GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

Molecular analysis of White Galloway coat colour variations

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Coat colour of livestock

- Coat colour variations have always been fascinating for man
- In early mythology and art, coat colour of animals played an important role
  (Siebel, 1997; Forbis, 1980)
- Coat colour variations have been one of the earliest selection criteria
  (Schmutz, 2002)

(www.onlinekunst.de, Das Rind in der Kunst)

(www.lascaux.culture.fr)

Coat colour variations in White Galloway and White Park cattle








WWM - White Well Marked

WSM - White Strongly Marked

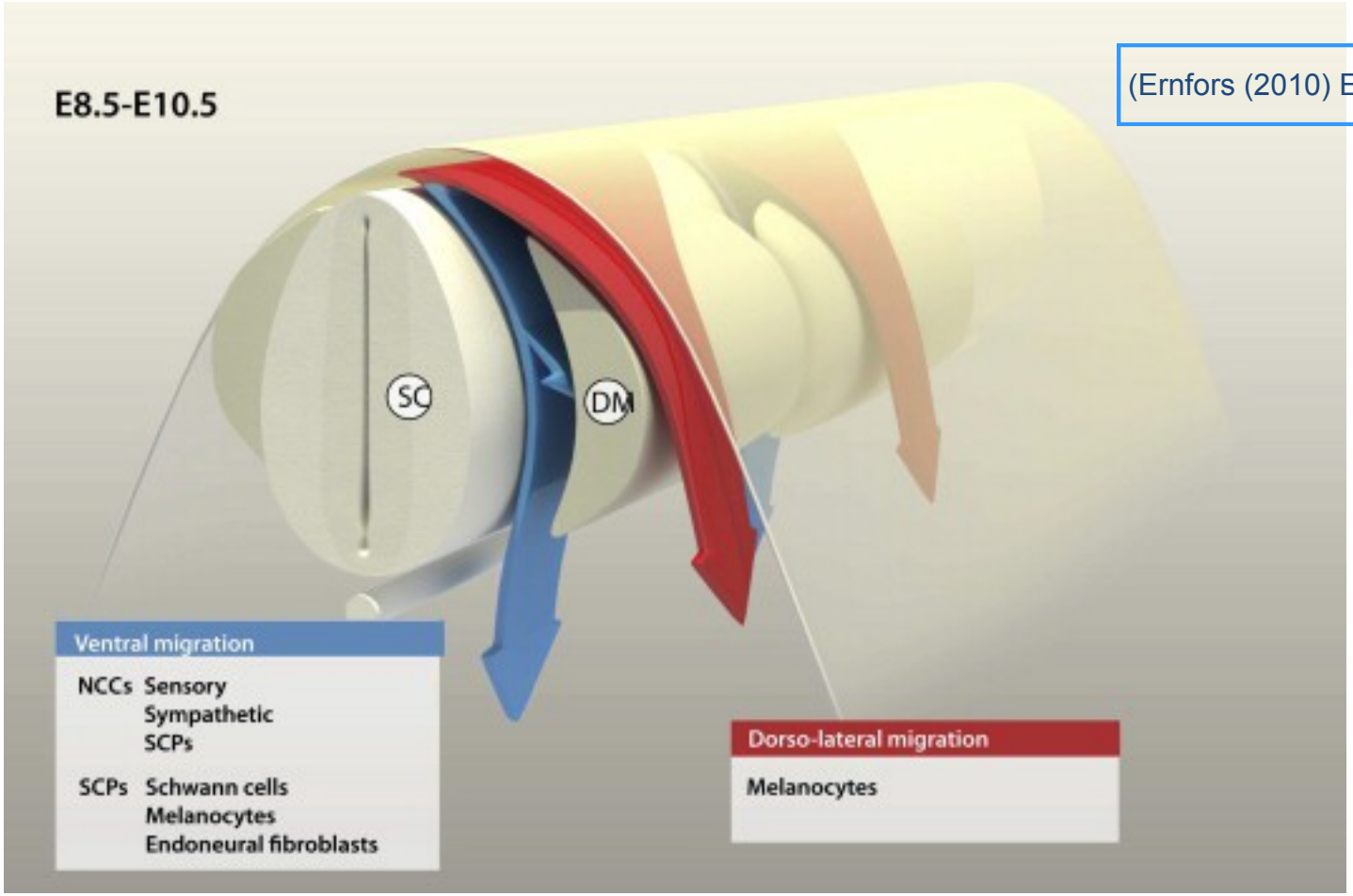
WPM - White Poorly Marked

WFB - White Fully Black

Aims of the study

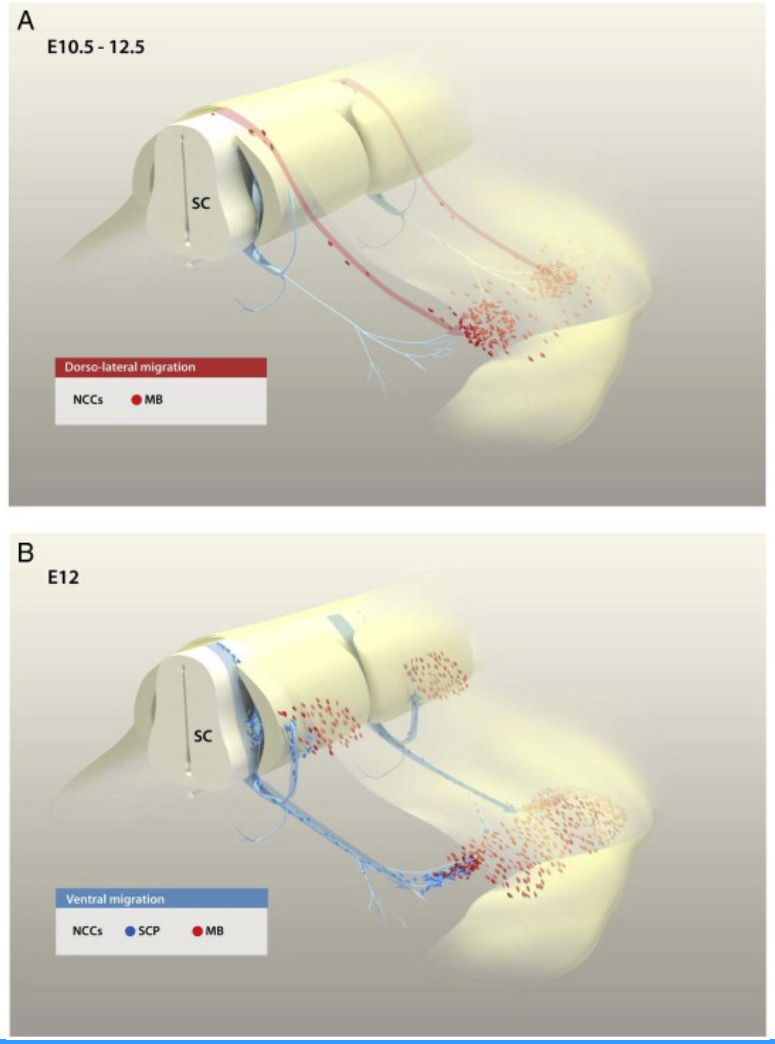
- Elucidation of the genetic background of the different coat colour variations
- Identification of DNA variants associated with the coat colour variations
- Development of a DNA –based test for breeding

Coat colour pathways



(Ernfors (2010) Exp Cell Res 316, 1397-1407)

Coat colour pathways



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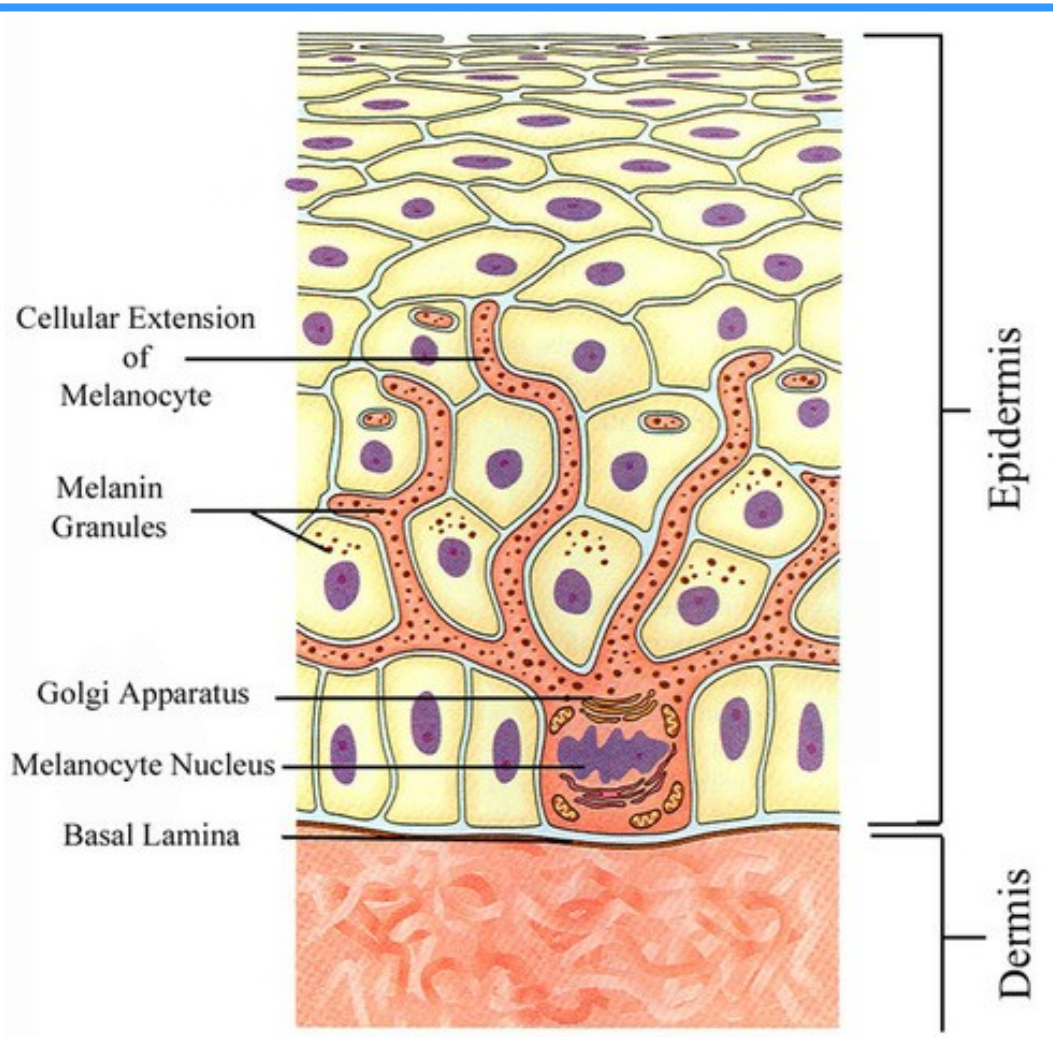
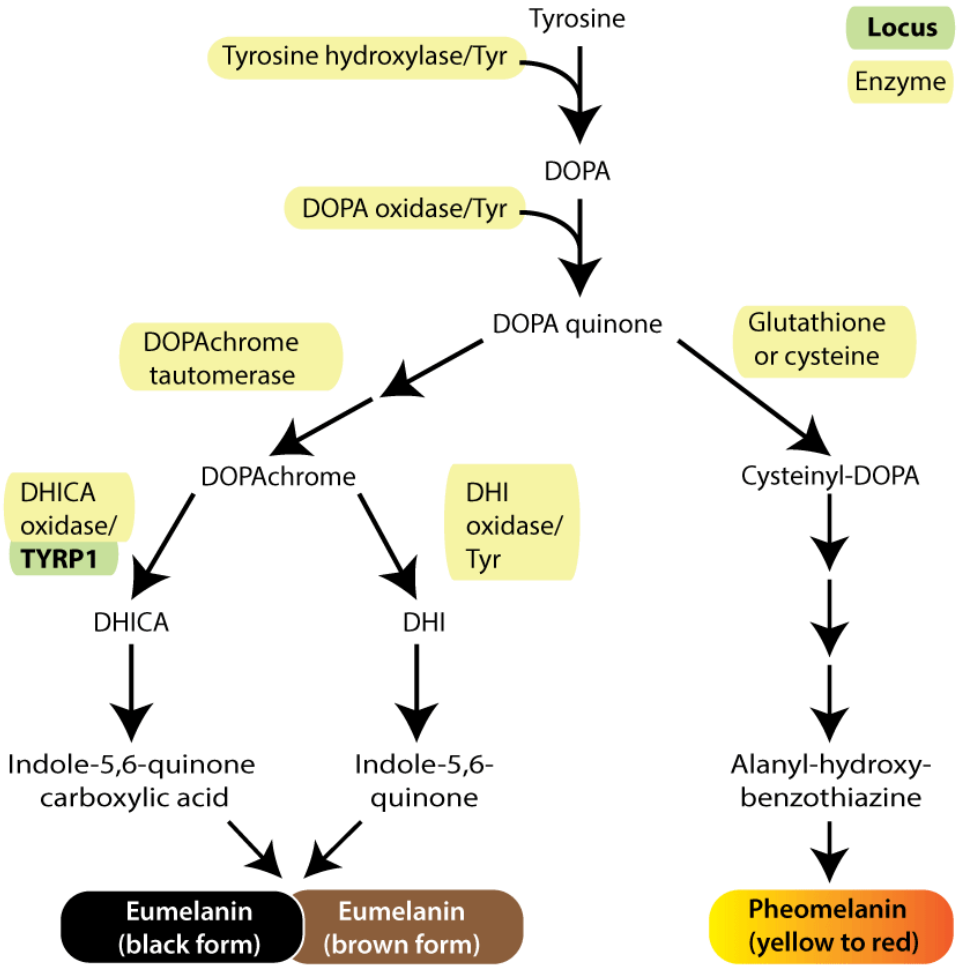
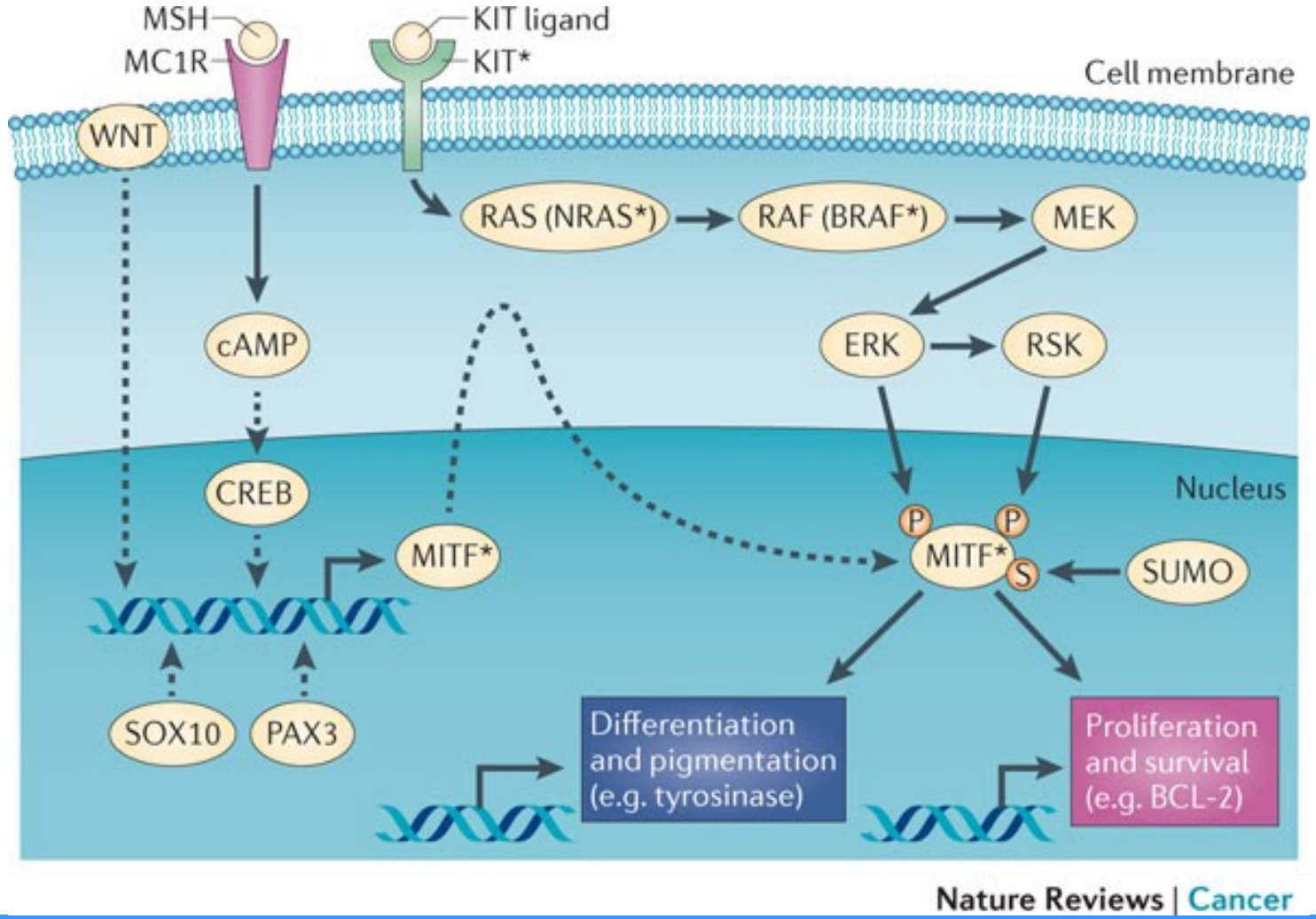


Coat colour pathways



(www.adapaproject.org)

Coat colour pathways



Coat colour genetics

- Different genes regulate
  - Melanin synthesis
  - Timing of pigmentation
  - Pigment deposition in hairs
  - Pigment distribution in the skin
- Gene interactions modify basic colours
- > 120 genes for coat colours are known in mice

Molecular genetics of bovine coat colours

- 8 genes and corresponding alleles have been described:
  – ***KIT*** (Mast/stem cell growth factor receptor): Colour-sided, White spotting
  – ***KITLG*** (KIT ligand): Roan
  – ***MC1R*** (Melanocortin 1 receptor): Extension
  – ***MITF*** (Microphthalmia-associated transcription factor): Dominant White, Piebaldism
  – ***TYR*** (Tyrosinase): Albinism
  – ***ASIP*** (Agouti signaling protein): Agouti
  – ***PMEL*** (Premelanosome protein): Dilution
  – ***TYRP1*** (Tyrosinase-related protein 1): Brown

(Quelle: Durkin *et al.*, 2012, S. Schmutz, 2010, RSH eG)

Identification of causative genes in White Galloway cattle

- Candidate gene analysis *KITLG*, *KIT*, *TYR* and *MITF*
  - 3 animals of each phenotype
  - Exons, splice-donor/acceptor-sites, UTR
  - Identification of functional SNPs
  - Genotyping of all animals (n=179)
- Genotyping of the *MC1R* locus
  - E^D, E^+, e
  - n=184

Results of candidate gene-analysis

Gene	Position	Polymorphism	Protein	Genotype	Number			
					wsch (n=27)	wsg (n=102)	wsü (n=10)	wss (n=40)
KITLG	5‘ UTR	g.221755T>C		T/T	12	60	4	24
				T/C	12	38	4	13
				C/C	3	3	2	3
	5‘ UTR	g.221761G>A		G/G	12	60	4	24
				G/A	12	38	4	13
				A/A	3	3	2	3
	Exon 7	g.45568C>A (roan)	p.A193D	C/C	27	102	10	40
				C/A	0	0	0	0
				A/A	0	0	0	0
TYR	Exon 1	g.66288G>C	p.R255P	G/G	17	84	7	33
				G/C	10	17	3	6
				C/C	0	1	0	1
KIT	Exon 5	g.71877602T>C	p.M258T	T/T	0	0	0*	0*
				T/C	7	14	2*	10*
				C/C	20	88	7*	29*
MITF	Exon 1	g.136G>A	p.M1I	G/G	4*	6*	2*	6*
				G/A	9*	6*	4*	6*
				A/A	0*	0*	3*	0*

**KIT*: wsü (n=9), wss (n=39); *MITF*: wsch (n=13), wsg (n=12), wsü (n=9), wss (n=12)

Results of genotyping

- *KITLG*, *TYR*, *KIT, and MITF*

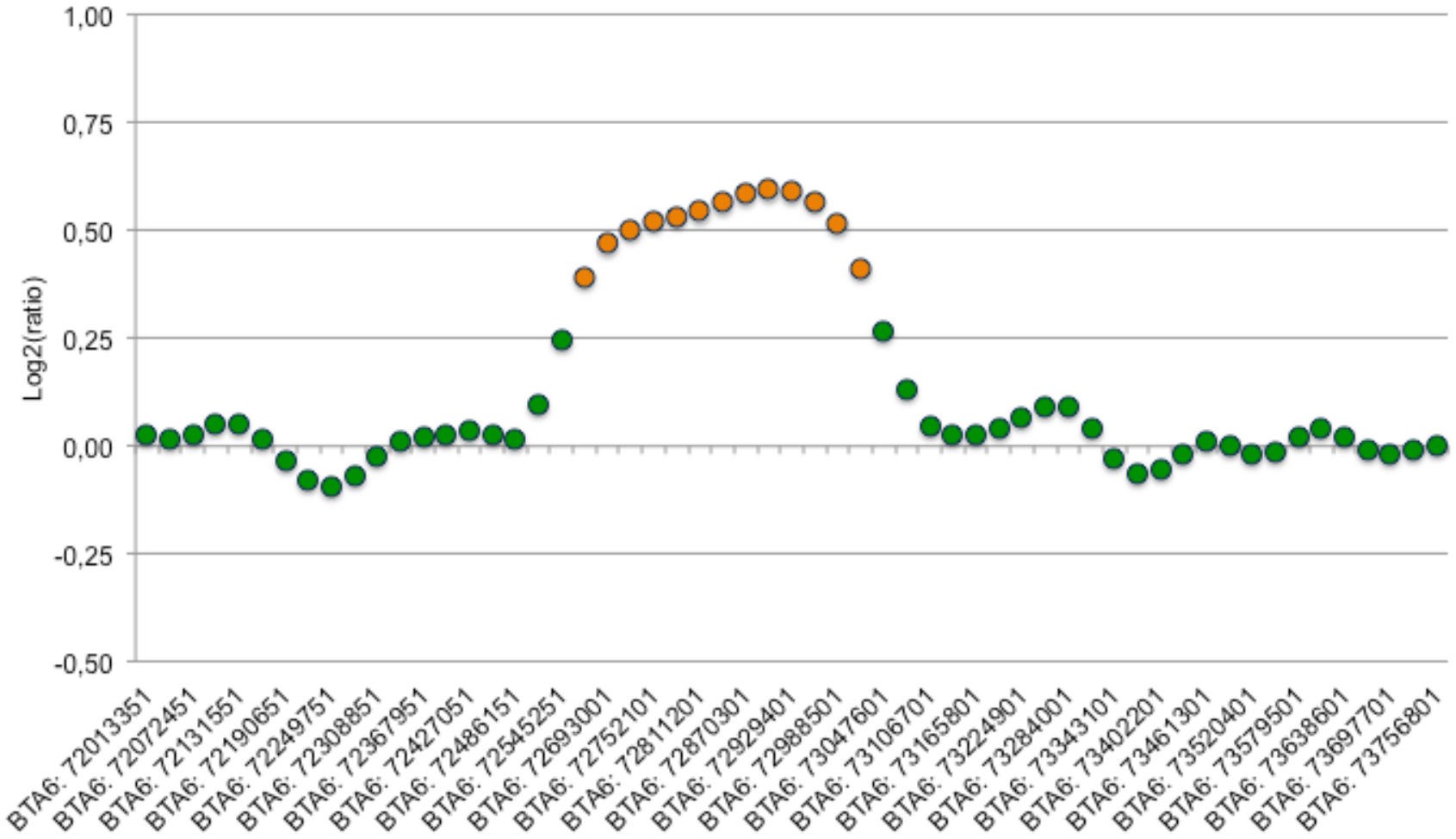
  - SNPs within the candidate genes do not show association with White Galloway coat colours

- *MC1R*

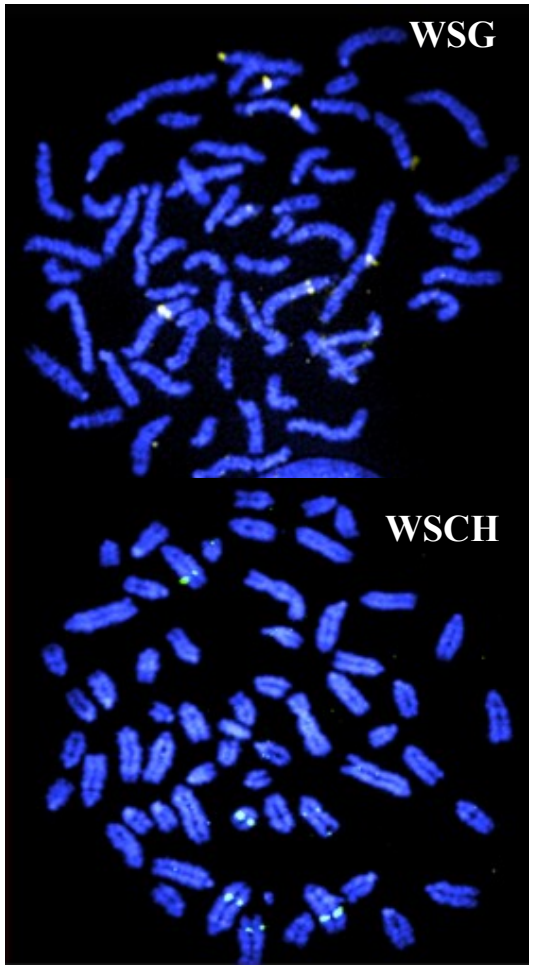
  - 182 black Galloway cattle: e/e, E_D/E_D; e/e, E_D/E_+

  - 2 red Galloway cattle: e/e, E_+/E_+

Whole Genome Sequencing



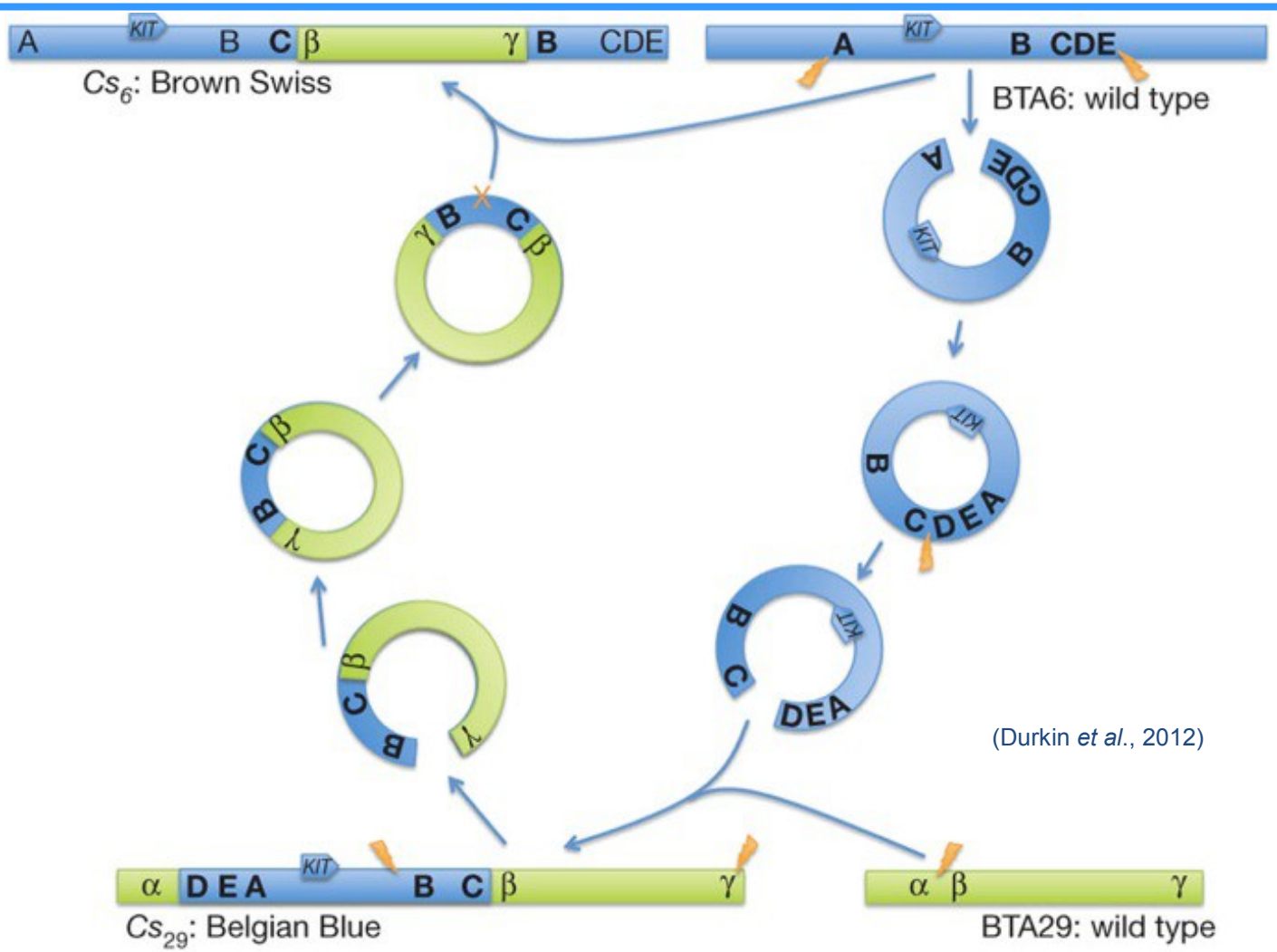
FISH analysis of White Galloway cattle



BAC harbouring *KIT* gene

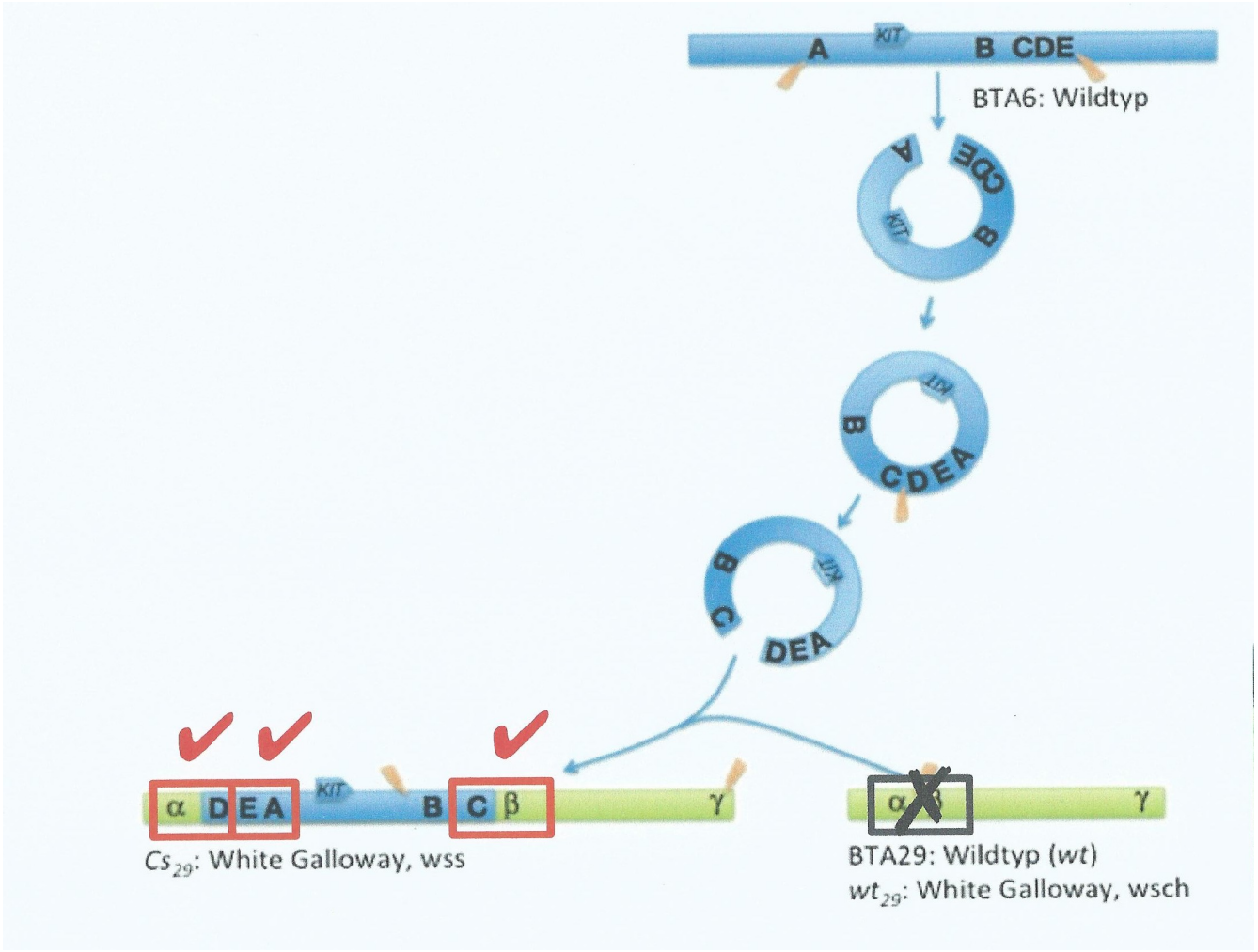
- Signals on
  - wildtype BTA6 (green)
  - translocation BTA29 (red)
  - BTA3 (yellow)
- Signals on
  - wildtype BTA6 (green)
  - BTA3 (yellow)

KIT gene translocation



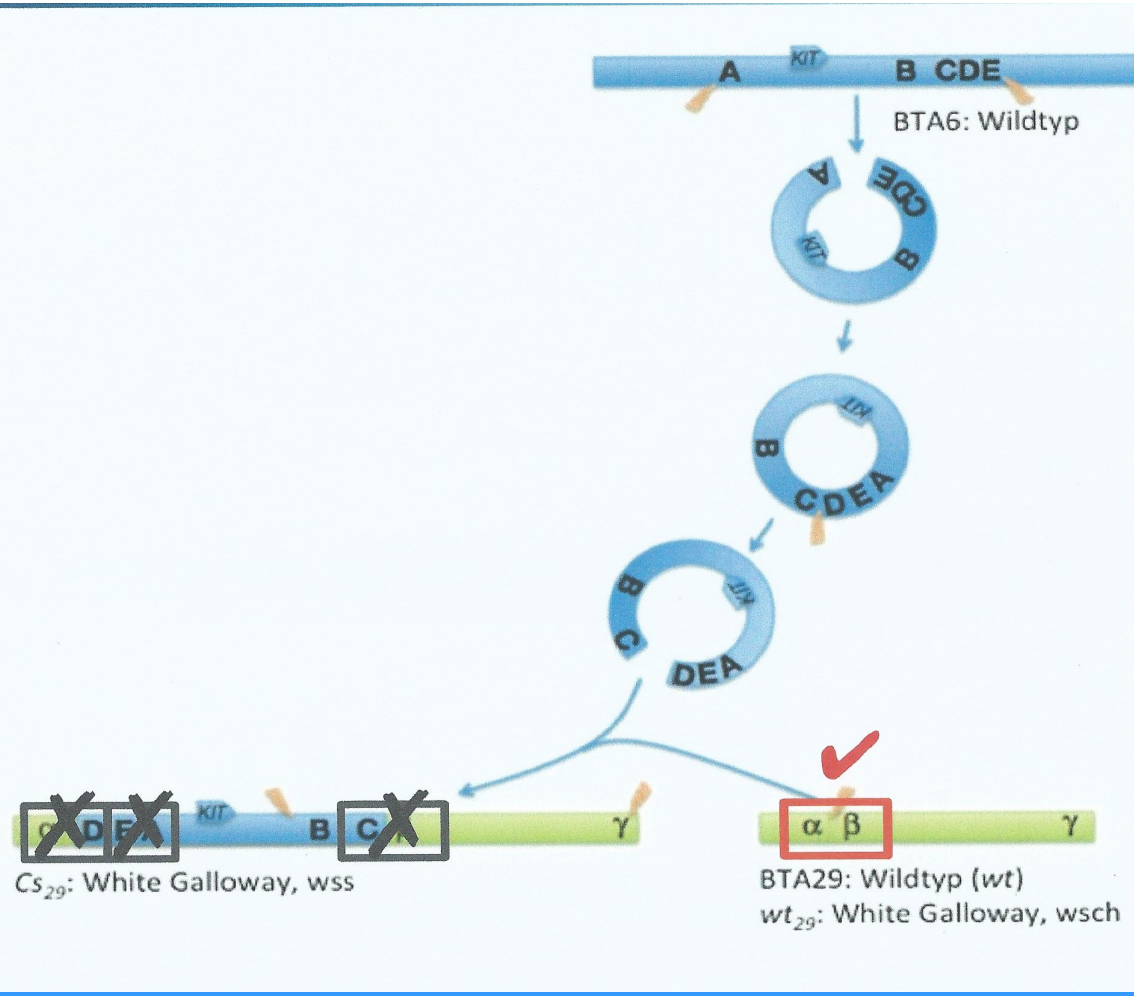
(Durkin *et al.*, 2012)

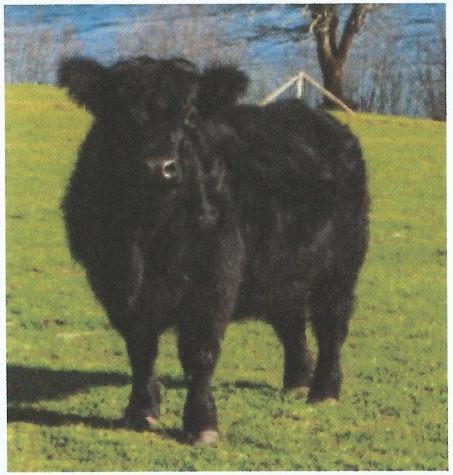
Genotype of mismarked White Galloway (WPM)



homozygous
(*Cs29* / *Cs29*)

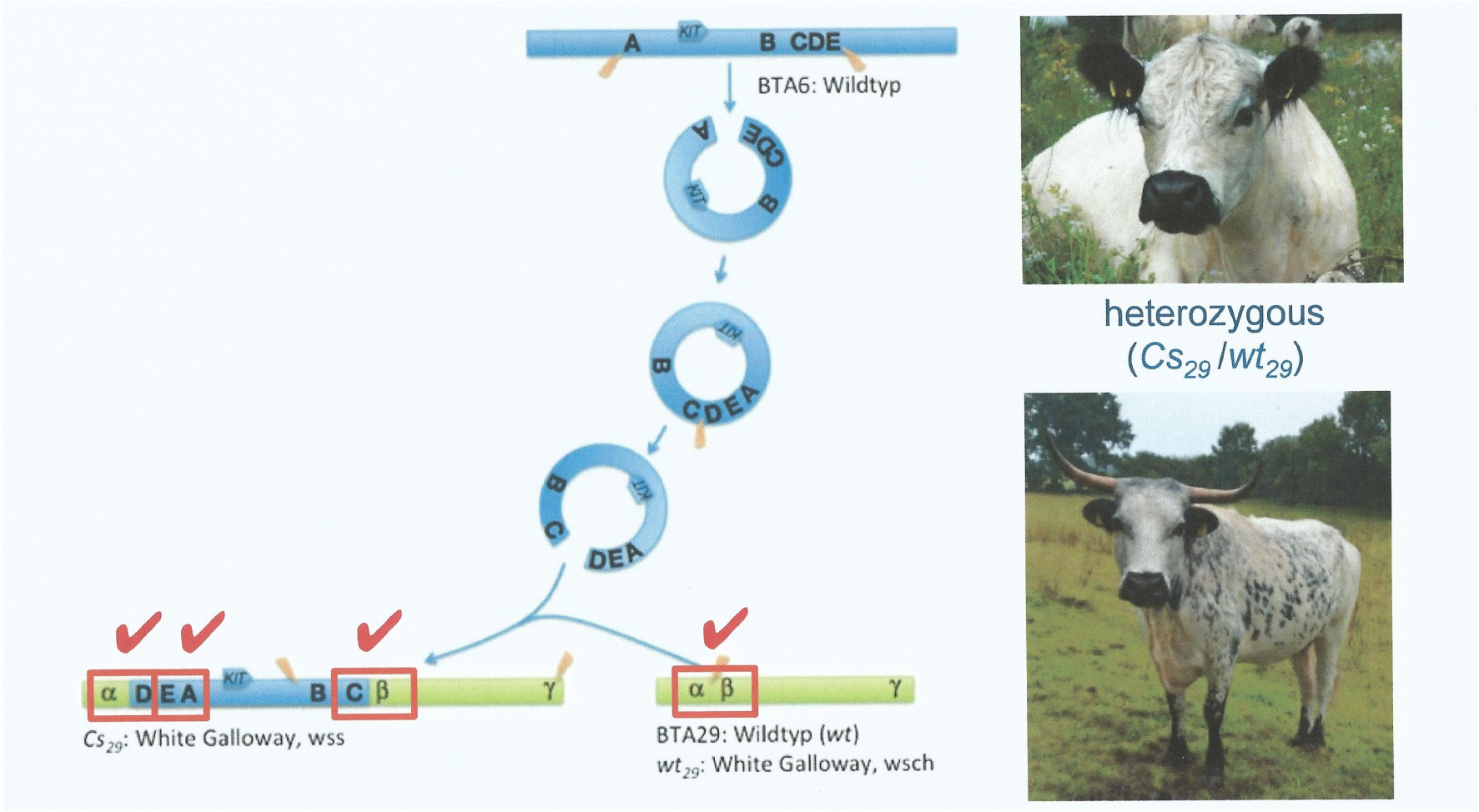
Genotype of fully black White Galloway (WFB)





homozygous
(wt_{29}/wt_{29})

Genotype of well marked and strongly marked White Galloway (wwm, wsm)



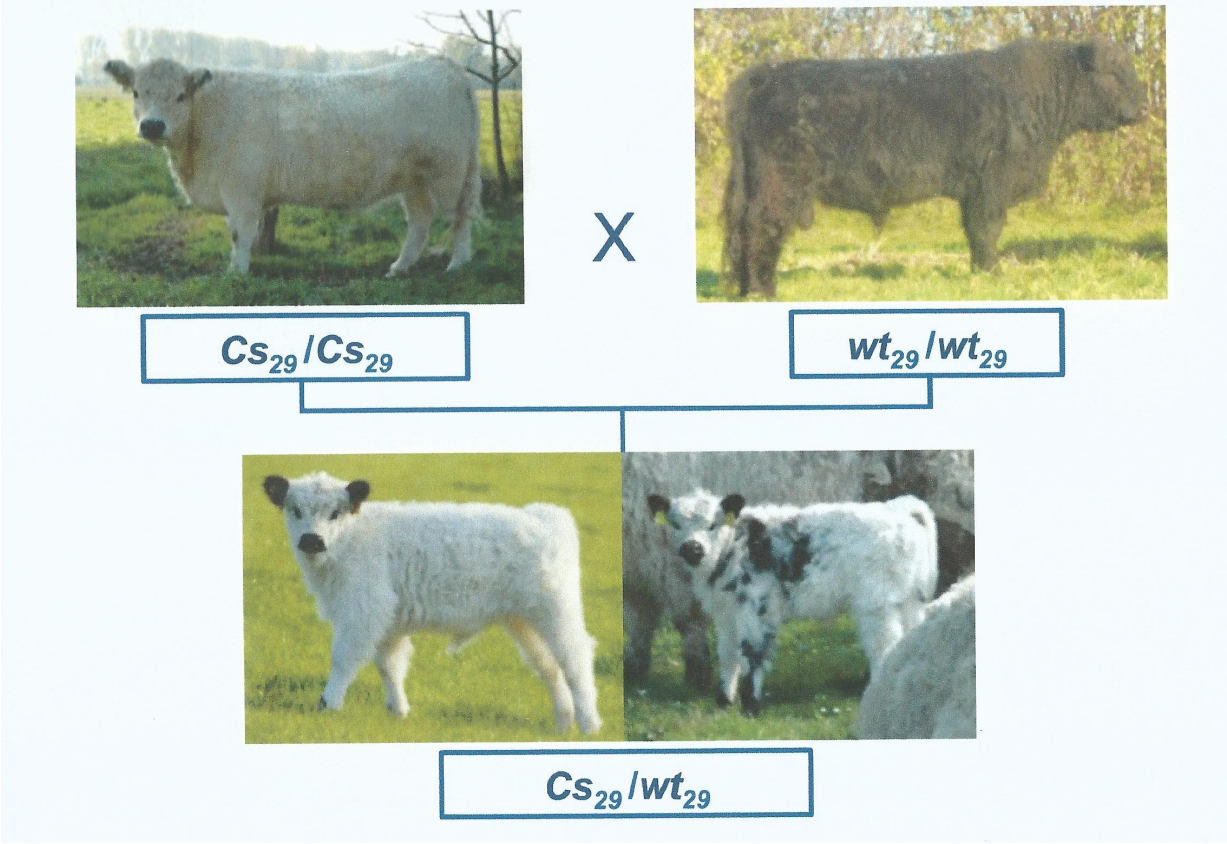
heterozygous
(Cs_{29}/wt_{29})

Translocation breakpoint analysis

White Galloway Phenotype	n (total)	BTA29 (wt_{29}) $\alpha\beta^{*)}$	BTA29 (Cs_{29}) $\alpha D^{*)}$	BTA29 (Cs_{29}) $EA^{*)}$	BTA29 (Cs_{29}) $C\beta^{*)}$	BTA6 (Cs_6) $\gamma B^{*)}$
well marked (wsg)/strngly marked (wsü)	162	162	162	162	162	0
mismarked (wss)	59	0	59	59	59	0
fully black (wsch)	42	42	0	0	0	0

Experimental matings

- 18 matings of White Galloway (wpm x wfb)



Conclusions

- Coat colours are inherited in a Mendelian fashion
- Coat colours are caused by a duplication and translocation of the *KIT* gene on chromosome 29 (BTA29, Cs_{29})
- Coat colours depend on the number of translocated *KIT* gene copies (dosage effect)
- Development of well marked (wwm) and strongly marked (wsm) phenotypes is still unclear

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