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Genetic Engineering and Biotechnology Research Division

Cell Biology Department

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

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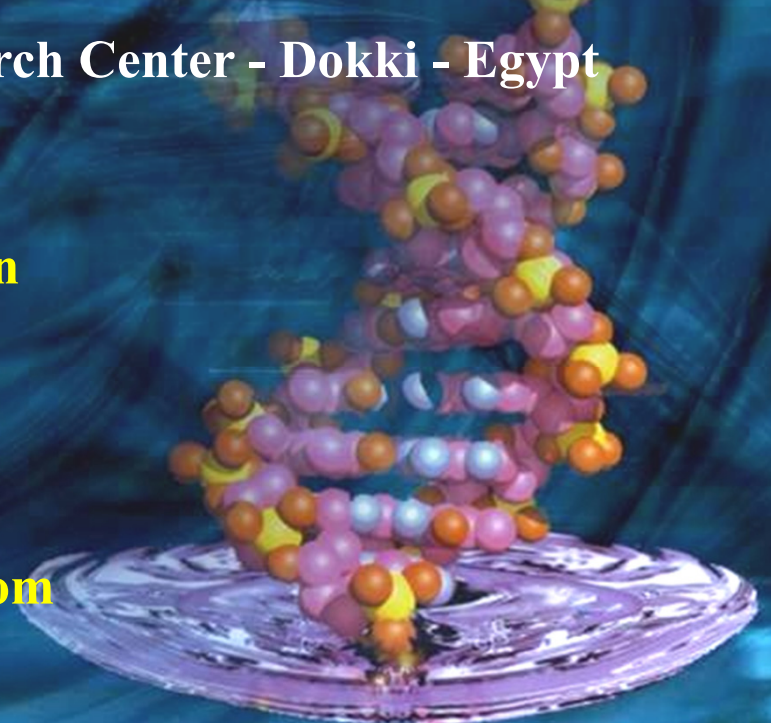
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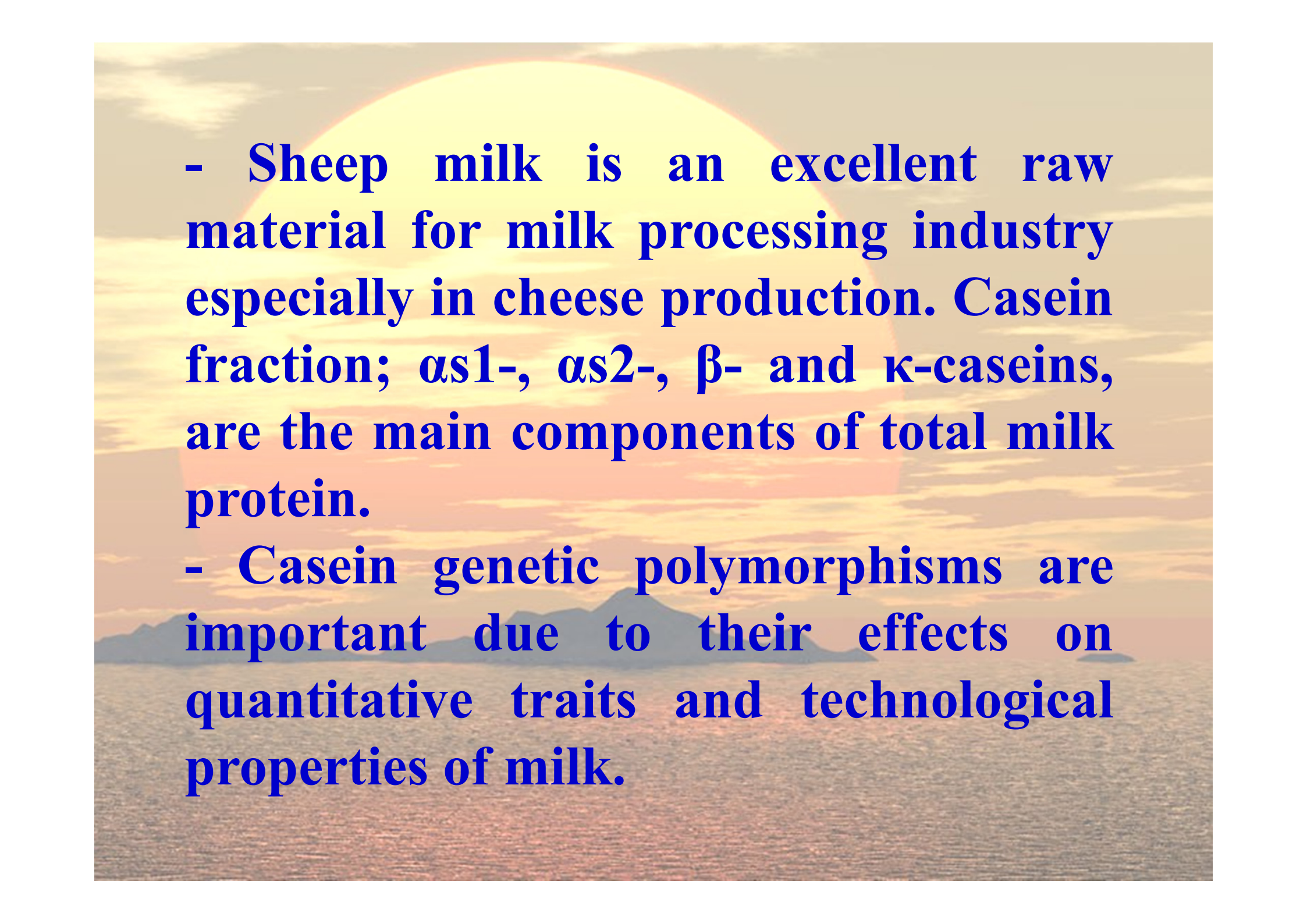
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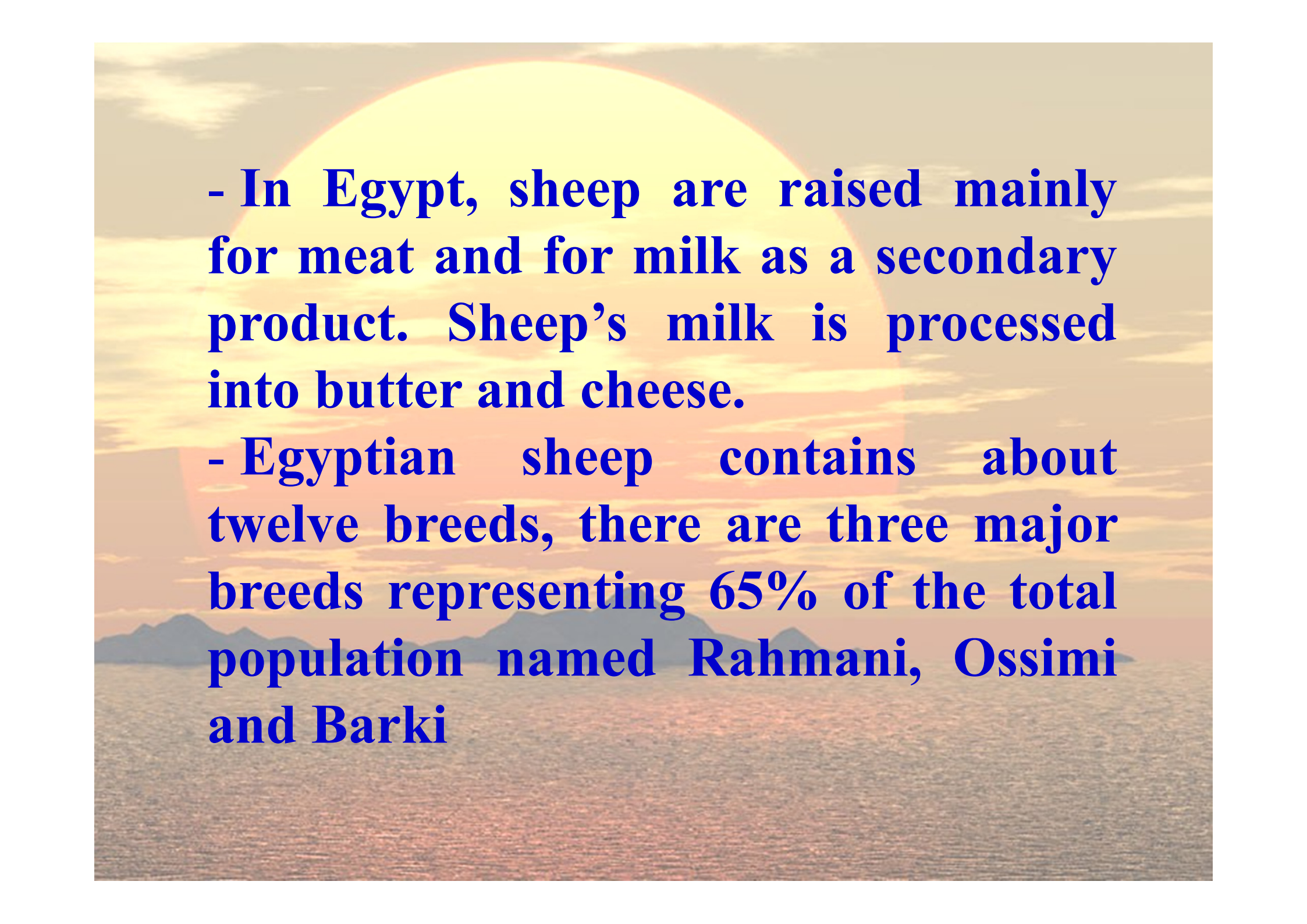
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A sunset over a body of water with mountains in the distance. The sun is a large, bright yellow-orange circle in the upper center, partially obscured by a thin layer of clouds. The sky is a mix of orange, yellow, and light blue. The water in the foreground is dark and reflects the light from the sun. In the background, there are dark, silhouetted mountains.

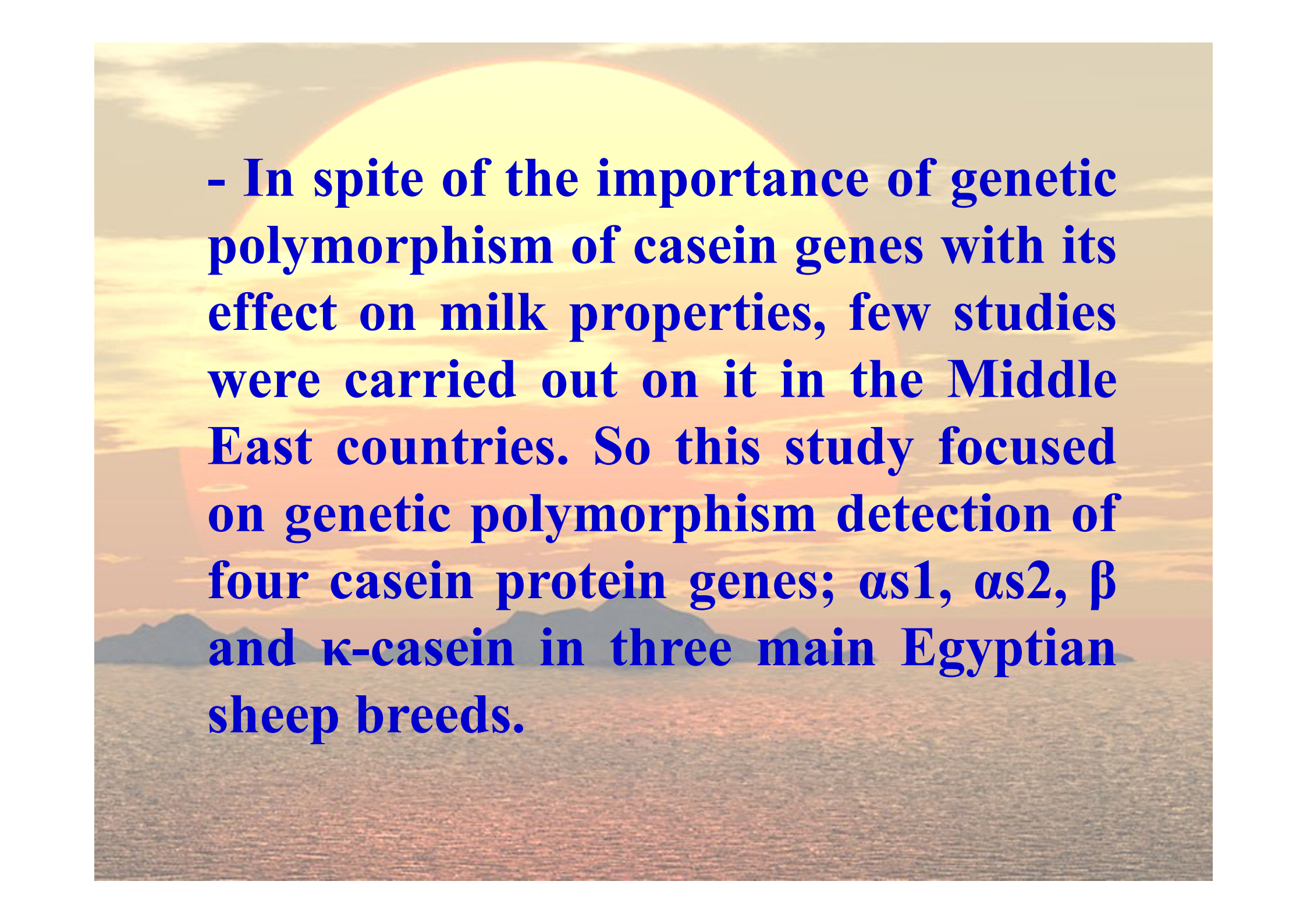
- Sheep milk is an excellent raw material for milk processing industry especially in cheese production. Casein fraction; α s1-, α s2-, β - and κ -caseins, are the main components of total milk protein.

- Casein genetic polymorphisms are important due to their effects on quantitative traits and technological properties of milk.

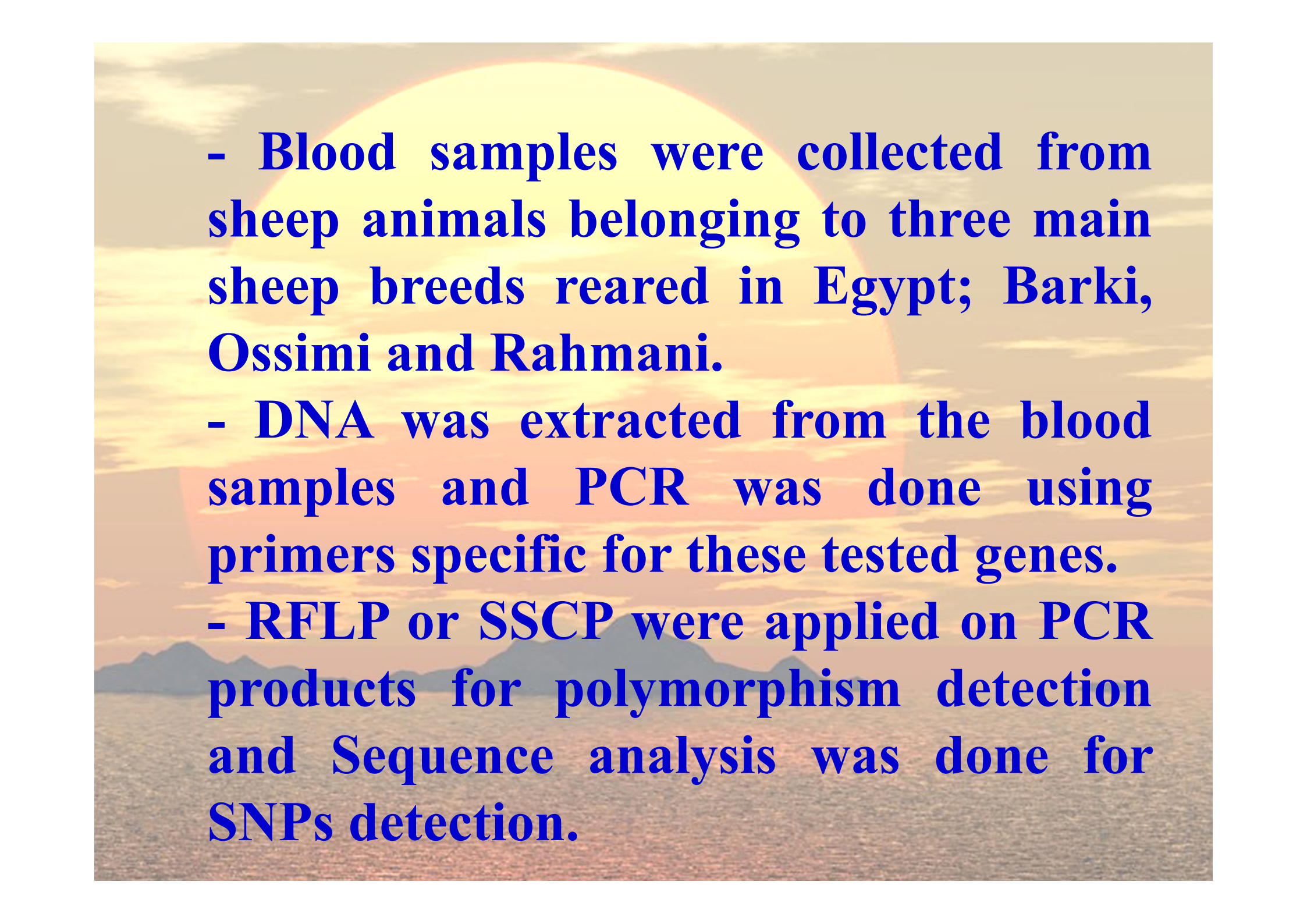
A sunset scene with a large, bright sun low on the horizon, casting a warm glow over a body of water. In the distance, there are silhouettes of mountains. The sky is filled with soft, orange and yellow clouds.

- 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

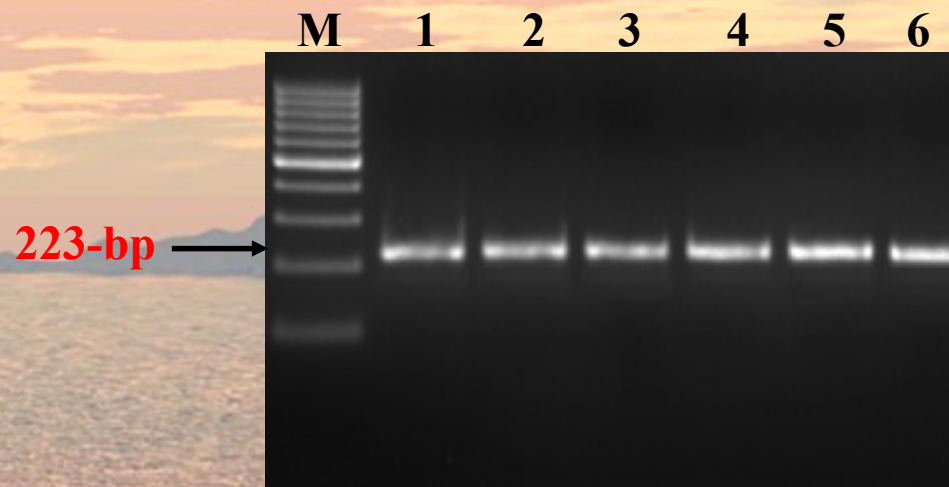
A sunset scene with a large, bright sun low on the horizon, casting a warm glow over a body of water. In the distance, there are silhouettes of mountains or hills. The sky is filled with soft, wispy clouds.

- 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; $\alpha s1$, $\alpha s2$, β and κ -casein in three main Egyptian sheep breeds.

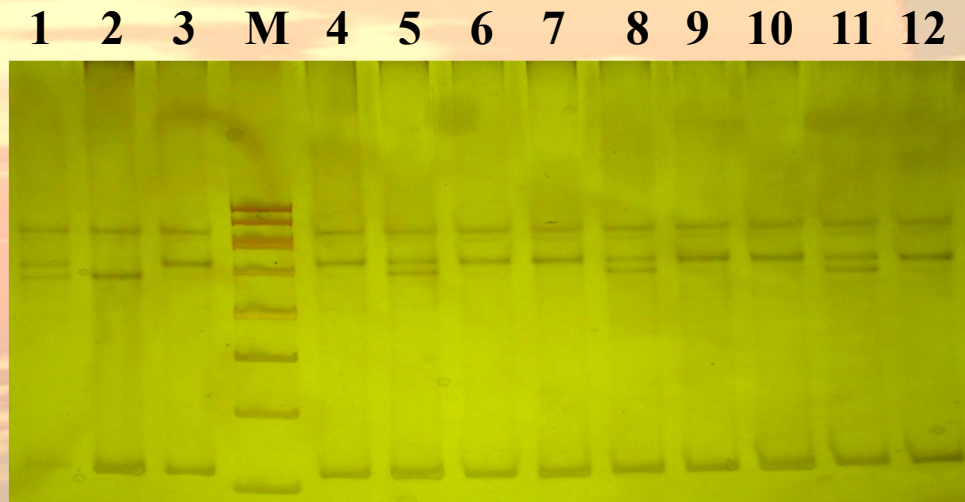
- 
- **Blood samples were collected from sheep animals belonging to three main sheep breeds reared in Egypt; Barki, Ossimi and Rahmani.**
 - **DNA was extracted from the blood samples and PCR was done using primers specific for these tested genes.**
 - **RFLP or SSCP were applied on PCR products for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

α s1-Casein Gene

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



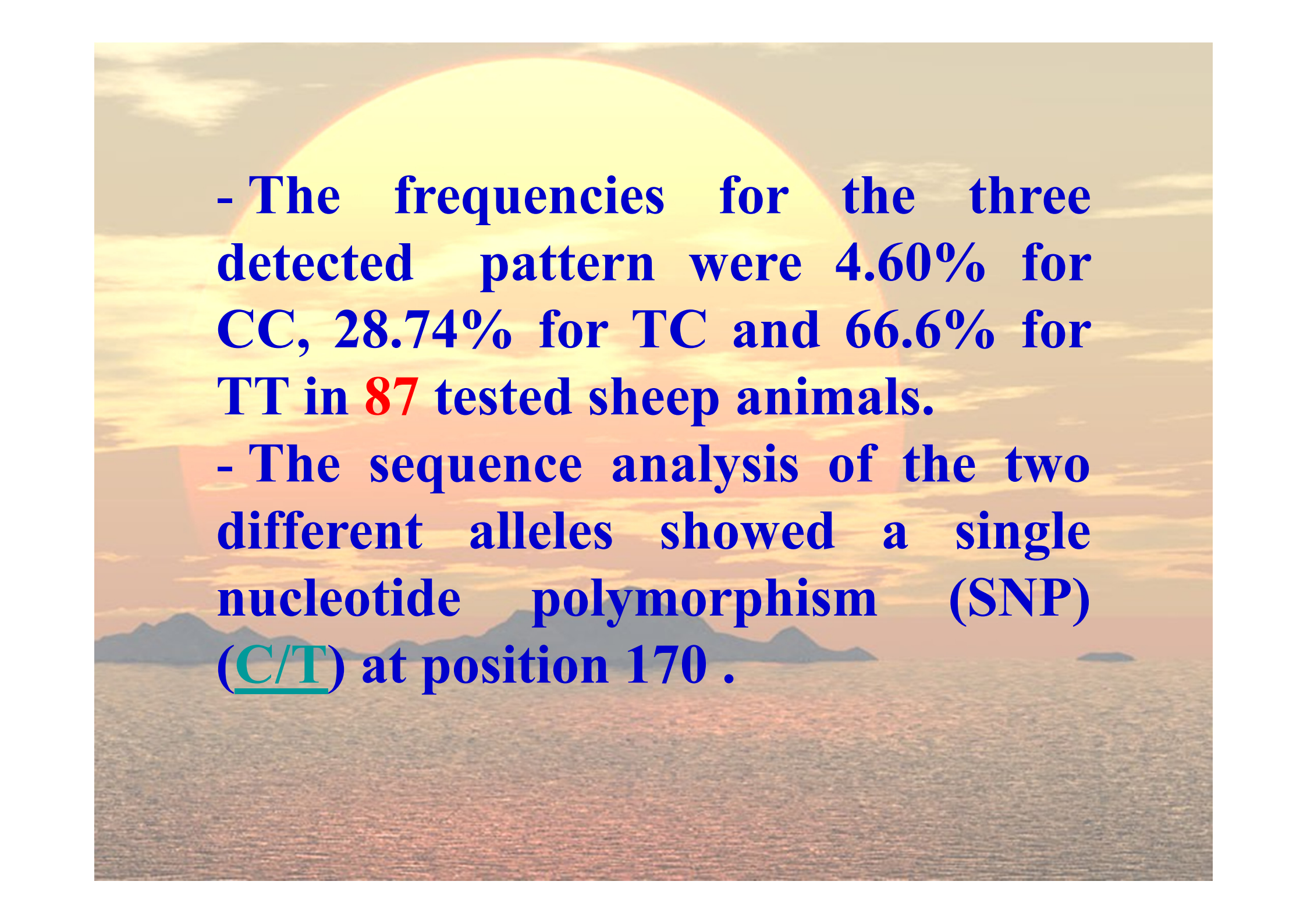
- **SSCP** result recorded the presence of three different patterns. **CC**, **CT** and **TT**



Lane 2: pattern **CC**

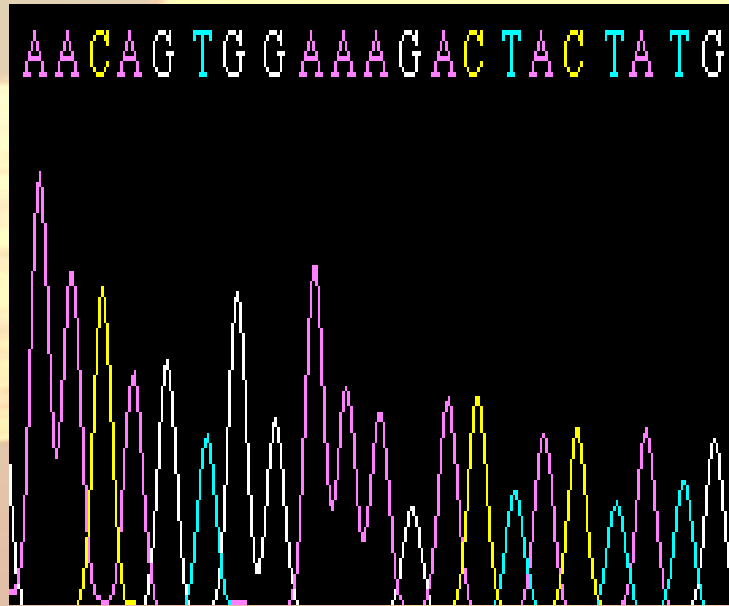
Lanes 1, 5, 8 and 11: pattern **CT**

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

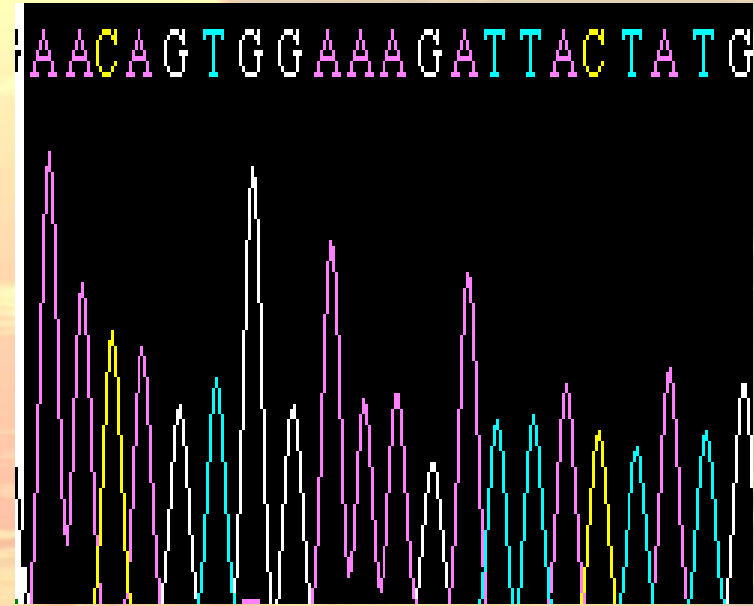


- The frequencies for the three detected patterns were 4.60% for CC, 28.74% for TC and 66.6% for TT in **87** tested sheep animals.

- The sequence analysis of the two different alleles showed a single nucleotide polymorphism (SNP) (C/T) at position 170 .



Allele C



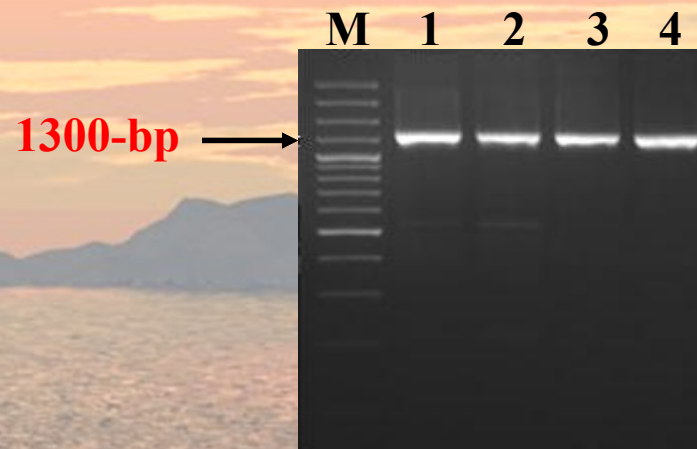
Allele T

A sunset scene with a large, bright sun low on the horizon, casting a golden glow over a body of water. In the distance, there are silhouettes of mountains or hills. The sky is filled with soft, wispy clouds.

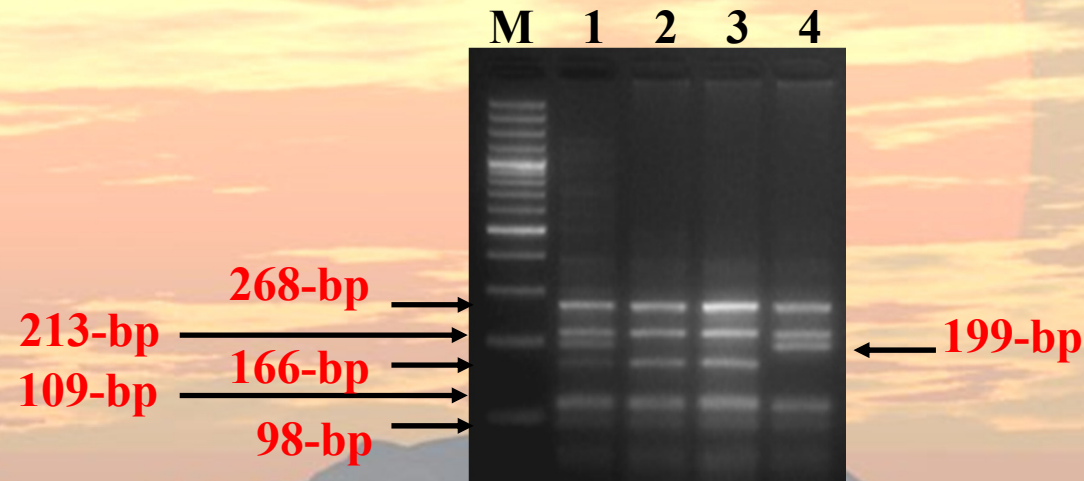
- The nucleotide sequences of **C** and **T** alleles of **as1-CN** gene in Egyptian sheep were submitted to GenBank with the accession numbers **KF018340** and **KF018339**, respectively

α s2-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 *TruII* endonuclease enable us to differentiate between three different genotypes; AA, AG and GG.



M: 100-bp plus ladder

Lanes 1: AG genotype

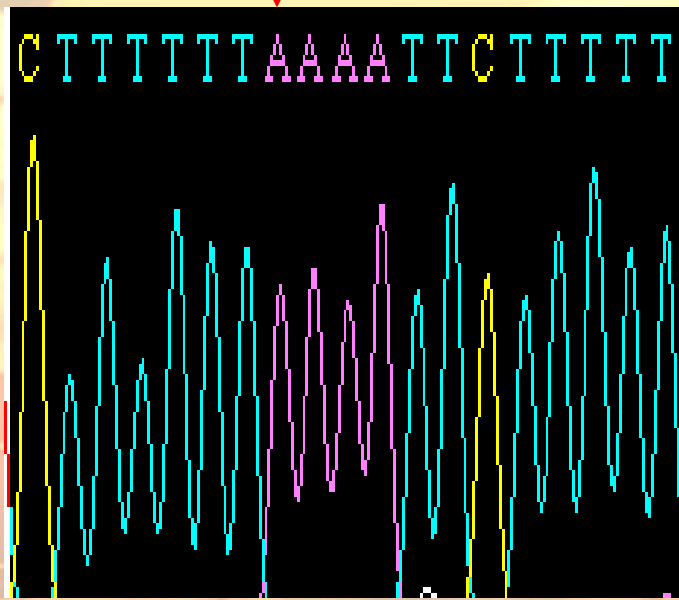
Lanes 2 and 3: AA genotype

Lane 4: GG genotype

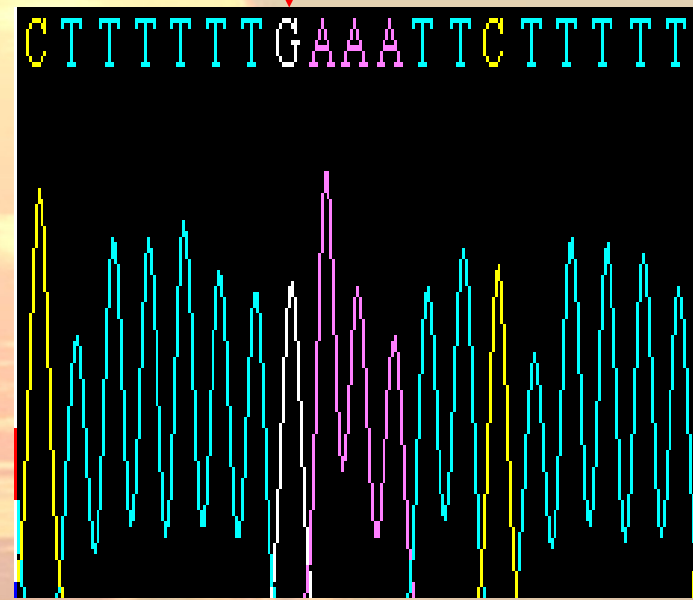


- The frequencies for the three detected genotypes were 86.05% for AA, 12.79% for AG and 1.16% for GG in 86 tested sheep animals.

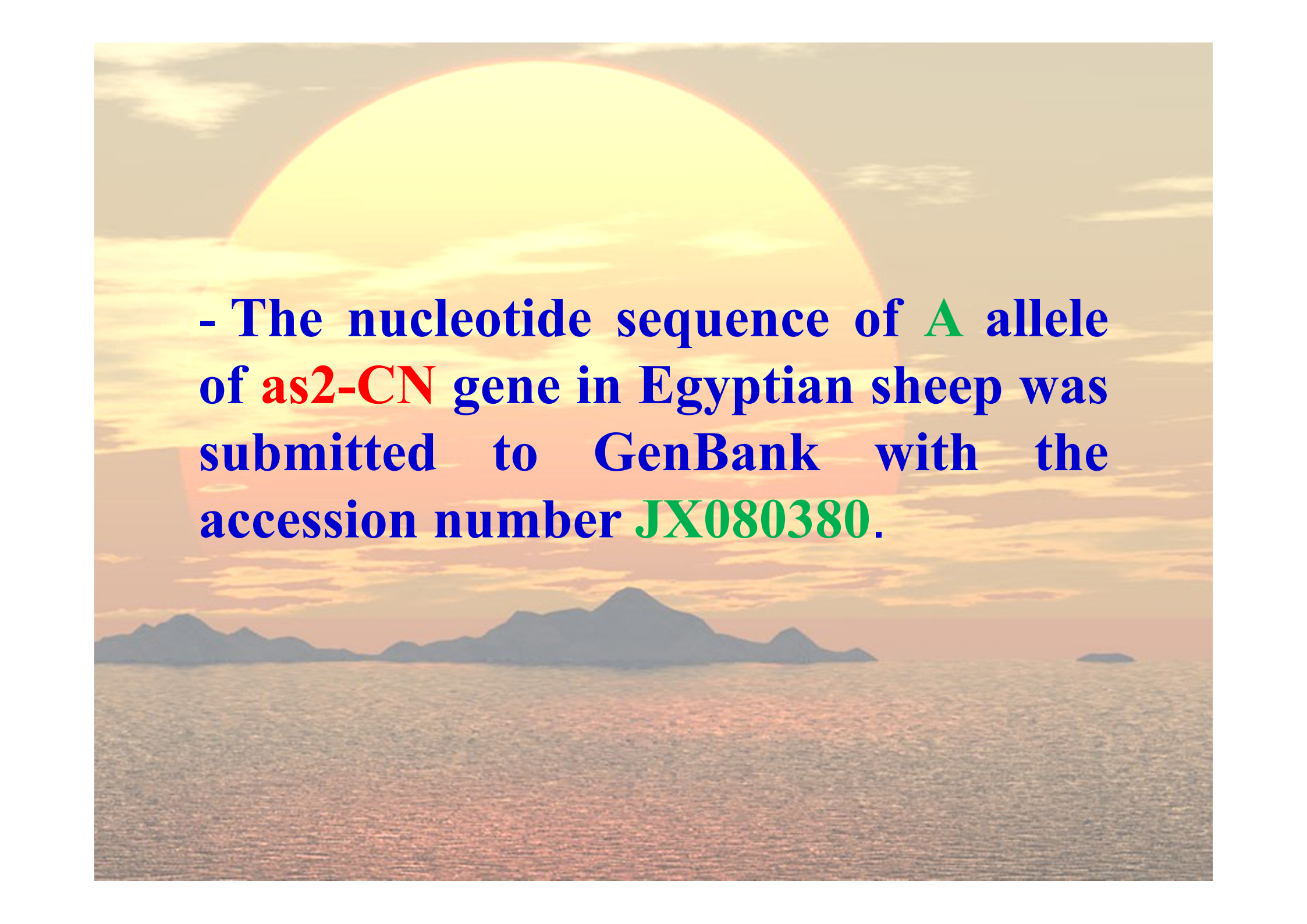
- The sequence analysis of the two different alleles showed a single nucleotide polymorphism (SNP) (A→G) at position 600 in the amplified fragment.



Allele A



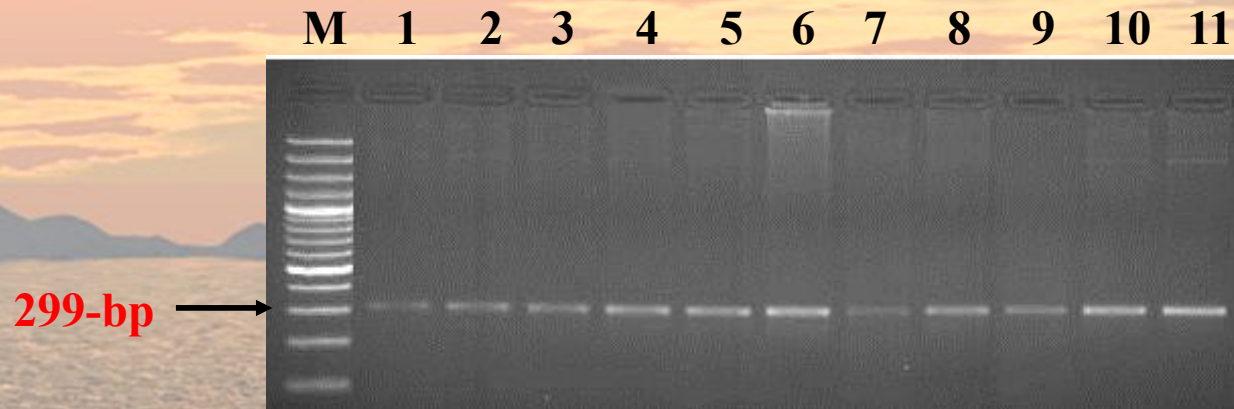
Allele G

A sunset scene with a large, bright sun partially obscured by a large, semi-transparent white circle. The sun is low on the horizon, casting a golden glow over the sky and the water below. The water is dark with some ripples, and there are silhouettes of mountains or hills in the distance. The overall color palette is warm, with yellows, oranges, and browns.

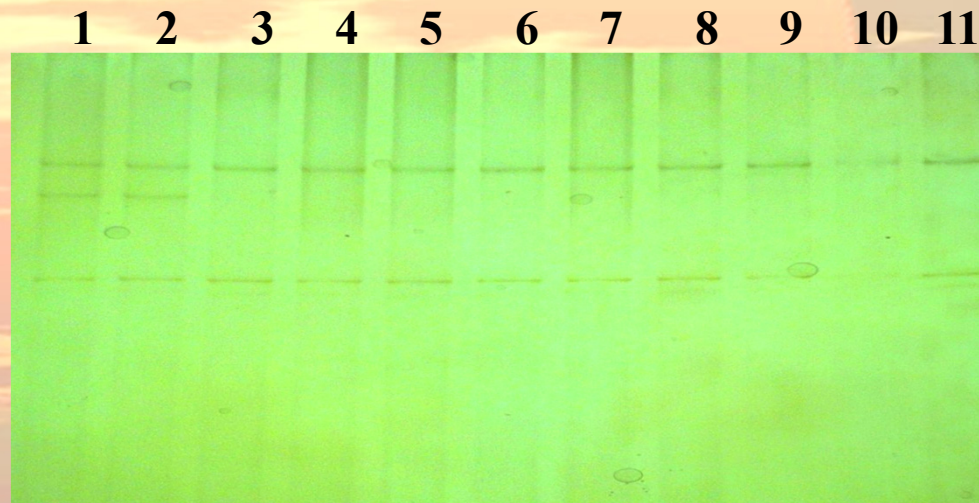
- The nucleotide sequence of **A** allele of **as2-CN** gene in Egyptian sheep was submitted to GenBank with the accession number **JX080380**.

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

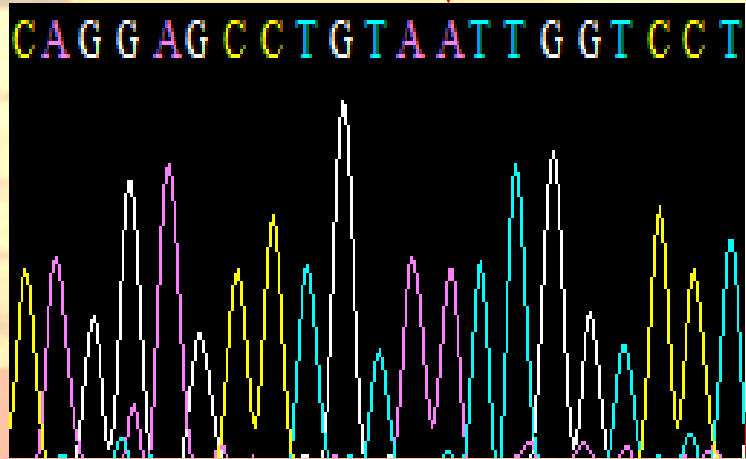


Lanes 1 and 2: **pattern I**

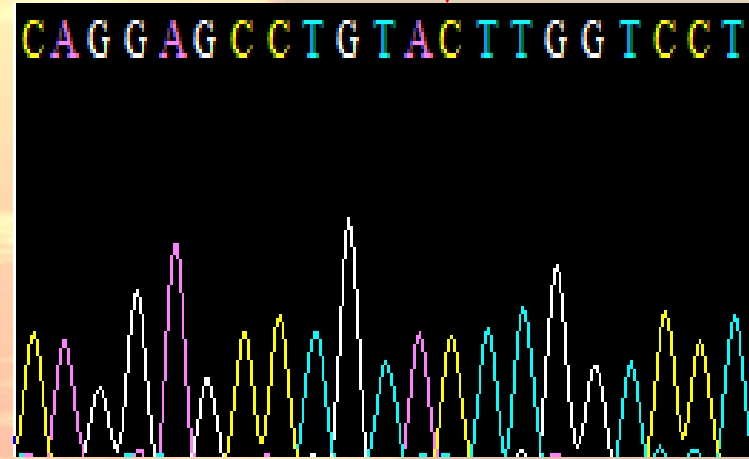
Lanes 3-11: **pattern II**

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

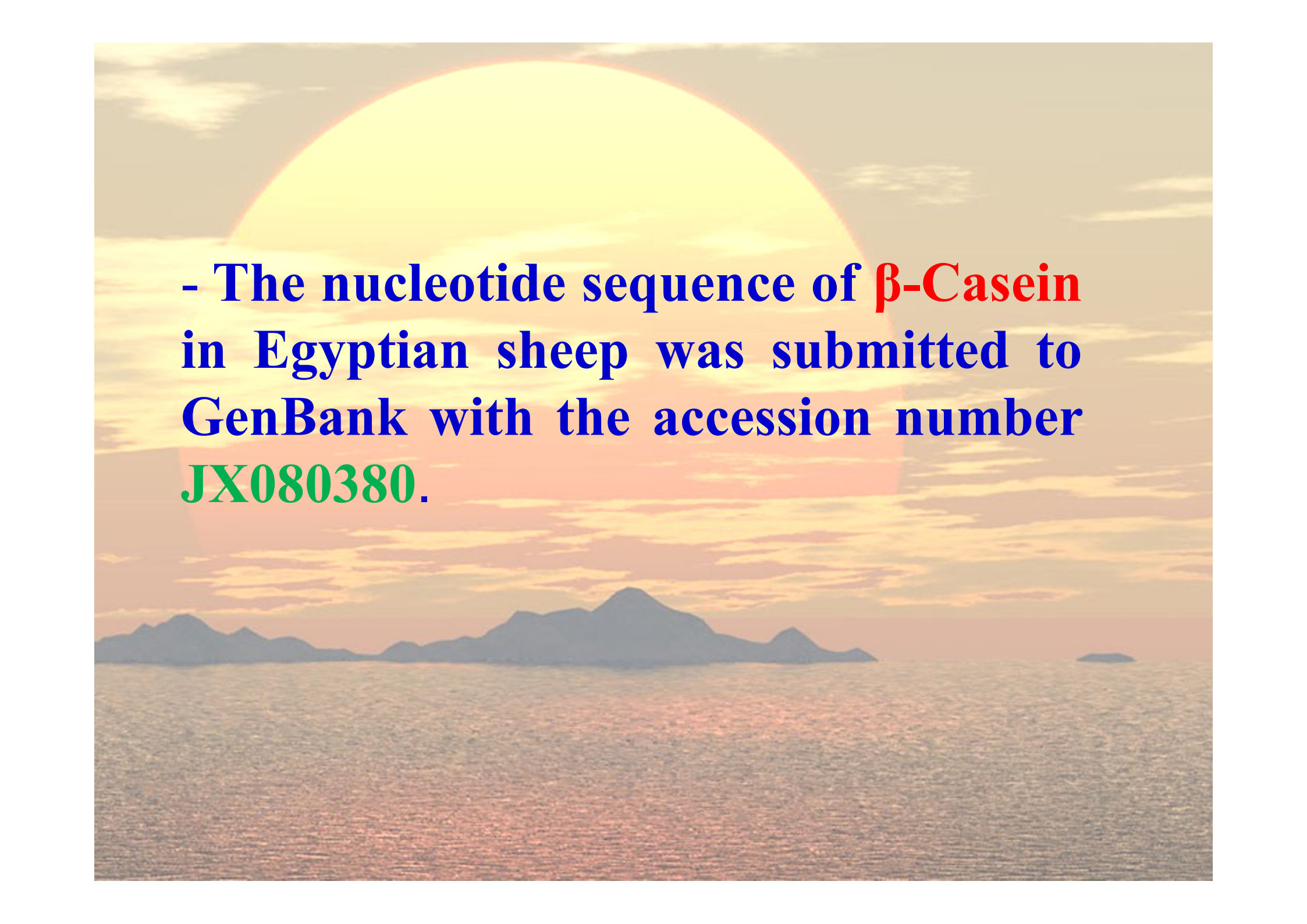
- The sequence analysis of the two different alleles showed the presence of two nucleotide substitutions: A→C and C→T at positions 104 and 193, respectively.



Allele A



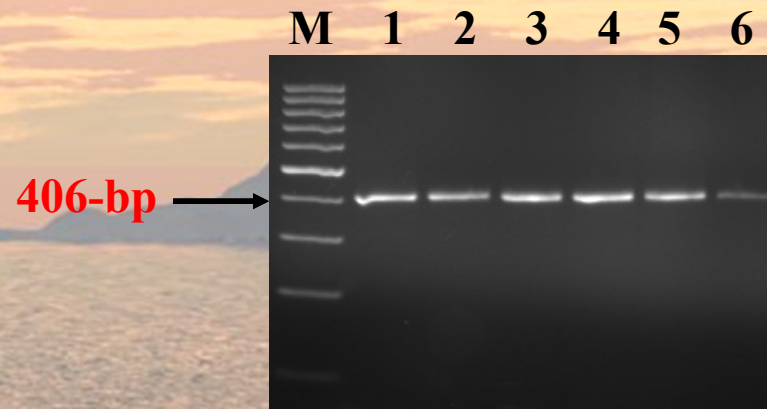
Allele C

A large, bright sun is setting behind a range of mountains, casting a golden glow over the sky and reflecting on the water in the foreground. The sun is partially obscured by a semi-transparent white box containing text.

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

K-Casein Gene

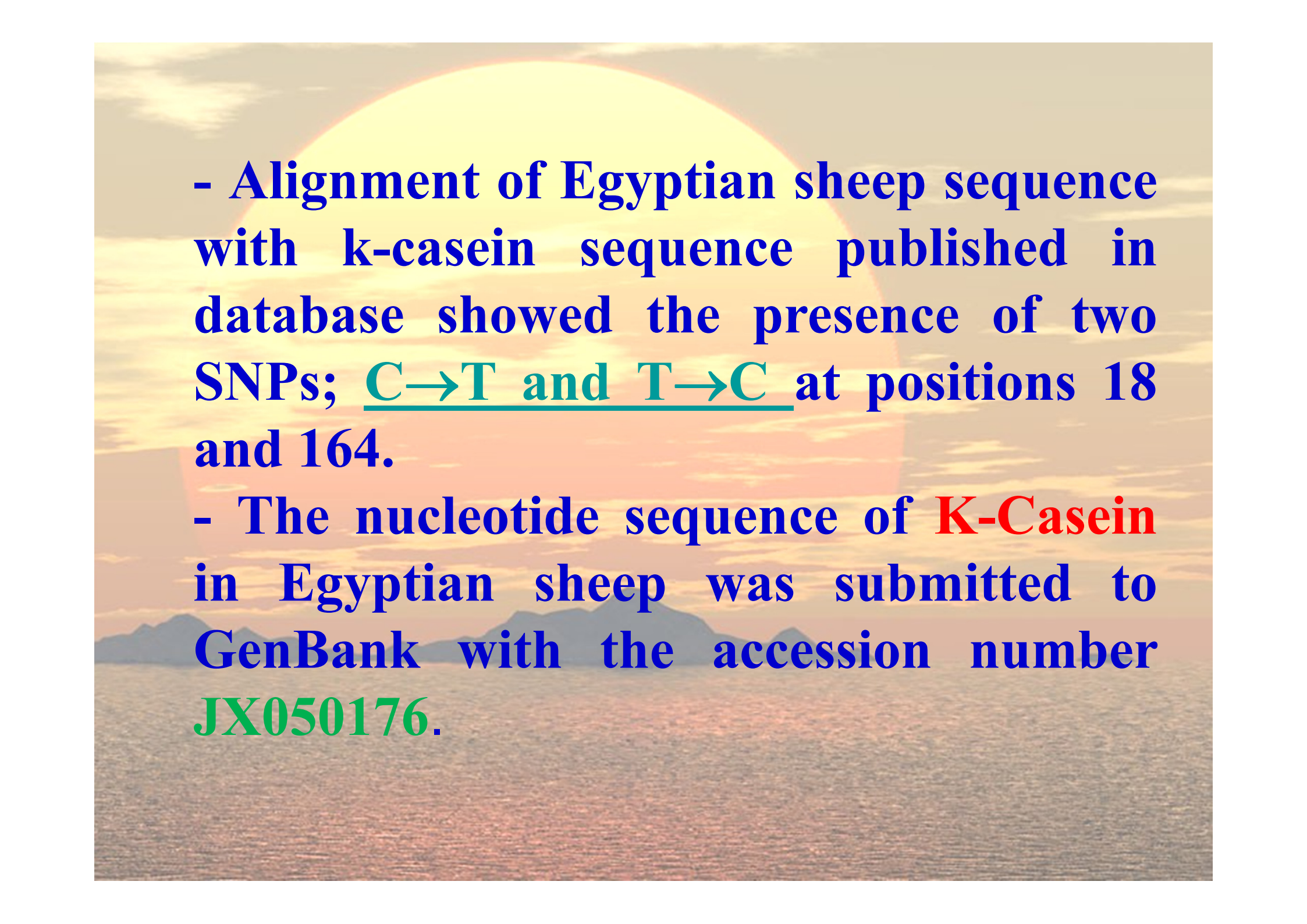
- The polymorphism of this gene was detected using PCR-SSCP technique. A 406-bp fragment was amplified by polymerase chain reaction



- 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