

PCR-RFLP protocol used for the genotyping of the Myostatin promoter according to Crisà et al. (2003)¹

PCR Mix:

Primer 1 (10 µM)	4 µl
Primer 2 (10 µM)	4 µl
PCR buffer 10x	5 µl
MgCl ₂ (25 mM)	3 µl
dNTPs (2 mM)	2,5 µl
Taq DNA polymerase (5 U/µl)	0,4 µl
H ₂ O	
ADN	100 ng
Total volume	50 µl

Primer GDF8-DRA-F : 5'-CTgAgggAAAAgCATATCAAC-3'

Primer GDF8-DRA-R : 5'-CCAgCAACAATCAgCATAAATAg-3'

Primer GDF8-SPE-F : 5'-gCTCCCAgACCTTACCCCAAATC-3'

Primer GDF8-SPE-R : 5'-gTTgATATgCTTTTCCCTCAg-3'

Cycling conditions :

One cycle of DNA denaturation for 2 min at 94 °C;

35 three-step cycles 94 °C for 1 min

56 °C for 1 min

and 72 °C for 1 min 30 s

Last extension for 10 min at 72 °C

Digestion of the PCR product :

Buffer 10x	2 µl
Enzyme (10 U/µl)	0,25 µl
H ₂ O	7,75 µl
PCR products	10 µl
Total volume	20 µl

Restriction endonuclease *Dra*I, incubation at 37 °C for 1 h

Restriction endonuclease *Spe*I, incubation at 37 °C for 1 h

Electrophoresis on an agarose gel (2,5 %)

¹ Sequence analysis of myostatin promoter in cattle (2003). Crisà A., Marchitelli C., Savarese M.C. and Valentini A. Cytogenetic and Genome Research 102 :48-52.

PCR-RFLP protocol used for the detection of the E, E^d and e alleles of the MC1R gene according to Rolando and Di Stasio (2006)²

PCR Mix :

Primer M1 (5 µM)	1,6 µl
Primer M2 (5 µM)	1,6 µl
PCR buffer 10x	5 µl
MgCl ₂ (25 mM)	3 µl
dNTPs (2 mM)	5 µl
Taq DNA polymerase (5 U/µl)	0,4 µl
H ₂ O	
ADN	100 ng
Total volume	50 µl

Primer M1 : 5'-AAgAACCgCAACCTgCACT-3'

Primer M2 : 5'-CTCgTTggCCTCTTCATAgC-3'

Cycling conditions :

One cycle of DNA denaturation for 5 min at 94 °C;

35 three-step cycles 94 °C for 30 sec

61 °C for 30 sec

and 72 °C for 1 min

Last extension for 5 min at 72 °C

Digestion of the PCR product with *MspAII* :

Buffer 10x	2 µl
Enzyme (10 U/µl)	0,25 µl
H ₂ O	7,75 µl
PCR products	10 µl
Total volume	20 µl

Incubate at 37 °C for 1 h

Digestion of the PCR product with *MspI* :

Buffer 10x	2 µl
Enzyme (100 U/µl)	0,12 µl
H ₂ O	7,88 µl
PCR products	10 µl
Total volume	20 µl

incubate at 37 °C for 1 h 30 min

Electrophoresis on an agarose gel (2,5 %)

² MC1R gene analysis applied to breed traceability of beef (2006). Rolando A. and Di Stasio L. Italian Journal of Animal Science, 5:87-91.

Original PCR protocol developed by CRA-W and INRA for the detection of the E₁ allele of the MC1R gene

PCR mix:

Primer M3-F (5 µM)	2 µl
Primer Parth2-R (5 µM)	2 µl
PCR buffer 10x	2 µl
MgCl ₂ (25 mM)	2 µl
dNTPs (2 mM)	2 µl
Taq DNA polymerase (5 U/µl)	0,1 µl
H ₂ O	
ADN	100 ng
Total volume	20 µl

Primer M3-F : 5'-CATAgCTATgCTggCCCTgAT-3'

Primer Parth2-R : 5'-gCgCTgCCTCTTCTggAg-3'

Cycling conditions :

One cycle of DNA denaturation for 4 min at 95 °C;

50 three-step cycles 94 °C for 15 sec

60 °C for 15 sec

and 72 °C for 15 sec

Last extension for 6 min at 72 °C

Electrophoresis on an agarose gel (3 %)

PCR protocol used for the detection of the A, A^{br} alleles of the Agouti gene

PCR mix:

Primer 1 (10 µM)	1 µl
Primer 2 (10 µM)	1 µl
PCR buffer 10x	2,5 µl
MgCl ₂ (25 mM)	3 µl
dNTPs (2 mM)	2,5 µl
Taq DNA polymerase (5 U/µl)	0,4 µl
H ₂ O	
ADN	100 ng
Total volume	25 µl

A allele: Primer AREP16 : 5'-AggCAACACTAAgTCACAACCCTggTCTTg-3'
Primer AREP25 : 5'-gATTggAgCAAgAgggTTAAgTgAAACTCT-3'

A^{br} allele : Primer AREP2 : 5'-AACCTCCACCCAgAgTCATCCATATT-3'
Primer AREP8 : 5'-CgAgCgCCAATTgCTCTCCCaggCgAgCgC-3'

Cycling conditions :

One cycle of DNA denaturation for 2 min at 94 °C;

35 three-step cycles 94 °C for 30 sec
61 °C for 30 sec
and 72 °C for 1 min

Last extension for 3 min at 72 °C

Electrophoresis on an agarose gel (2,5 %)

Original PCR protocol developed by CRA-W and INRA for the detection of the Sv, 2.1 and 2.2 alleles of the PAR2 gene

PCR Mix:

Primer PAR2-DIR (5 µM)	2 µl
Primer PAR2-REV (5 µM)	2 µl
PCR buffer 10x	5 µl
MgCl ₂ (25 mM)	3 µl
dNTPs (2 mM)	5 µl
Taq DNA polymerase (5 U/µl)	0,4 µl
H ₂ O	
ADN	100 ng
Total volume	50 µl

Primer PAR2-DIR : 5'-CTCAgTCgTTTCgCTTCTgC-3'

Primer PAR2-REV : 5'-gATCgTgTAGACAACCTggAAgAAAg-3'

Cycling conditions :

One cycle of DNA denaturation for 2 min at 94 °C;

35 three-step cycles 94 °C for 30 sec

61 °C for 30 sec

and 72 °C for 1 min

Last extension for 3 min at 72 °C

Digestion of the PCR product with *HindIII* :

Buffer 10x	2 µl
Enzyme (10 U/µl)	1 µl
H ₂ O	7 µl
PCR products	10 µl
Total volume	20 µl

Incubate at 37 °C for 1 h 30 min

Digestion of the PCR product with *NlaIII* :

Buffer 10x	2 µl
Enzyme (10 U/µl)	0,75 µl
H ₂ O	7,25 µl
PCR products	10 µl
Total volume	20 µl

incubate at 37 °C for 1 h 30 min

Electrophoresis on an agarose gel (2,5 %)

Table 1. Genotypes of the Belgian Blanc Bleu animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
BBB 01	$E^d E^d$	AA^{br}	Sv 2.1	A/A	G/G
BBB 02	$E^d E^d$	AA	Sv 2.1	T/A	G/G
BBB 03	$E^d E^d$	AA	Sv 2.1	T/A	G/G
BBB 04	$E^d E^d$	AA^{br}	Sv 2.1	T/T	G/G
BBB 05	$E^d E^d$	AA^{br}	2.1 2.1	T/T	G/G
BBB 06	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 07	$E^d E^d$	AA	2.1 2.1	T/A	G/G
BBB 08	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 09	$E^d E^d$	AA	Sv 2.1	A/A	G/G
BBB 10	$E^d E^d$	AA	Sv 2.2	T/T	G/G
BBB 11	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 12	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 13	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 14	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 15	$E^d E^d$	AA^{br}	Sv 2.1	A/A	G/G
BBB 16	$E^d E^d$	AA	Sv 2.1	T/A	G/G
BBB 17	$E^d E^d$	AA	Sv 2.1	A/A	G/G
BBB 18	$E^d E^d$	AA	Sv 2.1	T/A	G/G
BBB 19	$E^d E^d$	AA	2.1 2.1	A/A	G/G
BBB 20	$E^d E^d$	AA	Sv 2.1	T/A	G/G
BBB 21	$E^d E^d$	AA	2.1 2.1	T/A	G/G
BBB 22	$E^d E^d$	AA	Sv 2.1	A/A	G/G

Table 2. Genotypes of the Salers animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
SAL 01	ee	AA	Sv 2.2	T/A	G/C
SAL 02	ee	AA	Sv 2.2	T/A	G/G
SAL 03	ee	AA	2.1 2.1	T/T	G/G
SAL 04	ee	AA	2.1 2.1	T/A	G/G
SAL 05	ee	AA	2.1 2.1	T/A	G/C
SAL 06	ee	AA	2.1 2.1	T/A	G/G
SAL 07	ee	AA	2.1 2.1	T/T	G/G
SAL 08	ee	AA	Sv 2.1	T/A	G/G
SAL 09	ee	AA	Sv Sv	A/A	G/C
SAL 10	ee	AA	2.1 2.1	T/T	G/G
SAL 11	ee	AA	Sv 2.1	A/A	G/G
SAL 12	$E e$	AA	Sv Sv	T/A	G/G
SAL 13	ee	AA	Sv 2.1	T/A	G/G
SAL 14	ee	AA	2.1 2.1	T/T	G/C
SAL 15	ee	AA	Sv 2.1	T/T	G/G
SAL 16	ee	AA	Sv Sv	A/A	G/C
SAL 17	ee	AA	Sv 2.1	T/T	G/G
SAL 18	ee	AA	Sv 2.2	T/A	G/G
SAL 19	ee	AA	2.1 2.1	T/T	G/G
SAL 20	ee	AA	2.1 2.1	T/T	G/G

Table 3. Genotypes of the Limousine animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
LIM 01	ee	AA	2.1 2.1	T/T	G/G
LIM 02	ee	AA	2.1 2.1	T/T	G/G
LIM 03	ee	AA	2.1 2.1	T/T	G/G
LIM 04	ee	AA	Sv Sv	T/T	G/G
LIM 05	ee	AA	Sv 2.1	T/T	G/G
LIM 06	ee	AA	Sv 2.1	T/T	G/G
LIM 07	ee	AA	Sv 2.1	T/T	G/G
LIM 08	ee	AA	2.1 2.1	T/T	G/G
LIM 09	ee	AA	2.1 2.1	T/T	G/G
LIM 10	ee	AA	2.1 2.1	T/T	G/G
LIM 11	ee	AA	Sv 2.1	T/T	G/G
LIM 12	ee	AA	Sv 2.1	T/T	G/G
LIM 13	ee	AA	Sv 2.1	T/T	G/G
LIM 14	ee	AA	2.1 2.1	T/T	G/G
LIM 15	ee	AA	Sv 2.1	T/T	G/G
LIM 16	E e	AA	Sv 2.1	T/T	G/G
LIM 17	ee	AA	2.1 2.1	T/T	G/G
LIM 18	ee	AA	Sv 2.1	T/T	G/G
LIM 19	ee	AA	2.1 2.2	T/T	G/G
LIM 20	ee	AA	Sv 2.2	T/T	G/G

Table 4. Genotypes of the Parthenaise animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
PAR 01	E, E	AA	2.1 2.1	T/A	G/G
PAR 02	E, E	AA	Sv 2.1	A/A	G/G
PAR 03	E, E	AA	2.1 2.1	T/A	G/G
PAR 04	E E	AA	2.1 2.1	A/A	G/G
PAR 05	E, E	AA	2.1 2.1	A/A	G/G
PAR 06	E ₁ E ₁	AA	Sv 2.1	T/A	G/G
PAR 07	E ₁ E	AA	2.1 2.1	A/A	G/G
PAR 08	E E	AA	Sv 2.1	A/A	G/G
PAR 09	E ₁ E ₁	AA	2.1 2.1	T/A	G/G
PAR 10	E ₁ E	AA	2.1 2.1	T/A	G/G
PAR 11	E E	AA	2.1 2.1	T/A	G/G
PAR 12	E ₁ E ₁	AA	2.1 2.1	T/A	G/G
PAR 13	E ₁ E	AA	2.1 2.1	A/A	G/G
PAR 14	E ₁ E	AA	Sv 2.1	A/A	G/G
PAR 15	E ₁ E ₁	AA	Sv 2.1	T/A	G/G

Table 5. Genotypes of the Blonde d'Aquitaine animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
BLO 01	E ^d E ^d	AA	Sv 2.1	T/T	G/C
BLO 02	ee	AA	2.1 2.1	T/T	G/G
BLO 03	ee	AA	Sv 2.1	T/T	G/G
BLO 04	ee	AA	Sv 2.1	T/T	G/G
BLO 05	ee	AA	Sv 2.1	T/T	G/G
BLO 06	ee	AA	Sv 2.1	T/T	G/G
BLO 07	ee	AA	Sv Sv	T/T	G/G
BLO 08	ee	AA	2.1 2.1	T/T	G/G
BLO 09	ee	AA	Sv Sv	T/T	G/G
BLO 10	ee	AA	Sv Sv	T/T	G/G
BLO 11	ee	AA	2.1 2.1	T/T	G/G
BLO 12	ee	AA	Sv 2.1	T/T	G/G
BLO 13	ee	AA	Sv 2.1	T/T	G/G
BLO 14	ee	AA	Sv Sv	T/T	G/G
BLO 15	ee	AA	Sv 2.2	T/T	G/G
BLO 16	ee	AA	Sv 2.1	T/T	G/G
BLO 17	ee	AA	Sv 2.2	T/T	G/G
BLO 18	ee	AA	Sv Sv	T/T	G/G
BLO 19	ee	AA	Sv Sv	T/T	G/G
BLO 20	ee	AA	Sv Sv	T/T	G/G

Table 6. Genotypes of the Maine animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
MAI 01	ee	AA ^{br}	2.1 2.1	T/T	G/C
MAI 02	ee	AA ^{br}	Sv 2.1	T/T	G/G
MAI 03	ee	AA ^{br}	2.1 2.1	T/T	G/C
MAI 04	ee	AA ^{br}	2.1 2.1	T/T	G/G
MAI 05	ee	AA ^{br}	Sv 2.1	T/T	G/C
MAI 06	E e	AA ^{br}	2.1 2.1	T/A	G/G
MAI 07	ee	AA ^{br}	2.1 2.1	T/T	G/C
MAI 08	ee	AA ^{br}	Sv 2.1	T/T	G/G
MAI 09	E e	A ^{br} A ^{br}	2.1 2.1	T/T	G/C
MAI 10	ee	AA ^{br}	2.1 2.1	T/T	G/G
MAI 11	ee	A ^{br} A ^{br}	Sv 2.1	T/T	G/C
MAI 12	ee	AA ^{br}	2.1 2.1	T/T	G/C
MAI 13	ee	A ^{br} A ^{br}	2.1 2.1	T/T	G/C
MAI 14	ee	A ^{br} A ^{br}	2.1 2.1	T/T	G/C
MAI 15	ee	A ^{br} A ^{br}	Sv 2.1	T/T	G/C
MAI 16	ee	AA ^{br}	2.1 2.1	T/A	G/C
MAI 17	ee	AA ^{br}	2.1 2.1	T/T	G/C
MAI 18	ee	A ^{br} A ^{br}	Sv 2.1	T/T	G/G
MAI 19	ee	A ^{br} A ^{br}	2.1 2.1	T/T	G/G
MAI 20	ee	AA	2.1 2.1	T/T	G/G

Table 7. Genotypes of the Romagnola animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
ROM 01	EE	AA	2.1 2.1	T/A	G/G
ROM 02	EE	AA	Sv 2.1	T/T	G/G
ROM 03	EE	AA	Sv 2.1	T/T	G/G
ROM 04	EE	AA	Sv Sv	T/A	G/G
ROM 05	EE	AA	2.1 2.1	T/A	G/G
ROM 06	Ee	AA	2.1 2.1	T/T	G/G
ROM 07	EE	AA	Sv Sv	T/T	G/G
ROM 08	EE	AA	Sv Sv	T/T	G/G
ROM 09	EE	AA	Sv Sv	T/T	G/C
ROM 10		AA	2.1 2.1	T/T	G/G
ROM 11	EE	AA	Sv Sv	T/T	G/C
ROM 12	EE	AA	Sv 2.1	T/T	G/G
ROM 13	EE	AA	Sv 2.1	T/T	G/C
ROM 14	EE	AA	Sv 2.1	T/T	G/C
ROM 15	EE	AA	Sv 2.1	T/A	G/G
ROM 16	EE	AA	Sv 2.1	T/T	G/G
ROM 17	EE	AA	Sv 2.1	T/T	G/G
ROM 18	EE	AA	2.1 2.1	T/T	G/G
ROM 19	Ee	AA	Sv Sv	T/T	G/G
ROM 20	EE	AA	2.1 2.1	T/T	G/G
ROM 21	EE	AA	2.1 2.1	T/T	G/C
ROM 22	EE	AA	Sv 2.1	T/T	G/G

Table 8. Genotypes of the Marchigiana animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
MCG 01	EE	AA	2.1 2.2	T/T	G/G
MCG 02	EE	AA	Sv 2.1	T/A	G/G
MCG 03	EE	AA	2.1 2.1	T/T	G/G
MCG 04	EE	AA	Sv 2.1	T/T	G/G
MCG 05	EE	AA	Sv Sv	T/T	G/G
MCG 06	EE	AA	2.1 2.1	T/T	G/G
MCG 07	EE	AA	2.1 2.1	T/T	G/G
MCG 08	EE	AA	2.1 2.1	T/A	G/G
MCG 09	EE	AA	Sv 2.1	T/A	G/G
MCG 10	EE	AA	2.1 2.1	T/T	G/G
MCG 11	EE	AA	Sv 2.1	T/T	G/G
MCG 12	EE	AA	2.1 2.1	T/A	G/G
MCG 13	EE	AA	2.1 2.2	T/A	G/G
MCG 14	EE	AA	2.1 2.1	T/A	G/G
MCG 15	EE	AA	2.1 2.1	T/T	G/G
MCG 16	EE	AA	2.1 2.1	T/A	G/C
MCG 17	EE	AA	Sv 2.1	T/T	G/G
MCG 18	EE	AA	Sv 2.1	T/T	G/G
MCG 19	EE	AA	2.1 2.2	T/T	G/G
MCG 20	EE	AA	Sv 2.1	T/T	G/C
MCG 21	EE	AA	Sv Sv	T/A	G/G
MCG 22	EE	AA	Sv 2.1	T/T	G/G

Table 9. Genotypes of the Chianina animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
CHI 01	EE	AA	2.1 2.2	T/T	G/C
CHI 02	EE	AA	Sv 2.1	T/A	G/G
CHI 03	EE	AA	2.1 2.2	T/T	G/C
CHI 04	EE	AA	2.1 2.2	T/A	G/C
CHI 05	EE	AA	Sv 2.2	T/T	G/G
CHI 06	EE	AA	2.1 2.2	T/T	G/G
CHI 07	EE	AA	Sv 2.2	T/T	G/G
CHI 08	EE	AA	2.1 2.2	T/T	G/G
CHI 09	EE	AA	2.1 2.1	T/T	G/C
CHI 10	EE	AA	2.1 2.2	T/T	G/G
CHI 11	EE	AA	2.2 2.2	T/T	G/G
CHI 12	EE	AA	Sv 2.1	T/T	G/G
CHI 13	EE	AA	2.1 2.2	T/T	G/G
CHI 14		AA	2.1 2.2	T/T	G/C
CHI 15	EE	AA	Sv 2.1	T/A	G/G
CHI 16	EE	AA	2.1 2.2	A/A	G/C
CHI 17	EE	AA	Sv 2.2	T/T	G/G
CHI 18	EE	AA	2.1 2.2	T/T	G/G
CHI 19	EE	AA	2.1 2.2	T/T	G/G
CHI 20	EE	AA	Sv 2.2	T/T	G/G
CHI 21	EE	AA	Sv Sv	T/T	G/C
CHI 22	EE	AA	2.1 2.2	T/T	G/G

Table 10. Genotypes of the Piedmontese animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
PIM 01	EE	AA	Sv 2.1	T/T	G/G
PIM 02	EE	AA	2.1 2.1	T/T	G/G
PIM 03	EE	AA	Sv 2.1	T/T	G/G
PIM 04	EE	AA	Sv 2.1	T/T	G/G
PIM 05	EE	AA	Sv 2.1	T/T	G/G
PIM 06	EE	AA	2.1 2.1	T/T	G/G
PIM 07	EE	AA	2.1 2.1	T/T	G/G
PIM 08	EE	AA	2.1 2.1	T/T	G/G
PIM 09	EE	AA	Sv 2.1	T/T	G/G
PIM 10	EE	AA	Sv 2.2	T/T	G/G
PIM 11	EE	AA	Sv 2.1	T/T	G/G
PIM 12	EE	AA	2.1 2.1	T/T	G/G
PIM 13	EE	AA	Sv Sv	T/T	G/G
PIM 14	EE	AA	Sv 2.1	T/T	G/G
PIM 15	EE	AA	Sv 2.1	T/T	G/G
PIM 16	EE	AA	Sv 2.1	T/T	G/G
PIM 17	EE	AA	2.1 2.1	T/T	G/G
PIM 18	EE	AA	2.1 2.1	T/T	G/G
PIM 19	EE	AA	2.1 2.1	T/T	G/G
PIM 20	EE	AA	Sv 2.1	T/T	G/C
PIM 21	EE	AA	2.1 2.1	T/T	G/G
PIM 22	EE	AA	2.1 2.1	T/T	G/G

Table 11. Genotypes of the Italian Brown animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
IBR 01	E E	AA	2.1 2.1	T/A	G/G
IBR 02	E ₁ E ₁	AA	2.1 2.1	T/T	G/G
IBR 03	E ₁ E	AA	2.1 2.1	T/T	G/G
IBR 04	E E	AA	2.1 2.1	T/T	G/G
IBR 05	E ₁ E ₁	AA	2.1 2.1	T/T	G/G
IBR 06	E E	AA	2.1 2.1	T/T	G/G
IBR 07	E ₁ E	AA	2.1 2.1	A/A	G/G
IBR 08	E ₁ E ₁	AA	2.1 2.1	T/T	G/G
IBR 09	E E	AA	2.1 2.1	T/T	G/G
IBR 10	E ₁ E	AA	2.1 2.1	T/T	G/G
IBR 11	E E	AA	2.1 2.1	T/T	G/G
IBR 12	E E	AA	Sv Sv	T/A	G/G
IBR 13	E ₁ E	AA	2.1 2.1	T/A	G/G
IBR 14	E E	AA	2.1 2.1	T/T	G/G
IBR 15	E ₁ E	AA	2.1 2.1	T/A	G/G
IBR 16	E E	AA	Sv 2.1	T/A	G/G
IBR 17	E ₁ E	AA	2.1 2.1	T/T	G/G
IBR 18	E ₁ E	AA	2.1 2.1	T/T	G/G
IBR 19	E E	AA	2.1 2.2	T/T	G/C
IBR 20	E ₁ E	AA	2.1 2.1	A/A	G/G
IBR 21	E ₁ E ₁	AA	2.1 2.1	T/A	G/G
IBR 22	E E	AA	2.1 2.1	T/T	G/G

Table 12. Genotypes of the Maremmana animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
MAM 01	E E	AA	2.1 2.1	T/A	G/C
MAM 02	E ₁ E ₁	AA	2.1 2.1	T/T	G/G
MAM 03	E E	AA	2.1 2.1	T/T	G/C
MAM 04	E E	AA	2.1 2.1	T/T	G/C
MAM 05	E E	AA	2.1 2.1	T/T	G/C
MAM 06	E ₁ E	AA	2.1 2.1	A/A	G/C
MAM 07		AA	Sv 2.1	T/T	G/C
MAM 08	E ₁ E	AA	2.1 2.1	T/T	G/C
MAM 09	E E	AA	2.1 2.1	T/T	G/C
MAM 10	E E	AA	2.1 2.1	T/T	G/C
MAM 11	E ₁ E	AA	2.1 2.1	A/A	G/G
MAM 12	E ₁ E	AA	2.1 2.1	T/T	G/C
MAM 13	E ₁ E	AA	2.1 2.1	T/A	G/G
MAM 14	E ₁ E ₁	AA	2.1 2.1	T/A	G/C
MAM 15	E ₁ E	AA	2.1 2.1	T/T	G/C
MAM 16	E E	AA	2.1 2.1	T/T	G/C
MAM 17	E E	AA	2.1 2.1	T/T	G/G
MAM 18	E E	AA	2.1 2.1	T/A	G/G
MAM 19	E E	AA	2.1 2.1	T/T	G/G
MAM 20	E E	AA	2.1 2.1	T/T	G/G
MAM 21	E E	AA	2.1 2.1	T/T	G/G
MAM 22	E E	AA	Sv 2.1	T/T	G/G

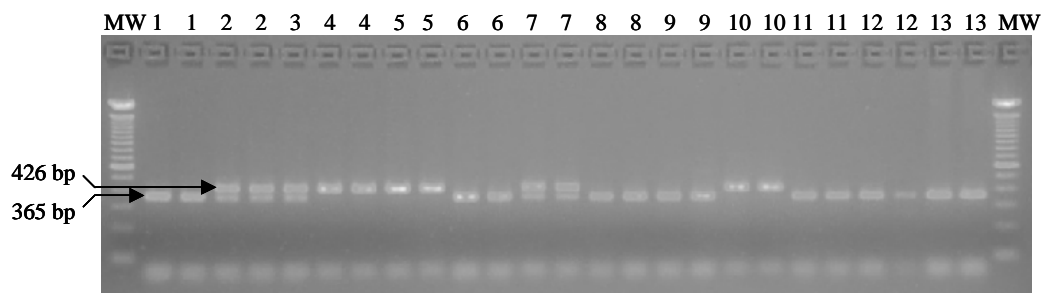
Table 13. Genotypes of the Pezzata Rossa Italiana animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
PRI 01	ee	AA ^{br}	2.1 2.1	T/T	G/C
PRI 02	ee	AA	2.1 2.1	T/T	G/C
PRI 03	ee	AA	2.1 2.1	T/T	G/C
PRI 04	ee	AA	Sv 2.1	T/T	G/G
PRI 05	ee	AA	2.1 2.1	T/A	G/C
PRI 06	ee	AA	Sv Sv	T/T	G/C
PRI 07	ee	AA	2.1 2.1	A/A	G/C
PRI 08	ee	AA ^{br}	2.1 2.1	T/T	G/C
PRI 09	ee	AA	2.1 2.1	T/T	G/G
PRI 10	ee	AA	2.1 2.1	T/T	G/C
PRI 11	ee	AA	Sv 2.1	T/T	G/C
PRI 12	ee	AA	Sv 2.1	T/T	G/G
PRI 13	ee	AA	Sv 2.1	T/T	G/C
PRI 14	ee	AA	2.1 2.1	T/T	G/C
PRI 15	ee	AA	2.1 2.1	T/T	G/C
PRI 16	ee	AA	Sv 2.1	T/T	G/G
PRI 17	ee	AA	2.1 2.1	T/T	G/G
PRI 18	ee	AA	2.1 2.1	T/T	G/C
PRI 19	ee	AA	2.1 2.1	T/T	G/C
PRI 20	ee	AA	2.1 2.1	T/T	G/C
PRI 21	ee	AA	Sv 2.1	T/T	G/G
PRI 22	ee	AA ^{br}	Sv 2.1	T/A	G/G

Table 14. Genotypes of the Italian Friesian animals for MC1R, Agouti, PAR2 and Myostatin Promoter genes

Animal	MC1R	Agouti	PAR2	Myostatin locus 1	Myostatin locus 2
IFR 01	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 02	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 03	E ^d E ^d	AA	Sv 2.1	T/T	G/C
IFR 04	ee	AA	Sv 2.1	T/T	G/G
IFR 05	E ^d E ^d	AA	Sv 2.1	T/T	G/G
IFR 06	E ^d E ^d	AA	2.1 2.1	T/T	G/C
IFR 07	E ^d E ^d	AA	2.1 2.1	T/T	G/C
IFR 08	E ^d E ^d	AA	Sv 2.1	T/T	G/C
IFR 09	E ^d E ^d	AA	Sv 2.1	T/T	G/C
IFR 10	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 11	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 12	E ^d E ^d	AA	Sv 2.1	T/T	G/G
IFR 13	E ^d E ^d	AA	2.1 2.1	T/T	G/C
IFR 14	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 15	E ^d E ^d	AA	Sv 2.1	T/T	G/G
IFR 16	E ^d E ^d	AA	2.1 2.1	T/A	G/G
IFR 17	E ^d E ^d	AA	Sv 2.1	T/T	G/G
IFR 18	E ^d E ^d	AA	Sv 2.1	T/T	G/G
IFR 19	E ^d E ^d	AA	Sv Sv	T/T	G/C
IFR 20	E ^d E ^d	AA	2.1 2.1	T/T	G/G
IFR 21	E ^d E ^d	AA	Sv 2.1	T/T	G/G

FR 22	$E E^d$	AA	2.1 2.1	T/T	G/C
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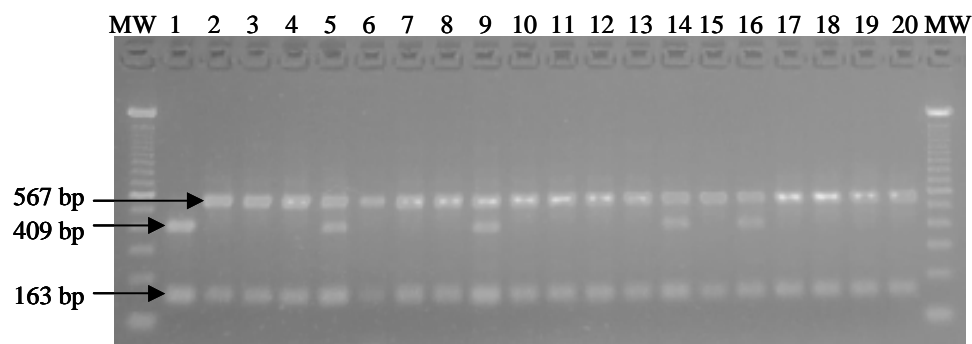


Legend : MW : 100 pb DNA ladder
 1-13 : Blanc Bleu Belge samples

Profiles : T/T : 426 bp : samples 4-5-10
 A/A : 365 bp : samples 1-6-8-9-11-12-13
 T/A : 426 and 365 bp : samples 2-3-7

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Figure 1.a. Profiles obtained by the digestion with DraI of an amplicon (561 bp) in the Myostatin Promoter amplified according to the protocol described by Crisà et al.(2003) for the genotyping of the locus 1

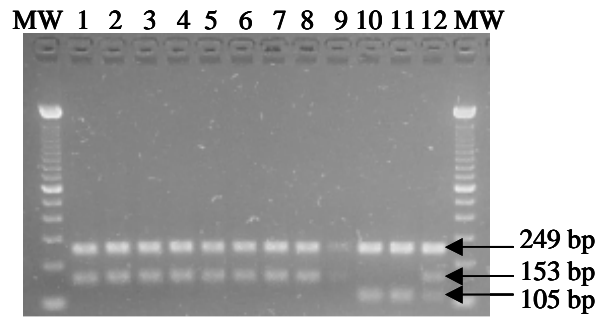


Legend : MW : 100 pb DNA ladder
 1-20 : Salers samples

Profiles : G/G : 567 bp and 163 bp : samples 2-3-4-6-7-8-10-11-12-13-15-17-18-19-20
 C/C : 409 bp and 163 bp : sample 1
 G/C : 567, 409 and 163 bp : samples 5-9-14-16

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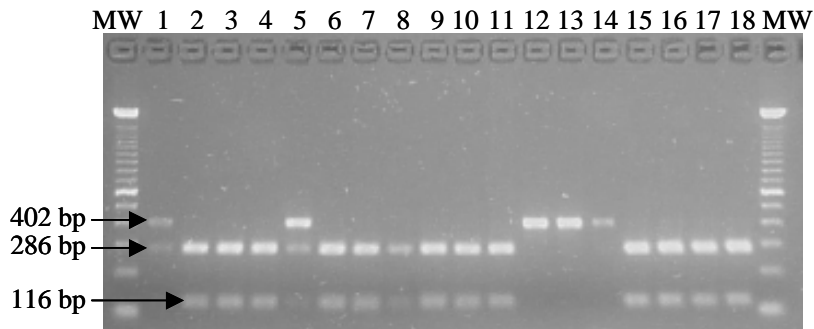
Figure 1.b. Profiles obtained by the digestion with SpeI of an amplicon (561 bp) in the Myostatin Promoter amplified according to the protocol described by Crisà et al.(2003) for the genotyping of the locus 2



Legend : MW : 100 pb DNA ladder
1-12 : Various samples

Profiles : 249 and 153 bp : samples 1-2-3-4-5-6-7-8-9
249 and 105 bp : samples 10-11
249, 153 and 105 bp : samples 12

Figure 2.a. Profiles obtained by the digestion with MspAII of an amplicon (402 bp) in the MC1R gene



Legend : MW : 100 pb DNA ladder
1-13 : various samples

Profiles : 402 bp : samples 12-13-14
286 and 116 bp : samples 2-3-4-6-7-8-9-10-11-15-16-17-18
402, 286 and 116 bp : samples 1-5

Figure 2.b. Profiles obtained by the digestion with MspI of an amplicon (402 bp) in the MC1R gene

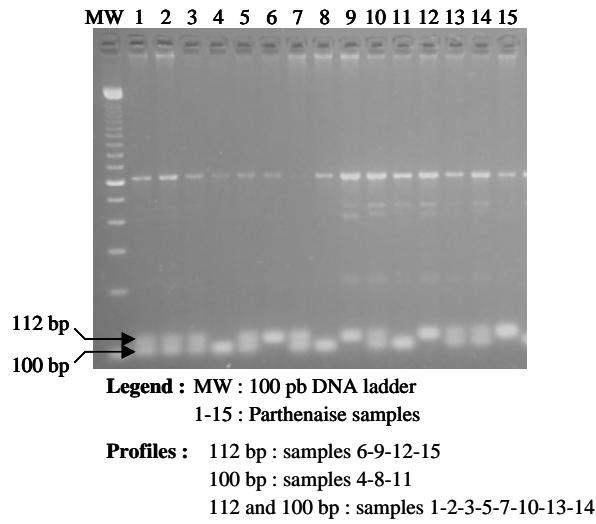


Figure 2.c. Profiles obtained by the amplification of an amplicon (100 and 112 bp) in the MC1R gene

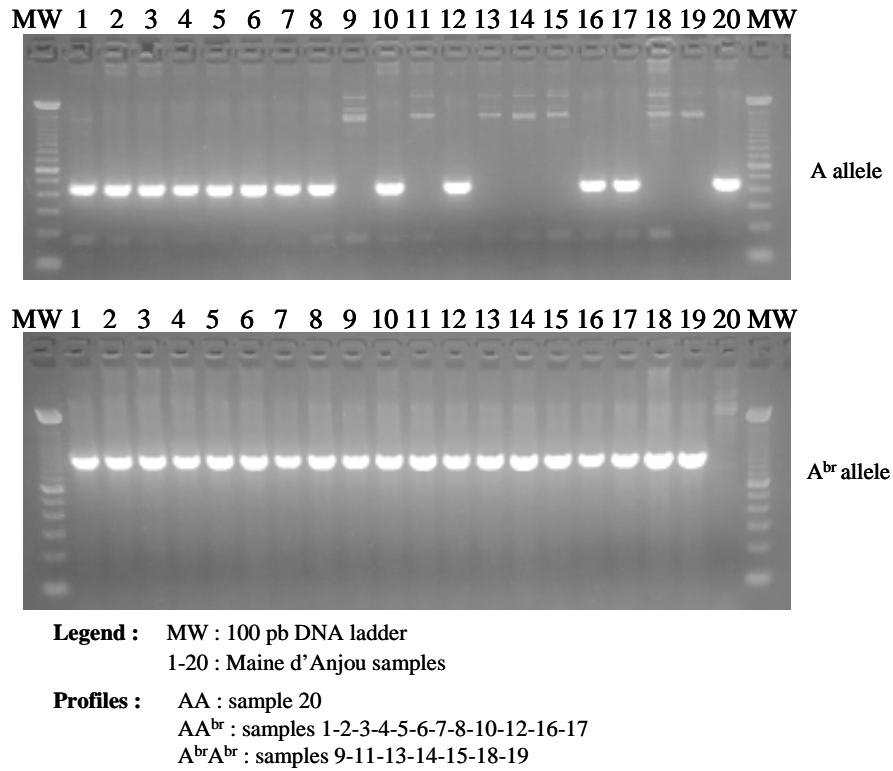


Figure 3. Profiles obtained by the amplification of an amplicon in the Agouti gene

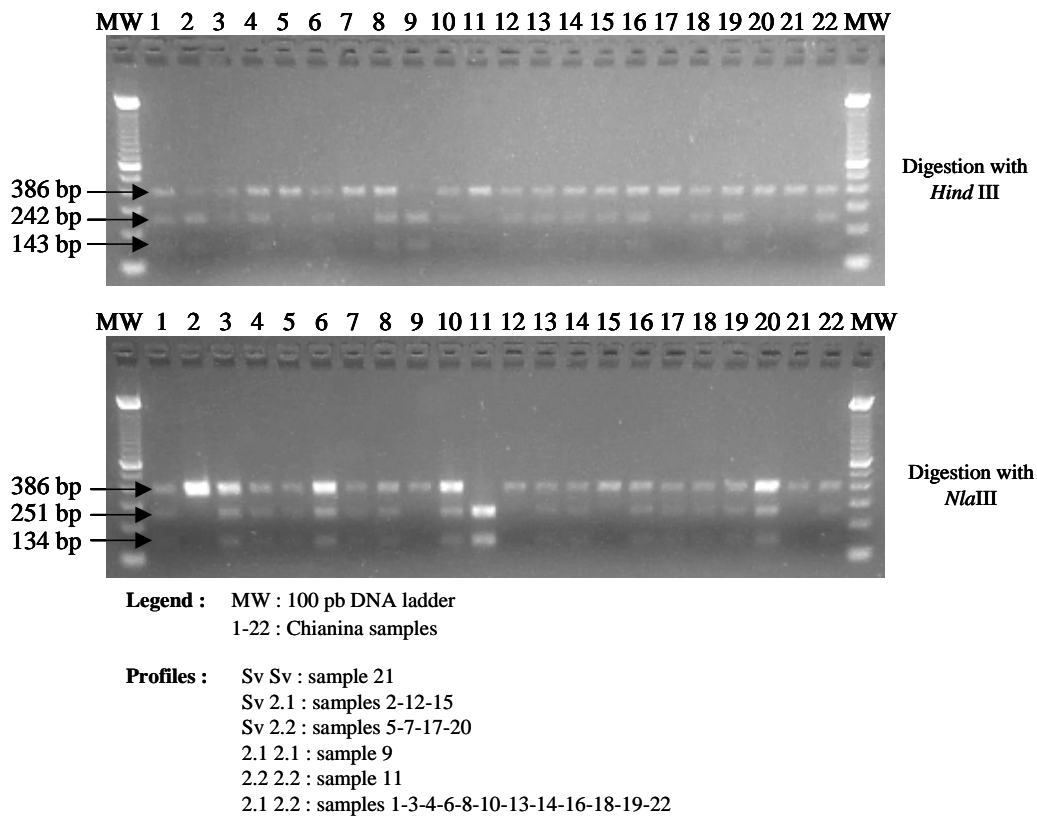


Figure 4. Profiles obtained by the digestion with HindIII and NlaIII of an amplicon (386 bp) in the PAR2 gene