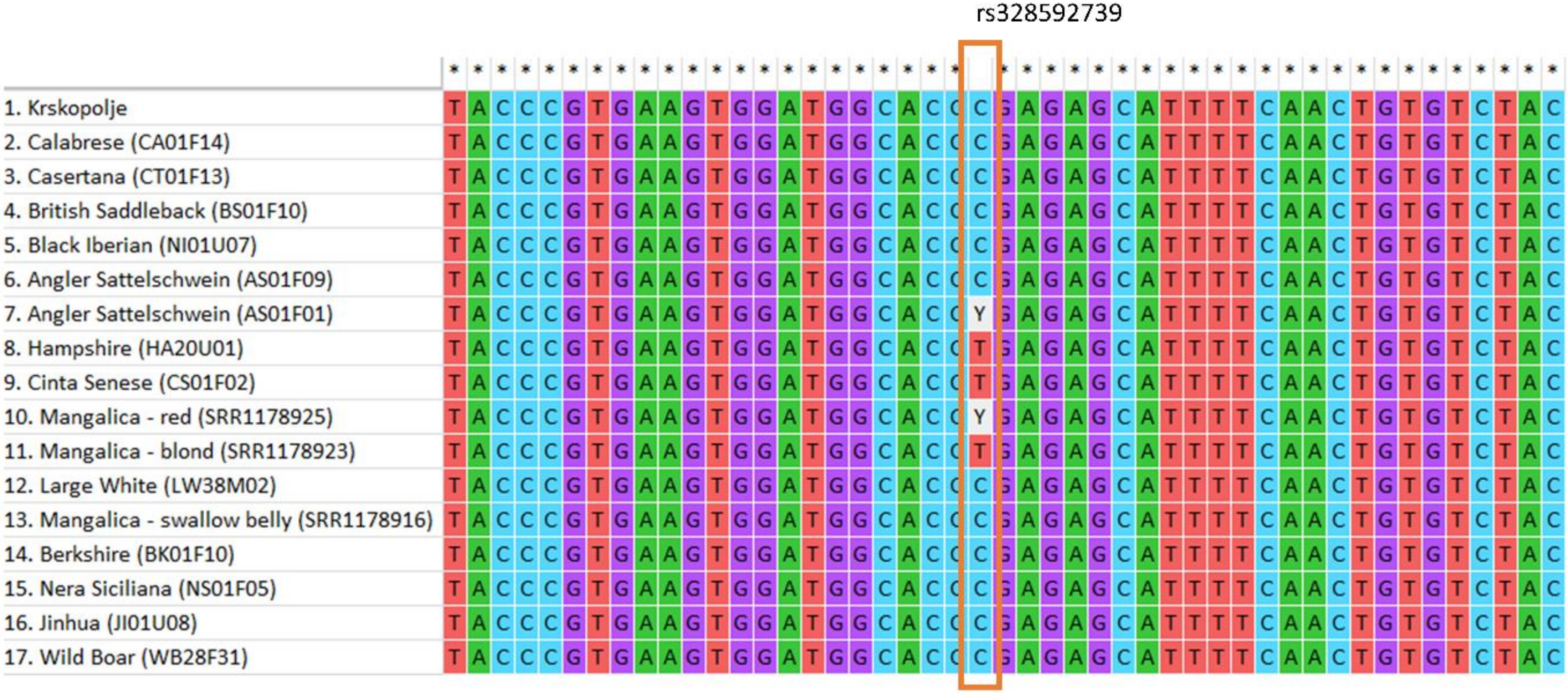

		rs328592739	
	**********************		************************
1. Krskopolje	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
2. Calabrese (CA01F14)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
3. Casertana (CT01F13)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
4. British Saddleback (BS01F10)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
5. Black Iberian (NI01U07)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
6. Angler Sattelschwein (AS01F09)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
7. Angler Sattelschwein (AS01F01)	TACCCGTGAAGTGGATGGCACC	Y	GAGAGCATTTTCAACTGTGTCTAC
8. Hampshire (HA20U01)	TACCCGTGAAGTGGATGGCACC	T	GAGAGCATTTTCAACTGTGTCTAC
9. Cinta Senese (CS01F02)	TACCCGTGAAGTGGATGGCACC	T	GAGAGCATTTTCAACTGTGTCTAC
10. Mangalica - red (SRR1178925)	TACCCGTGAAGTGGATGGCACC	Y	GAGAGCATTTTCAACTGTGTCTAC
11. Mangalica - blond (SRR1178923)	TACCCGTGAAGTGGATGGCACC	T	GAGAGCATTTTCAACTGTGTCTAC
12. Large White (LW38M02)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
13. Mangalica - swallow belly (SRR1178916)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
14. Berkshire (BK01F10)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
15. Nera Siciliana (NS01F05)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
16. Jinhua (JI01U08)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC
17. Wild Boar (WB28F31)	TACCCGTGAAGTGGATGGCACC	C	GAGAGCATTTTCAACTGTGTCTAC

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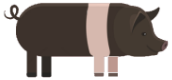
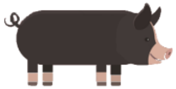
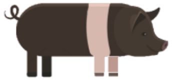
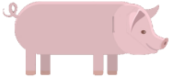
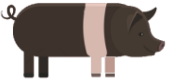
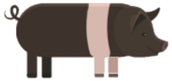
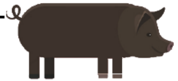
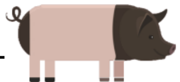
TREASURE

rs328592739 genotype in 22 pigs of different breeds determined from publicly available whole-genome sequencing data.

Wild boar - Italy		CC	
Angler Sattelschwein		CT	
		CC	⇦
Berkshire		CC	
		CC	
Black Iberian		CC	
British Saddleback		CC	⇦
Large White		CC	
Calabrese		CC	
Casertana		CC	
		CC	
Cinta Senese		TT	
Hampshire		TT	
		TT	
Mangalica		CT	⇦
		TT	⇦
		TT	⇦
		CT	⇦
		TT	⇦
		CC	
Nera Siciliana		CC	
Jinhua		CC	⇦

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Martien A. M. Groenen, 2016
A decade of pig genome sequencing: a window on pig domestication and evolution

TREASURE

Pig whole-genome resequencing: overview of pig short read Illumina sequences deposited in the European Nucleotide Archive

Accession	Species	Pig breeds/populations	Number of individuals
PRJEB1683	Ssc, Svr, Sba, Sce, Scb, Paf	EWB, AWB, DU, HA, PI, LR, LW, XI, JQ, MS	77
PRJNA144099	Ssc	WZ	1
PRJNA41185	Ssc	DU	1
PRJNA176189	Ssc	GM	1
PRJNA231897	Ssc	RC	6
PRJNA186497	Ssc	AWB, TWB, PZ, WJ, YN, NJ, JH	49
PRJNA238851	Ssc	TC	5
PRJNA260763	Ssc	DU, LR, YM, KWB, LW	70
PRJEB9115	Ssc	DU[a]	1
PRJNA213179	Ssc	AWB, BX, EH, HT, LA, LU, MI, GA, SC, TP, YN, WZ	69
PRJNA221763	Ssc	BK	3
PRJNA239399	Ssc	MA, DU	4
PRJNA190683	Ssc	IB	1
PRJNA255085	Ssc	EWB, GC, IB, MP	4
PRJEB9326	Ssc, Scb	PI	18
PRJEB9922	Ssc, Svr	EWB, ASW, WS, ZA, LS, AS, BB, BK, BS, CM, CS, CA, CT, GO, HA, LB, LI, MA, MW, IB, NS, TA, RE, JQ, XI	102
PRJNA281548	Ssc	BK	10

∑ 422

- Ssc, *Sus scrofa*
- Svr, *Sus verrucosus*
- Sba, *Sus barbatus*
- Sce, *Sus celebensis*
- Scb, *Sus cebifrons*
- Paf, *Phacochoerus africanus*

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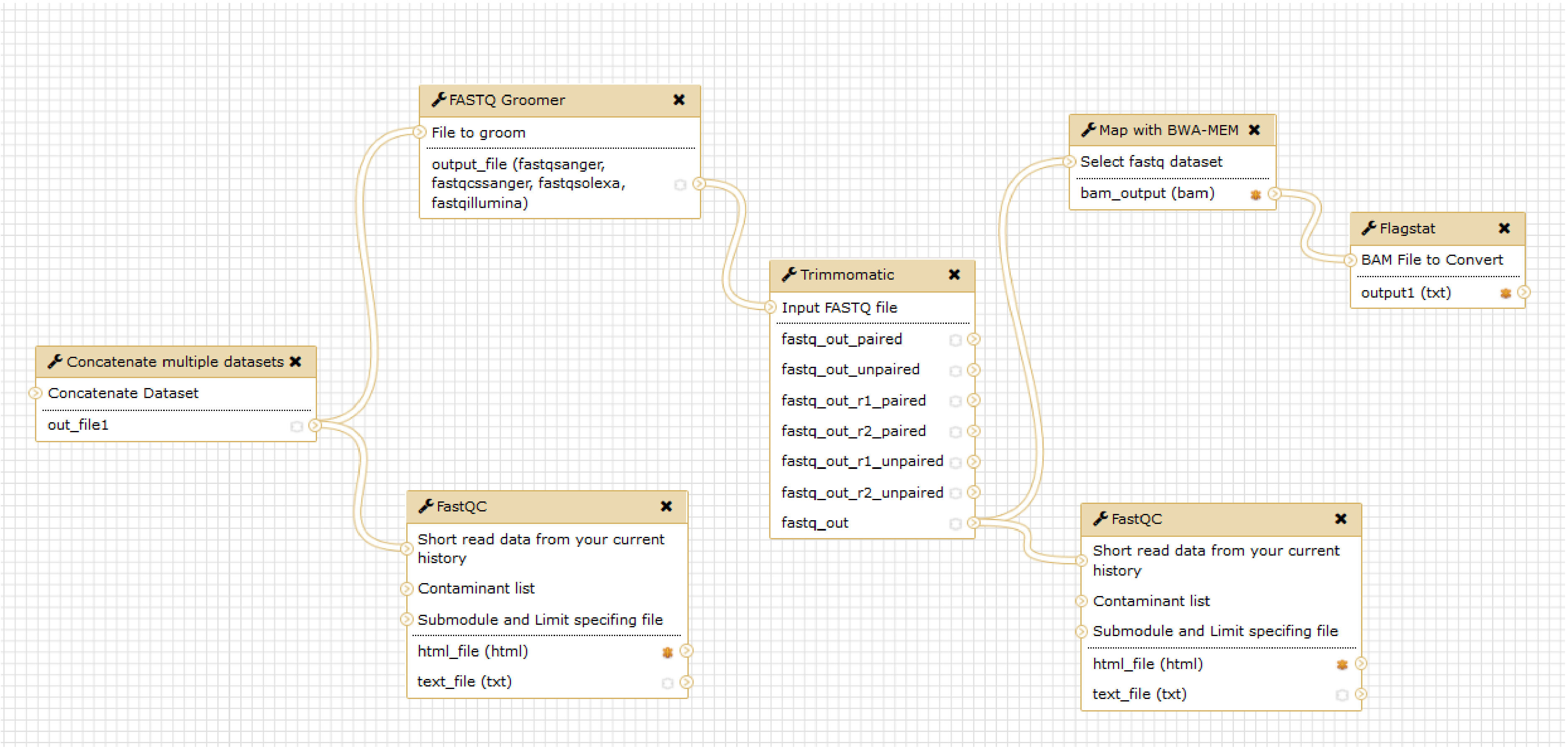
Galaxy Workflow



Concatenate multiple datasets
Concatenate Dataset
out_file1

FASTQ Groomer
File to groom
output_file (fastqsanger, fastqcssanger, fastqsolexa, fastqillumina)

FastQC
Short read data from your current history
Contaminant list
Submodule and Limit specifing file
html_file (html)
text_file (txt)

Trimmomatic
Input FASTQ file
fastq_out_paired
fastq_out_unpaired
fastq_out_r1_paired
fastq_out_r2_paired
fastq_out_r1_unpaired
fastq_out_r2_unpaired
fastq_out

Map with BWA-MEM
Select fastq dataset
bam_output (bam)

Flagstat
BAM File to Convert
output1 (txt)

FastQC
Short read data from your current history
Contaminant list
Submodule and Limit specifing file
html_file (html)
text_file (txt)

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rs328592739

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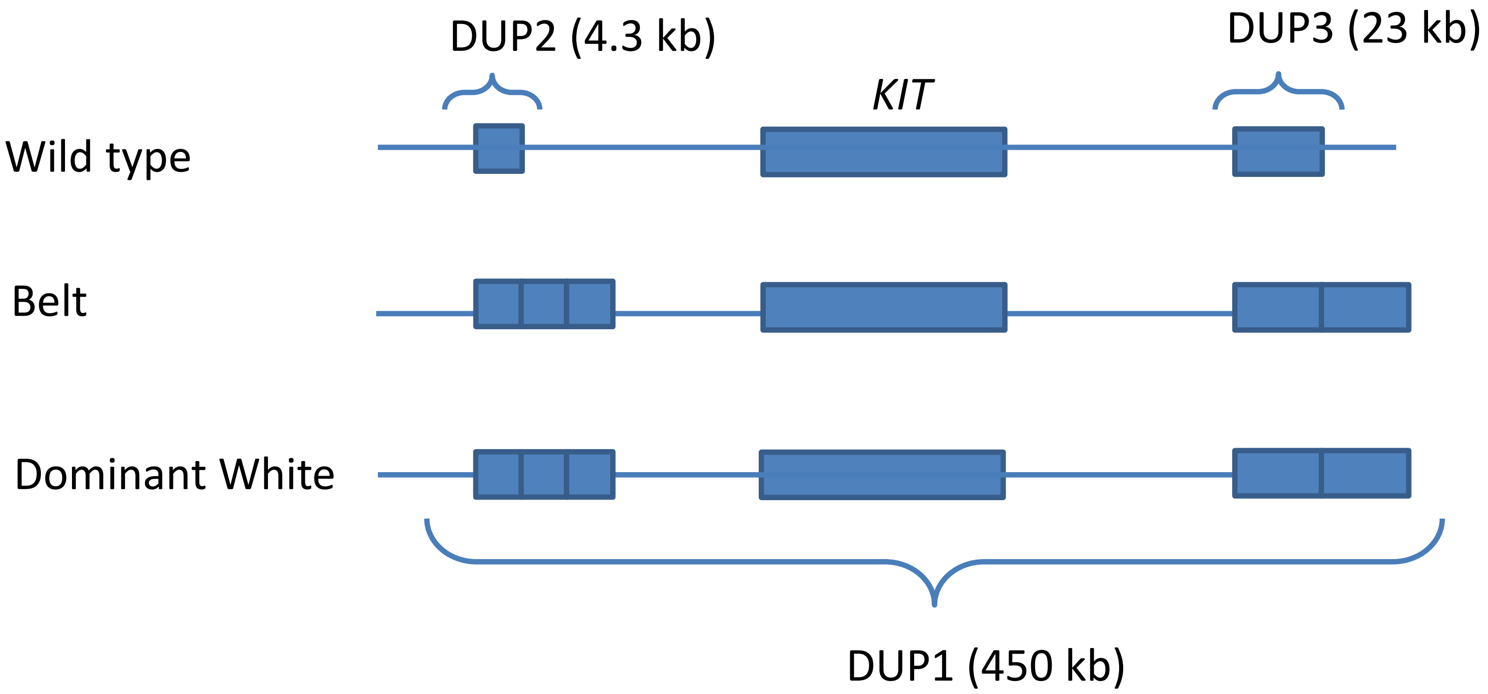
Rubin 2012



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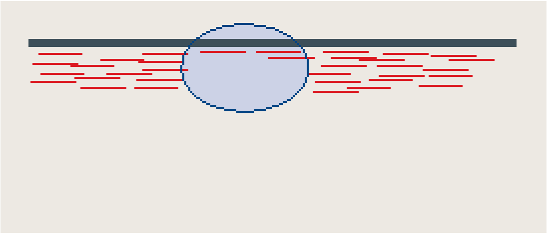
Sequence-based analytical approaches used to detect structural variation

Deletion

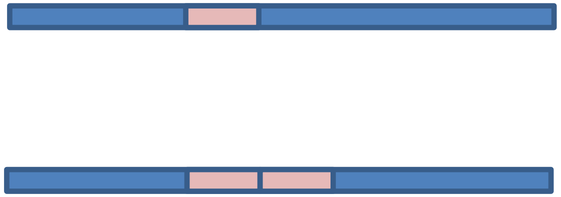
Reference genome

Subject‘s genome

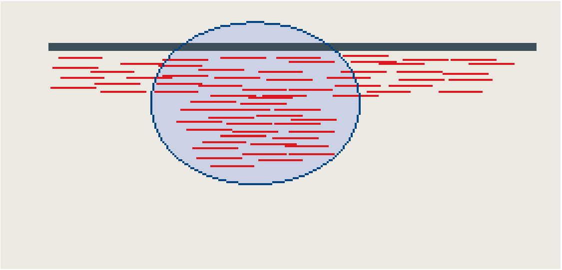


Duplication

Reference genome



Subject‘s genome



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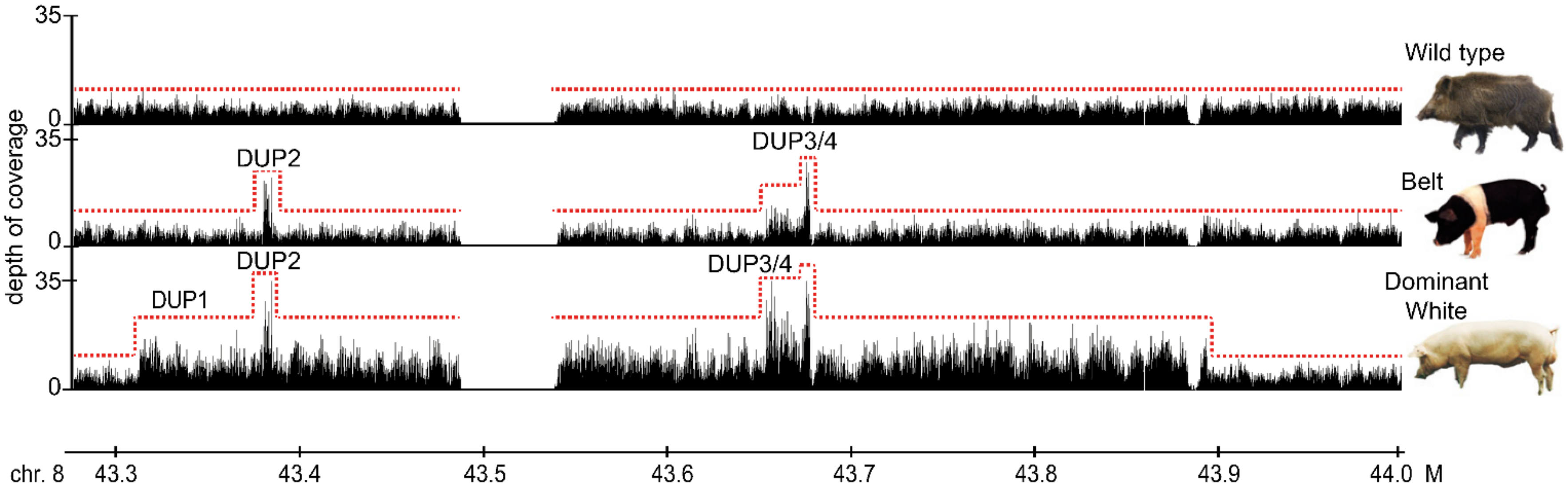
Rubin 2012



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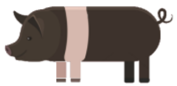
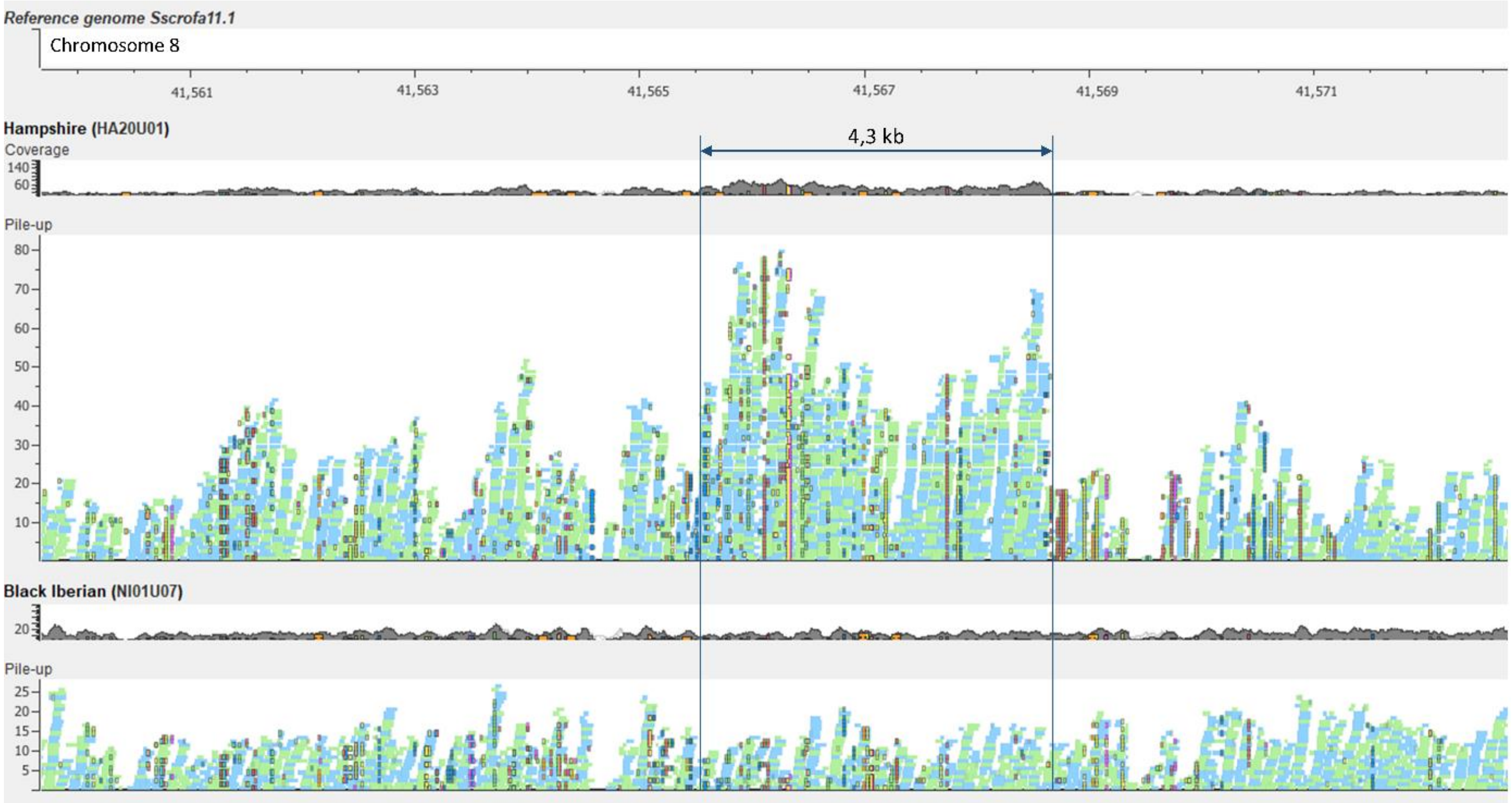
Duplication





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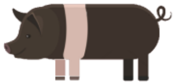
Duplication



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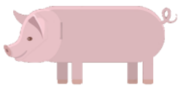
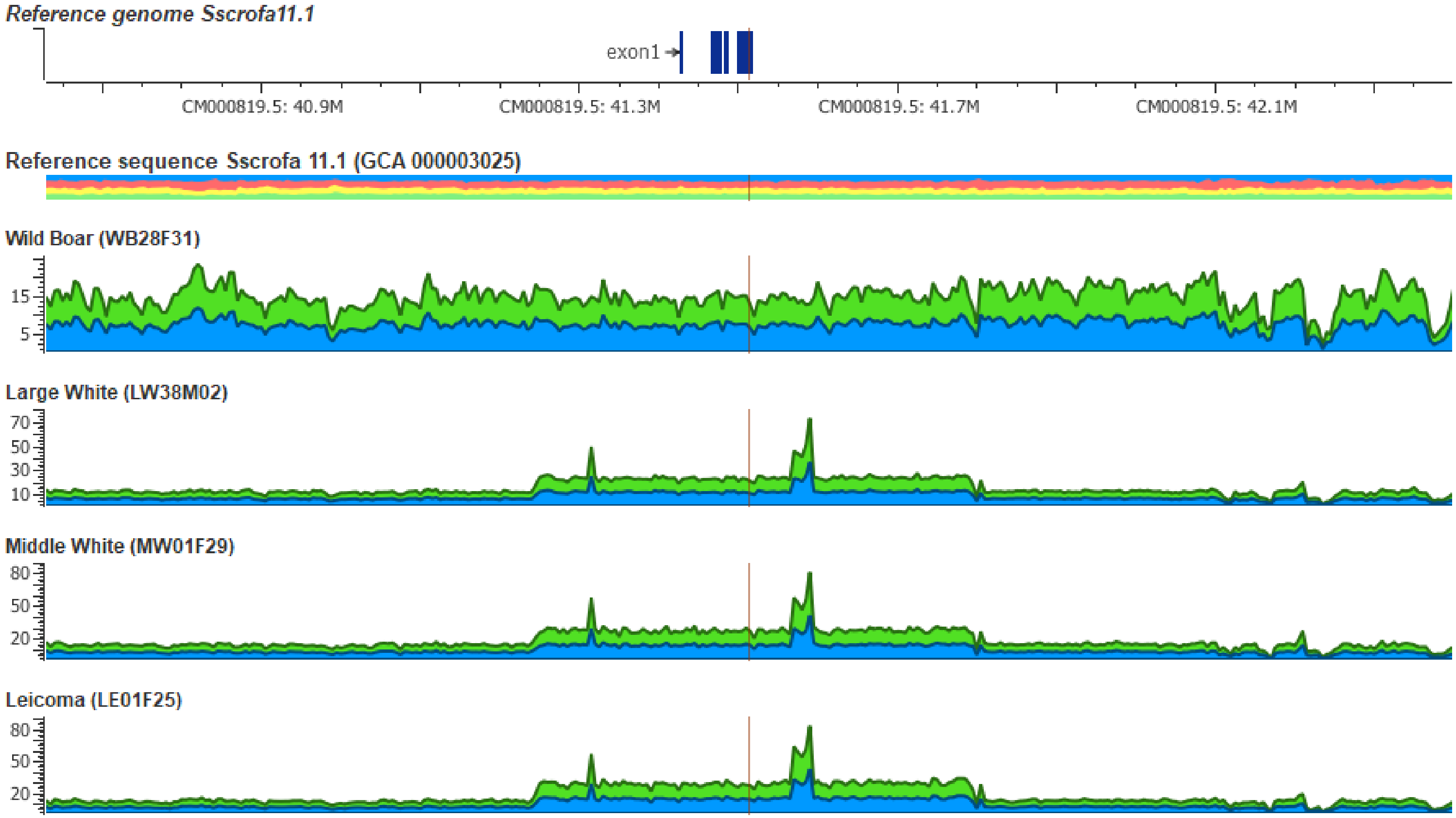
CNV





DUP1

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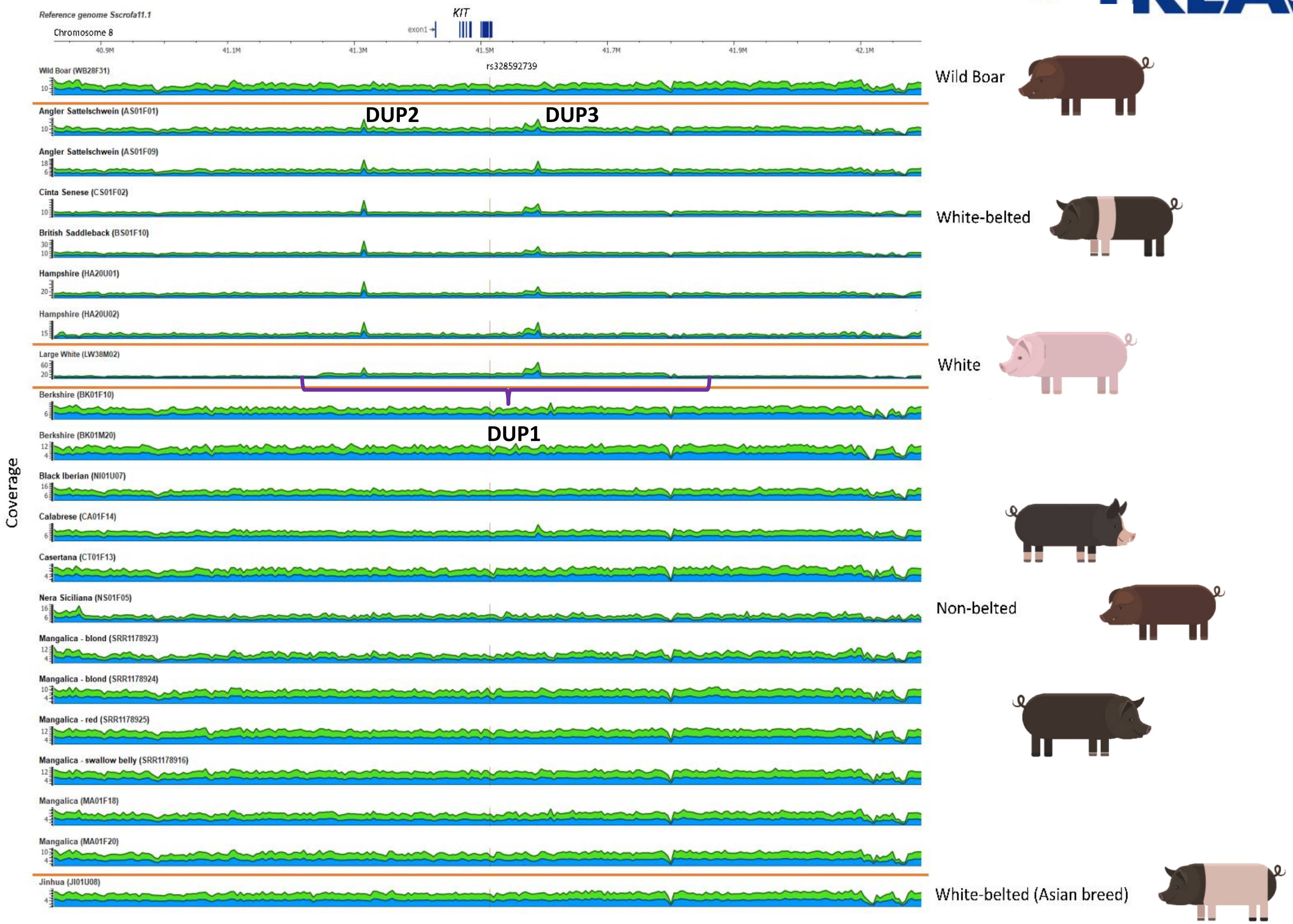

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Conclusions

- The alignment of genomic sequences confirmed 3 structural mutations:
    - 112 kb upstream
    - 160 kb downstream of the KIT locus and
    - the duplication of the region including the entire KIT gene.

- The 4.3 kb duplication located upstream of the KIT locus seems to be associated with belted phenotype in at least some belted pig breeds.

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- ARRS (P4-0220)

and for your attention.

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