Genomic: A Revolutionary Approach to Explore the Potential of Camel

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Domestic Animal Resources in Pakistan

Domestic Animals	Population (M)	Breeds
Cattle	48	15
Buffalo	40	5
Sheep	31	30
Goat	76	36
Camel	1.1	20

- 2nd highest buffalo population
- 4th largest milk producing country
- 3rd largest goat producing country
- Among top ten camel producing countries

(Eco. Surv. Pak: 2019-20)

Camel has unique qualities

- Camel with many unique qualities has the potential to battle with increasing desertification
- Can endure prolonged water shortage, up to 14 days
- A best suited animal in extreme weathers under current global climate changes

Camel in Pakistan

(20 camel breeds)

(Isani and Baluch, 2000)

Unique features of Camel invite us to explore its genomics

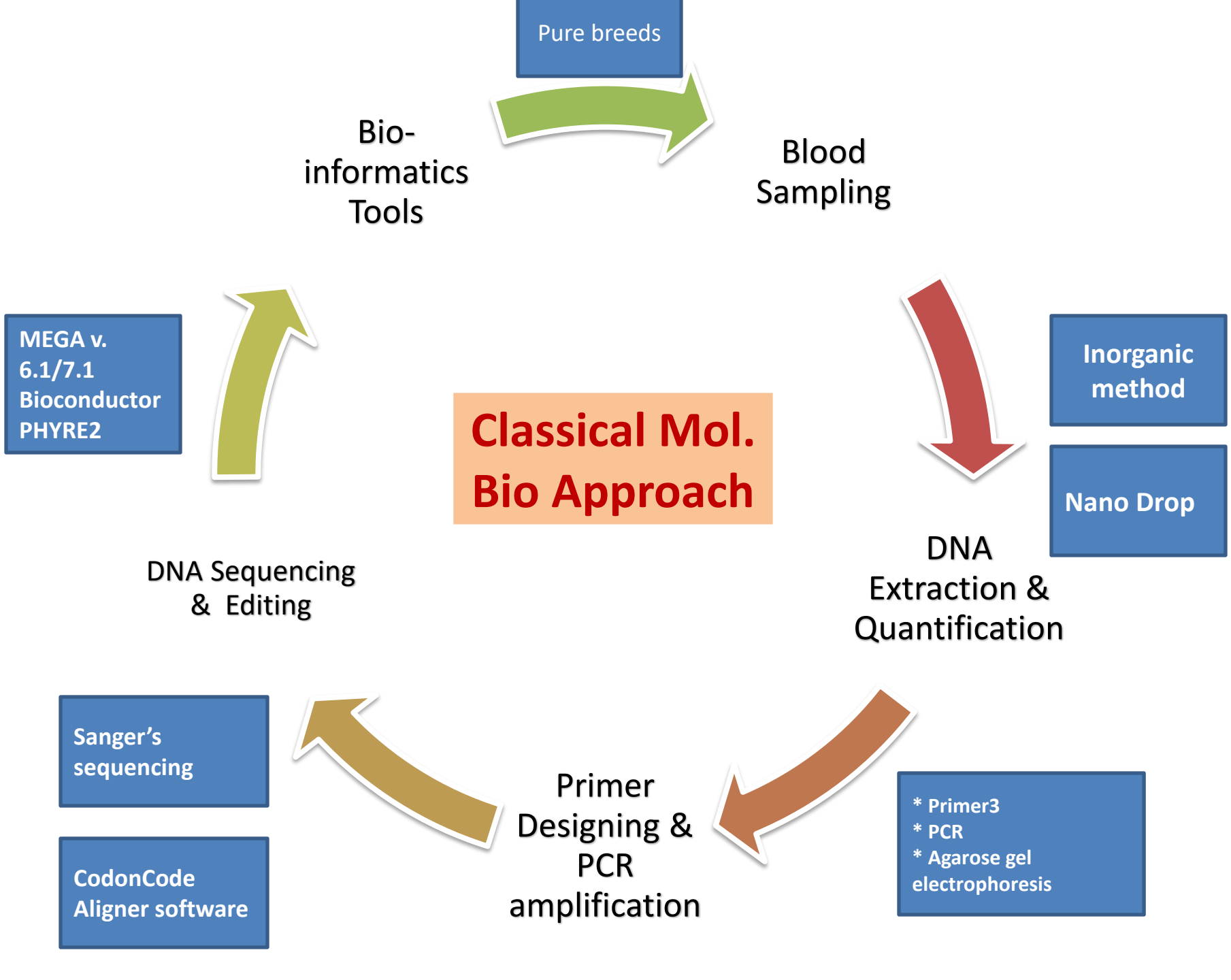
Characterization of Animal Resources in Pakistan

FAO & ISAG recommended molecular markers

✓Mitochondrial markers

✓Microsatellite markers

- Buffalo
- Cattle
- Sheep
- Goat
- **Camel**
- Yak
- Horses
- Chicken


Pure breeds
Blood Sampling
Inorganic method
Nano Drop
DNA Extraction & Quantification
* Primer3
* PCR
* Agarose gel electrophoresis
Primer Designing & PCR amplification
Sanger's sequencing
CodonCode Aligner software
DNA Sequencing & Editing
MEGA v. 6.1/7.1
Bioconductor
PHYRE2
Bio-informatics Tools
Classical Mol. Bio Approach

Diversity Analysis of Pakistani Camel Breeds

Done by Sequencing and genotyping:

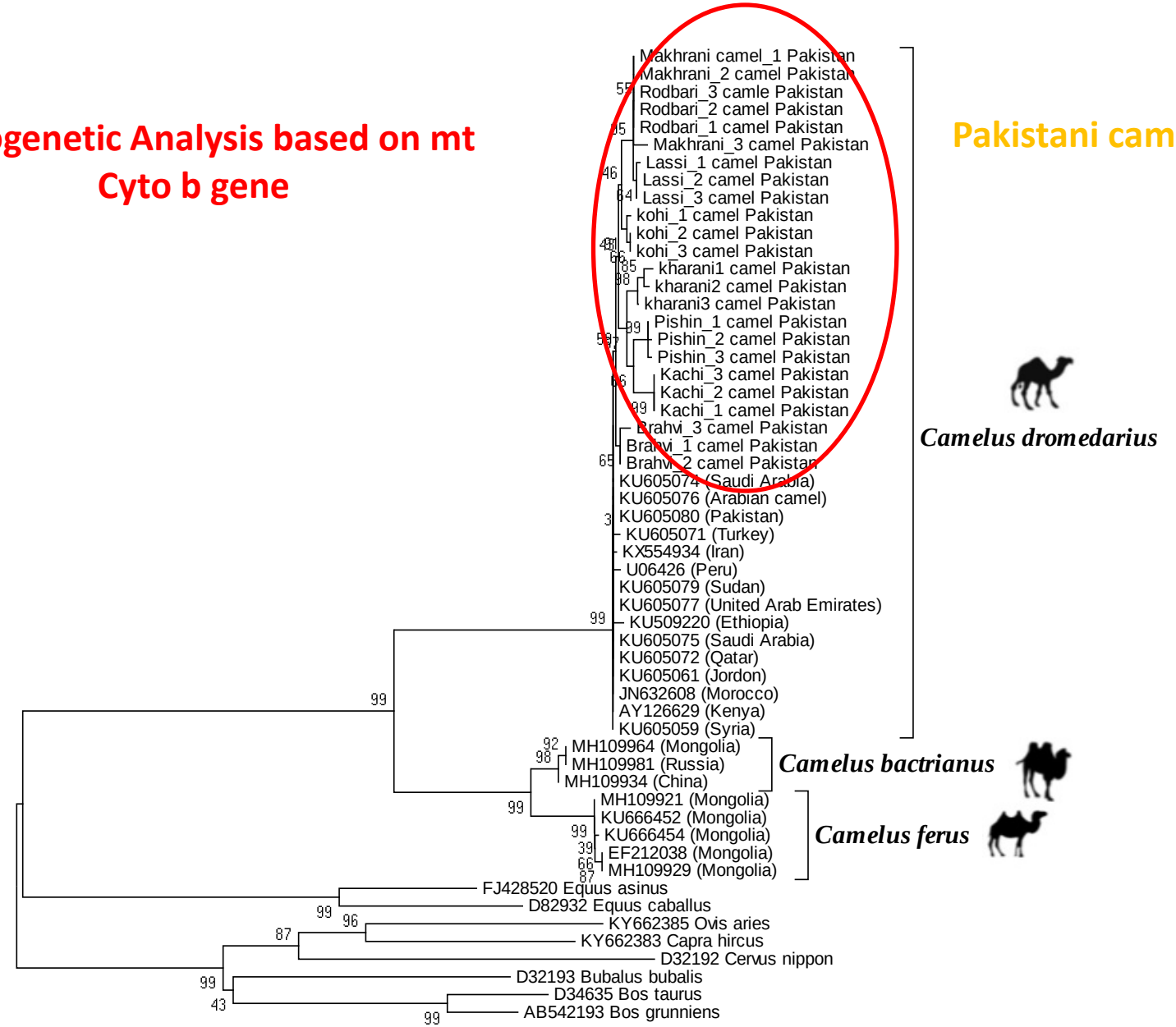
- Mitochondrial D-Loop
- Cytochrome b
- ATPase 6-8 genes
- Microsatellite markers
- Prion protein gene

Complete mt Cytochrome b gene sequencing in 8 breeds

- Eight Camel breeds with 210 samples
- Complete *Cyto b* gene (1140 bp) was amplified
- Low haplotype (Hd= 0.484±0.051) and nucleotide diversity (π=0.00272) was found
- High genetic similarity among breeds was observed

Phylogenetic Analysis based on mt Cyto b gene

Pakistani camels

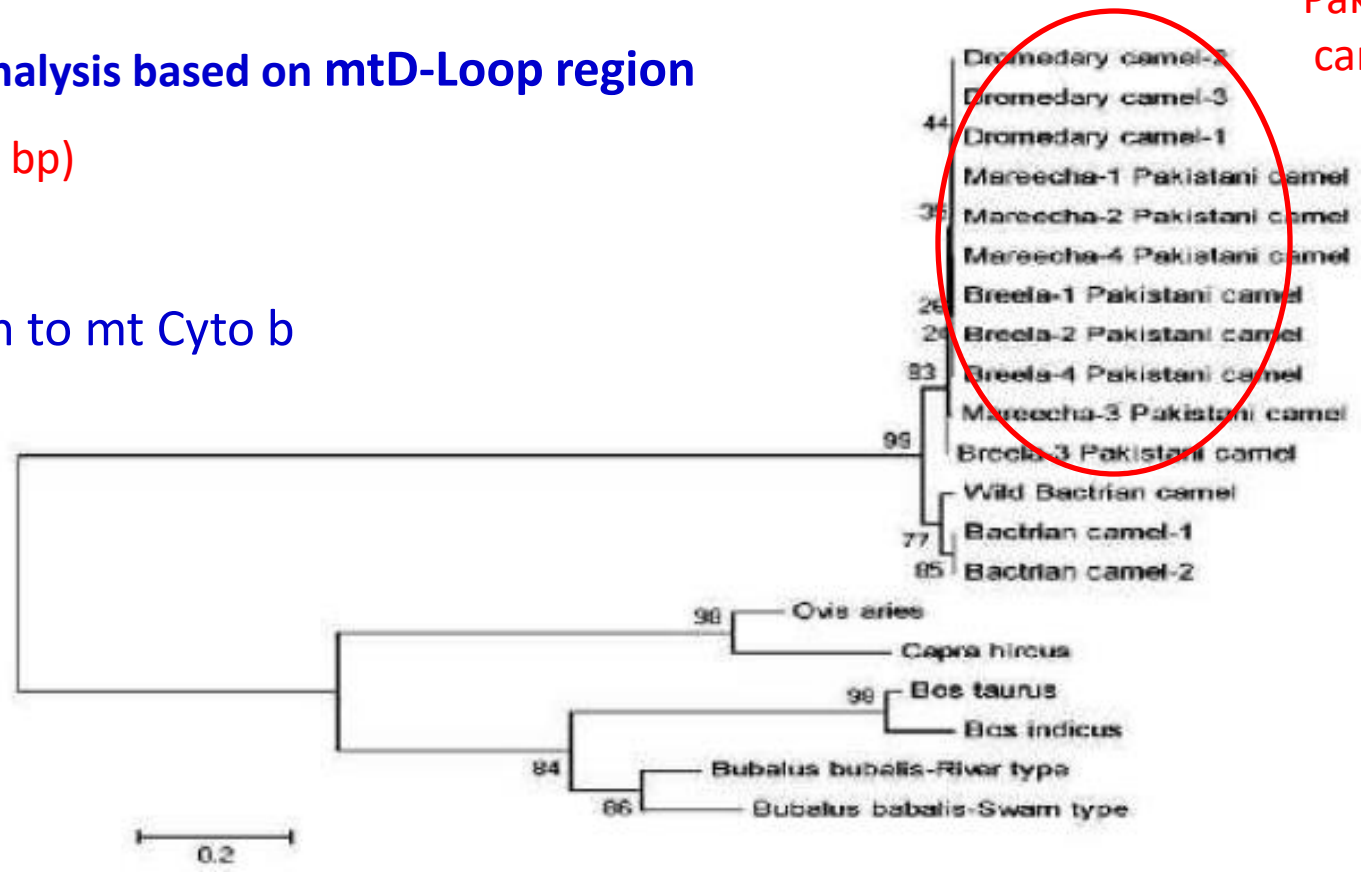


Phylogenetic Analysis based on mtD-Loop region

(complete 1214 bp)

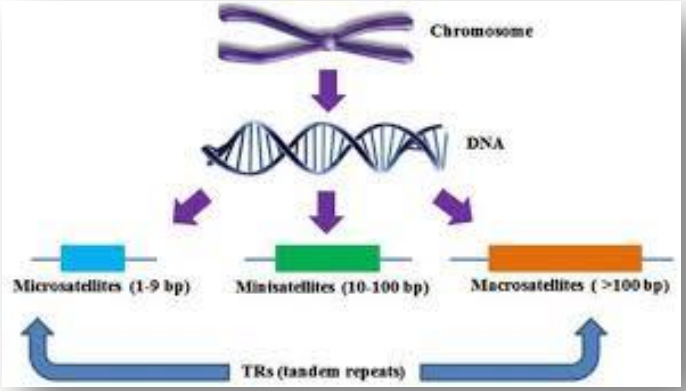
Similar pattern to mt Cyto b

Pakistani camels



Microsatellites markers based Diversity Study

12 Microsatellite Markers (dye labelled) Set for Genotyping using Genetic Analyzer ABI 3130 xl



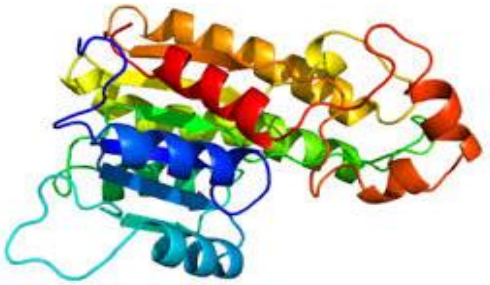
Data of studied markers in camel Breeds

Breeds	Mareecha					Barela				
Locus	Na	Ne	Ho	He	PIC	Na	Ne	Ho	He	PIC
YWLL38	9.00	6.38	0.88	0.86	0.83	7.00	3.23	1.00	0.72	0.66
LCA66	9.00	4.11	0.60	0.77	0.72	5.00	3.90	0.91	0.78	0.70
CVRL02	8.00	4.79	0.88	0.81	0.77	6.00	3.41	1.00	0.74	0.66
CMS17	6.00	3.54	0.96	0.73	0.68	4.00	3.14	0.91	0.71	0.63
CMS15	13.00	5.53	1.00	0.84	0.80	10.00	6.05	1.00	0.87	0.82
CMS13	10.00	3.94	0.68	0.76	0.72	7.00	5.38	0.73	0.85	0.79
VOLP032	3.00	1.73	0.32	0.43	0.59	2.00	1.94	0.45	0.51	0.50
VOLP67	8.00	3.48	0.96	0.73	0.67	5.00	3.32	1.00	0.73	0.65
YWLL44	9.00	4.43	0.80	0.79	0.75	7.00	6.72	0.82	0.89	0.83
VOLP08	9.00	4.03	0.96	0.77	0.72	8.00	5.38	0.91	0.85	0.79
VOLP03	9.00	5.87	0.96	0.85	0.81	8.00	6.21	0.55	0.88	0.82
VOLP10	10.00	5.81	0.76	0.84	0.81	7.00	5.50	0.73	0.86	0.79
Mean	8.50	4.47	0.81	0.76	0.72	6.33	4.51	0.83	0.78	0.70
St. Dev	2.24	1.30	0.20	0.11		2.10	1.53	0.19	0.11	

Screening of Pakistani Camels for Prion Diseases

- Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are rare progressive neurodegenerative disorders that affect both humans and animals.

  - Fetal disorders
  - Zoonotic in nature

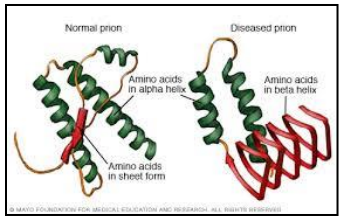


  - ✓ Scrapie in sheep and goats
  - ✓ Mad-cow in Cattle/ Bovine spongiform encephalopathy

Our group has screened almost all livestock breed in Pakistan to identify resistance/ susceptibility to Prion

Camel PrP differences from other mammals
might be responsible for PrP resistance

We sequenced eight camel breeds of Pakistan and found that camels are resistant to prions as compared to other mammals



Amino Acid Position	Camel	Other mammals
102	-	glycine
109	alanine	serine
11	glycine	serine
122	Serine	asparagine
245	tyrosine	serine
250	serine	tyrosine
252	glycine	glutamine

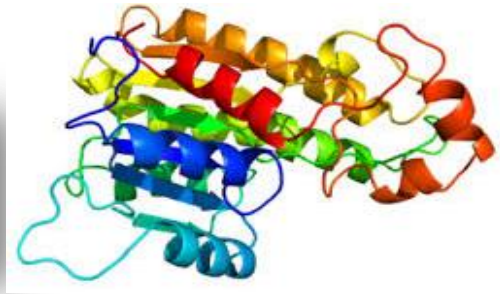
Kappa casein gene variations in different breads of camel of Pakistan

Sr#	Position	Sequence change	Type of change	Sr #	Position	Sequence change	Type of change
Kachi breed				**Watni breed**			
1	c.1-1075	del. A	Deletion	1	c.1-1075	del. A	Deletion
2	c.1-1046	GT	Heterozygous	2	c.1-1046	GT	Heterozygous
3	c.1-707	del. G	Deletion	3	c.1-982	GC	Heterozygous
4	c.1-684	A>T	Transversion	4	c.1-707	del. G	Deletion
5	c.1-683	T>G	Transversion	5	c.1-1156	AT	Insertion
6	c.1-402	Ins. T	Insertion				
7	c.1-340	Ins. T	Insertion	**Kharani breed**			
8	c.1-321	Ins. T	Insertion	1	c.1-1046	GT	Heterozygous
9	c.1-1085	del. A	Deletion	2	c.1-982	GC	Heterozygous
10	c.1-1081	Ins. A	Insertion	3	c.1-707	del. G	Deletion
11	c.1-1036	A>G	Transition				
12	c.1-1035	A>G	Transition	**Thari breed**			
13	c.1-682	del. G	Deletion	1	c.1-707	del. G	Deletion
14	c.1-525	GA	Heterozygous				
Mareecha breed				**Pahari breed**			
1	c.1-1075	del. A	Deletion	1	c.1-1075	del. A	Deletion
2	c.1-1046	GT	Heterozygous	2	c.1-1046	GT	Heterozygous
3	c.1-982	GC	Heterozygous	3	c.1-982	GC	Heterozygous
4	c.1-707	del. G	Deletion	4	c.1-707	del. G	Deletion
5	c.1-684	A>T	Transversion	5	c.1-684	A>T	Transversion
6	c.1-683	T>G	Transversion	6	c.1-683	T>G	Transversion
7	c.1-1088	G>T	Transversion	7	C1-1057	ins T	Insertion
8	c.1-1087	A>G	Transition				
9	c.1-1054	C>T	Transition				
Mixed Breed 3500-4000				**Breela Breed**			
1	c.1-1075	del. A	Deletion	1	c.1-1075	del. A	Deletion
2	c.1-1046	GT	Heterozygous	2	c.1-1046	GT	Heterozygous
3	c.1-982	GC	Heterozygous	3	c.1-982	GC	Heterozygous
4	c.1-707	del. G	Deletion	4	c.1-707	del. G	Deletion
5	c.1-684	A>T	Transversion	5	c.1-684	A>T	Transversion
6	c.1-683	T>G	Transversion	6	c.1-1081	ins. A	insertion
				7	c.1-690	ins. G	insertion

Camel as a model animal to study Heat Tolerance

- Most of dromedary camels live in extreme desert conditions
- It can be a good model to study Heat Tolerance
- Heat shock proteins (HsP) genes family HsP40, 60, 70, 90, 100 can be explored
- HsPs protect the cell against exposures to lethal heat shocks and stress
- Has critical role in the development of thermo-tolerance and Immunity

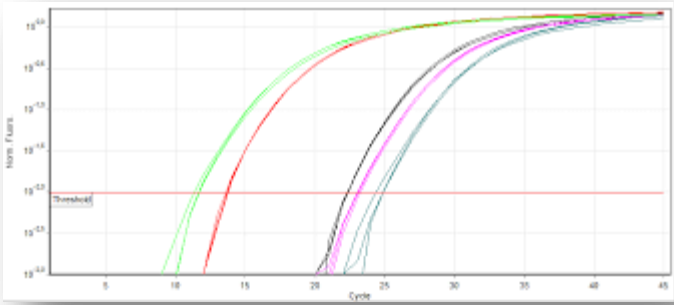
MOLECULAR MARKERS



Ongoing research in HSP genes..

- Gene Expression Study in different camel breeds is going on to see level of HsP genes expression using RT-PCR

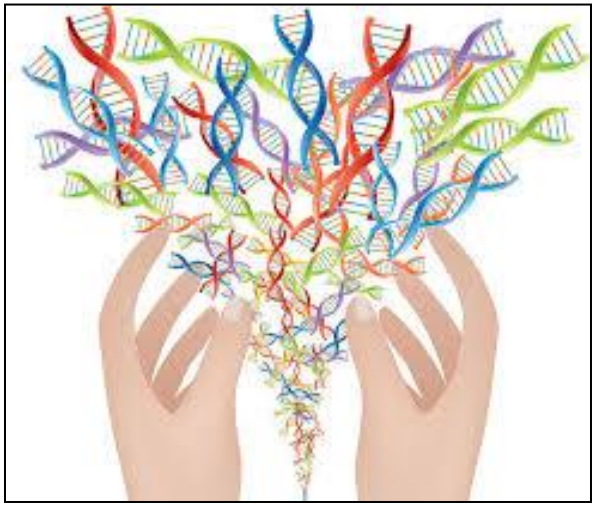
- Expression level in different season will give us some idea about these sets of genes



Modern Genomics Techniques

Modern robust techniques should be applied to explore Camel's Genome:

- Next Generation Sequencing
- Whole Genome Sequencing
- Genome Wide Associate Studies using large data sets
- Genotyping by Sequencing (GBS)
- Microarray techniques
- Droplet Digital PCR (dd PCR)
- Double Digest Restriction Associated DNA Seq (ddRAD)
- illumina HiSeq
- RNA-Seq /Whole Transcriptome Sequencing
- **And many more are coming….**



These techniques would be helpful for better analyzing the camel's genomics

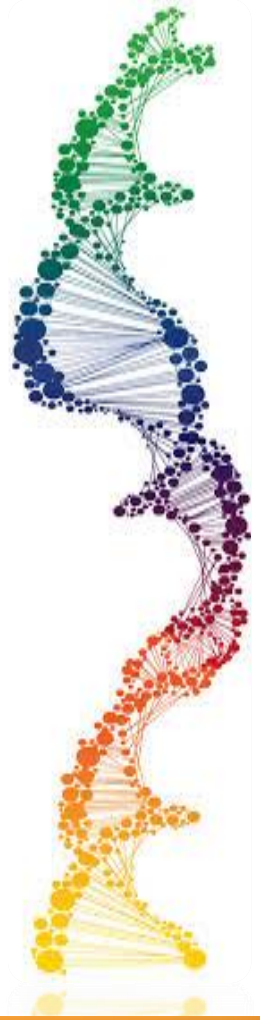
Genomic Areas to work on..

areas to be explored in Camel

1. QTL Mapping of Camel's genotypes
2. Genotype-phenotype association studies
3. Population, ecological and evolutionary studies
4. Genomic as well as Epigenomic approaches to study camel in different environments
5. Expression studies of different important genes of camel under stressed conditions like heat, aridity, UV light and dust and comparison with other mammalian species
6. Exploring the camel immune responses
7. Genes related to lungs development, sight, blood glucose levels, transportation of sodium and potassium to explore its characteristics
8. And many more…

Camel Genomics Research Projects

Title	Status
Genotyping By Sequencing (GBS) Based Genome Exploration of Pakistani Camel to Determine their Genetic Potentials as the Animal of Future	Ongoing
Whole Genome Sequencing of Famous Camel breeds of Pakistan	Ongoing
Transcriptome Analysis of Milk Production Genes for Developing Libraries of Marecha Camel breed of Pakistan	Ongoing



Thank you, for being an audience