

BOVINE KAPPA-CASEIN PCR-RFLP PROTOCOL FOR ALLELES A AND B

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PCR Reaction (Volume 25 μ l)

dH ₂ O	19.125 μ l
10x buffer	2.5 μ l
25mM MgCl ₂	1.5 μ l
10mM dNTP	0.25 μ l
10pmol Primers	0.25 μ l each
Taq polymerase 5UI	0.125 μ l
DNA 50ng	1 μ l

Cycling

35X: 94°C 30sec; 60°C 30sec; 72°C 30sec.

Enzymatic digestion: with *HinfI*

Recognition site: GANT↓C

dH ₂ O	4.65 μ l
Buffer NEB 2	2.25 μ l
<i>HinfI</i>	0.6 μ l (6 units)
PCR product	15 μ l

Incubation: 37 °C at least 2 hrs.

PCR Polymorphisms and Genotypes (Agarose gel 3%)

	AA	AB	BB	Uncut
350				—
266		—	—	
132/134	—	—		
84	—	—	—	

Primers:

JK5: 5` ATCATTATGGCCATTCCACCAAAG 3`

JK3: 5` GCCATTTCGCCTTCTCTGTAACAGA 3`

Genbank sequence

Accession number: X14908, NM_174294

DEFINITION Bovine gene for kappa-casein exons 3-5.

ACCESSION X14908 X14326

VERSION X14908.1 GI:180

Exons 3-5

>gi|27881411|ref|NM_174294.1| Bos taurus casein kappa (CSN10), mRNA

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1 TTCACTTACAGTGGAAAGGCCAACTGAACCTACTGCCAAGCAAGAGCTGACGGTCACAAGGAAAGGTGCA
73 ATGATGAAGAGTTTTTTCCTAGTTGTGACTATCCTGGCATTAAACCCTGCCATTTTTGGGTGCCCAGGAGC
147 AAAACCAAGAACAACCAATACGCTGTGAGAAAGATGAAAGATTCTTCAGTGACAAAATAGCCAAATATAT
201 CCCAATTCAGTATGTGCTGAGCAGGTATCCTAGTTATGGACTCAATTACTACCAACAGAAACCAGTTGCA
272 CTAATTAATAATCAATTTCTGCCATACCCATATTATGCAAAGCCAGCTGCAGTTAGGTCACCTGCCAAA
345 TTCTTCAATGGCAAGTTTTGTCAAATACTGTGCCTGCCAAGTCCTGCCAAGCCCAGCCAACCTACCATGGC
419 ACGTCACCCACACCCACATTTATCATTATGGCCATTCCACCAAAGAAAAATCAGGATAAAACAGAAATC
591 CCTACCATCAATACCATTGCTAGTGGCGAGCCTACAAGTACACCTACCACCGAAGCAGTAGAGAGCACTG
664 TAGCTACTCTAGAAGATTCTCCAGAAGTTATTGAGAGCCCACCTGAGATCAACACAGTCCAAGTTACTTC
735 AACTGCAGTCTAAAACTCTAAGGAGACATCAAAGAAGACAACGCAG-----INTRON IV-----
-----TCTGTTACAGAGAAGCGCAAATGGGC-----
783GTCTAGCTGAAACCAAATGACTACTTCAAACCTTTCTTTGGCCAGTTGTCTGCCTTCGGTGAACAGAGAAT
ATGATTTTCACAGATCTGGCTCCTTCCTCGTCTCCTTACATTTTACTTTTATGCCAGATTTAATTTTTTGAT
TCCTGCATAATAAAGCCAATCAAATGCA

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Genetic variant	Exon	Nucleotide position	Change	Amino acid position	Change
A → B	IV	5345	A → C	148	Asp → Ala

Reference:

Medrano, J.F. and E. Aguilar-Cordova. 1990. Genotyping of bovine kappa-casein loci following DNA sequence amplification. *Bio/Technology*. 8:144-146.