Genotyping of White Coat Color in Taiwan Water Buffalo (Bubalus bubalis)

Pi-Hua, Chuang

Hualien Animal Propagation
Station, COA-TLRI, Taiwan.
Date:2009/12/17

Subject

White swamp-type water buffalo

- **They are characterized with their white coat, which is very distinguished from gray/black buffalo**

White water buffalo=Albinism???

What caused the white buffalo??

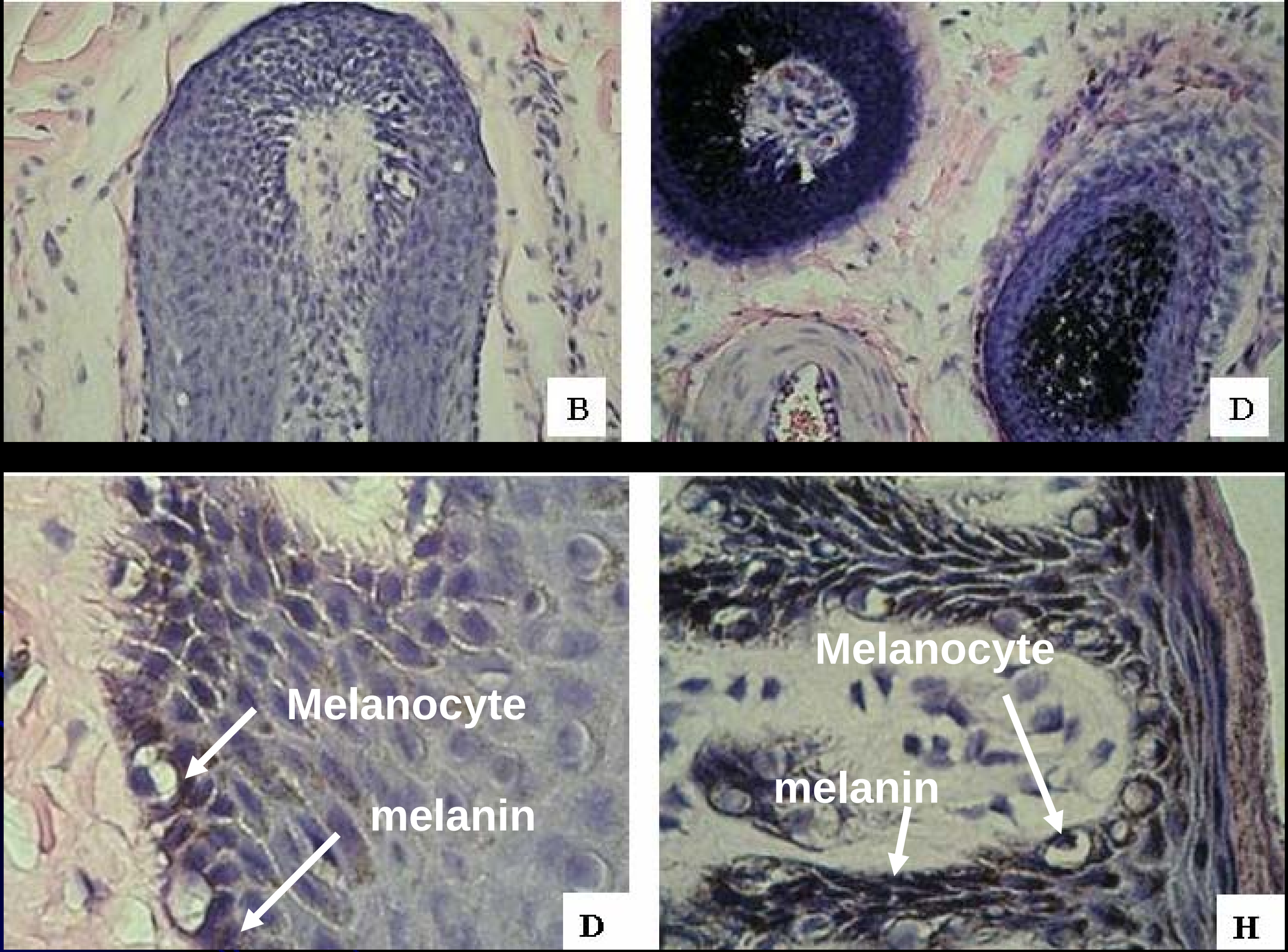
Phenotypes

B

W

Pasture all-weather

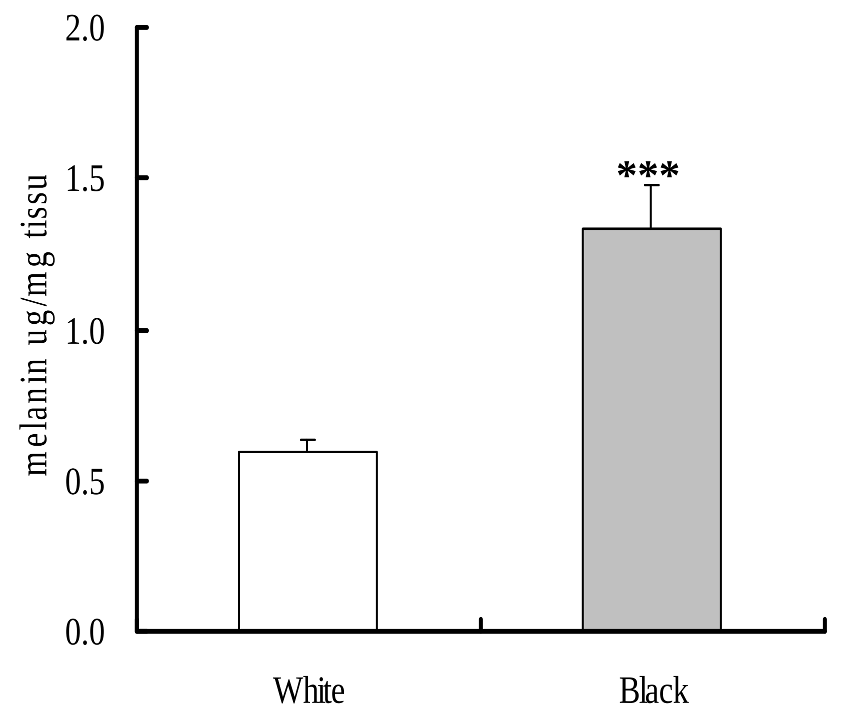
Histological sections of the ear skin and hair

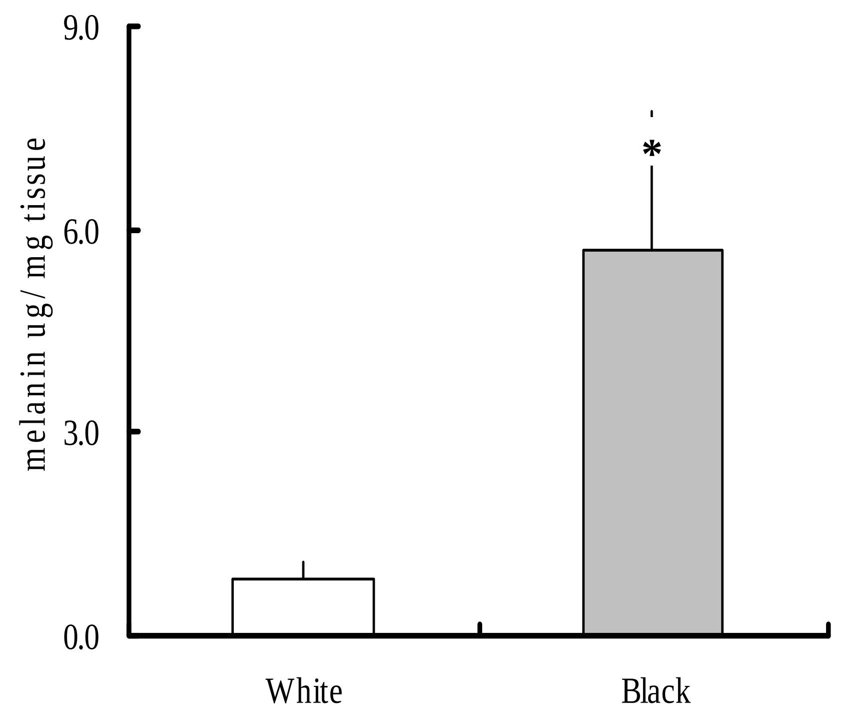


W (H & E Stain) B

X1000

Melanin content in skin and hair





Skin

W:0.595±0.125 ug/mg (n=9)
B:1.337±0.492 ug/mg (n=12)
***** p<0.001**

Hair

W:0.848±0.638 ug/mg (n=6)
B:5.696±5.028 ug/mg (n=6)
***p<0.05**

Coat Genes of Cattle

	Locus Name	Gene	Action	Cattle	Human
	A agouti	agouti peptide	shading?	13	20q11
v	B brown	TYRP1	dun brown in Dexter	8	9p23
v	C albino	tyrosinase	White Park pattern	29	11
v	E extension	MC1R	red versus black	18	16q24
		MSH/ POMC	melanocyte stimulating hormone	?	?
v	slaty	TYRP2	greying in mice	12q23	13q32
	underwhite	MATP	palamino in horse	20?	5p
	R roan/steel	KITLG or MGF	roan in cattle	5	12q22
		KIT	spotting	6q23	4q12
	piebald	EDNRB	overo in horses	12q22	13q22-q31
	lethal spotting	EDN3		13	20q13
	patch	PDGFRA	belt in mice	6	4q
	attractin	ATRN	mahogany in mice	6	20p13
	microphthalmia-associated transcription factor	MITF	spotting in mice	22	3p12-14

Test Method

Step 1

DNA Extraction (blood)

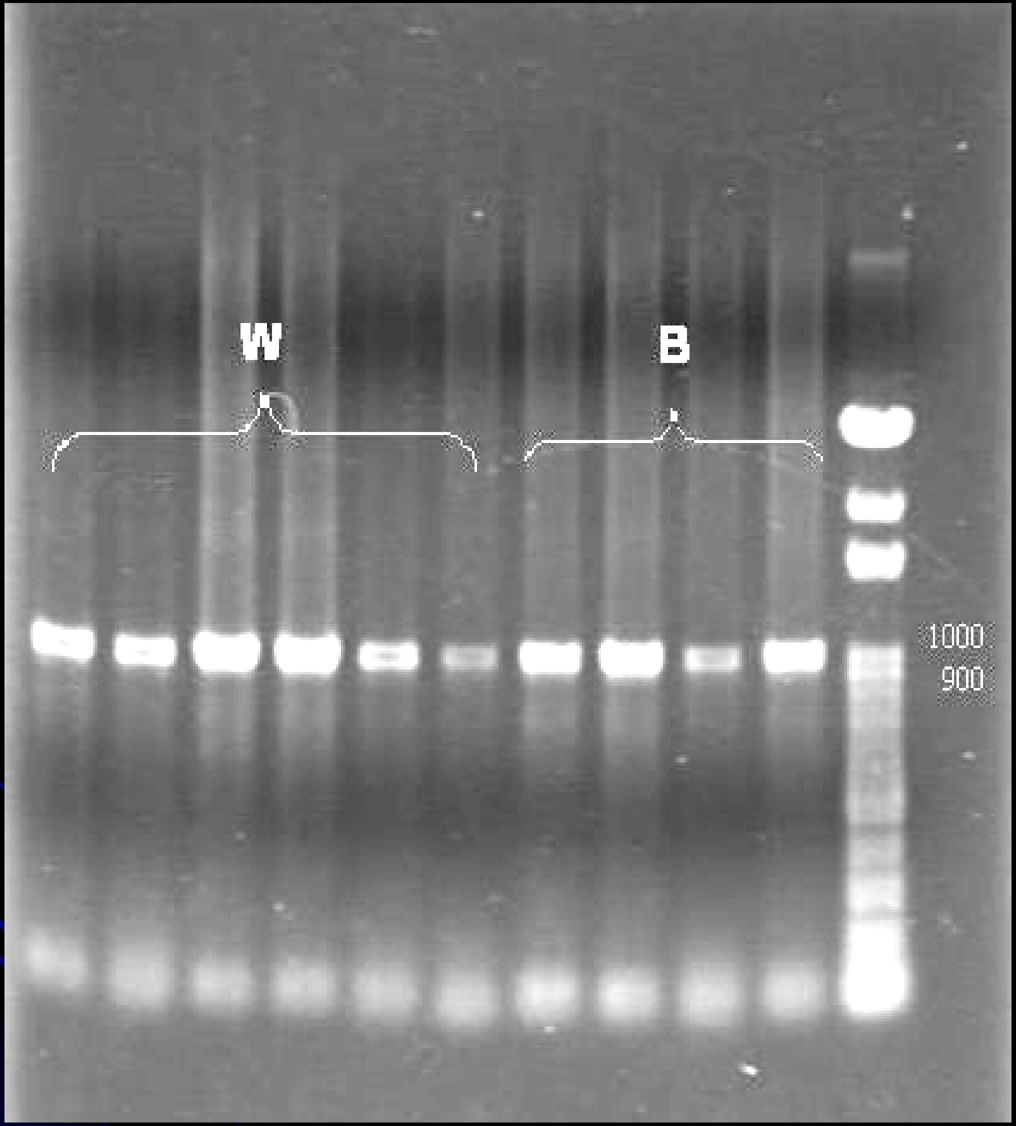
Step 2

PCR (Polymerase chain reaction)

Step 3

Cloning and sequencing

MC1R agarose gel electrophoresis



MC1R DNA Sequence

```
Buffalo     ATGCCTGCACTCGGCTCCCAGAGACGGCTGCTGGGTTCCCTTAACTGCACGCCCCCAGCC   60
Cattle      -----------T------------------------------------------------
Buffalo     ACCCTCCCCCTCACTCC-GGCCCCCAACCGGACGGGGCCCCAGTGCCTGGAGGTGTCCAT   119
Cattle      ---------T----.--T------------------------------------------
Buffalo     CCCTGATGGGCTCTTTCTCAGCCTGGGGCTGGTGAGTCTCGTGGAGAACGTGCTGGTAGT   179
Cattle      ------------------------------------------------------------
Buffalo     GGCTGCCATCGCCAAGAACCGCAACCTGCACTCCCCCATGTACTACTTAATCTGCTGCTT   239
Cattle      ---------T--------------------------------------T---------C-
Buffalo     GGCTGTGTCTGACCTGCTGGTGAGCGTCAGCAACGTGCTGGAGACGGCAGTCATGCTGCT   299
Cattle      -------------T----------------------------------------------
Buffalo     GCTGGAGGCCGGTGTCCTGGCCACCCAGGCGGCCGTGGTGCAGCAGCTGGACAATGTCAT   359
Cattle      ------------------------------------------------------------
Buffalo     CGACGTGCTCATCTGCAGCTCCATGGTGTCCAGCCTCTGCTTCCTGGGTGCCATTGCTGT   419
Cattle      ----------------G-A-----------------------------------------
Buffalo     GGACCGCTACATCTCCATCTTCTACGCCCTGCGGTACCACAGCGTTGTGACGCTGTCCCG   479
Cattle      ------------------------------------------T--------A---C----
Buffalo     AGCGTGGAGGATCATTGCGGCCATCTGGGTGGCCAGCATCCTCACCAGCCTGCTCTTCAT   539
Cattle      ------------------------------------------------------------
Buffalo     CACCTACTACAACCACAAGGTCGTACTGCTGTGCCTCGTTGGCTTCTTCGTAGCTATGCT   599
Cattle      ----------------------A-C------------------C-----A----------
Buffalo     GGCCCTGATGGCCATCCTGTACGTCCACATGCTGGCCCGGGCCTGCCAGCATGCaCCGGGG  659
Cattle      -------------G----C-----------------------------------------
Buffalo     CATCGCCCGGCTCCAGAAGAGGCAGCGCCCCATTCATCAGGGCTTTGGCCTCAAGGGCGC   719
Cattle      ---T--------------------------------------------------------
Buffalo     TGCCACCCTCACCATCCTGCTGGGCGTCTTCTTCCTCTGCTGGGGCCCCTTCTTCTTGCA   779
Cattle      -------------------------------------------------------C----
Buffalo     CCTCTCGCTCATCGTCCTCTGCCCCCAGCACCCCACCTGTGGCTGCATCTTCAAGAACTT   839
Cattle      ------------------------------------------------------------
Buffalo     CAACCTCTTCCTGGCCCTCATCATTTGCAATGCCATTGTGGACCCCCTCATCTATGCCTT   899
Cattle      ------------------------------C-----------------------------
Buffalo     CTGCAGCCAGGAGCTCCGGAAGACACTCCAAGAGGTGCTGCAGTGCTCCTGGTGA        954
Cattle      -C----------------------G------------------------------
```

Identities ：97% (Cattle)

Buffalo MC1R

- 954 bp, 318 amino acids
- MC1R gene sequences was 100% identical between black and white buffalos
- The similarity between the other species

Identities (%)	Cattle	Human
nucleotide sequence	97	85
amino acid sequence	96	81

Tyrosinase family genes

Test Method

Step 1

cDNA extraction (ear tissue)

Step 2

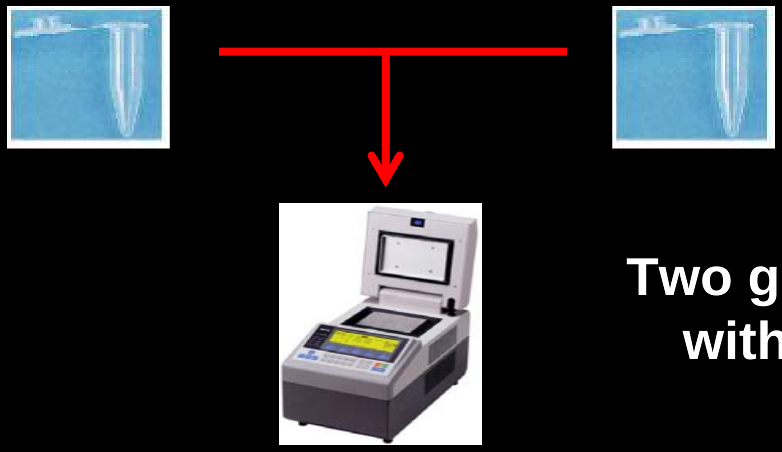
Semiquantitative RT-PCR

Step 3

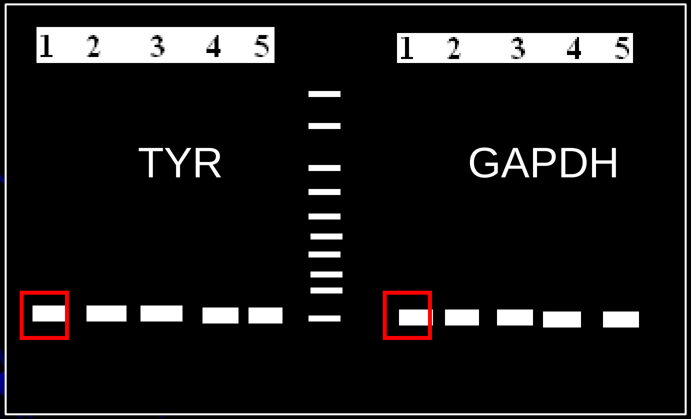
Phoretix ID standard software

Semiquantitative RT-PCR

TYR GAPDH **(internal control)**



Two genes in each vial with same reaction condition

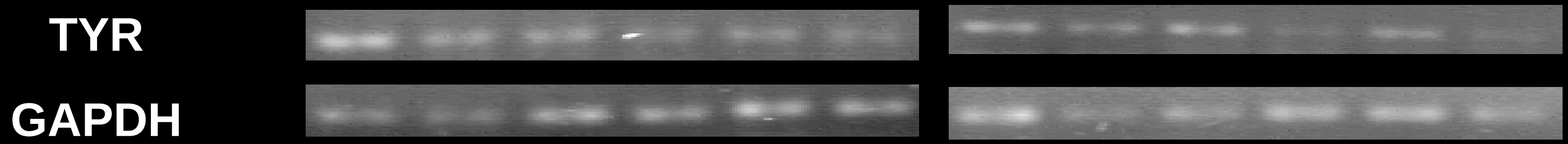


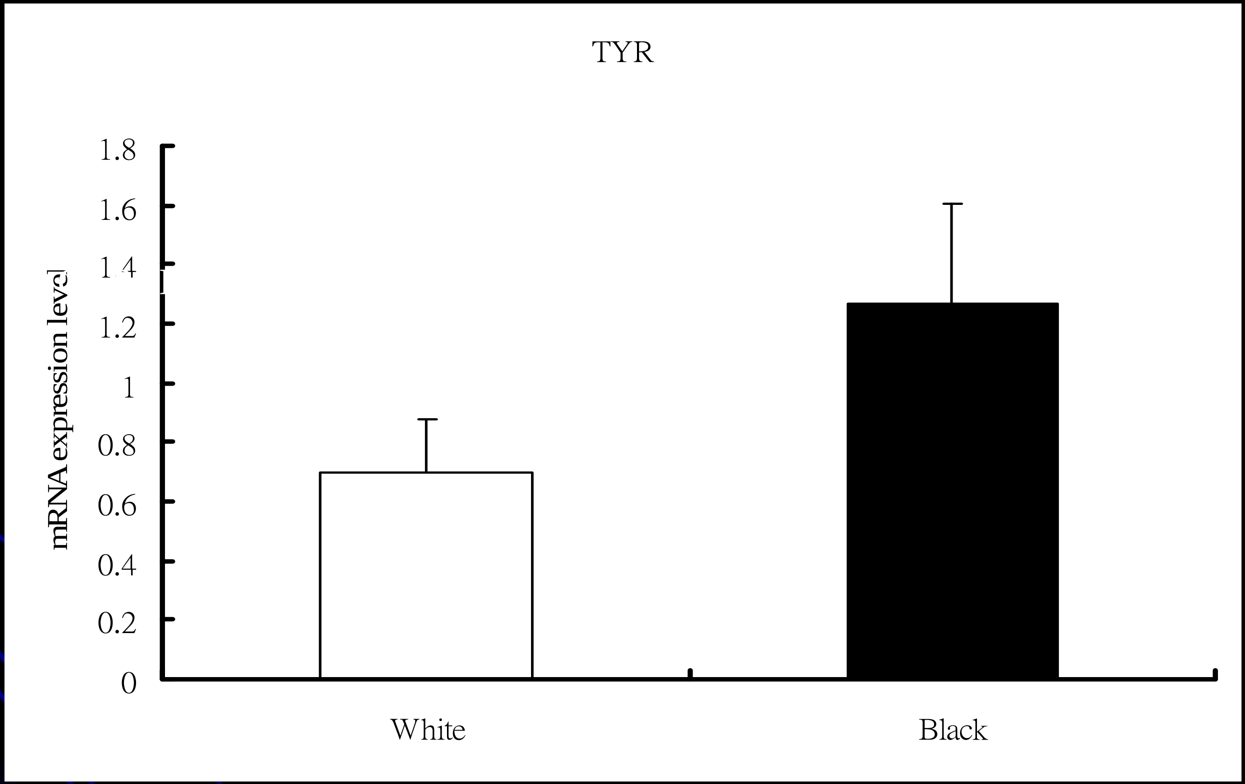
Phoretix ID standard software

The values of gene expression were then divided by the values of GAPDH (internal control).

Tyrosinase family genes

Tyrosinase Family gene

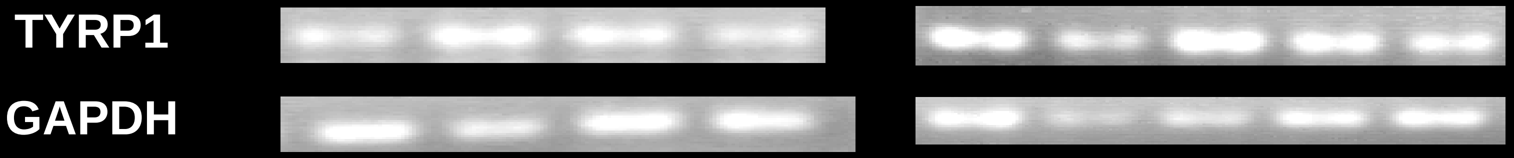


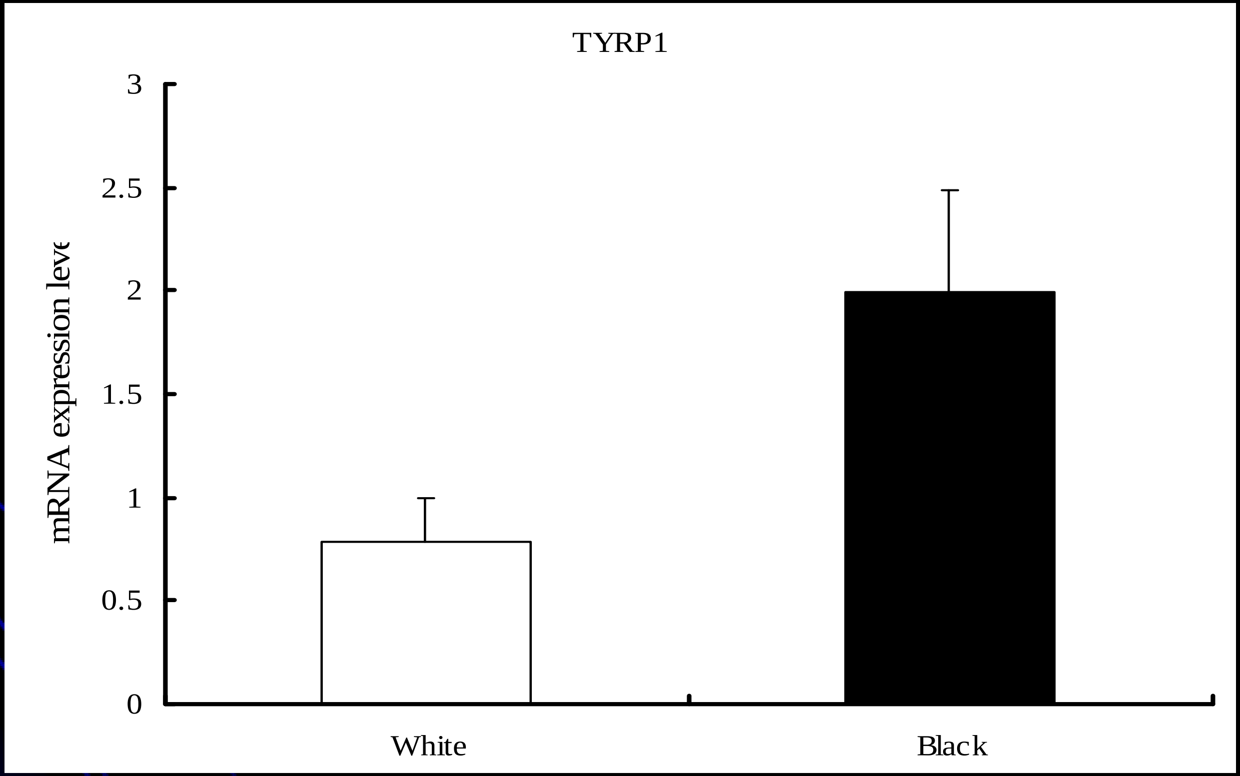


0.697±0.178 (n=6)

1.265±0.338 (n=6)

Tyrosinase Family gene



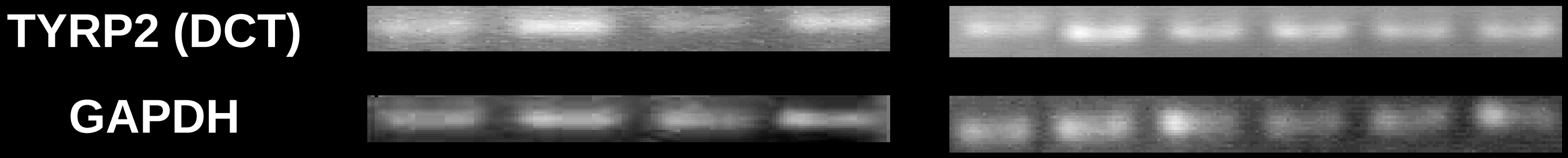


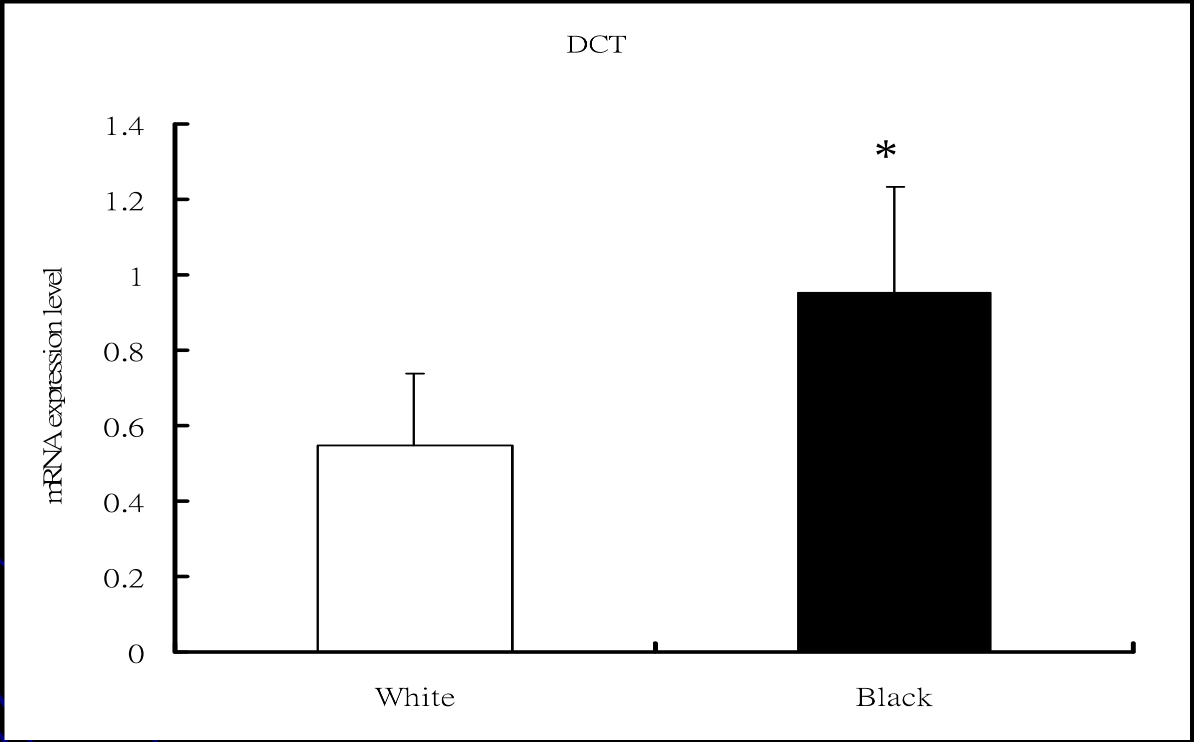
0. 787±0.414 (n=4)

1.995±1.210 (n=5)

Tyrosinase family genes

Tyrosinase Family gene





0.545±0.194 (n=4) 0.952±0.280 (n=5)

*** P<0.05**

Conclusion

- Melanin synthesis occurred in back and white buffalos.
- There was no significant difference in MC1R gene between black and white buffalos.
- The similarity of MC1R nucleotide sequence with cattle was 97% .
- The expression of tyrosinase family genes in white buffalos was lower than that of black buffalos.
- The expression of TYRP2 (DCT) showed statistically different ($P < 0.05$), while the other two genes were not.

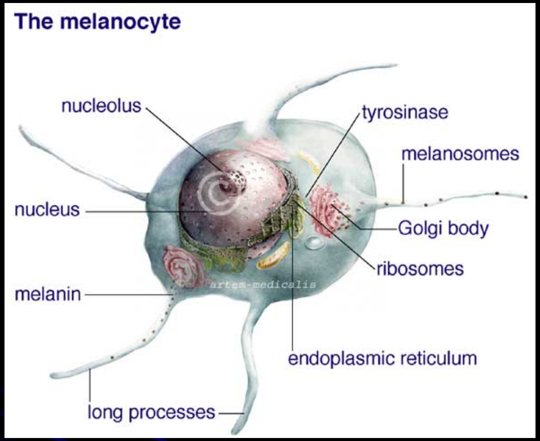
Acknowledges

Many thanks to Dr. Ching-Feng Weng, the professor of the Institute of Biotechnology, National Dong Hwa University, Taiwan, who conducted the research.

This work was supported by Hualien Animal Propagation Station, COA-TLRI, Taiwan.

Uncommitted neural crest cell

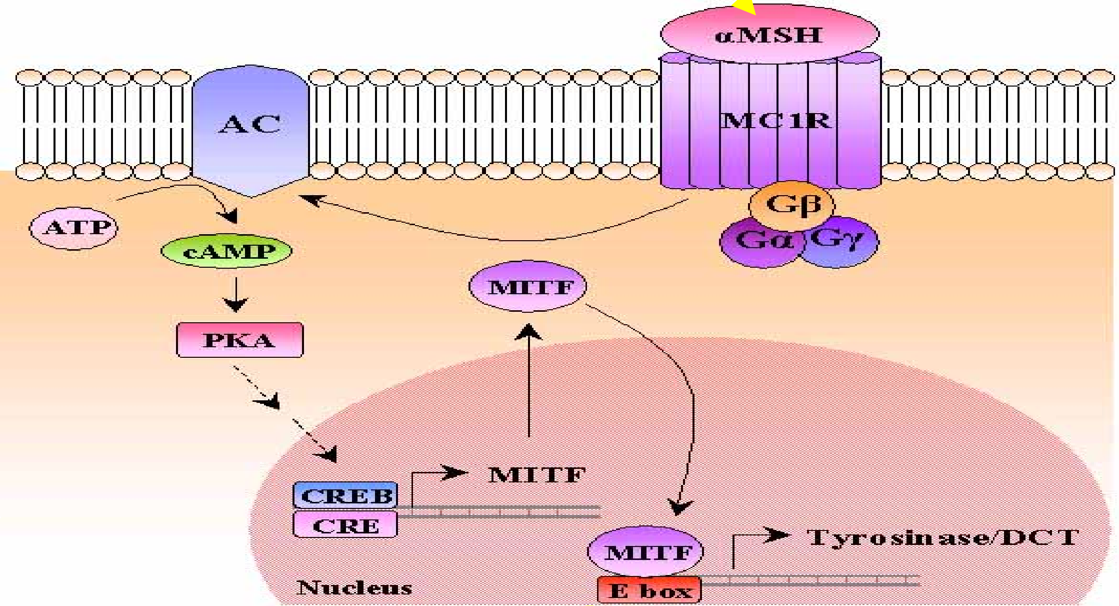
Migration and differentiation



Melanocyte

Pro-opiomelanocortin (POMC/腦下垂體)

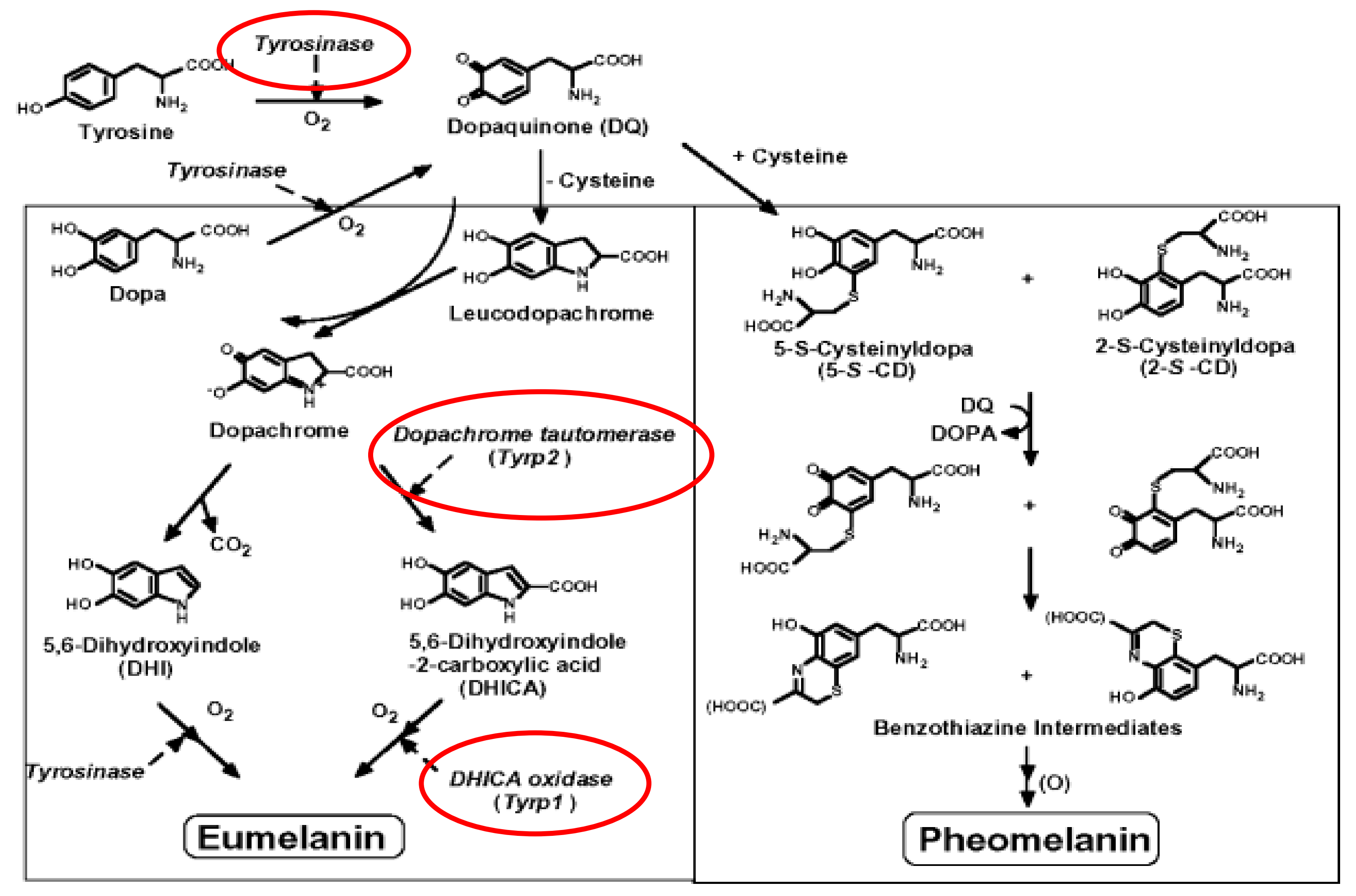
α-melanocyte-stimulating hormone (α-MSH)



Melanogenesis

http://www.artem-medicalis.com/poster.htm

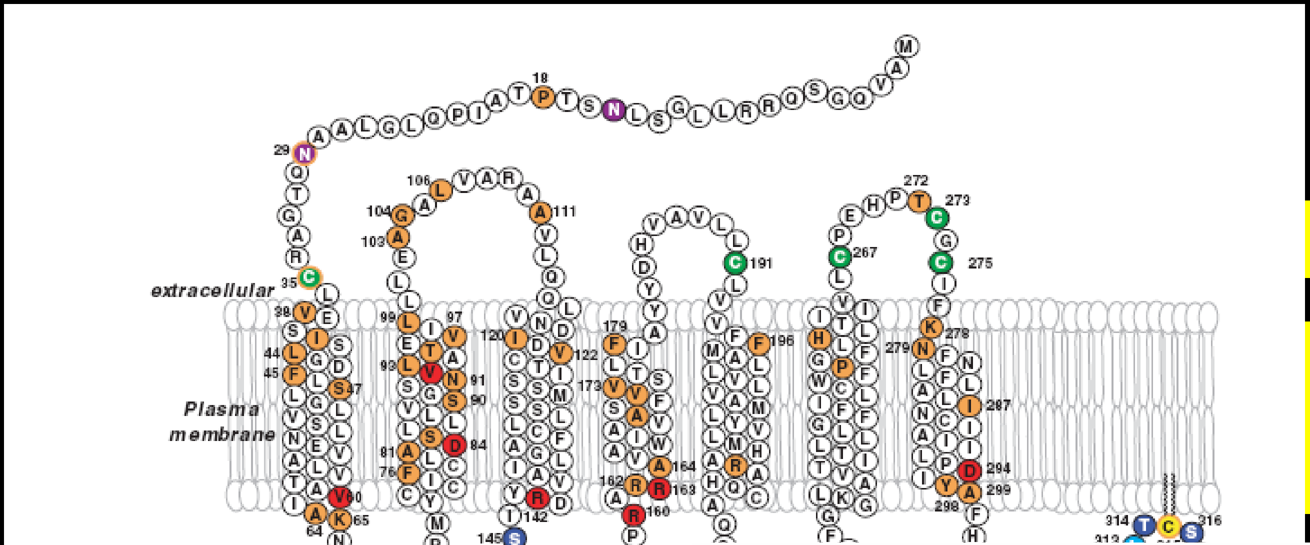
Melanin Synthesis (within melanocyte)



⬛ 🟫 🟨 🟥 🟥

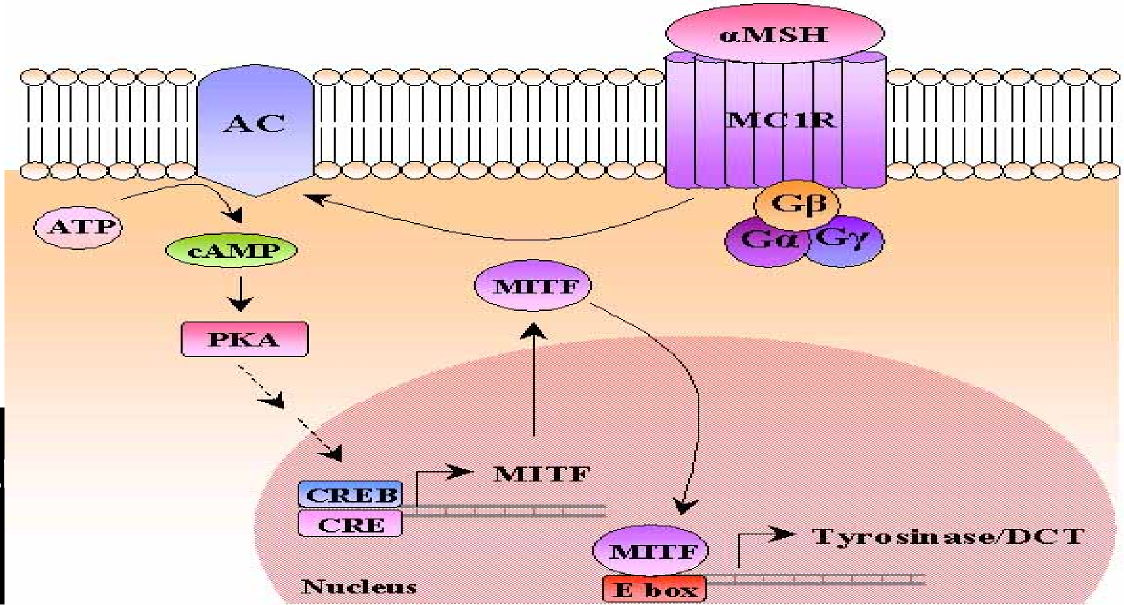
Melanocortin-1 receptor (MC1R)

Cattle : Chr18 / Extension locus



Extracellular loop

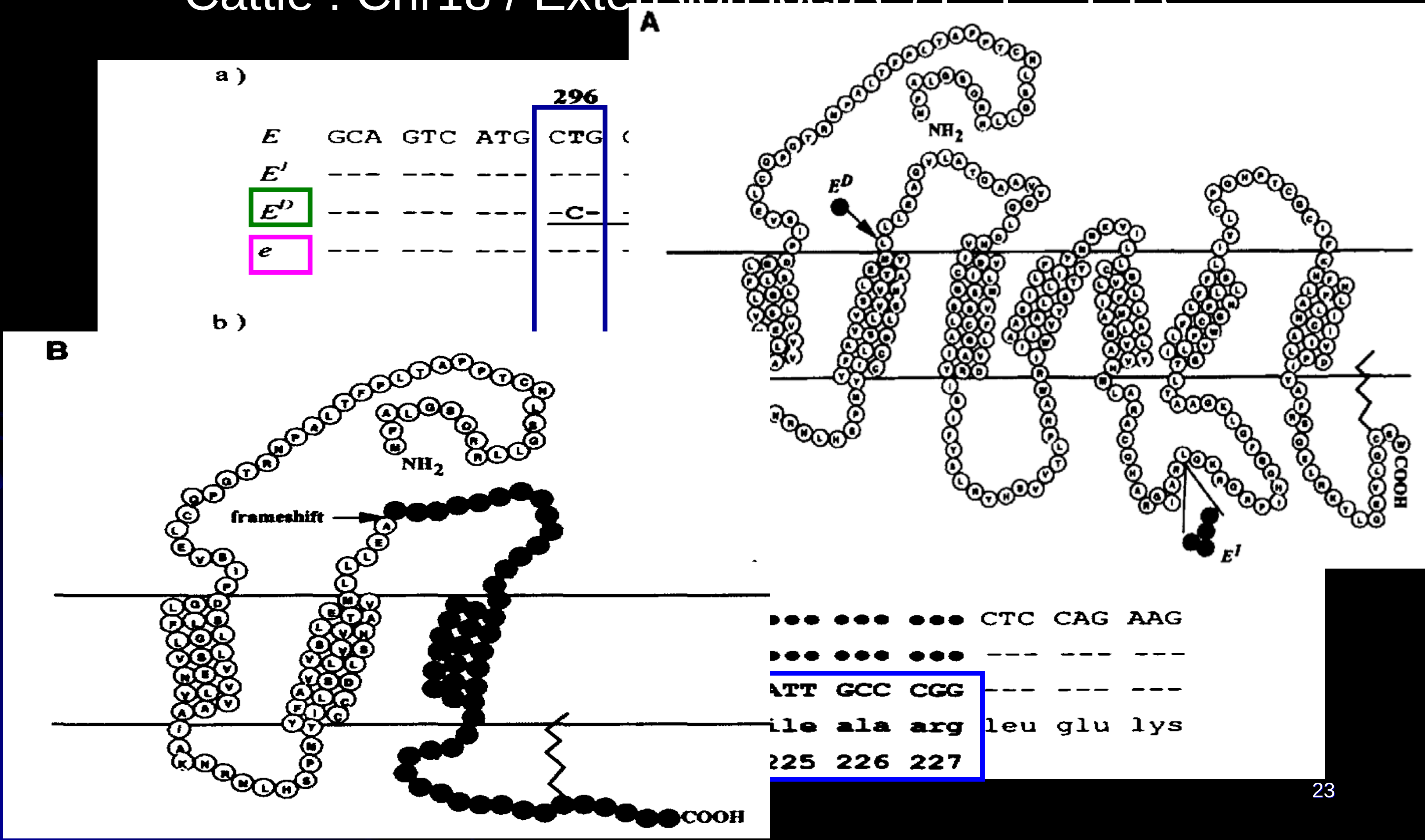
Transmembrane-Spanning domains



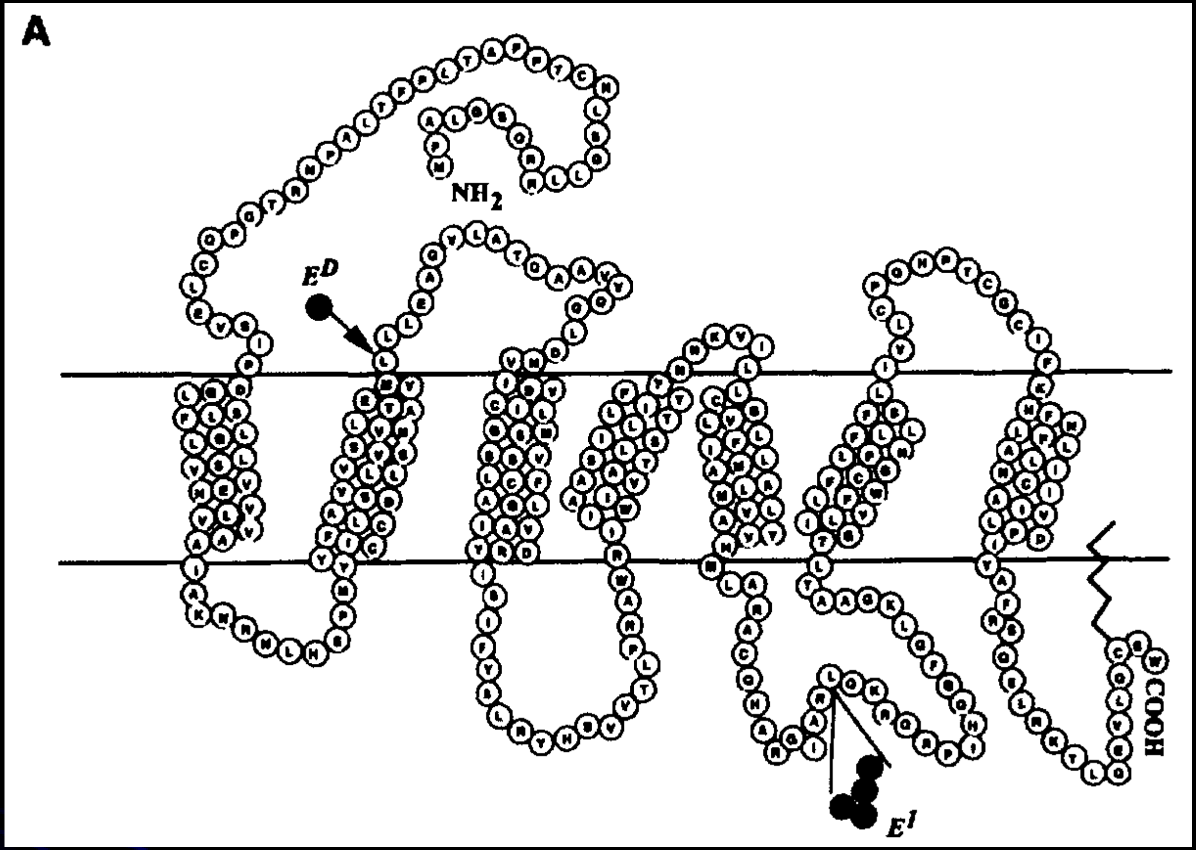
約有950 bp, 317~319 a

Melanocortin-1 receptor (MC1R)

Cattle : Chr18 / Extension locus / E E^D E1 e



Rouzaud et al(2000) Genet Sel. Evol 32, 511-520



E^D/E^D: Holstein

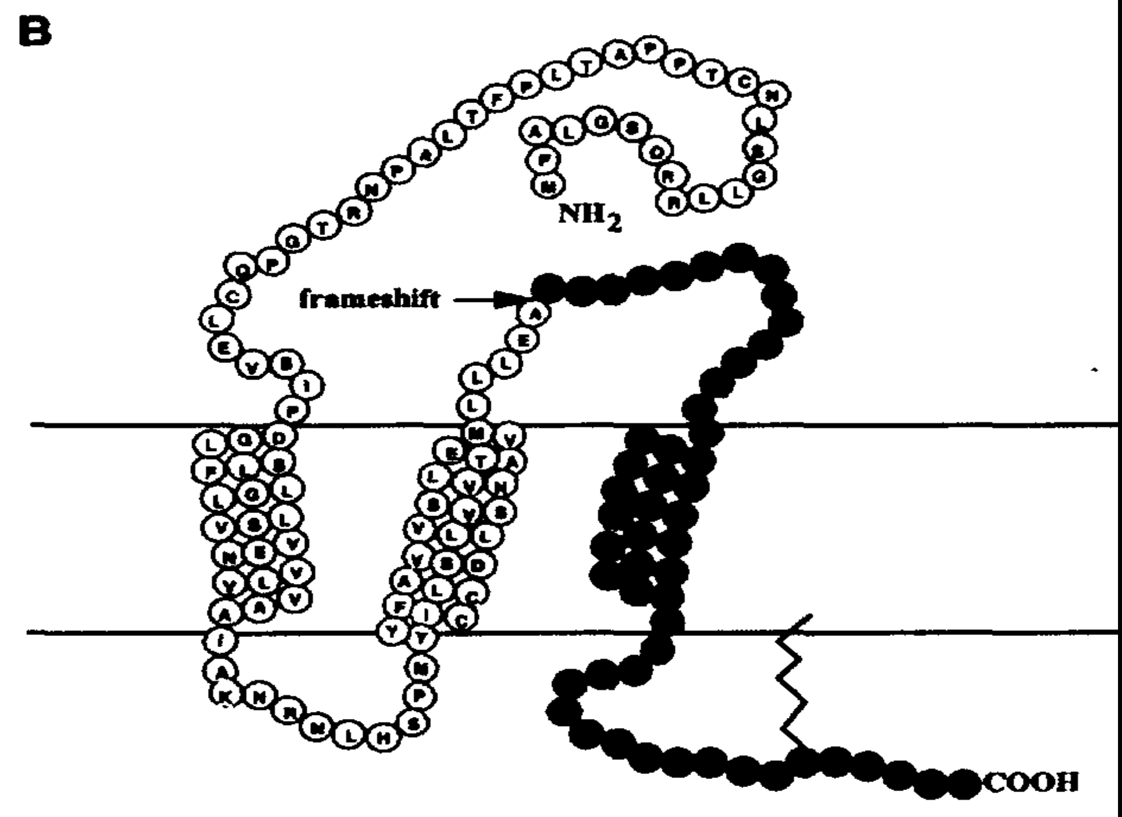
E^1/E^1: Aubrac

(E1/E)

E^1/E^1: Gasconne

(E1/E)

Rouzaud et al(2000) Genet Sel. Evol 32, 511-520



e/e

Limousine

Salers

Charolaise

Blonde d’Aquitaine

Rouzaud et al(2000) Genet Sel. Evol 32, 511-520