

# Patterns and mechanisms of recombination at the barley *VRN-H1* locus

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# Talk Outline:

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Project background

Genetic markers used

Homologous and non-homologous recombination within *BM5A*

Putative mechanism for creation of spring *BM5A* alleles

Summary

# Flowering time

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- ! Wild plants need to flower when chances of pollination, seed development and dispersal are best
- ! This is also the case for crops – good pollination & seed development → high yield
- ! To achieve ideal flowering time, plants must flower at optimal time of year – use cues from the environment

# Two major classes of genes controlling barley flowering time

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## ! Photoperiod response (day length)

LD plants:        flowering accelerated by LD  
SD plants:        flowering accelerated by SD

## ! Vernalization response (low temperature)

Vern responsive / non vern responsive

# Vernalization – the induction of flowering by long periods of cold temperature

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**Spring barley**

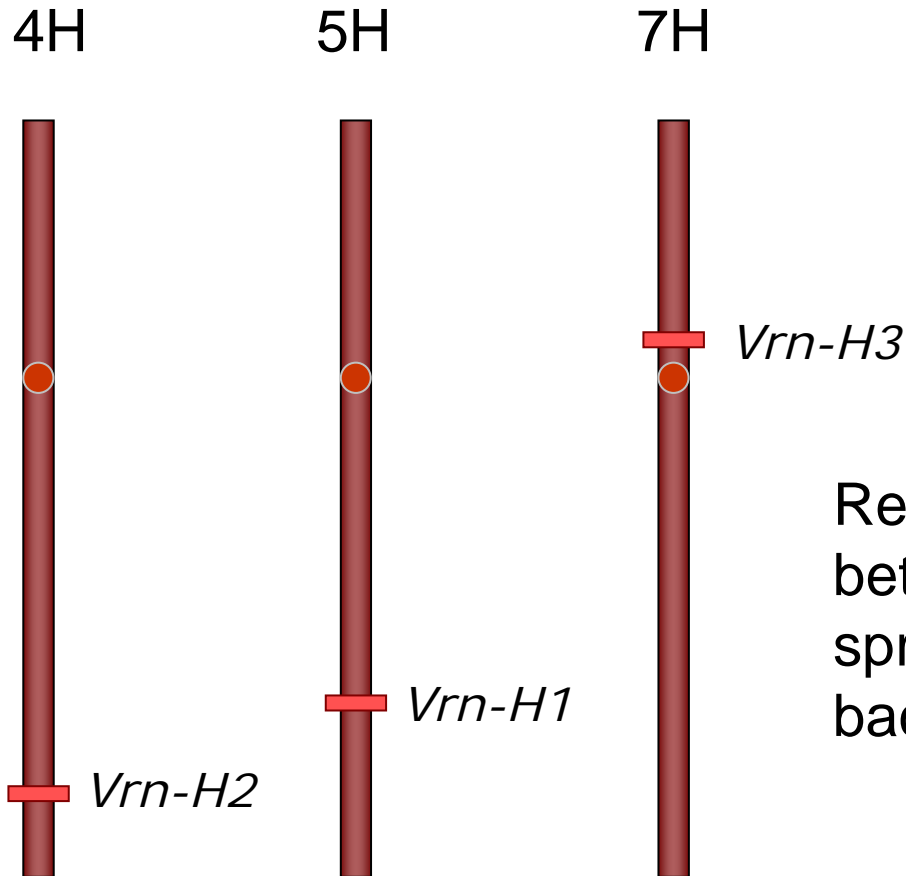
**Winter barley**



- ! Some cereal varieties are delayed in flowering unless exposed to a period of low temperature (vernalization)
- ! A vernalization response allows autumn sowing of winter barley by preventing initiation of cold susceptible floral organs until onset of favourable conditions in spring.
- ! Differences in vernalization requirement have been bred into many crops producing winter/spring sown varieties and adapting them to a wide range of agro-environments

# Vernalization response loci

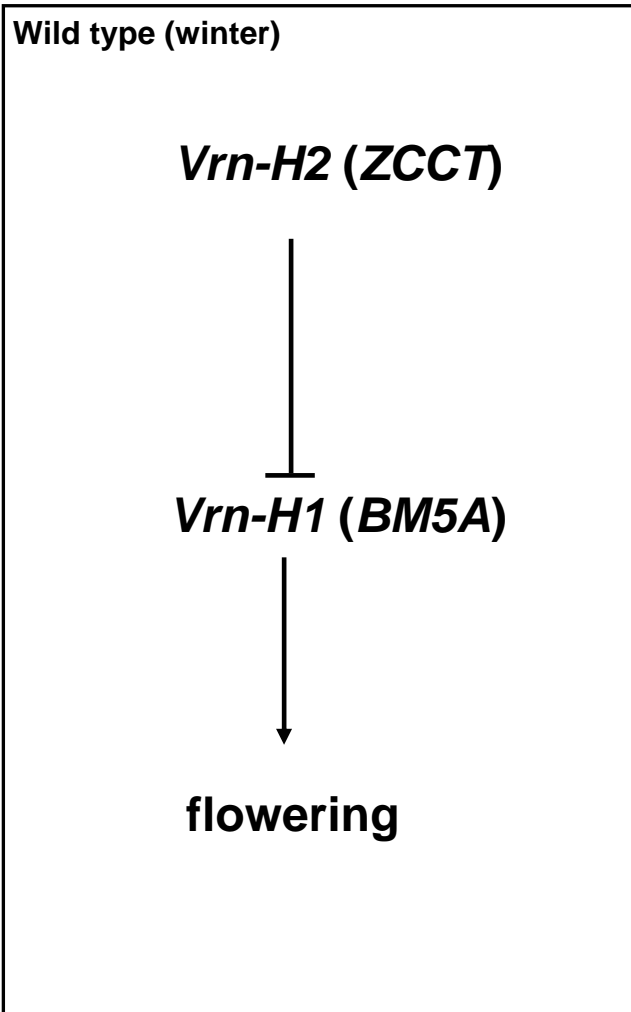
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Responsible for the distinction between winter/spring (Crossing spring allele into a winter background → spring type plant)

# Vernalization loci in the barley flowering pathway

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- ! Genes underlying colinear loci in diploid wheat cloned
- ! *VRN-H1* candidate: *BM5A* (*AtAP1*-like)
- ! *VRN-H2* candidates: *ZCCT-Ha -Hb -Hc* (contain CCT domain found in *AtCO*)
- ! Genetic / molecular model flowering pathway

# Recombination

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## ! Homologous recombination

DNA exchange between sister chromatids, meiosis

## ! Non-homologous recombination

Repair of ds chromosomal breaks

Transposition – target site recognition

Site-specific recombination eg removal of retrotransposons → solo LTRs)

# Questions

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- ! Can spring/winter Growth Habit in EU barley be explained by known alleles at *BM5A* (*VRN-H1*) and *ZCCT* (*VRN-H2*)?
- ! Are any previously un-described alleles present?
- ! Can we identify/validate diagnostic markers for Growth Habit
- ! How are spring *VRN-H1* alleles derived from winter alleles?

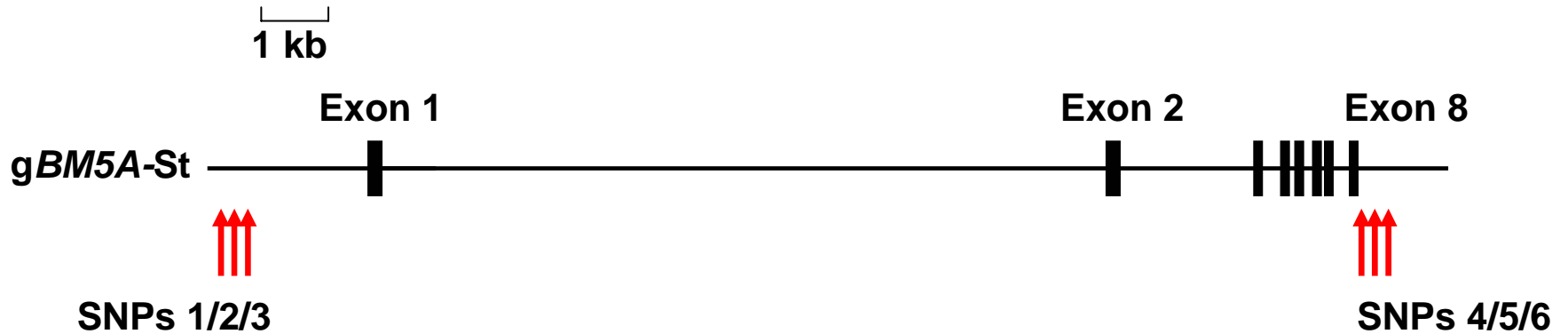
# *BM5A*



*BM5A* ~17 kb MADS-box gene

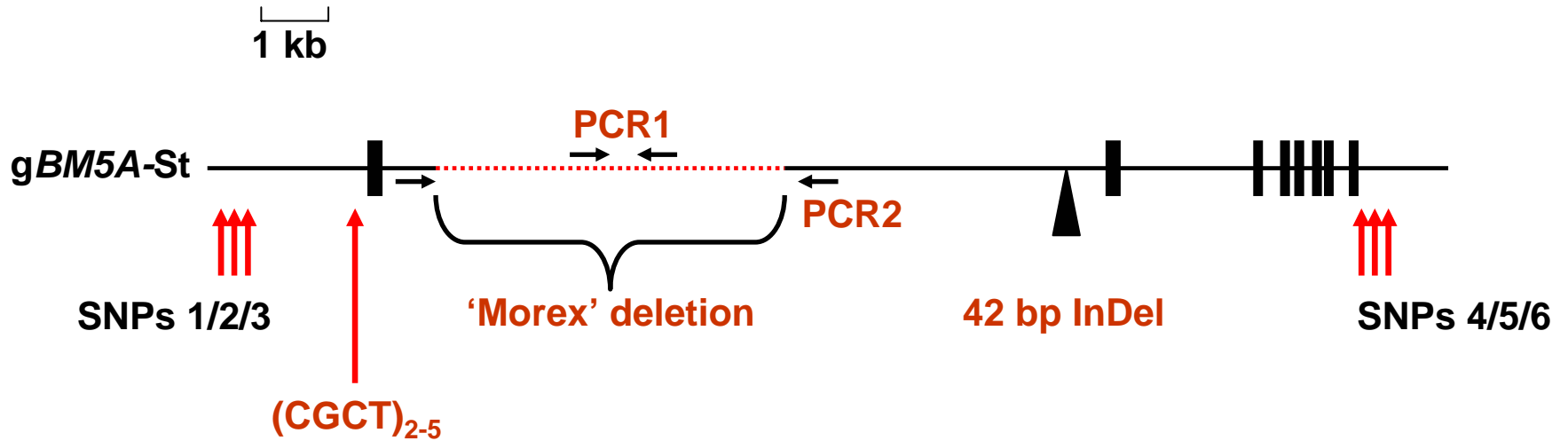
2 fully sequenced alleles

# BM5A assays



6 discriminatory SNPs selected for  
haplotyping across a collection of 430 barley varieties

# BM5A assays



10 *BM5A* markers assayed over complete varietal collection

# Haplotype analysis – 430 varieties

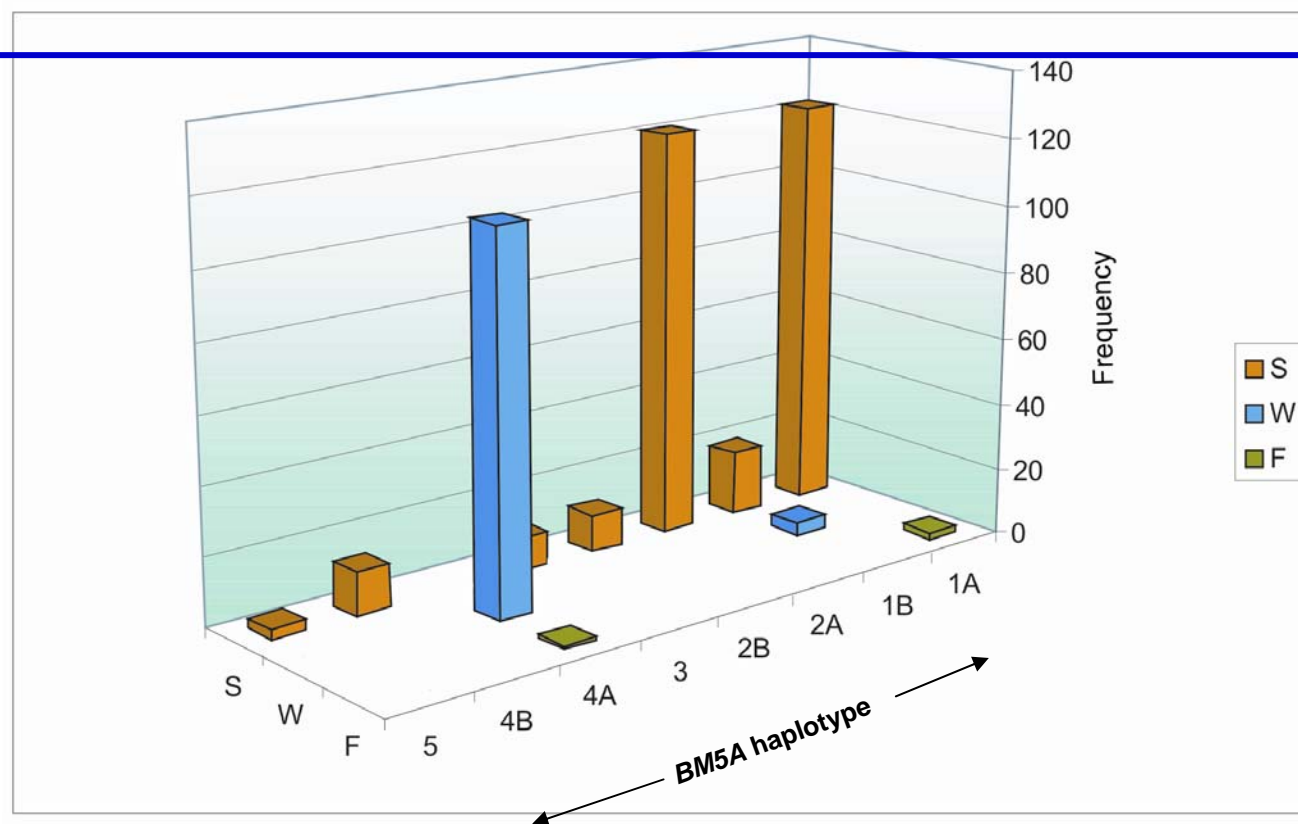
	GH	Hap	SHP1	SHP2	SHP3	SSR	St F3R3	MxF3R3	42bp	SHP4	SHP5	SHP6	N° varieties	Growth Habit
			-1948	-1881	-1684	-48			10878	14567	14585	14828		
			T/C	A/G	T/C	(CGCT)	no Del	Del	42 InDel	T/C	G/A	C/G		
1	S	1A	T	G	C	3	1	0	2	C	A	G	125	S=123, F=2
	S	1B	T	G	C	3	1	1	2	C	A	G	24	S=20, F=1, W=3
2	S	2A	C	G	C	1	1	1	2	C	G	C	125	S=121, U=4
	S	2B	C	G	C	1	1	0	2	C	G	C	11	S=11
3	S	3	T	G	C	2	1	1	2	C	A	C	10	S=10
4	W	4A	T	A	T	2	0	1	1	T	G	C	118	F=1, W=114, U=2
	S	4B	T	A	T	2	1	1	1	T	G	C	13	S=13
5	S	5	C	G	C	2	0	1	1	T	G	C	3	S=3



5 core haplotypes. Subdivided using wt/morex intron I assay

8 *BM5A* haplotypes (430 varieties)

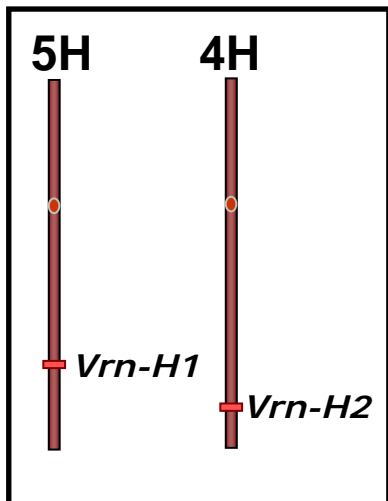
# BM5A Haplotype analysis



- ! 3 common haplotypes
- ! 4A haplotype 99 % predictive power for winter Growth Habit (4 exceptions: Athene, Almunia, Birgit, Express)
- ! Spring vars all carry 'spring' haplotypes at *BM5A* \*
- ! Identification of 2 possible homologous recombination events in *BM5A*

# VRN-H2 candidate gene (ZCCT) analysis

GH	Hap	SHP1	SHP2	SHP3	SSR	St F3R3	MxF3R3	42bp	SHP4	SHP5	SHP6	N <sup>o</sup> varieties	Growth Habit	Row number	Combined EMSA and ZCCT locus haplotype
		-1948	-1881	-1684	-48			10878	14567	14585	14828				
S	1A	T	G	C	3	1	0	2	C	A	G	125	S=123, F=2	2- <sub>row</sub> =117, 6- <sub>row</sub> =7 U=1	1A+Z = 14, 1A-Z = 111
S	1B	T	G	C	3	1	1	2	C	A	G	24	S=20, F=1, W=3	2- <sub>row</sub> =17, 6- <sub>row</sub> =7	1B+Z = 8, 1B-Z = 16
S	2A	C	G	C	1	1	1	2	C	G	C	125	S=121, U=4	2- <sub>row</sub> =104, 6- <sub>row</sub> =17, U=4	2A+Z = 15, 2A-Z = 110
S	2B	C	G	C	1	1	0	2	C	G	C	11	S=11	2- <sub>row</sub> =10, 6- <sub>row</sub> =1	2B+Z = 2, 2B-Z = 9
S	3	T	G	C	2	1	1	2	C	A	C	10	S=10	2- <sub>row</sub> = 10	3+Z = 3, 3-Z = 7
W	4A	T	A	T	2	0	1	1	T	G	C	118	F=1, W=114, U=2	2- <sub>row</sub> =68, 6- <sub>row</sub> =44, U=6	4A+Z = 115, 4A-Z = 3
S	4B	T	A	T	2	1	1	1	T	G	C	13	S=13	2- <sub>row</sub> =1, 6- <sub>row</sub> =11, U=1	4B+Z = 0, 4B-Z = 13
S	5	C	G	C	2	0	1	1	T	G	C	3	S=3	2- <sub>row</sub> =3	5+Z = 2, 5-Z = 1

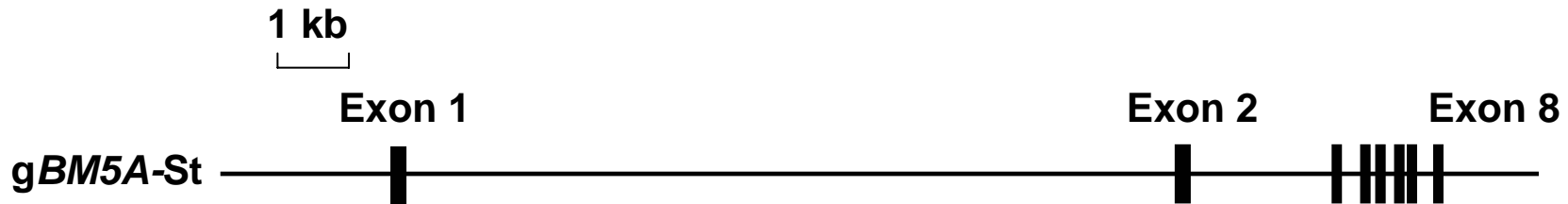


- ! 13 % of spring varieties carry ZCCT locus (winter allele?)
- ! Markers predict Growth Habit in all but 3 of the 430 barley vars
- ! Phenotyping to verify Growth Habit

# *BM5A* Haplotype analysis

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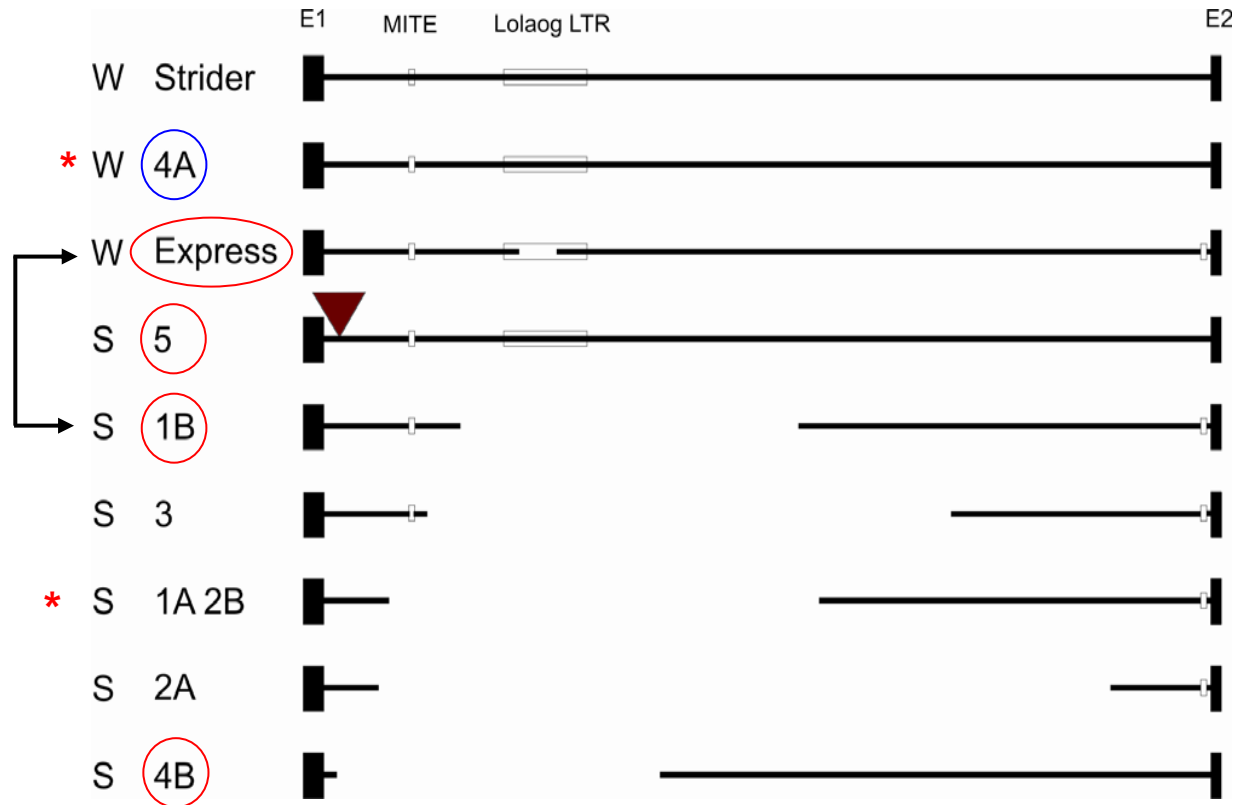
- ! Understand the patterns of recombination and the number of spring alleles present
- ! 3 varieties from each haplotype selected for full sequencing.



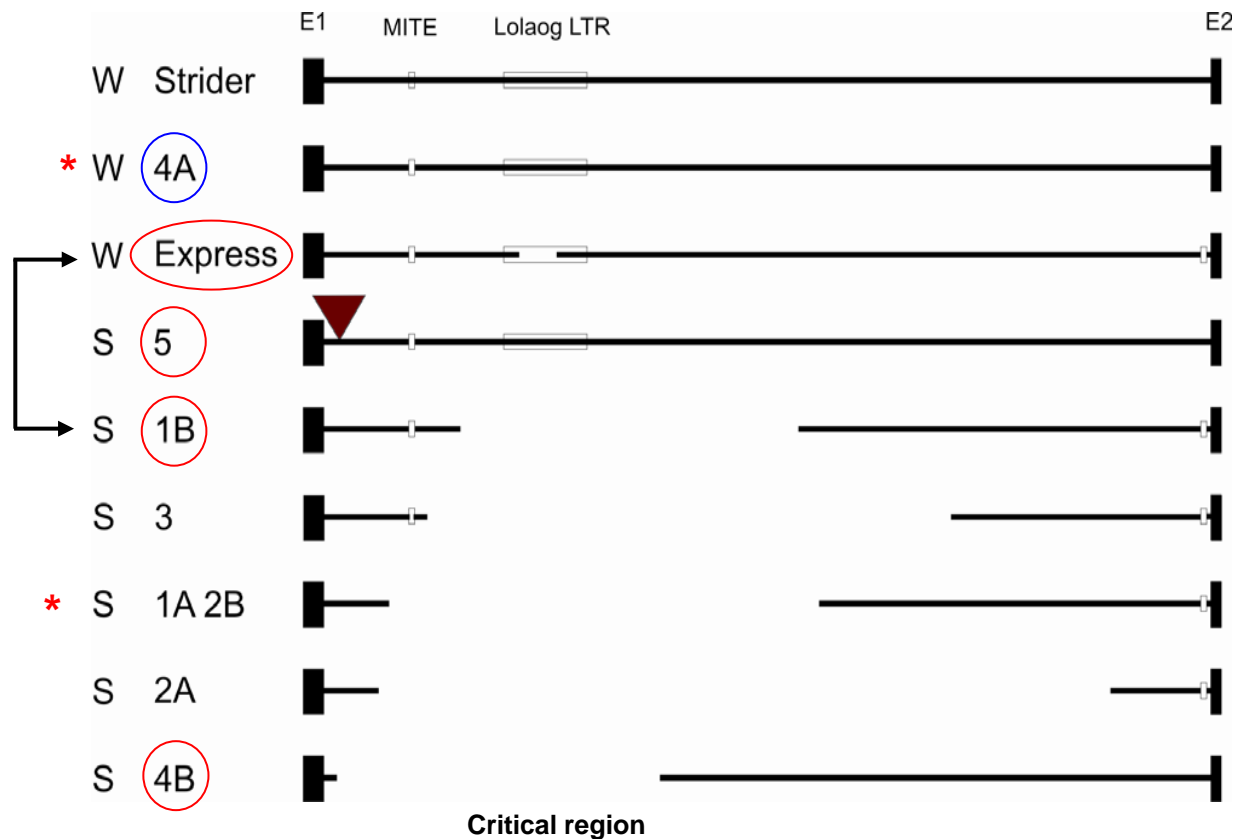
- ! Large intron I dels previously suggested responsible for spring *BM5A* alleles - 85 polymorphisms\*.



! 9 Intron I dels configurations, 4 novel intron configurations, plus full European winter allele sequence



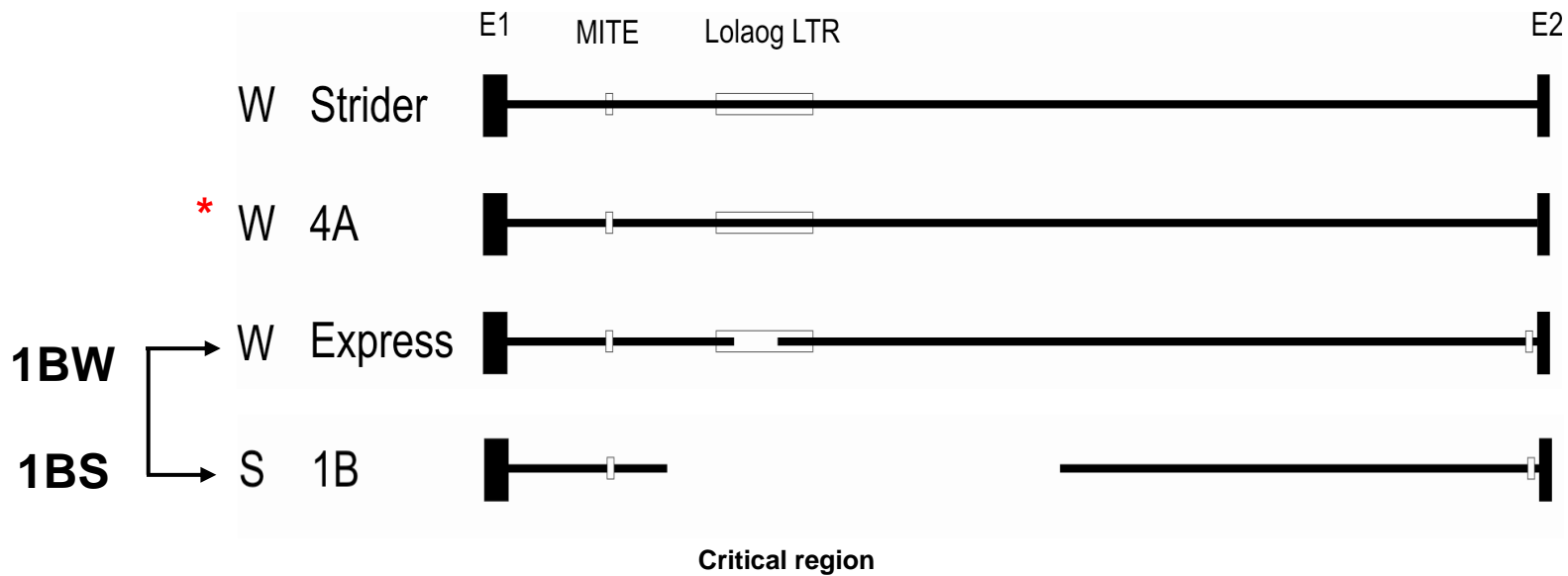
! 9 Intron I dels configurations, 4 novel intron configurations, plus full European winter allele sequence



! Hap 1B: 2 alternative deletions assoc with diff Growth Habit

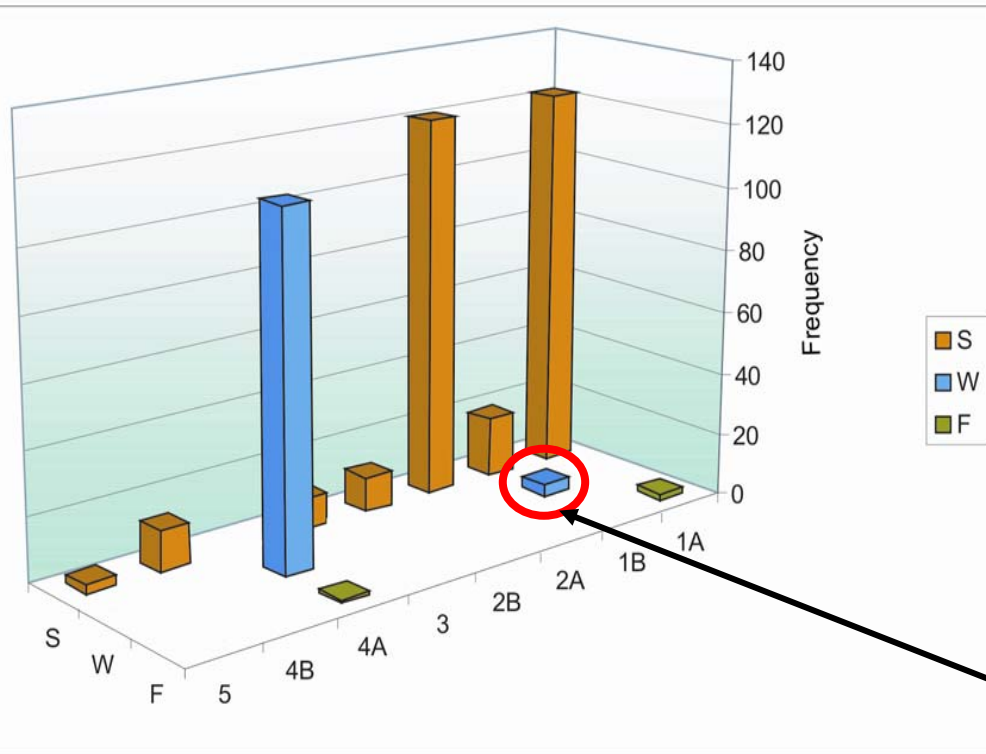
# BM5A Sequence analysis

! Express differentiated from haplotype 1B by small intron I deletion within Lolaog solo LTR. Winter GH retained

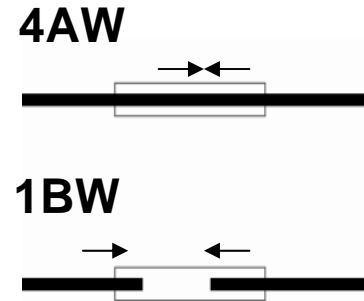


# BM5A Sequence analysis

! Express directly related to Athene + Birgit by pedigree. Almunia carries same del



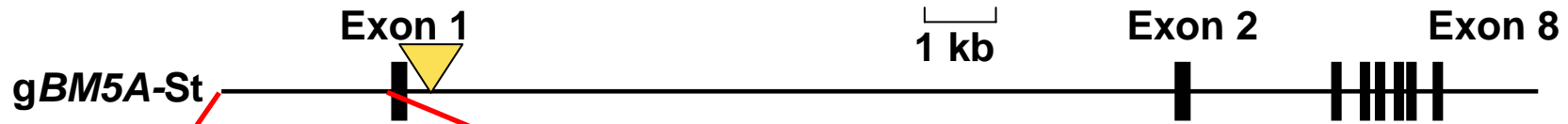
GH	Variety	BM5A Del	ZCCT
S	MORGENROT	Del-1B	-ZCCT
S	PROCTOR	Del-1B	-ZCCT
S	DIGGER	Del-1B	-ZCCT
S	KEG	Del-1B	-ZCCT
S	MOSANE	Del-1B	-ZCCT
S	PIROLINE	Del-1B	-ZCCT
S	MIRENA	Del-1B	-ZCCT
S	KORAL1	Del-1B	-ZCCT
S	ORTOLAN	Del-1B	-ZCCT
S	AMSEL	Del-1B	-ZCCT
S	PEGASUS	Del-1B	-ZCCT
S	ORIOLE	Del-1B	-ZCCT
S	TERN	Del-1B	-ZCCT
S	BINDER	Del-1B	-ZCCT
S	LEONESSA	Del-Ex	-ZCCT
S	ARK ROYAL	Del-1B	-ZCCT
S	SANDRA	Del-1B	-ZCCT
S	CRUSADER	Del-1B	-ZCCT
S	FRISIA	Del-1B	+ZCCT
S	ALBACETE	Del-1B	+ZCCT
S	WEIDER	Del-1B	+ZCCT
S	RESANA	Del-1B	+ZCCT
W	EXPRESS	Del-Ex	+ZCCT
W	BIRGIT	Del-Ex	+ZCCT
W	ATHENE	Del-Ex	+ZCCT
W	ALMUNIA	Del-Ex	+ZCCT



! Describe all winter alleles at *VRN-H1* \*

! Diagnostic PCR/Agarose gel-based marker for winter alleles at *VRN-H1* \*

# BM5A homologous recombination – Hap 5



		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
GOLF	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G	T
G PROMISE	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G	T
PHOTO	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G	T
ORIOLE	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G	T
PEGASUS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	G	A	C	T	G	T	
EXPRESS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G	T
ERKKI	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C
OPTIC	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C
TRIUMPH	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C
M CANNON	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C
HORA	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C
PRISMA	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	G	T	G	G	C	C	C	C
BETRICE	3	T	G	C	A	C	T	G	G	C	T	A	T	A	A	A	A	C	G	G	A	A	C	T	G	C	T	G	C	C	C	C	A	
CHARIOT	3	T	G	C	A	C	T	G	G	C	T	A	T	A	A	A	A	C	G	G	A	?	T	?	T	G	C	T	C	G	A	C	C	A
DANDY	3	T	G	C	A	C	T	G	G	C	T	A	T	A	A	A	A	C	G	G	A	A	C	T	G	C	T	C	G	A	C	C	A	
YARUNDA	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	T	G	G	C	C	C	A
MAZURKA	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	T	G	G	C	C	C	A
DRAM	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	T	G	G	C	C	C	A
XENIA	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
IGRI	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
PANDA	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
PEARL	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
SRTIDER	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
MEIKY	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
LISE	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
ARRA	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	
ETU	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	T	T	T	A	C	C	T	G	G	C	C	C	A	

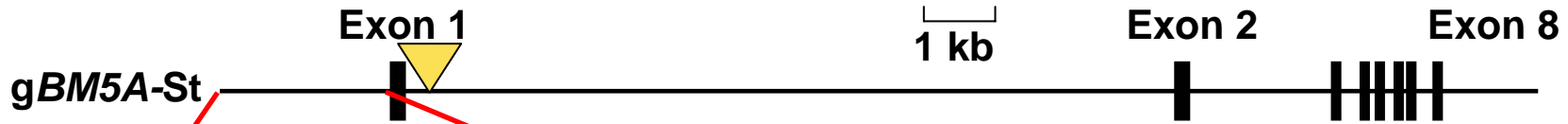
! Genomic sequence for haplotype 4A (winter) and 5 (spring) are identical apart from 2 features:

# BM5A homologous recombination – Hap 5



		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
GOLF	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
G PROMISE	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
PHOTO	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
ORIOLE	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
PEGASUS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
EXPRESS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	A	G	G	C	G	A	C	T	G		
ERKKI	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C	
OPTIC	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C	
TRIUMPH	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	?	?	G	G	C	C	C	C	
M CANNON	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	G	?	G	G	C	C	C	C	
HORA	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	G	?	G	G	C	C	C	C	
PRISMA	2B	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	G	G	T	G	G	C	C	C	C	
BETRICE	3	T	G	A	A	C	C	T	G	G	C	T	A	A	A	A	C	G	G	A	A	A	C	A	G	G	C	C	T	C	G	A	C	C	
CHARIOT	3	T	G	A	A	C	C	T	G	G	C	T	A	A	A	A	C	G	G	A	A	A	C	A	G	G	C	C	T	C	G	A	C	C	
DANDY	3	T	G	A	A	C	C	T	G	G	C	T	A	A	A	A	C	G	G	A	A	A	C	A	G	G	C	C	T	C	G	A	C	C	
YARUNDA	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	C	T	G	G	C	C	C	A
MAZURKA	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	C	T	G	G	C	C	C	A
DRAM	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	A	C	C	C	T	G	G	C	C	C	A
XENIA	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
IGRI	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
PANDA	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
PEARL	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
SRTIDER	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
MEIKY	4A	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
LISE	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
ARRA	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A
ETU	4B	T	A	G	A	T	C	G	G	C	T	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A

# BM5A homologous recombination – Hap 5



		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
GOLF	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	G	A	C	T	G	T	
G PROMISE	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	G	A	C	T	G	T	
PHOTO	1A	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	A	C	T	G	T		
ORIOLE	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	A	C	T	G	T		
PEGASUS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	A	C	T	G	T		
EXPRESS	1B	T	G	A	A	C	C	A	G	C	G	A	T	A	A	A	A	C	G	G	G	A	A	C	G	G	G	G	A	C	T	G	T		
ERKKI	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
OPTIC	2A	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
TRIUMPH		C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
MCC		C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
HYMA	2E	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
FRAMA	2E	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	?	?	G	G	C	C	C	C	
BETRICE	3	T	G	A	A	C	C	A	G	C	T	A	A	A	A	C	G	G	A	T	G	A	A	C	G	G	T	G	C	C	C	C	C		
ARIOT	3	T	G	A	A	C	C	A	G	C	T	A	A	A	A	C	G	G	A	T	G	A	A	C	G	G	T	G	C	C	C	C	C		
VA	3	T	G	A	A	C	C	A	G	C	T	A	A	A	A	C	G	G	A	T	G	A	A	C	G	G	T	G	C	C	C	C	C		
MAZDA	5	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	C	C	T	G	G	C	C	C	A
DRAM	6	C	G	T	T	C	C	G	A	T	T	A	A	A	T	T	G	G	A	T	G	A	A	C	G	G	C	C	T	G	G	C	C	C	A
XENIA	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
IGRI	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
PANDA	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
PEARL	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
SRTIDER	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
MEIKY	4A	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	
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ETU	4B	T	A	G	A	T	C	G	G	C	T	T	T	A	A	A	C	G	G	G	T	T	T	A	C	C	C	T	G	G	C	C	C	A	

! 'Spring' promoter recombined into 'winter' haplotype

! recombination event: 93 bp

! Recombination can be seen in *CStF50*, 1 cM distal

(GCCC)<sub>4-5</sub>

# BM5A homologous recombination– Hap 2B



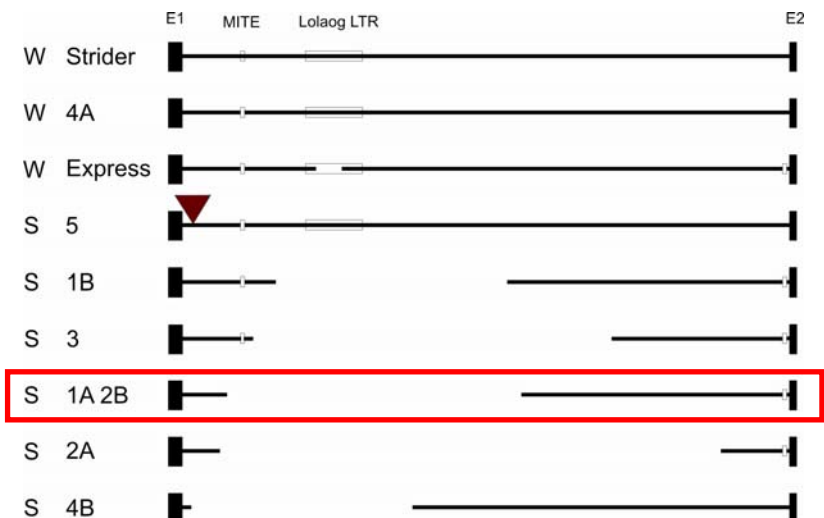
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G PROMISE	1A	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G			
PHOTO	1A	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G			
ORIOLE	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G			
PEGASUS	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G			
EXPRESS	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G			
ERKKI	2A	G	G	C	C	C	G	G	C	C	G	G	G	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
OPTIC	2A	G	G	C	C	C	C	G	C	C	G	G	G	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
TRUMPET	2A	G	G	C	C	C	C	G	C	C	G	G	G	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
M CANNON	2B	G	G	C	C	C	C	G	C	A	C	A	C	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
HORA	2B	G	G	C	C	C	C	G	C	A	C	A	C	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
PRISMA	2B	G	G	C	C	C	C	G	C	A	C	A	C	G	G	C	G	C	C	G	A	G	T	T	C	G	A	C	T	G	G	G	G			
BETRICE	3	C	G	C	C	C	A	C	T	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C			
CHARIOT	3	C	G	A	C	C	A	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C			
DANDY	3	C	G	A	C	C	A	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C			
YARUNDA	5	G	G	C	C	C	A	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
MAZURKA	5	G	G	C	C	C	A	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
DRAM	5	G	G	C	C	C	A	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
XENIA	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
IGRI	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
PANDA	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
PEARL	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
SRTIDER	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
MEIKY	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
LISE	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
ARRA	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				
ETU	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G				



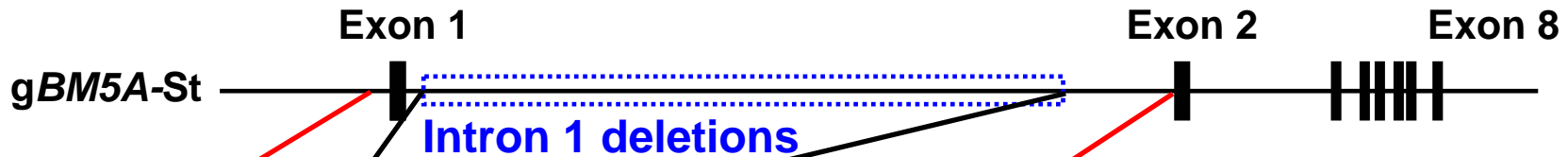
# BM5A homologous recombination– Hap 2B



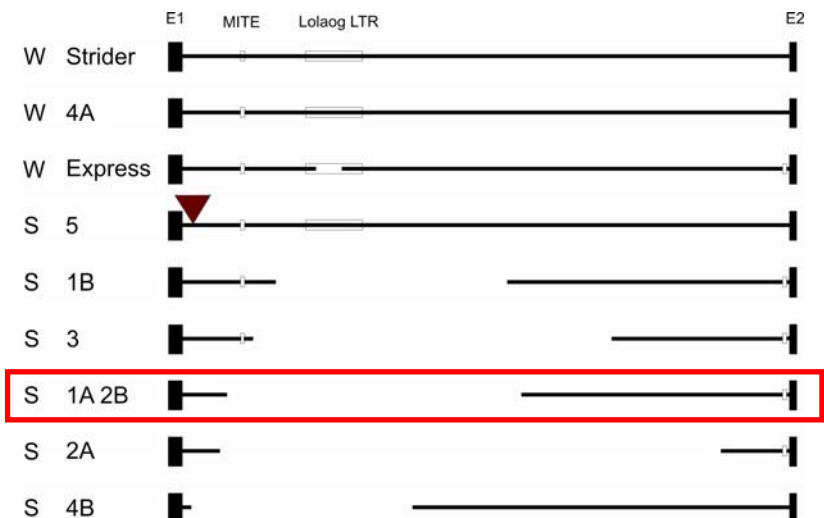
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G PROMISE	1A	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	
PHOTO	1A	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	
ORIOI	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	
PEGASUS	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	
EXPRESS	1B	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	
ERKKI	2A	G	G	C	C	C	G	G	C	C	G	G	G	G	G	C	G	C	G	A	A	G	T	T	C	G	A	C	T	G	G	G	G	
OPTIC	2A	G	G	C	C	C	C	G	C	C	G	G	G	G	G	C	G	C	C	G	A	A	G	T	T	C	G	A	C	T	G	G	G	G
TRUMPET	2A	G	G	C	C	C	C	G	C	C	G	G	G	G	G	C	G	C	C	G	A	A	G	T	T	C	G	A	C	T	G	G	G	G
M CANNON	2E	G	G	C	C	C	C	G	C	C	A	C	A	C	G	G	C	G	C	A	A	G	T	T	C	G	A	C	T	G	G	G	G	
HORA	2E	G	G	C	C	C	C	G	C	C	A	C	A	C	G	G	C	G	C	A	A	G	T	T	C	G	A	C	T	G	G	G	G	
PRISMA	2E	G	G	C	C	C	C	G	C	C	A	C	A	C	G	G	C	G	C	A	A	G	T	T	C	G	A	C	T	G	G	G	G	
BETRICE	3	C	G	C	C	C	A	C	T	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C	
CHARIOT	3	C	G	A	C	C	A	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C	
DANDY	3	C	G	A	C	C	A	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C	
YARUNDA	5	G	G	C	C	C	A	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
MAZURKA	5	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
DRAM	5	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
XENIA	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
IGRI	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
PANDA	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
PEARL	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
SRTIDER	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
MEIKY	4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
LISE	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
ARRA	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	
ETU	4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G	



# BM5A homologous recombination– Hap 2B

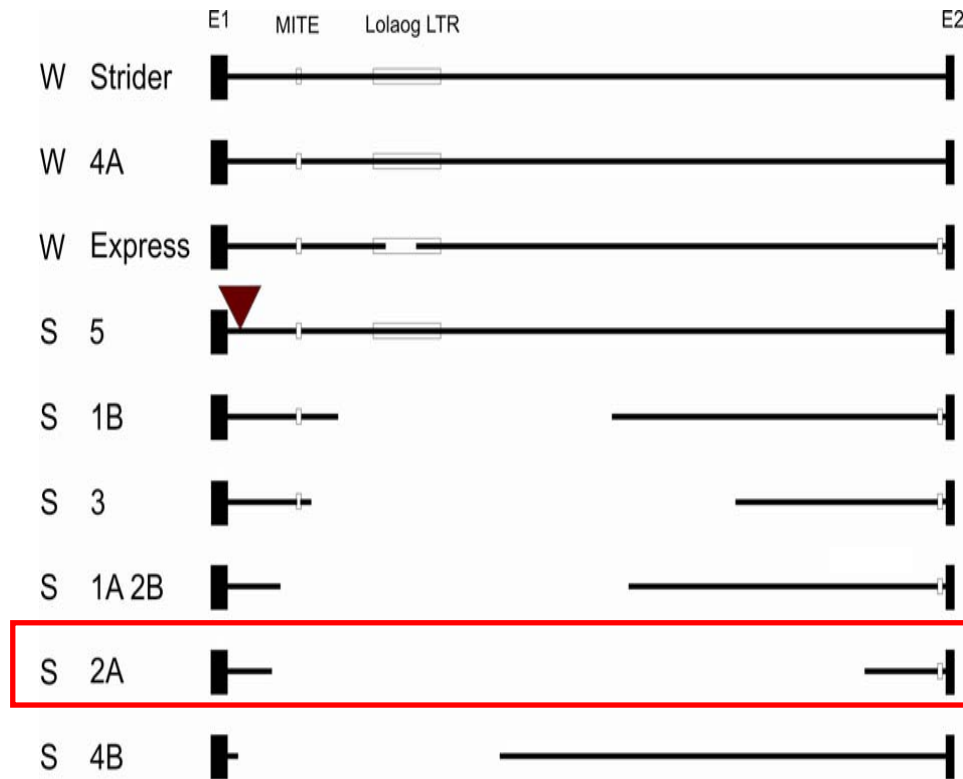


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PHOTO 1A	G	A	C	T	G	T	C	C	A	C	A	C	G	G	C	A	G	G	C	C	T	C	T	A	C	T	A	G	G	G	G		
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ERKKI 2A	G	G	C	C	C	G	G	C	C	G	G	G	G	G	C	G	C	C	A	G	T	T	C	G	A	C	T	G	G	G	G		
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TRUMPET 2A	G	G	C	C	C	C	G	C	C	G	G	G	G	G	C	G	C	C	A	G	T	T	C	G	A	C	T	G	G	G	G		
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HORA 2E	G	G	C	C	C	C	G	C	C	A	C	A	C	G	G	C	G	C	C	A	G	T	T	C	G	A	C	T	G	G	G	G	
PRISMA 2E	G	G	C	C	C	C	G	C	C	A	C	A	C	G	G	C	G	C	C	A	G	T	T	C	G	A	C	T	G	G	G	G	
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CHARIOT 3	C	G	A	C	C	A	C	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C
DANDY 3	C	G	A	C	C	A	C	C	C	C	C	G	G	G	G	C	G	C	A	A	A	G	T	T	A	T	A	C	T	G	G	A	C
YARUNDA 5	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
MAZURKA 5	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
DRAM 5	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
XENIA 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
IGRI 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
PANDA 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
PEARL 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
SRTIDER 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
MEIKY 4A	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
LISE 4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
ARRA 4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G
ETU 4B	G	G	C	C	C	A	C	C	C	C	G	G	G	G	G	T	A	T	A	G	A	G	T	A	C	T	G	T	A	G	A	G	G



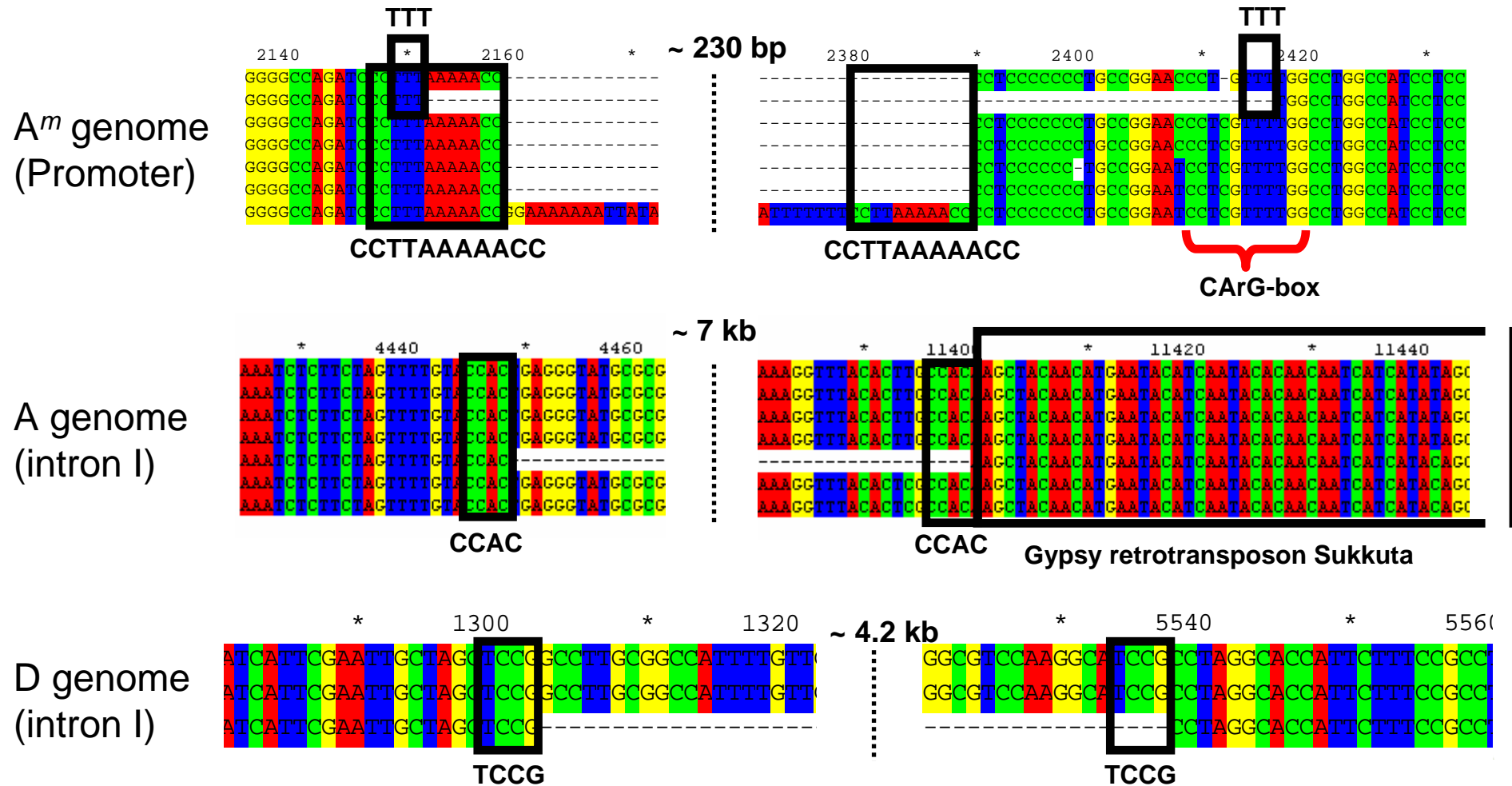
5': 105 bp\*      3' recombination region: 346 bp

# *BM5A* non-homologous recombination Intron I breakpoint analysis



# Wheat *AP1* non-homologous recombination Intron I analysis

Similar motifs can be seen flanking *AP1* deletion breakpoints in wheat



# Wheat/Barley *AP1* analysis

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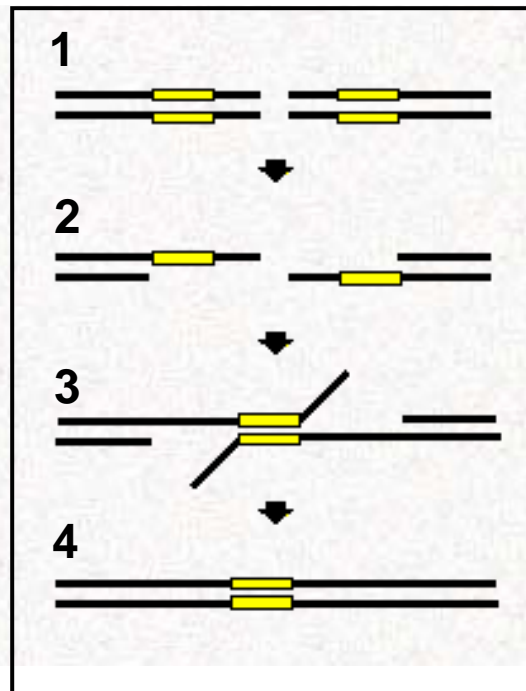
- ! Intron I (and promoter) breakpoints are flanked by endogenous repeated motifs in barley and diploid and hexaploid wheat (3 – 11 bp)
- ! Deletions in wheat and barley have been independently selected during domestication after the divergence of the 2 spp.
- ! Suggests common mechanism could be operating in both species
- ! Some deletion borders are flanked by retroelements in wheat, and possibly barley

# Hypothesis for breakpoint formation

- ! Deletions created by non-homologous recombination, principally by the Single-Strand Annealing (SSA) mechanism

SSA experimentally proven in plants in *Arabidopsis* and Tobacco (I-SceI rare cutter mediated restriction) DS breaks induced and the resulting rearrangements analyzed by sequencing.

Only small deletions (100s of bases) have been shown experimentally.



Similar short sequence homologies flanking deletions in transposable elements has been put forward as a method to counteract genome expansion by reduction and eventual deletion of these elements in Arab and rice

Here we show evidence of its occurrence in barley, and its possible involvement in the creation of functionally important novel alleles, which form the basis of the distinction between winter and spring barley

# Summary

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European barley characterised for *BM5A* alleles (*VRN-H1*)

Diagnostic marker for winter alleles at *VRN-H1*

Identification of novel *BM5A* alleles associated with spring/winter GH

Putative mechanism for intron I deletions, candidate mutations for the spring *Vrn-H1* allele, and selected for post-domestication

# Uses

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Diagnostic markers for GH in Distinctness Uniformity and Stability (DUS)

Uniformity of GH (DUS)

Characterise the effect of different *VRN* alleles and allele combinations on flowering time (in conjunction with *PPD* alleles)

→ Tailor flowering time to specific agro-environments

# Thanks to

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