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IDENTIFICATION OF BETA-LACTOGLOBULIN AND KAPPA-CASEIN GENOTYPES USING PCR-RFLP IN HOLSTEIN CATTLE

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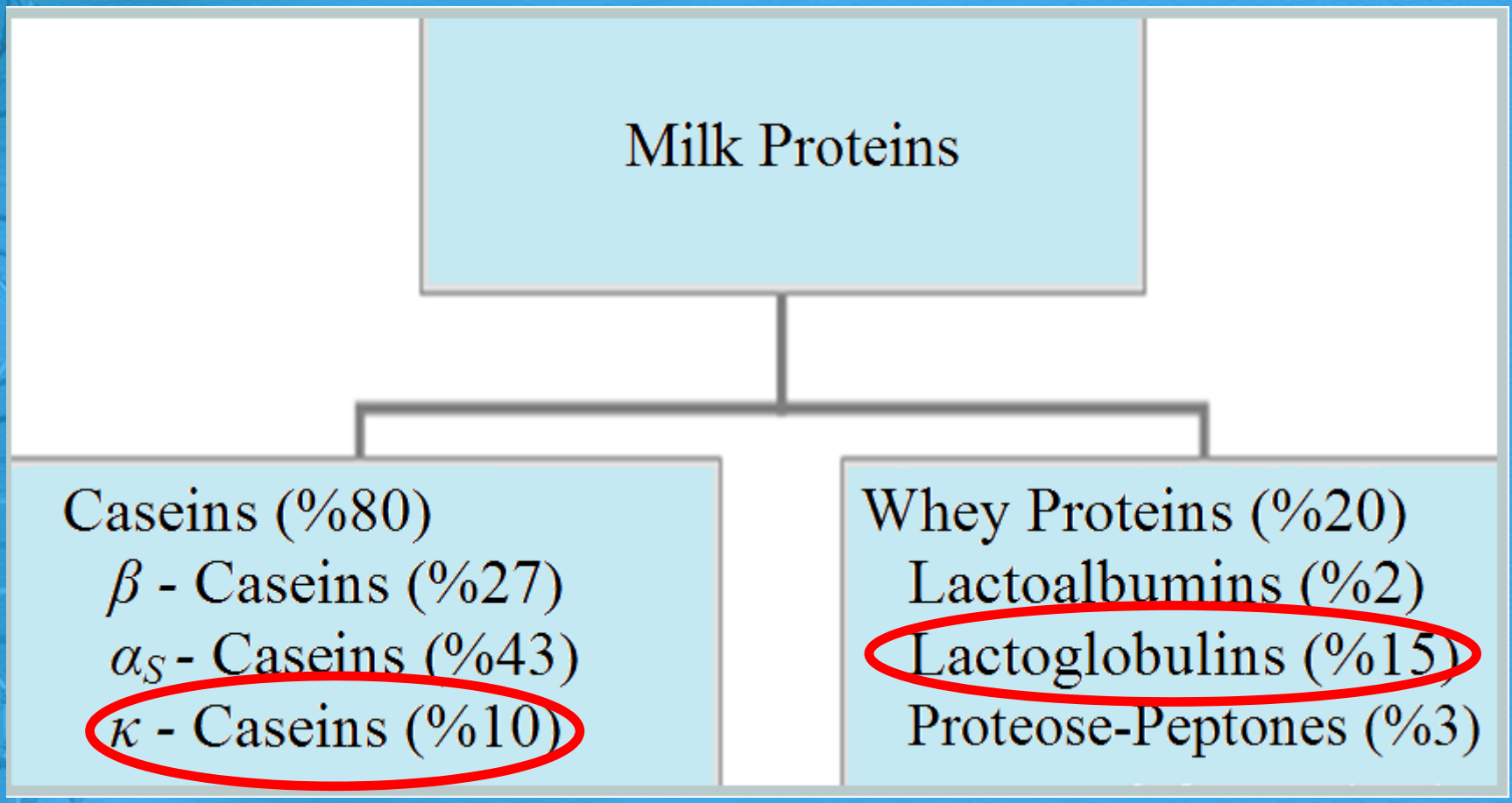
MILK

All mammals produce milk to feed their young. The milk is used to help the young mammal grow and develop, and so contains all necessary nutrients to enable maturation of the young until they are able to feed themselves.

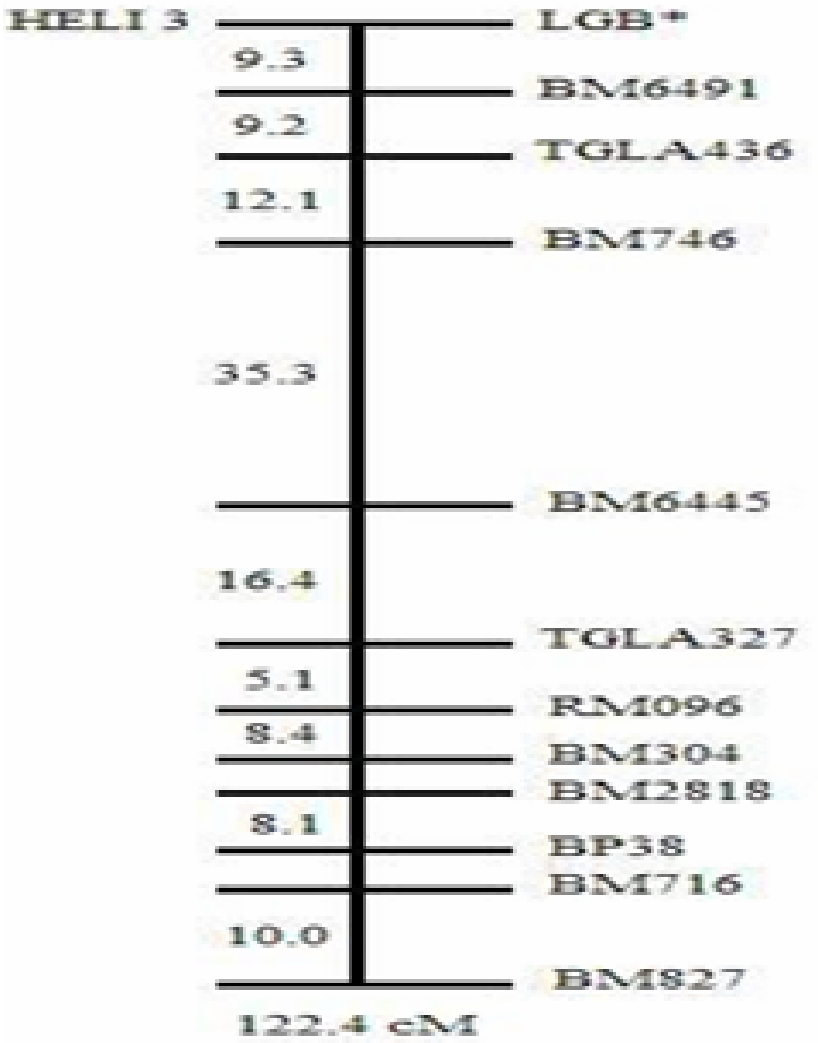
Milk proteins;



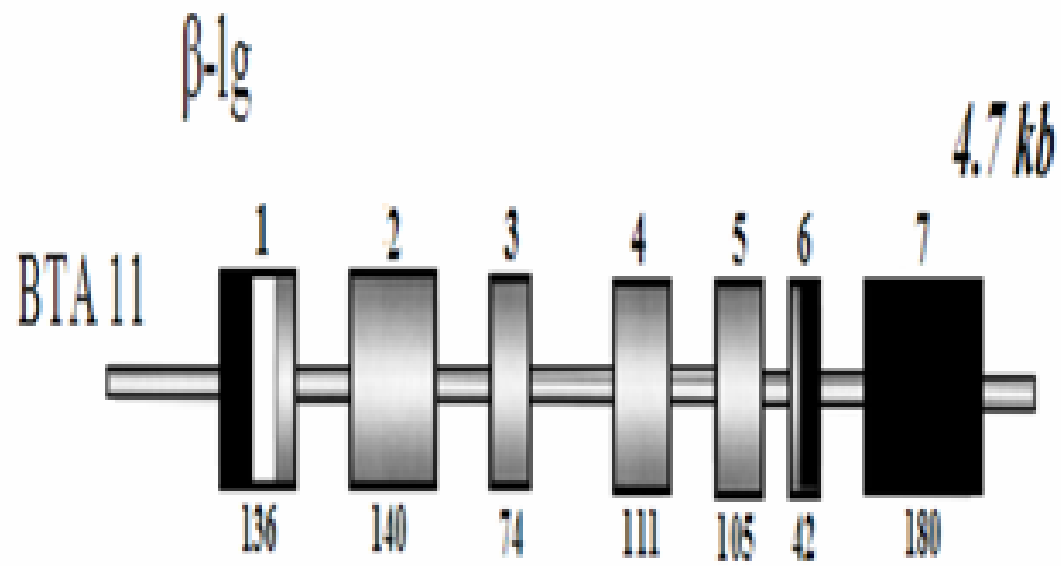
β-lactoglobulin;

- The major protein of bovine milk whey, is found in a number of genetic variants of which A & B predominant.
- A & B variants of *β-LG* may affect milk composition and properties.
- *β-LG* AA genotypes; ⇧ *β-LG,* ⇩ *caseins,* ⇩ *fat*
- *β-LG* BB genotypes; ⇧ caseins
- *β-LG* B allele and BB genotype mastitis resistance ⇧

- The gene encoding β-lactoglobulin has been mapped on chromosome 11 (BTA 11) in cattle.

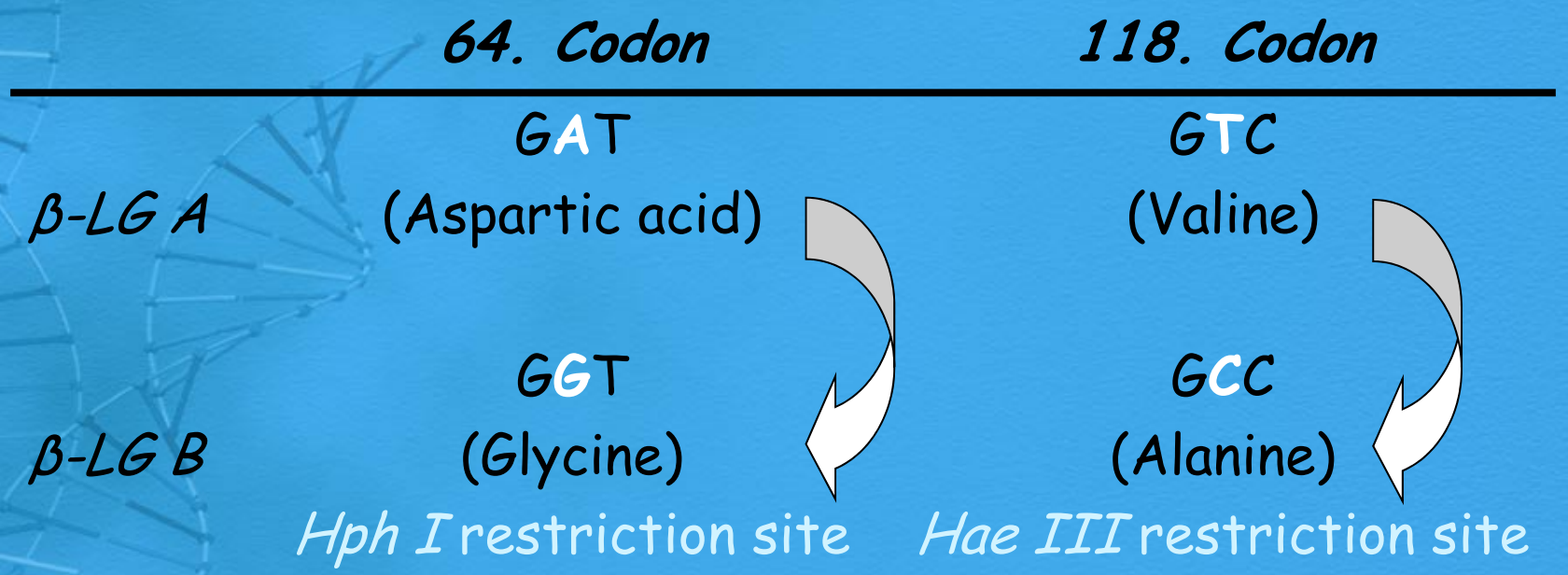


A



B

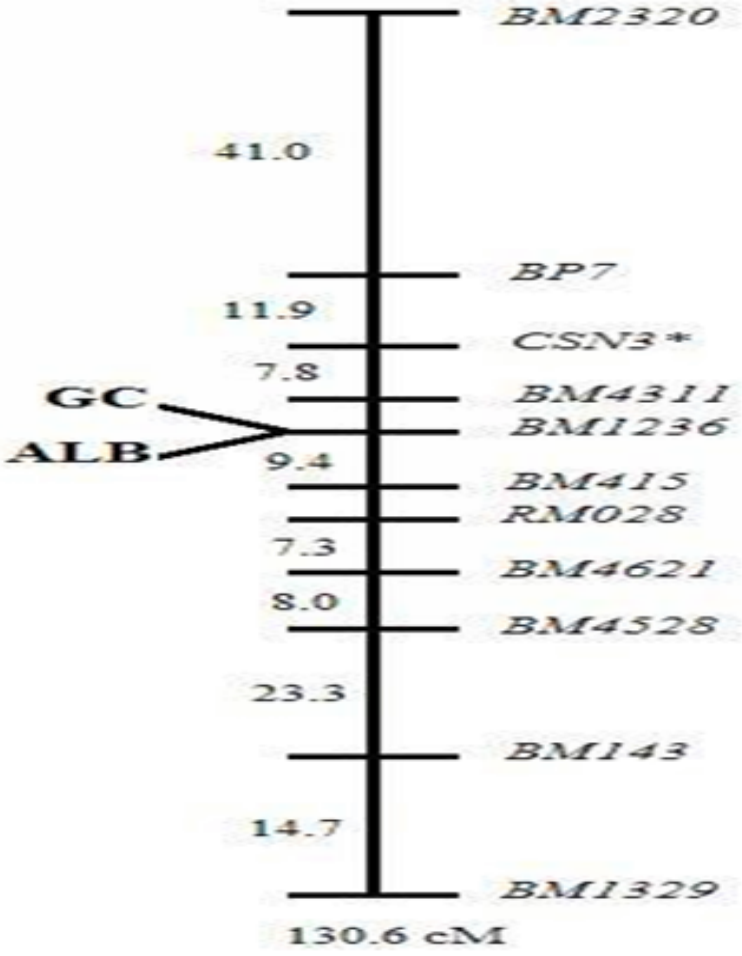
- *β-LG A* variant differs from *B* variant by two amino acid only Aspartic acid-64 and Valine-118.



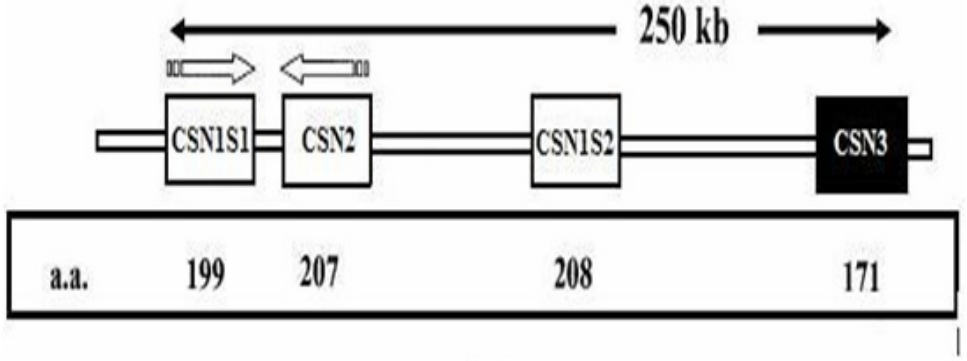
κ-casein;

- CSN3 plays an important role in preserving the other caseins from precipitation.
- Treatment of milk with rennin cleaves CSN3, resulting in curd formation.
- CSN3 variants affect the cheese making properties of milk.
- CSN3 B allele was reported to have favourable and significant effect on both milk and milk protein yield.

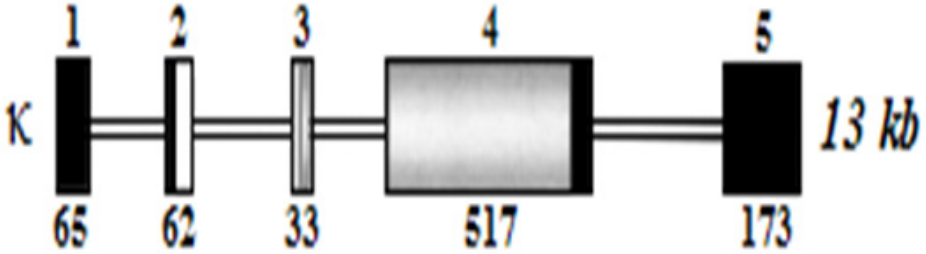
- κ-casein gene has been mapped on chromosome 6 (BTA 6).



A

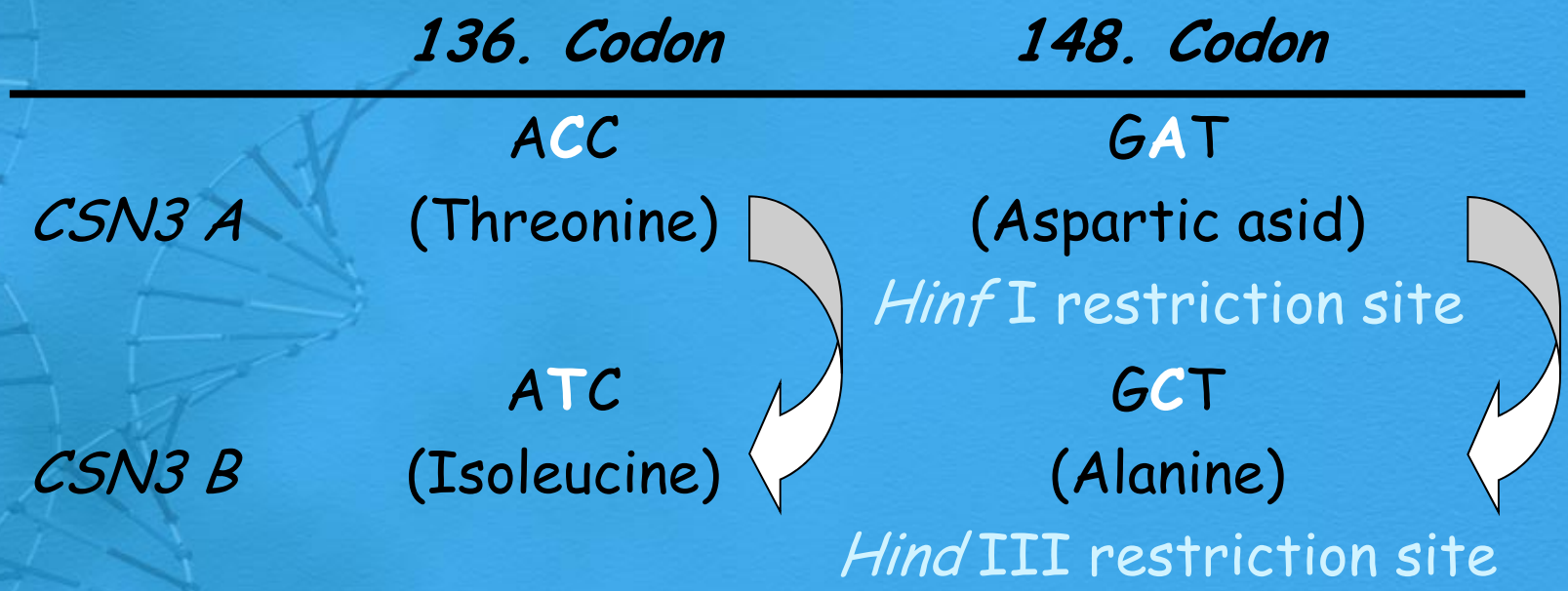


B



C

- *CSN3 A* variant differs from *B* variant by two amino acid only Threonine-136 and Aspartic acid-148.



AIM

The aim of this study was to determine genotypic and allelic frequencies of *β - LG* and *CSN3* in Holstein cattle populations in reared in Turkey.

MATERIALS & METHODS

ANIMAL MATERIAL

Province	Enterprise	Sample Size
Ankara	Bala Agricultural Enterprises	78
Şanlıurfa	Ceylanpınar Agricultural Enterprises	89
Total		167

Blood Samples

- Blood samples were collected by puncture of jugular vein into sterile tubes containing EDTA.

Genomic DNA Isolations

- Salting-out

  % 1 agarose gel electrophoresis

  spectrophotometer at A_{260} / A_{280} nm

Amplification of β - LG gene by PCR

***Forward* primer:**

5′ ACC TGG AGA TCC TGC TGC AGA AAT G 3′

***Reverse* primer:**

5′ CAT CGA TCT TGA ACA CCG CAG GGA T 3′

94	°C	→	3	min.	*Initial denaturation*
94	°C	→	1	min.	*Denaturation*
61	°C	→	30	sec.	*Annealing*
72	°C	→	2.5	min.	*Extension*
72	°C	→	5	min.	*Final extension*

30 cycles

Amplification of CSN3 gene by PCR

***Forward* primer:**

5' GTG CTG AG(T/C) AGG TAT CCT AG 3'

***Reverse* primer:**

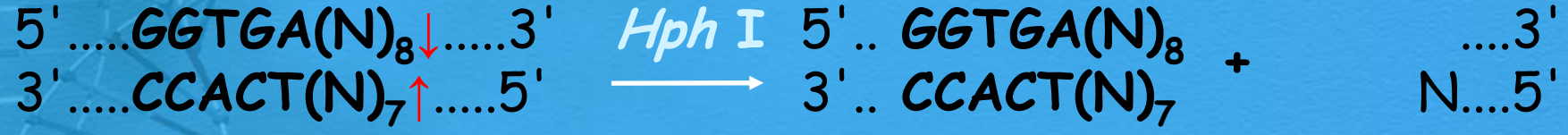
5' GTA GAG TGC AAC AAC ACT GG 3'

95 °C → 5 min.		*Initial denaturation*
94 °C → 1 min.	30 cycles	*Denaturation*
57 °C → 1 min.		*Annealing*
74 °C → 3 min.		*Extension*
72 °C → 5 min.		*Final extension*

Incubation of β-LG PCR products with *Hph I* restriction enzyme

15 µl PCR + 10 µl *Hph* I restriction mix

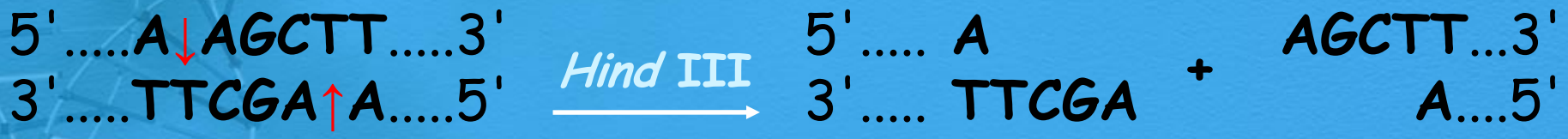
37 °C for 1 h.



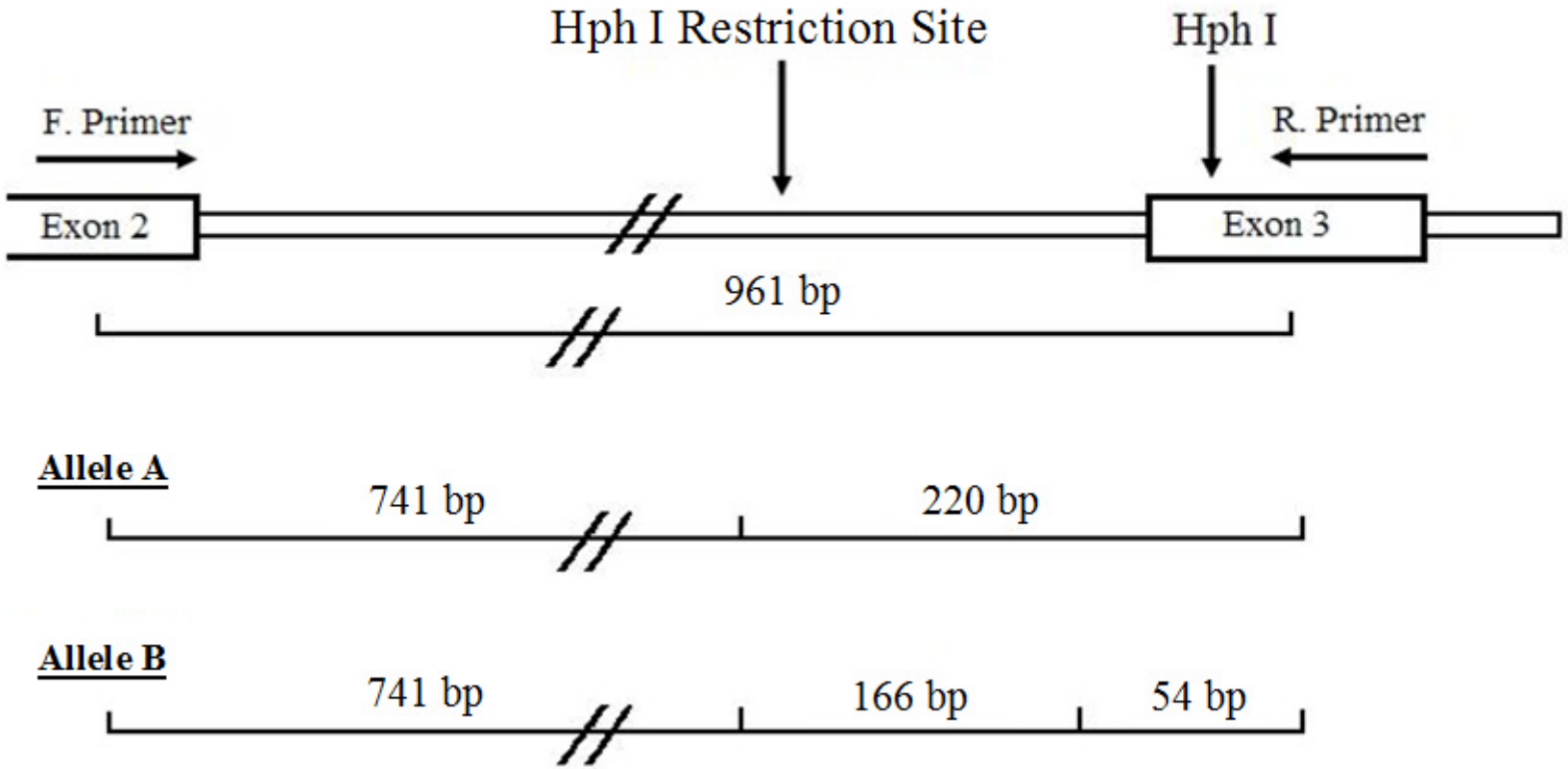
Incubation of CSN3 PCR products with *Hind* III restriction enzyme

10 µl PCR + 10 µl *Hind* III restriction mix

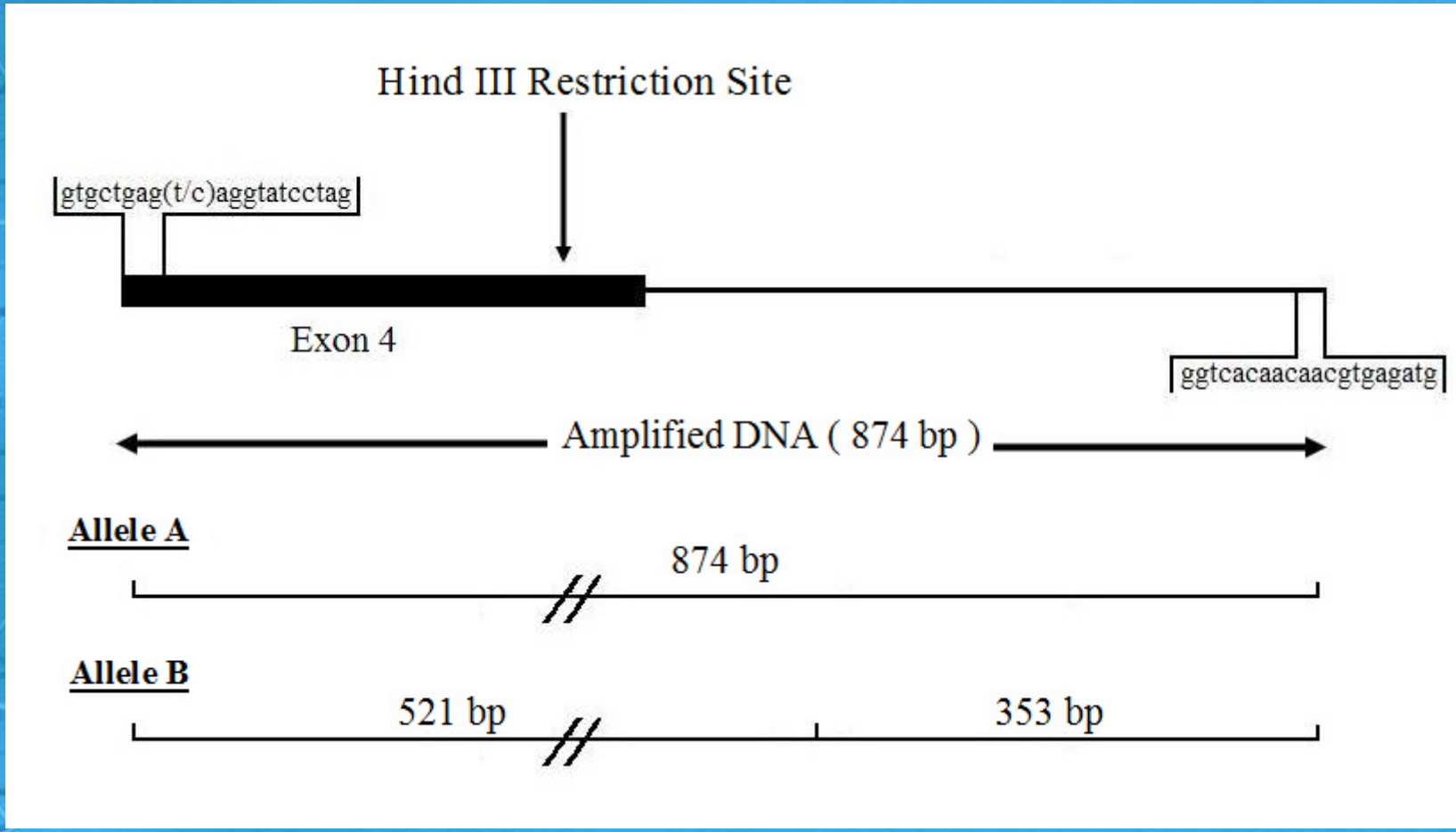
37 °C for 1 h.



Detection of alleles at the β-LG locus



Detection of alleles at the κ-CN locus

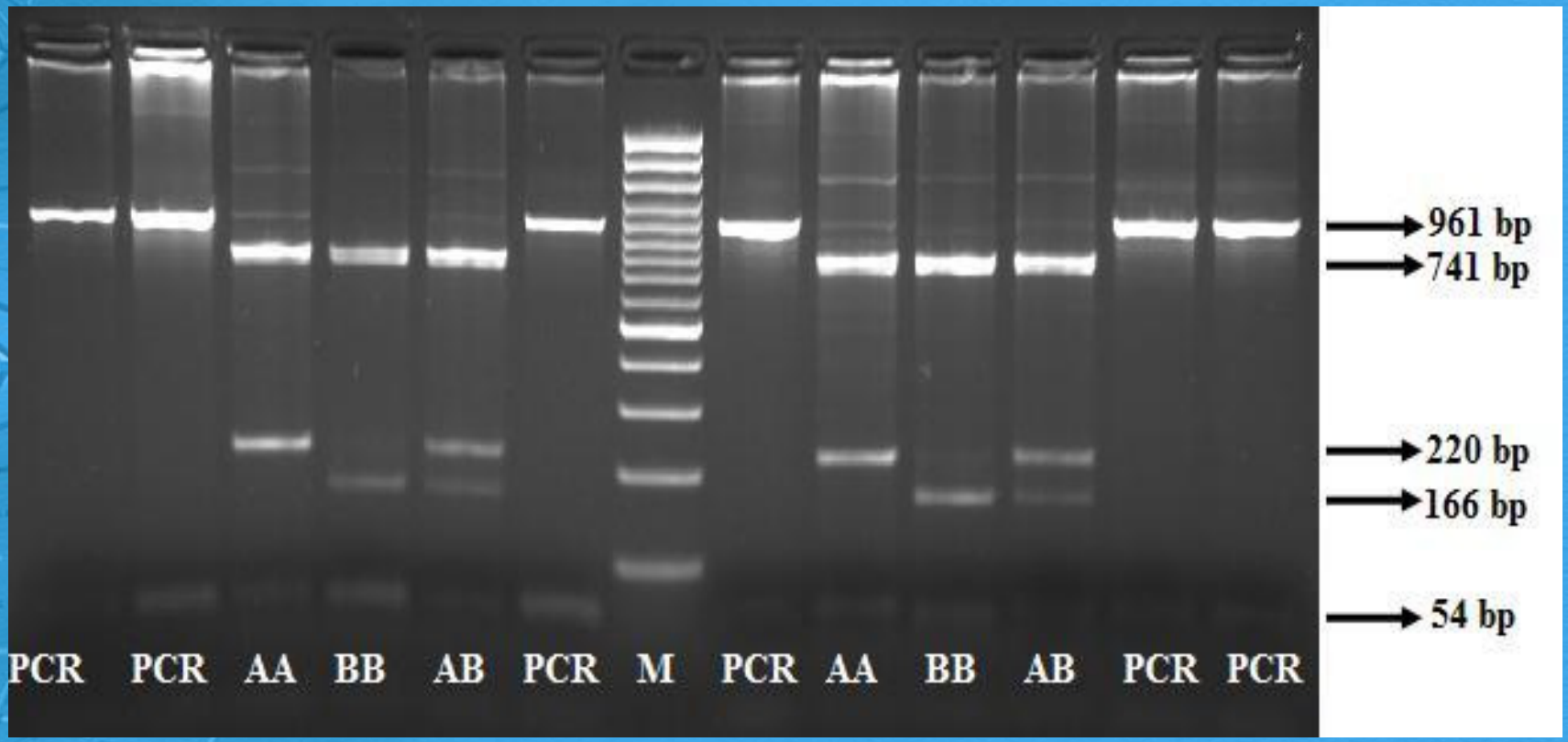


Statistical Analysis

Counting the number of gene was used to estimate gene and genotype frequencies of β-LG and CSN3. The χ^2 test was used to check whether the populations were in Hardy-Weinberg equilibrium or not.

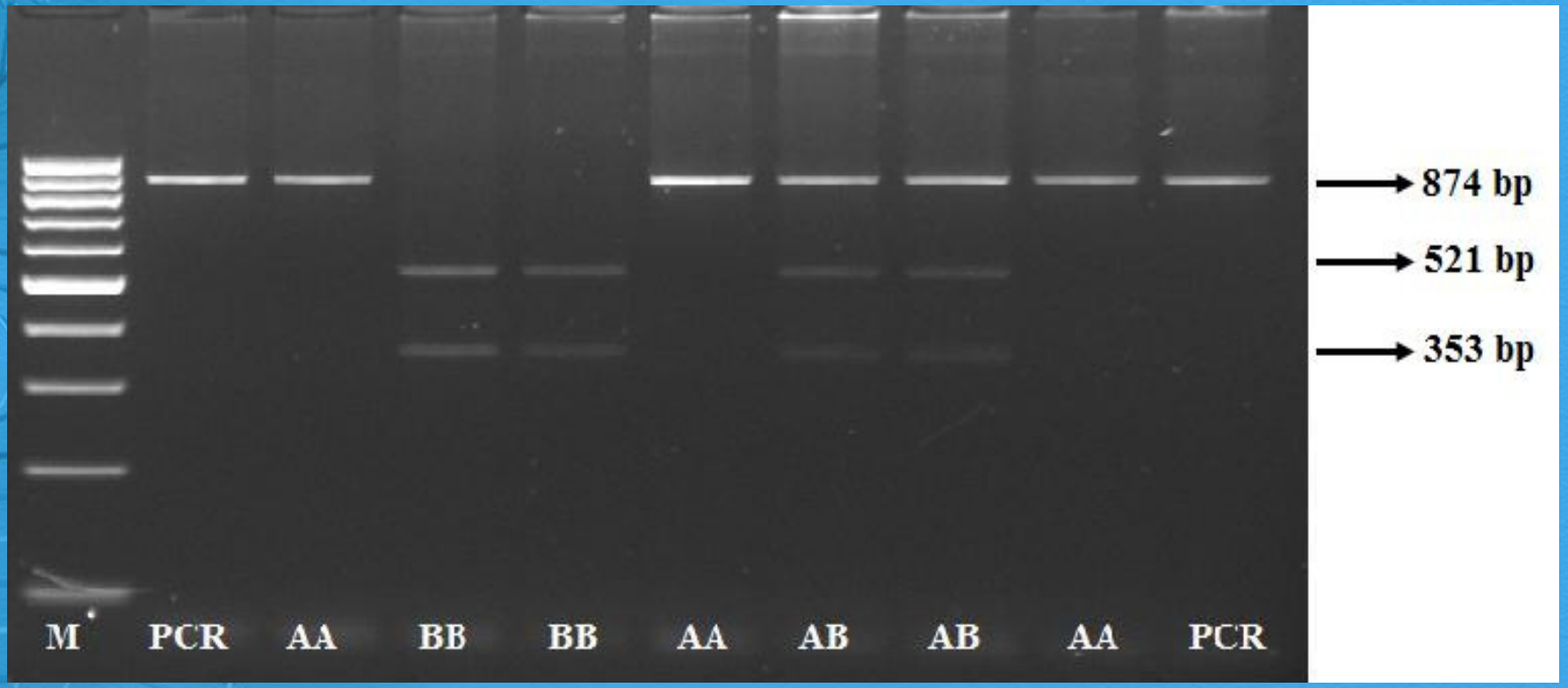
RESULTS

Identification of β-LG Genotypes



Identification of β-lactoglobulin genotypes on % 2 agarose gels by PCR-RFLP (M 100 bp DNA marker).

Identification of CSN3 Genotypes



Identification of κ-casein genotypes on % 2 agarose gels by PCR-RFLP (M 100 bp DNA marker).

Herd	No. of animals	Genotype						Allele frequency			
		β-LG			*CSN3*			*β-LG*		*CSN3*	
		AA	BB	AB	AA	BB	AB	A	B	A	B
Bala	78	17	15	46	50	3	25	0.51	0.49	0.80	0.20
Ceylanpınar	89	10	39	40	65	5	19	0.34	0.66	0.84	0.16
p<0.05											

DISCUSSION

- Bala and Ceylanpınar populations were found to be polymorphic in two loci.
- In the β-LG locus, both populations were in Hardy-Weinberg equilibrium, while in the CSN3 locus only one of the populations was in the Hardy-Weinberg equilibrium.

- This seemingly unexpected result might be, either due to the sampling error, or, due to that the bull who gives his sperm to the cows was a selected certain one, not randomly taken. Whereas in the case of bull being selected, not only one, but both loci are expected to be biased from the Hardy-Weinberg equilibrium.

- So we could say that the bias from Hardy-Weinberg equilibrium was a sampling error. If you have decided the rejection criteria as the probability $p<0.01$ instead of $p<0.05$, you have accepted the hypothesis that the population was in Hardy-Weinberg equilibrium.

- Further studies looking at the relation of the various yield and quality features of milk with the genetic variation in the milk proteins such as β-Lactoglobulin and K-casein can give rise to getting some molecular genetic markers as selection criteria for milk production.


THANK YOU…