National Research Centre

Genetic Engineering and Biotechnology Research Division

Cell Biology Department

Genetic characterization and polymorphism detection of casein genes in Egyptian sheep breeds

Othman E. Othman and Samia A. El-Fiky

Cell Biology Department - National Research Center - Dokki - Egypt

Corresponding author

Dr. Othman El Mahdy Othman

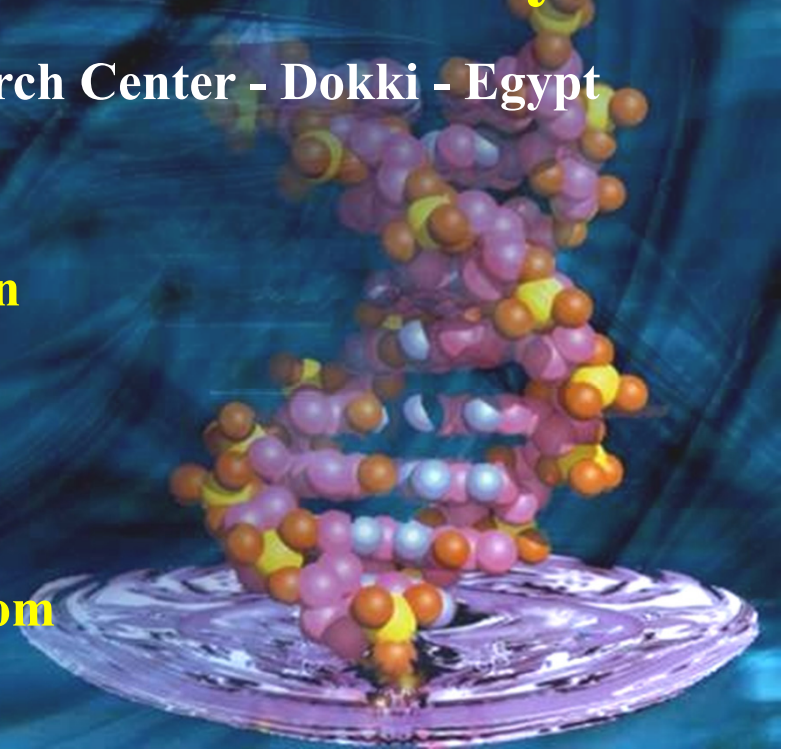
Cell Biology Department

National Research Center

Dokki - Giza - Egypt

Fax: 202 3370931

E-mail: othmanmah@yahoo.com



- Sheep milk is an excellent raw material for milk processing industry especially in cheese production. Casein fraction; αs1-, αs2-, β- and κ-caseins, are the main components of total milk protein.
- Casein genetic polymorphisms are important due to their effects on quantitative traits and technological properties of milk.

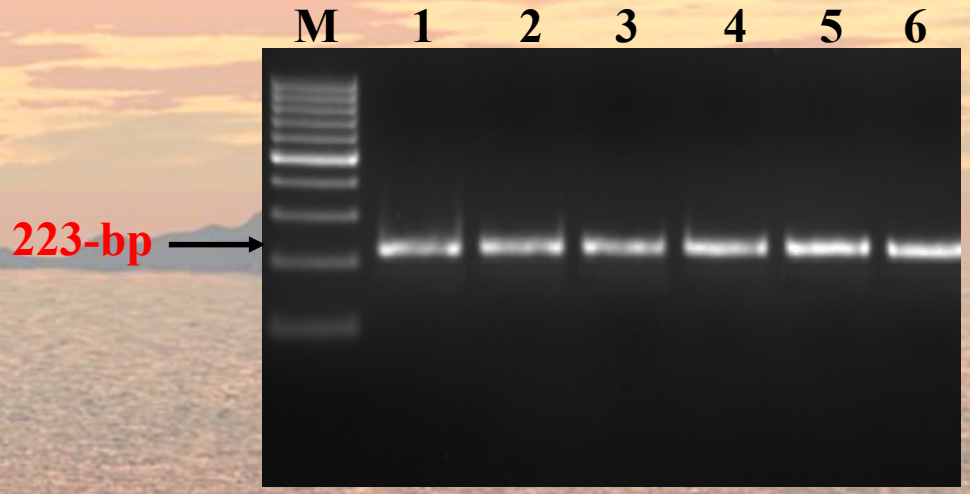
- In Egypt, sheep are raised mainly for meat and for milk as a secondary product. Sheep's milk is processed into butter and cheese.
- Egyptian sheep contains about twelve breeds, there are three major breeds representing 65% of the total population named Rahmani, Ossimi and Barki

- In spite of the importance of genetic polymorphism of casein genes with its effect on milk properties, few studies were carried out on it in the Middle East countries. So this study focused on genetic polymorphism detection of four casein protein genes; αs1, αs2, β and κ-casein in three main Egyptian sheep breeds.

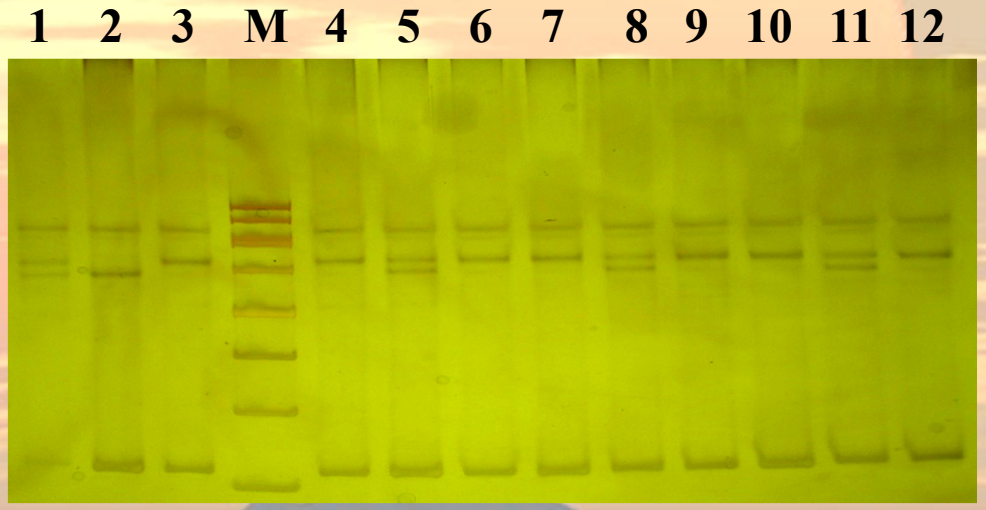
- Blood samples were collected from sheep animals belonging to three main sheep breeds reared in Egypt; Barki, Ossimi and Rahmani.
- DNA was extracted from the blood samples and PCR was done using primers specific for these tested genes.
- RFLP or SSCP were applied on PCR products for polymorphism detection and Sequence analysis was done for SNPs detection.

αs1-Casein Gene

- The polymorphism of this gene was detected using PCR-SSCP technique. A 223-bp fragment was amplified by polymerase chain reaction



- SSCP result recorded the presence of three different patterns. CC, CT and TT

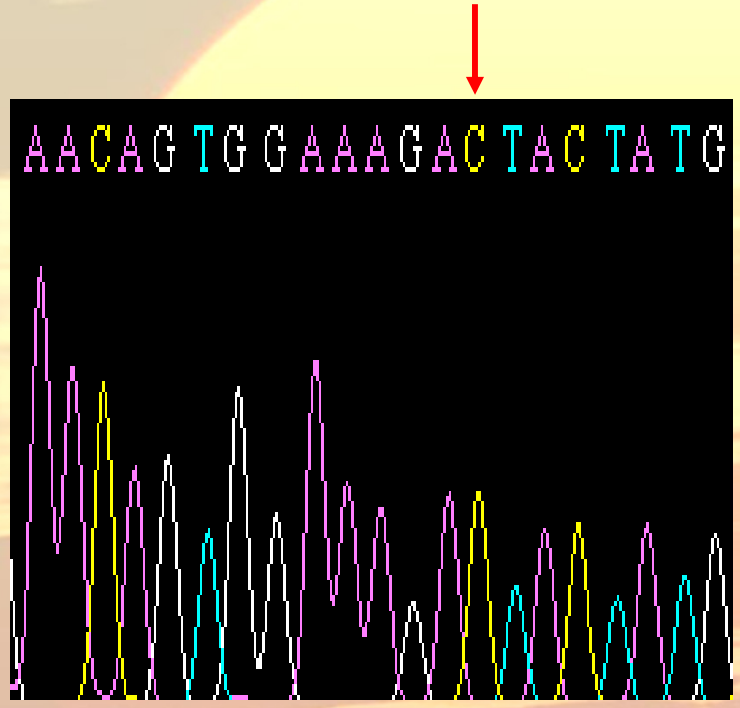


Lane 2: pattern CC

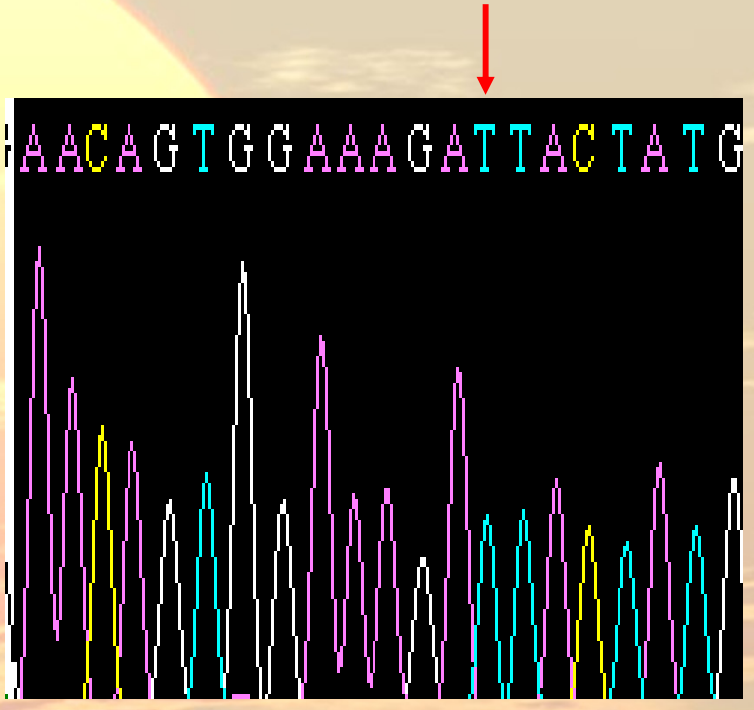
Lanes 1, 5, 8 and 11: pattern CT

Lanes 3, 4, 6, 7, 9, 10 and 12: pattern TT

- The frequencies for the three detected pattern were 4.60% for CC, 28.74% for TC and 66.6% for TT in 87 tested sheep animals.
- The sequence analysis of the two different alleles showed a single nucleotide polymorphism (SNP) (C/T) at position 170 .



Allele C

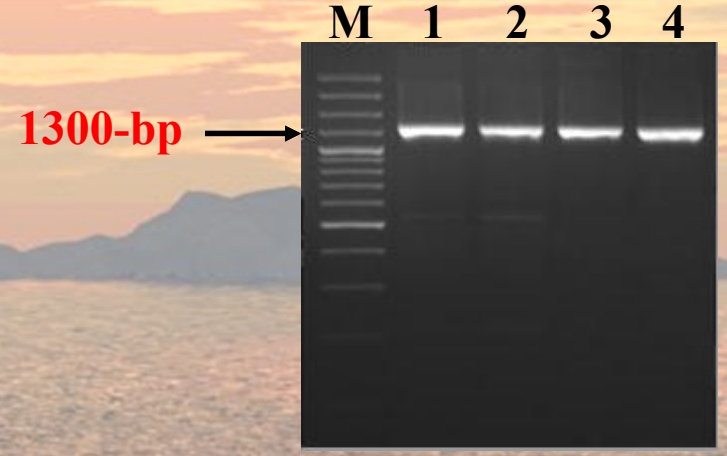


Allele T

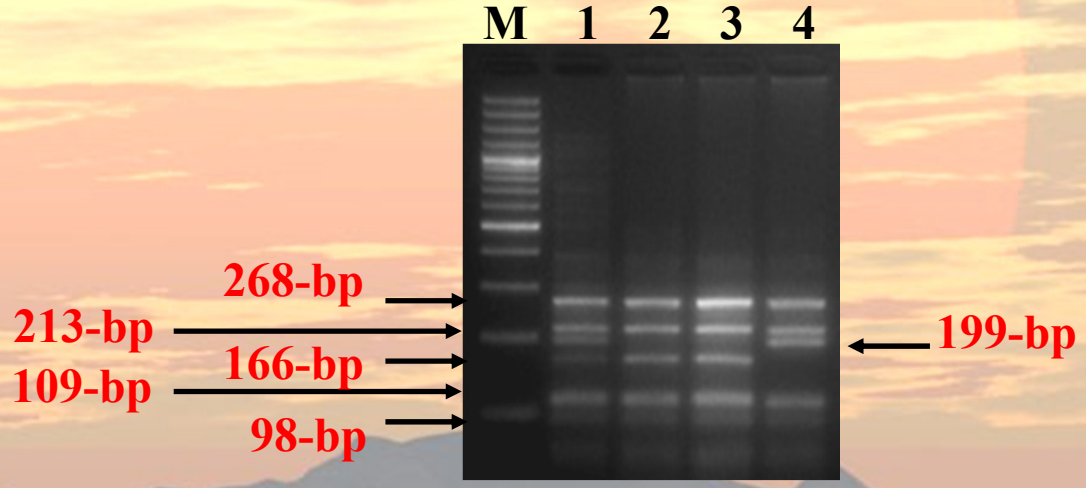
- The nucleotide sequences of C and T alleles of as1-CN gene in Egyptian sheep were submitted to GenBank with the accession numbers KF018340 and KF018339, respectively

αs2-Casein Gene

- The polymorphism of this gene was detected using PCR-RFLP technique. A 1300-bp fragment was amplified by polymerase chain reaction

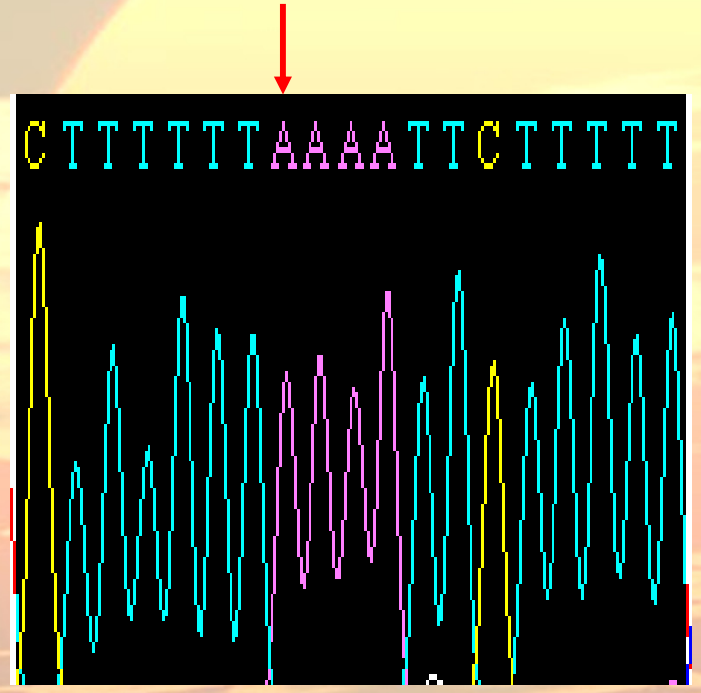


- The digestion of the PCR fragments by *Tru1*I endonuclease enable us to differentiate between three different genotypes; AA, AG and GG.

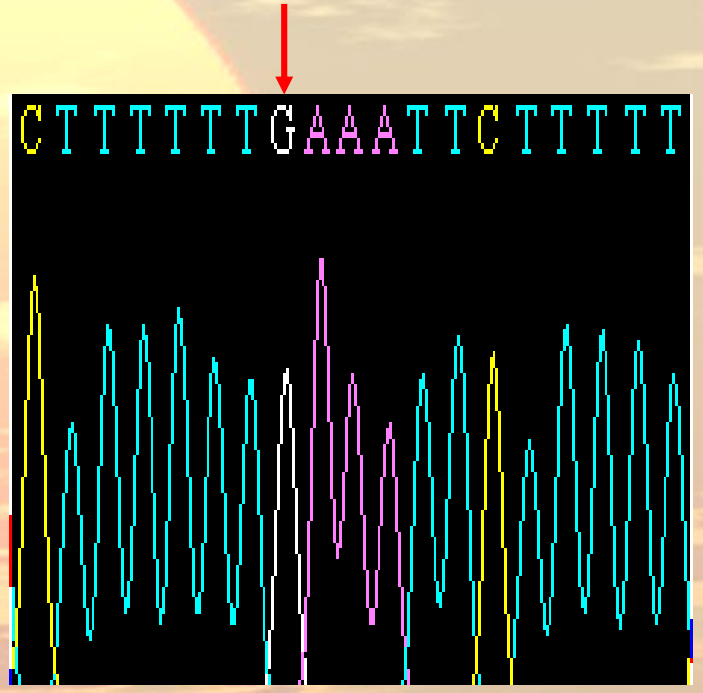


M: 100-bp plus ladder
Lanes 1: AG genotype
Lanes 2 and 3: AA genotype
Lane 4: GG genotype

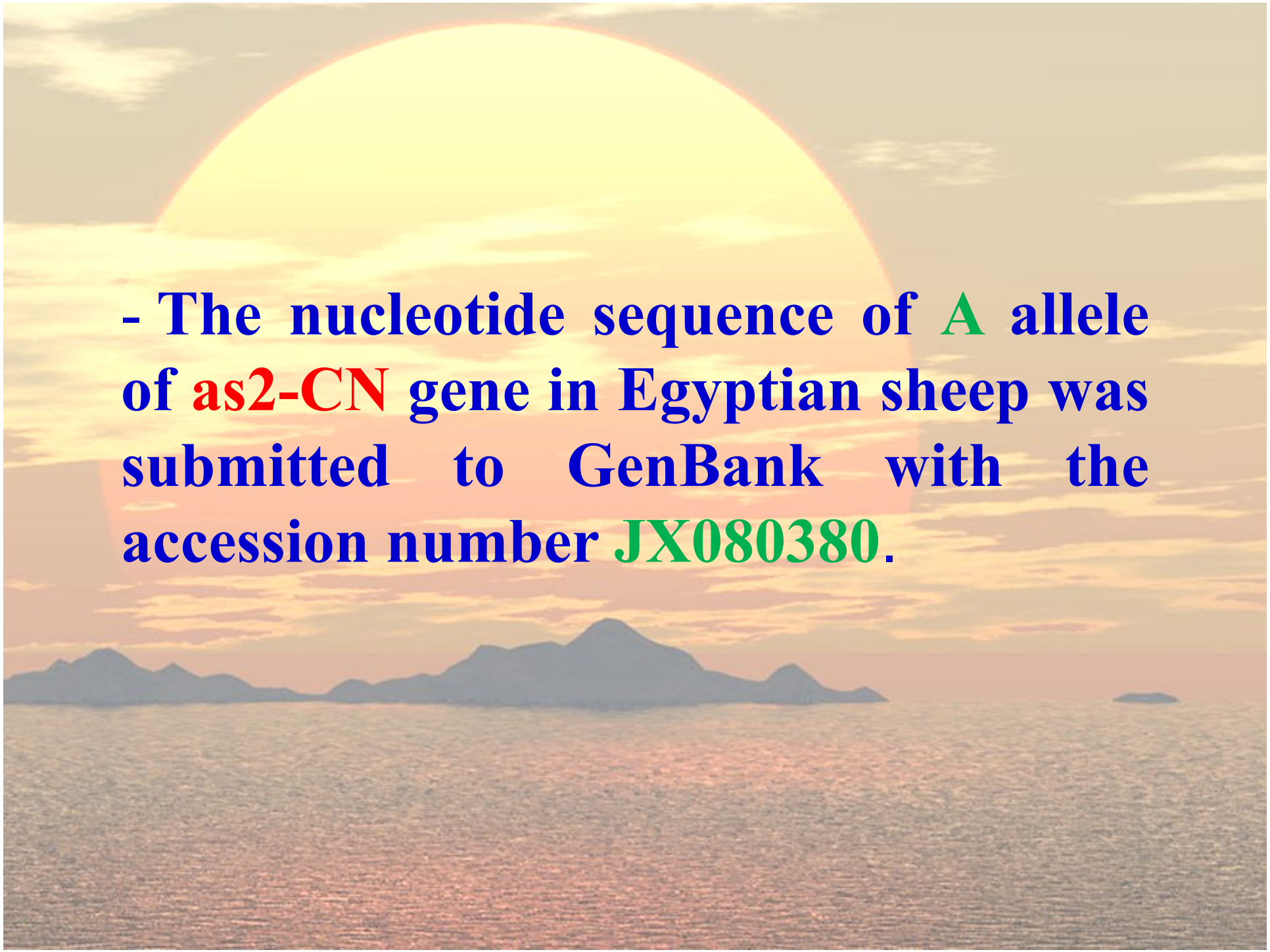
- The frequencies for the three detected genotypes were 86.05% for AA, 12.79% for AG and 1.16% for GG in 86 tested sheep animals.
- The sequence analysis of the two different alleles showed a single nucleotide polymorphism (SNP) (A→G) at position 600 in the amplified fragment.



Allele A

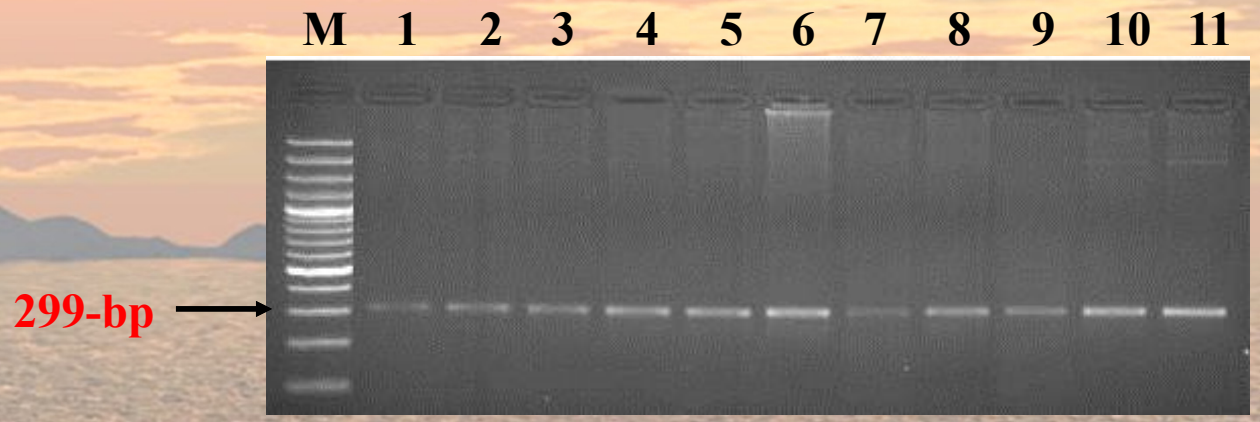


Allele G

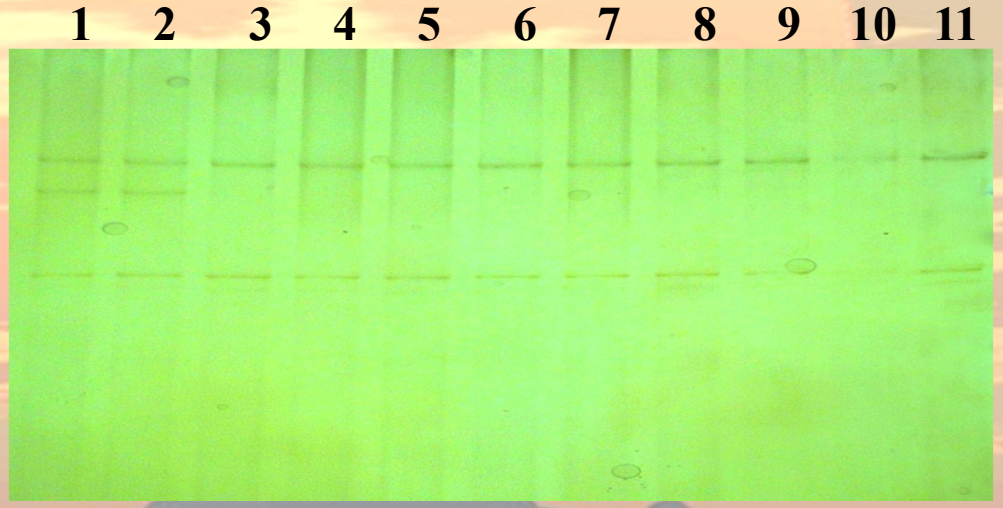

- The nucleotide sequence of A allele of as2-CN gene in Egyptian sheep was submitted to GenBank with the accession number JX080380.

β-Casein Gene

- The polymorphism of this gene was detected using PCR-SSCP technique. A 299-bp fragment was amplified by polymerase chain reaction



- SSCP result recorded the presence of two different patterns

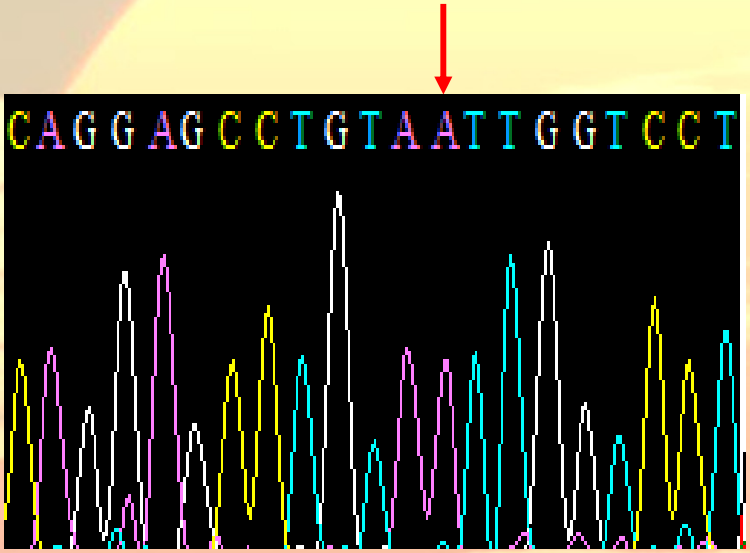


Lanes 1 and 2: pattern I

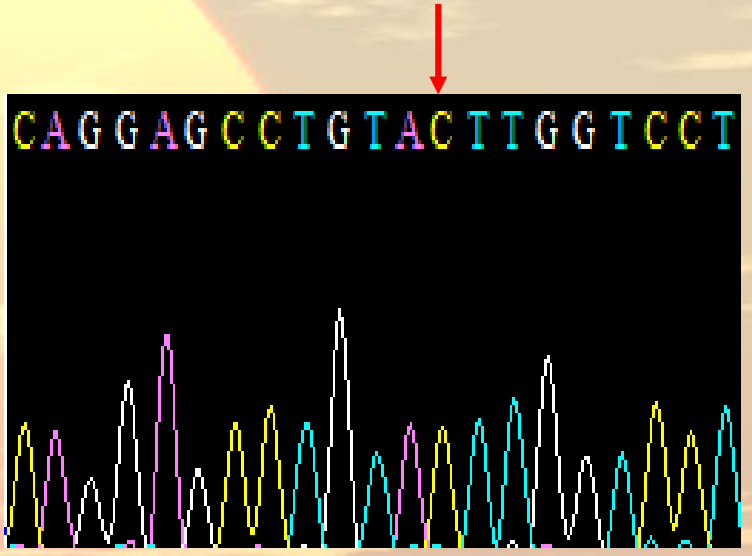
Lanes 3-11: pattern II

-The frequencies for the two detected patterns were 83.53% for pattern I and 16.47% for pattern II in 85 tested sheep animals.

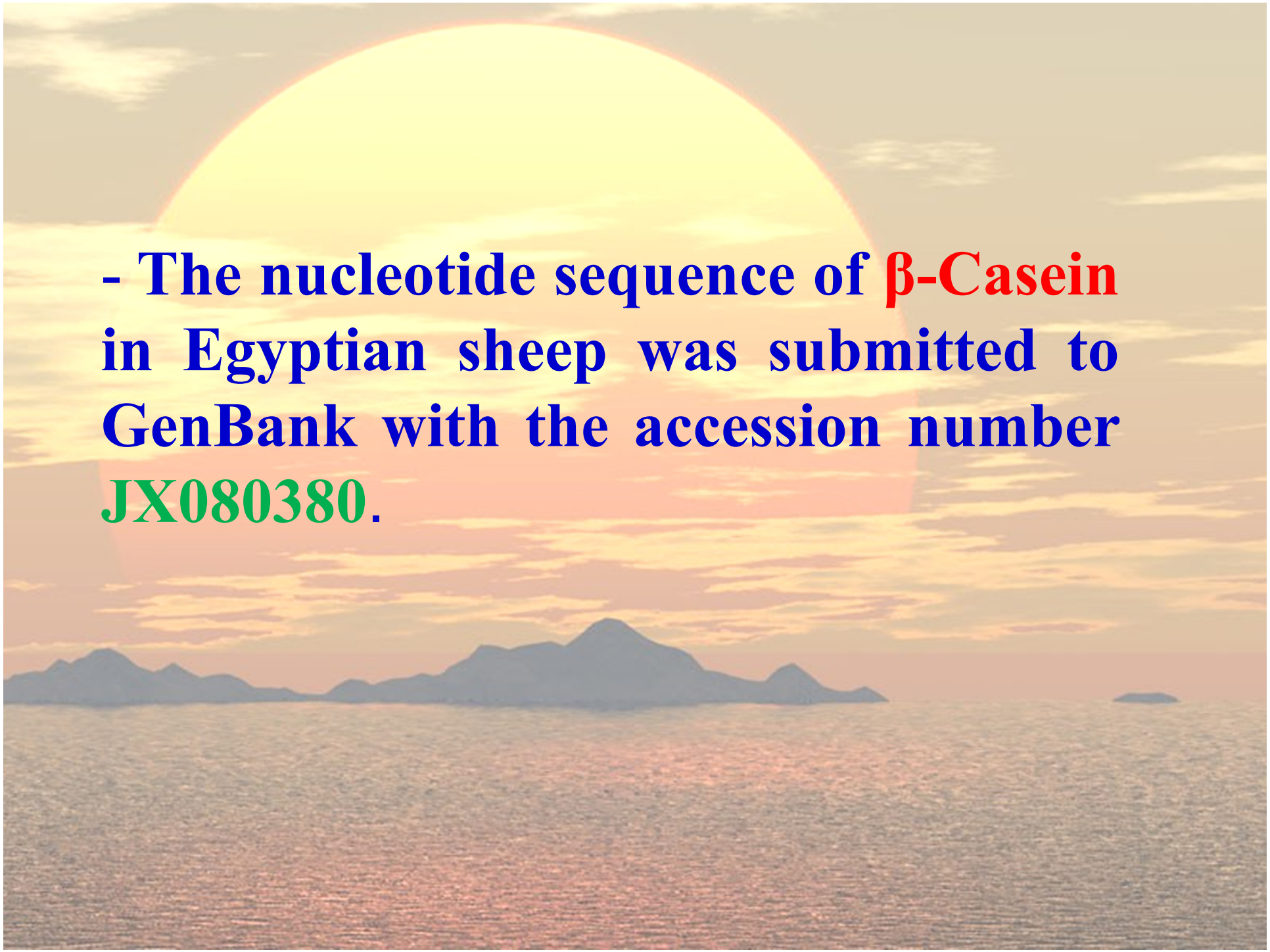
- The sequence analysis of the two different alleles showed the presence of two nucleotide substitutions: A→C and C→T at positions 104 and 193, respectively.



Allele A

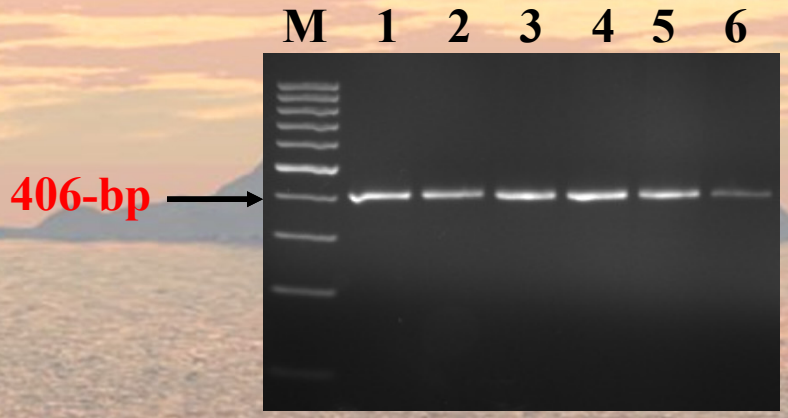


Allele C

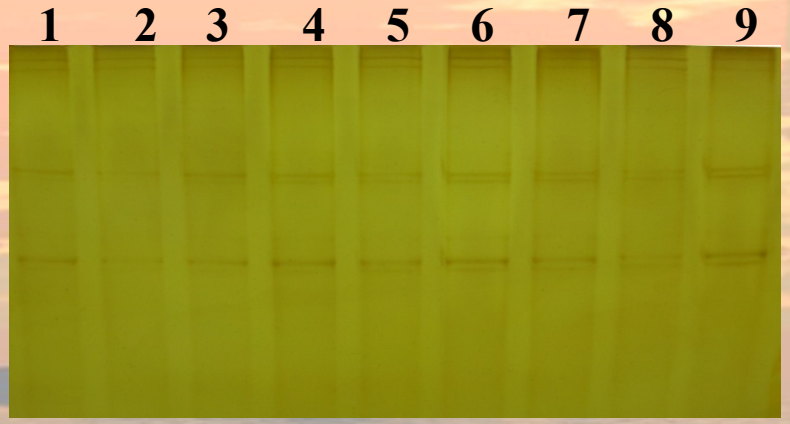

- The nucleotide sequence of β-Casein in Egyptian sheep was submitted to GenBank with the accession number JX080380.

K-Casein Gene

- The polymorphism of this gene was detected using PCR-SSCP technique. A 406-bp fragment was amplified by polymerase chain reaction



- SSCP result of κ-Casein gene showed that all 86 tested sheep animals are monomorphic and possess the same SSCP pattern



Lanes 1-9: The monomorphic pattern of κ-casein gene in Egyptian sheep

- Alignment of Egyptian sheep sequence with k-casein sequence published in database showed the presence of two SNPs; C→T and T→C at positions 18 and 164.

- The nucleotide sequence of K-Casein in Egyptian sheep was submitted to GenBank with the accession number JX050176.

THANK YOU