

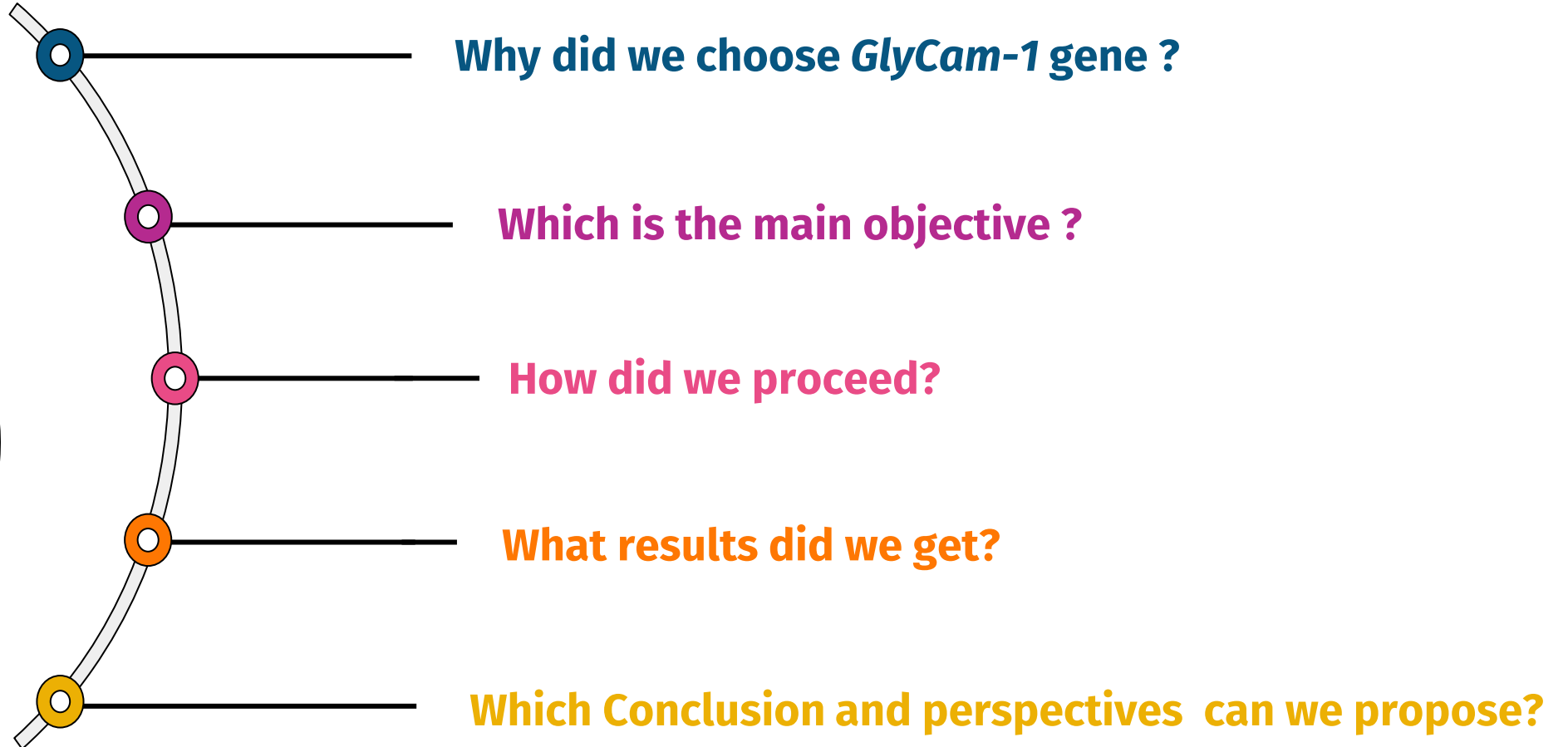
Glycosylation dependent cell adhesion molecule, first genetic diversity investigation in camelids

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Session 26: Production potential and adaptation of camelid species to extensive, semi-intensive and intensive production systems in arid and semi-arid environments



Plan



Introduction

MILK PROVIDES US:



Proteins



Fats



Lactose



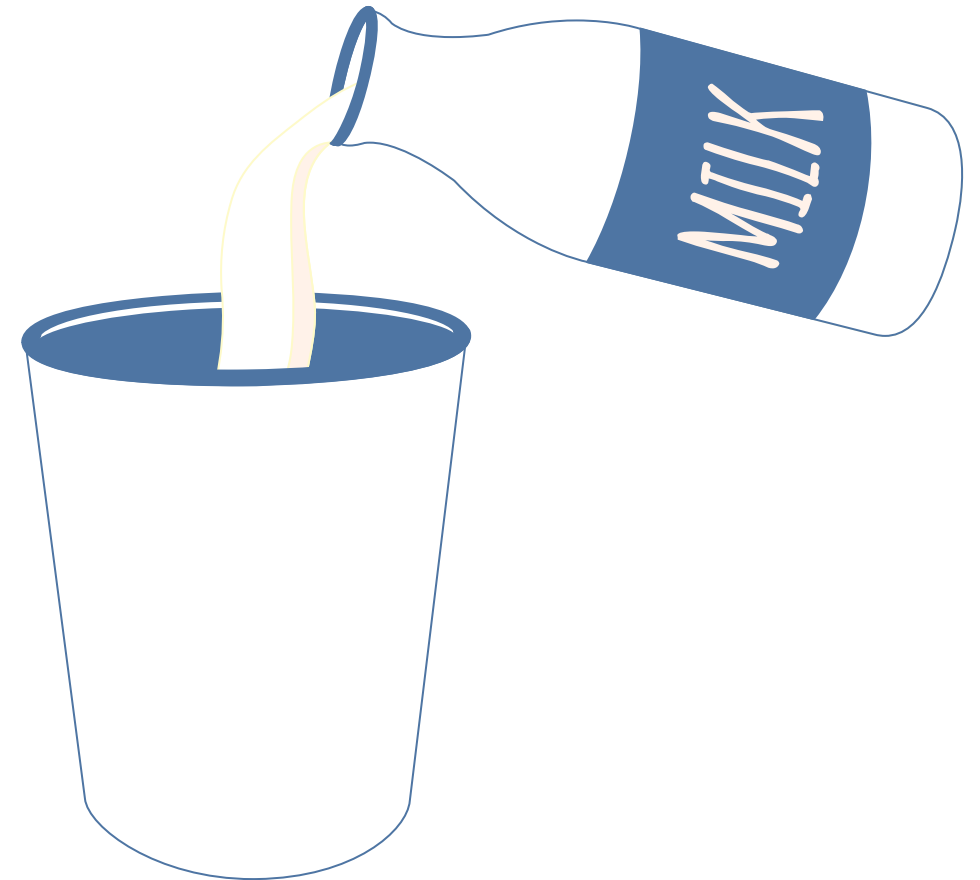
Vitamins



Minerals



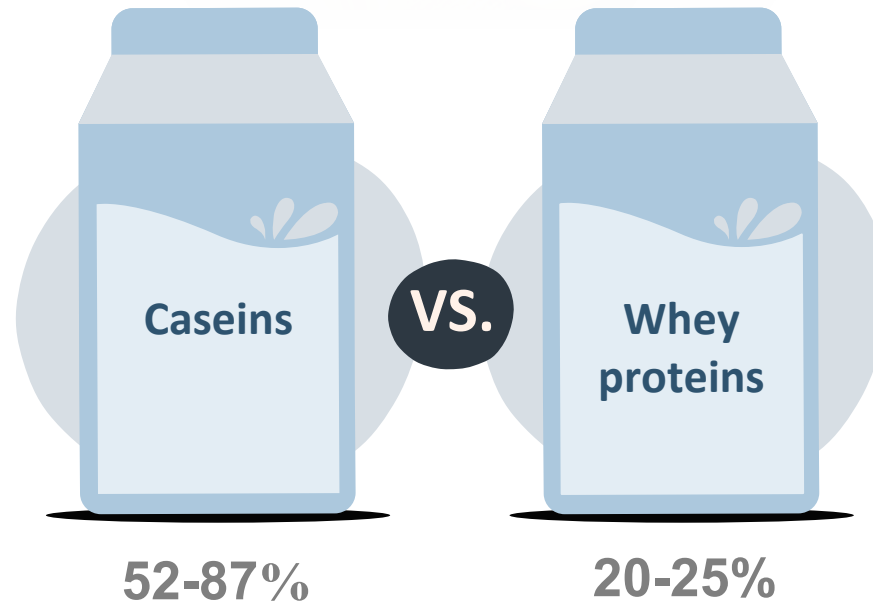
Water



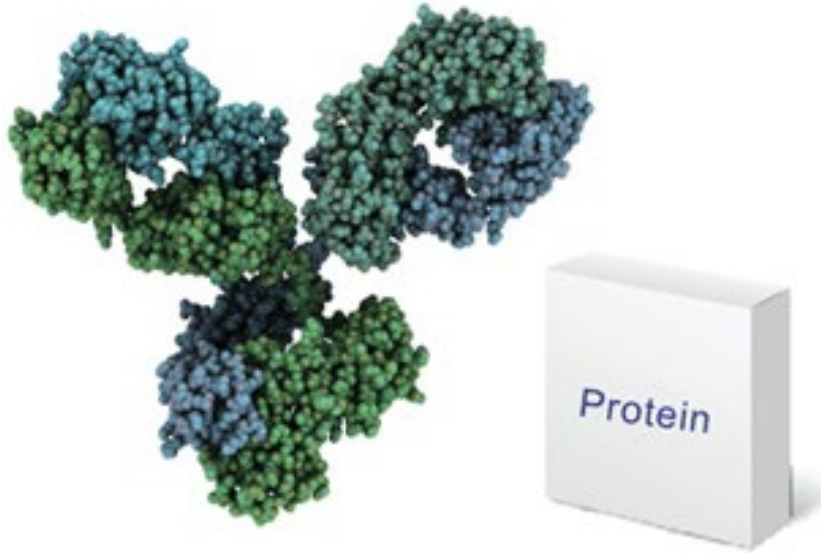


Caseins VS. Whey proteins

- β casein
- α S1 casein
- α S2 casein
- K casein



- α -lactalbumin
- IgG
- Lactoferrin
- **GlyCAM-1**
- serum albumin
- Camel whey basic protein



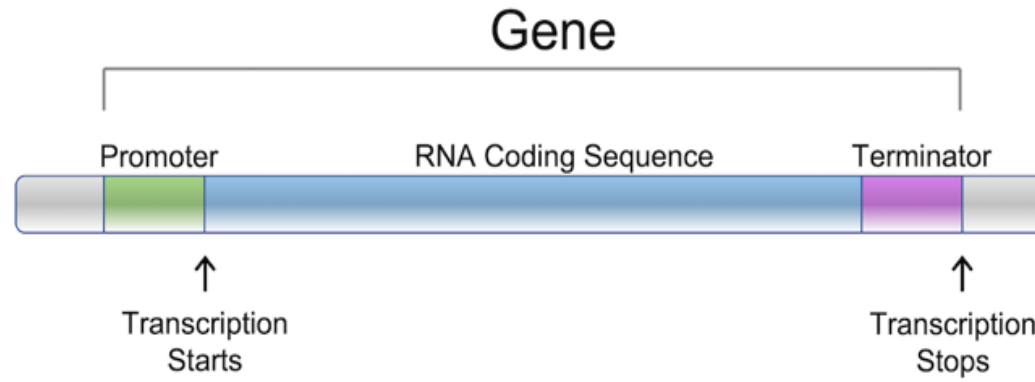
GlyCam-1

- Expressed in the lactating mammary gland during pregnancy and lactation
- Its concentration in camel milk is three times higher than in cow's milk, measuring 954 mg l^{-1} compared to 300 mg l^{-1} .
- Preventing fat globule aggregation during fat globules secretion into the alveolar lumen of the lactating udder (Kappeler et al, 1991)
- Preventing the occurrence of infections of the mastitis type in the lactating animals (Sørensen and Petersen 1993)



- Lactophorin or *GlyCAM-1* gene has been described in different species.
- Two non-allelic variants, A (156 aa) and B (141 aa) were identified in camel Lactophorin protein.
- In particular, the variant B was 15 amino acids shorter than variant A, likely as result of an alternative splicing of the exon 2

Objectives



Lactophorin



GlyCAM-1

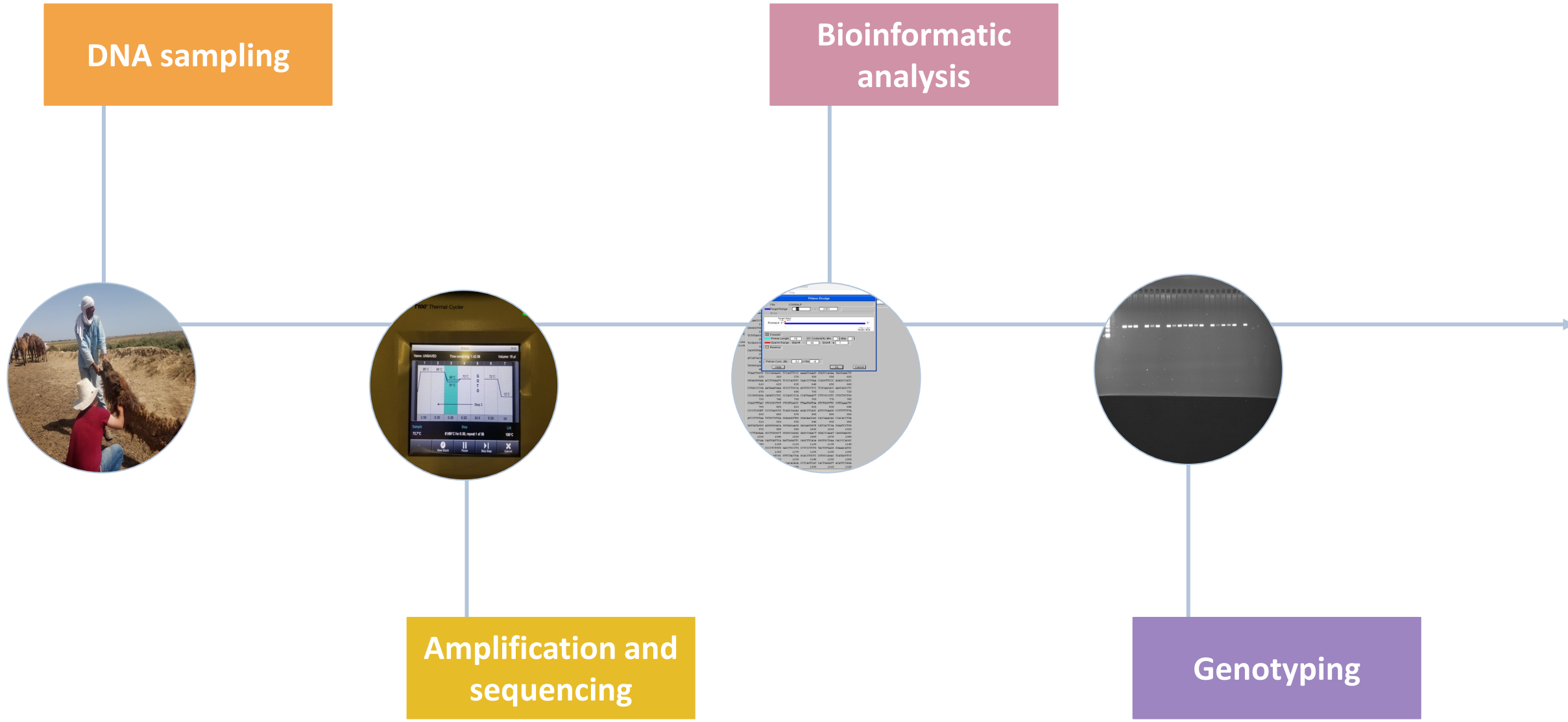


Old world camelids



New world camelids

Methodology



Gene structure



- Consists of 4 exons and 3 introns

- Open Reading Frame: 154 amino acids

- Stop codon: between nucleotides 130-134 of exon 4

- Polyadenylation signal: 95bp downstream from the stop codon

Entire gene: 2272 bp, 499 bp in exonic regions, 1773 bp in intronic regions

5' flanking region: 662/661 bp

3' flanking region: 274 bp

Genetic variability

Dromedary camel

- **14** polymorphic sites:
 - ✓ 1 in the promoter region
 - ✓ 4 in the exonic region
 - ✓ 9 in the introns
- The **4** mutations detected in the exons were **synonymous**

Bactrian camel

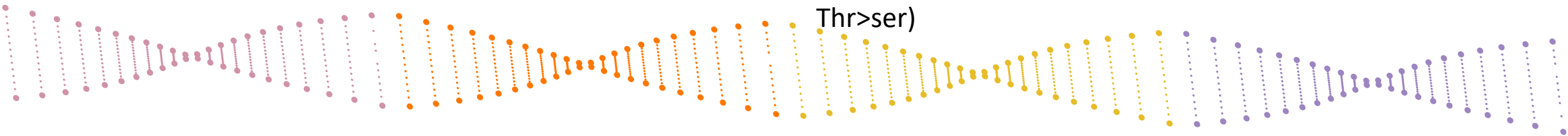
- **7** polymorphic sites:
 - ✓ 4 in the introns
 - ✓ 3 in the 3end region
- **No** mutations were detected in the promoter and in the exonic regions

Llama

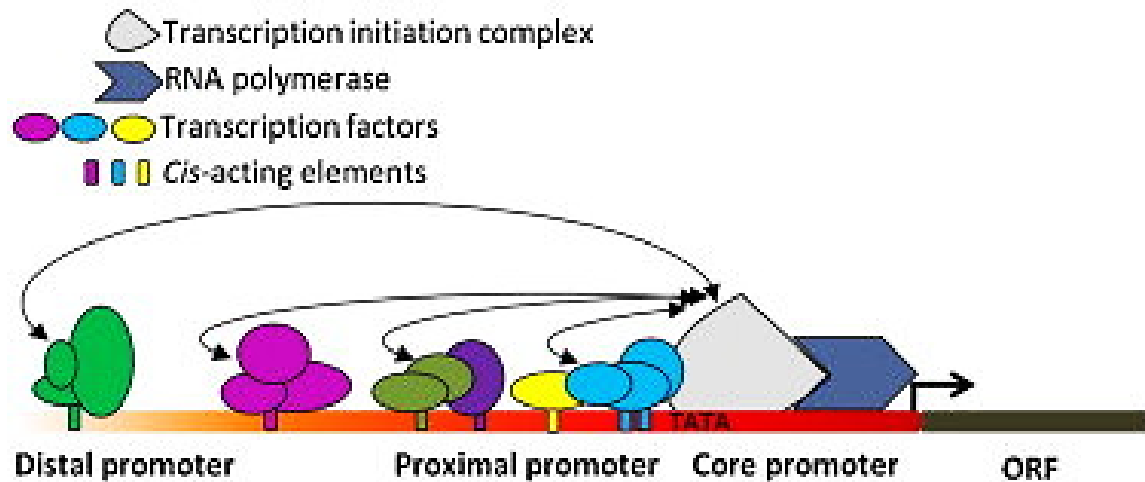
- **38** polymorphic sites:
 - ✓ 12 in the promoter
 - ✓ 3 in the exonic region
 - ✓ 18 in the introns
 - ✓ 6 in the 3end region
- **2** mutations detected in the exons were responsible for an AA change (G>A → Glu>Lys and G>A → Thr>ser)

Alpaca

- **31** polymorphic sites:
 - ✓ 7 in the promoter region
 - ✓ 5 in the exonic region
 - ✓ 19 in the introns
- **3** out of the 5 mutations detected in the exons were non-synonymous (G>A → Glu>Lys , G>A → Ser>asn and T>C → pro>ser)



GlyCam-1 Gene promoter



Gene promoters have transcription factor (TF) binding sites necessary for the regulation of gene expression.

Mutations in the promoters have been reported to modify the transcription rates or the mRNA stability, thus affecting the protein yield.

GlyCam-1 Gene promoter

Arabian camel

- The substitution of C by A was responsible for the creation of an additional transcription factor binding site for **GATA-1**

```
Sequence seq_277

=====
seq( 0.. 59)   ttatgaagacacctcacctccgtgaacatctgtttcttcttgataataacacacac
Segments:
=====
seq( 60.. 119) ctcacaacctcatgaattaataaagagcccatatgaacaactcaggacaatgcctgg
Segments:
=====
seq( 120.. 179) caggggctggaatccctttaactgcctgacccctccactcctgaacatgtgggagcct
Segments:
2.3.1.0      121 131   =====Sp1=====
2.2.1.1      177 186   =====
=====
seq( 180.. 239) tatctgtagcctagaggacagcctagccctcttattcattcattcgtctgtaagtt
Segments:
2.2.1.1      177 186   GATA-1=
2.1.1.2      192 201   =====PR=====
=====
seq( 240.. 299) attcggccctacgacacagagctcttcttaggcctggataaacaatgcaagtaaa
Segments:
2.3.1.0      243 252   =====Sp1=====
=====
seq( 300.. 359) gcagggtgaaccaggcactgtggaagtctcattctctgggaagatagctattaatta
Segments:
=====
seq( 360.. 419) aatcatcacaaaaaagtaaaattctaccagtaataagtcgaagtactgtggagc
Segments:
9.9.29       360 369   =====AP-1=====
3.3.2.0       369 378   =====HNF-3=====
9.9.539       399 408   =====NF-1=====
2.1.1.4       405 414   =====ER=====
1.6.1.0       417 426   =====AP=====
=====
seq( 420.. 479) ctggggcaggcggccaggcacatccgagcccgagggaataactaatgctgttctct
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2.3.1.0       429 438   =====Sp1=====
1.6.1.0       430 439   =AP-2alph=
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gene-regulation.com/cgi-bin/pub/programs/alibab2/webabab2.cgi 1/2
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Segments:
=====
seq( 60.. 119) ctcacaacctcatgaattaataaagagcccatatgaacaactcaggacaatgcctgg
Segments:
=====
seq( 120.. 179) caggggctggaatccctttaactgcctgacccctccactcctgaacatgtgggagcct
Segments:
2.3.1.0      121 131   =====Sp1=====
2.2.1.1      177 186   =====
=====
seq( 180.. 239) tatctgtagcctagaggacagcctagccctcttattcattcattcgtctgataagtt
Segments:
2.2.1.1      177 186   GATA-1=
2.1.1.2      192 201   =====PR=====
2.2.1.1      228 237   =====GATA-1=====
=====
seq( 240.. 299) attcggccctacgacacagagctcttcttaggcctggataaacaatgcaagtaaa
Segments:
2.3.1.0      243 252   =====Sp1=====
=====
seq( 300.. 359) gcagggtgaaccaggcactgtggaagtctcattctctgggaagatagctattaatta
Segments:
=====
seq( 360.. 419) aatcatcacaaaaaagtaaaattctaccagtaataagtcgaagtactgtggagc
Segments:
9.9.29       360 369   =====AP-1=====
3.3.2.0       369 378   =====HNF-3=====
9.9.539       399 408   =====NF-1=====
2.1.1.4       405 414   =====ER=====
1.6.1.0       417 426   =====AP=====
=====
seq( 420.. 479) ctggggcaggcggccaggcacatccgagcccgagggaataactaatgctgttctct
Segments:
1.6.1.0       417 426   -2alph=
2.3.1.0       422 431   =====Sp1=====
2.3.1.0       429 438   =====Sp1=====
1.6.1.0       430 439   =AP-2alph=
=====
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Segments:
2.3.1.0       494 503   =====Sp1=====
2.3.1.0       539 550   =====
=====
seq( 540.. 599) tggggggggaacctggaatgtccaggtccaacatgaaccaggttccagagcccatc
Segments:

gene-regulation.com/cgi-bin/pub/programs/alibab2/webabab2.cgi 1/2
```

GlyCam-1 Gene promoter

- Llama

01

A>G

Creation of new putative binding site for EmBP-1

02

C>T

Creation of new putative binding site for AP-2

03

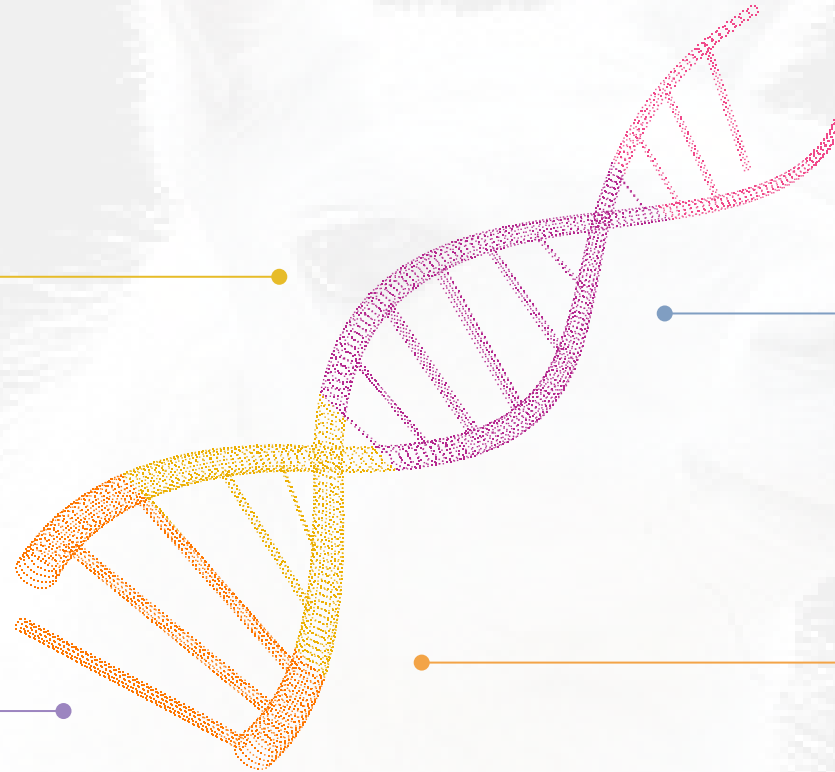
G>A

Creation of a new putative binding site for C/EBPalp

04

G>A

Removal of the binding site of AP-2alph



GlyCam-1 Gene promoter

- Alpaca

01

A>G

Creation of new putative
binding site for EmBP-1

02

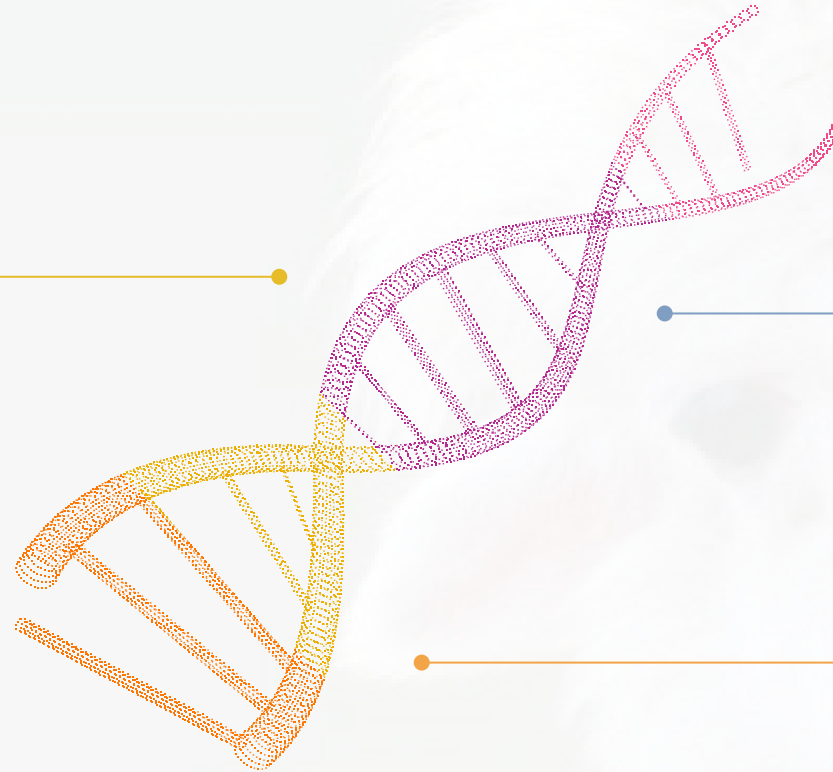
G>A

Removal of the binding site
of Sp1

03

G>A

Removal of the binding site
of Sp1 and AP-2alph



Genotyping

South American camelids promoter

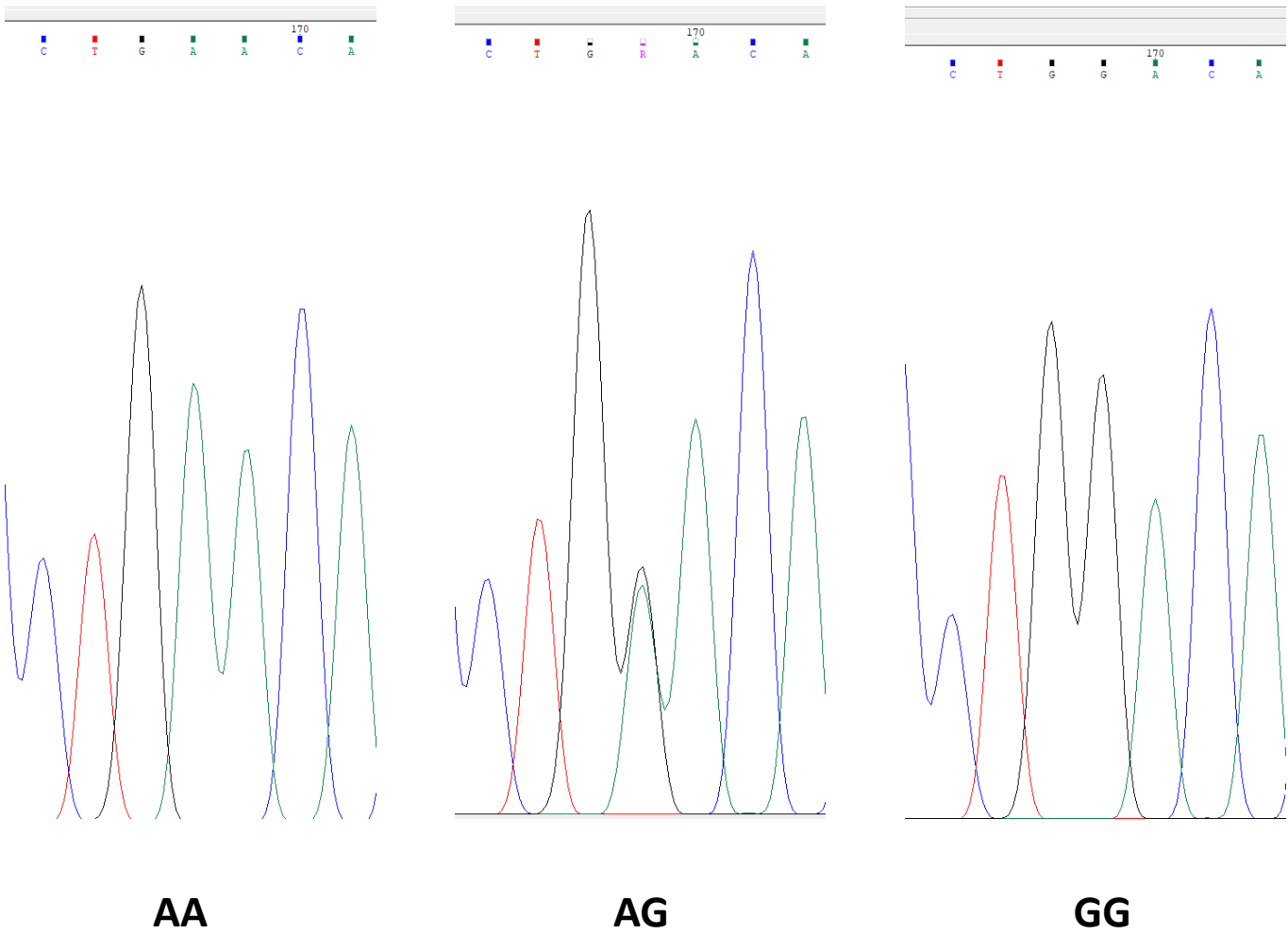


Table. Genotyping data and allele frequency in south American Camelids (Promoter)

Genotype Distribution					Allelic frequency	
	AA	AG	GG	Total	A	G
Observed	56	6	4	75	0.79	0.21
Expected	46.8	24.9	3.3			

Genotyping

South American camelids Ex3 G>A → responsible for Glu>Lys change

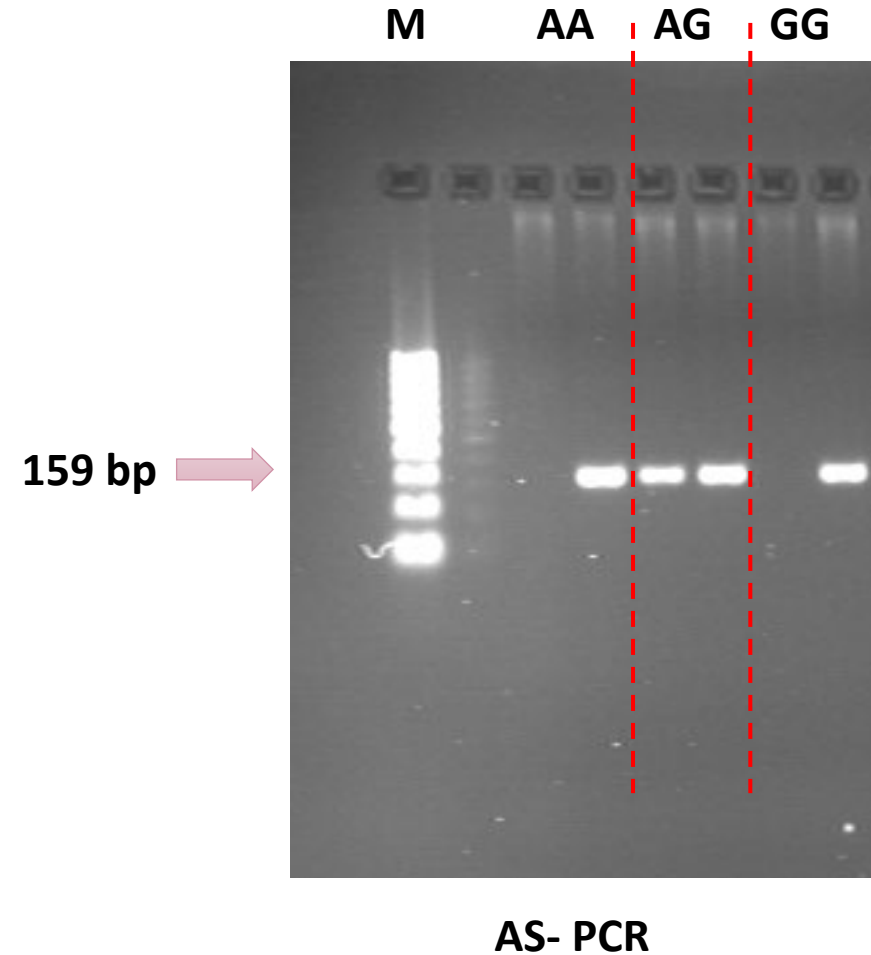


Table. Genotyping data and allele frequency in south American Camelids (Exon 3)

	Genotype Distribution				Allelic frequency	
	AA	AG	GG	Total	A	G
Observed	1	25	56	82	0.17	0.83
Expected	2.46	23.14	56.49			

Conclusion and perspectives

- *GlyCam-1* gene study and the identification of its genetic diversity are highly interesting due to its reported role in mastitis prevention, emulsification and bifidobacterial growth-promoting activity.
- Investigate the potential effects of all identified mutations on splicing process and expression levels.
- Explore the genetic diversity of the *GlyCam-1* gene within the dromedary camel population and across other camelid species could provide valuable insights and broaden our understanding of this gene.

Thank you for your attention



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