



FINAL YEAR RESEARCH PROJECT

Genetic Screening of β -Thalassemia HBB Gene Analysis

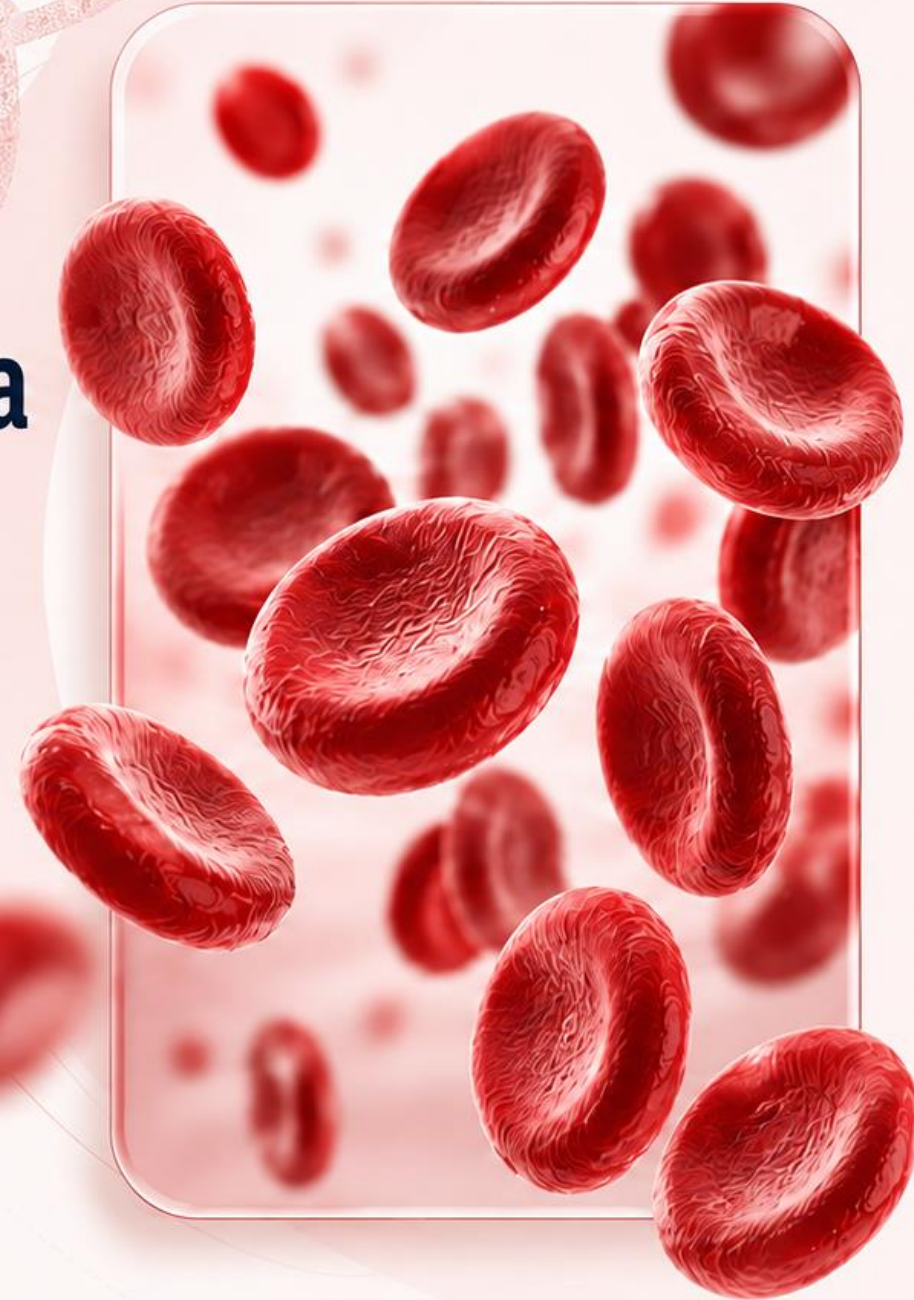
A molecular approach for mutation detection

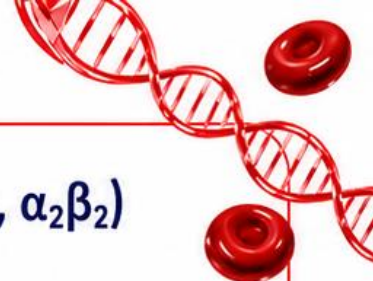
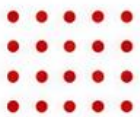


Prepared by

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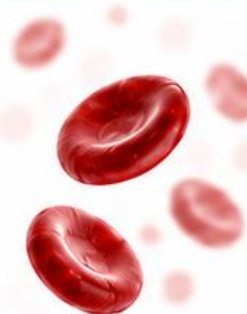
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BACKGROUND

- Red blood cells transport oxygen through hemoglobin.
- *HBB* gene is located on chromosome 11.



PROBLEM STATEMENT

- Pakistan carries a high β -thalassemia burden.



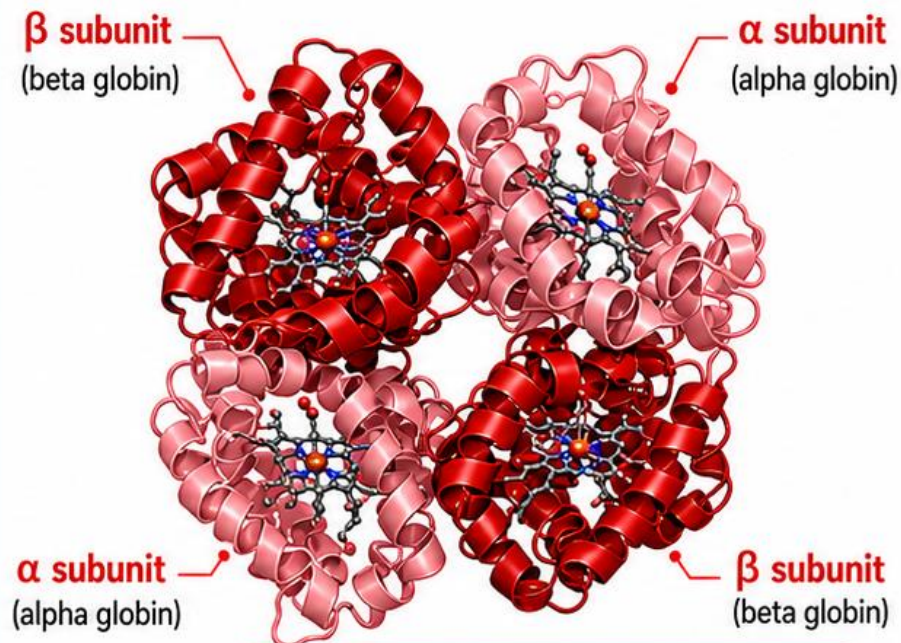
STUDY OBJECTIVE

- To study the *HBB* mutated gene.
- To raise awareness of molecular screening among locals.

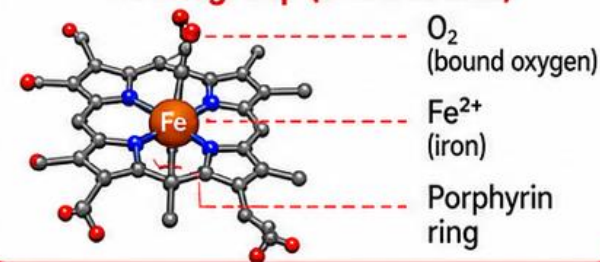


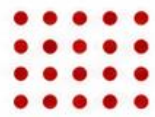
Human Hemoglobin (HbA, $\alpha_2\beta_2$)

TETRAMERIC STRUCTURE

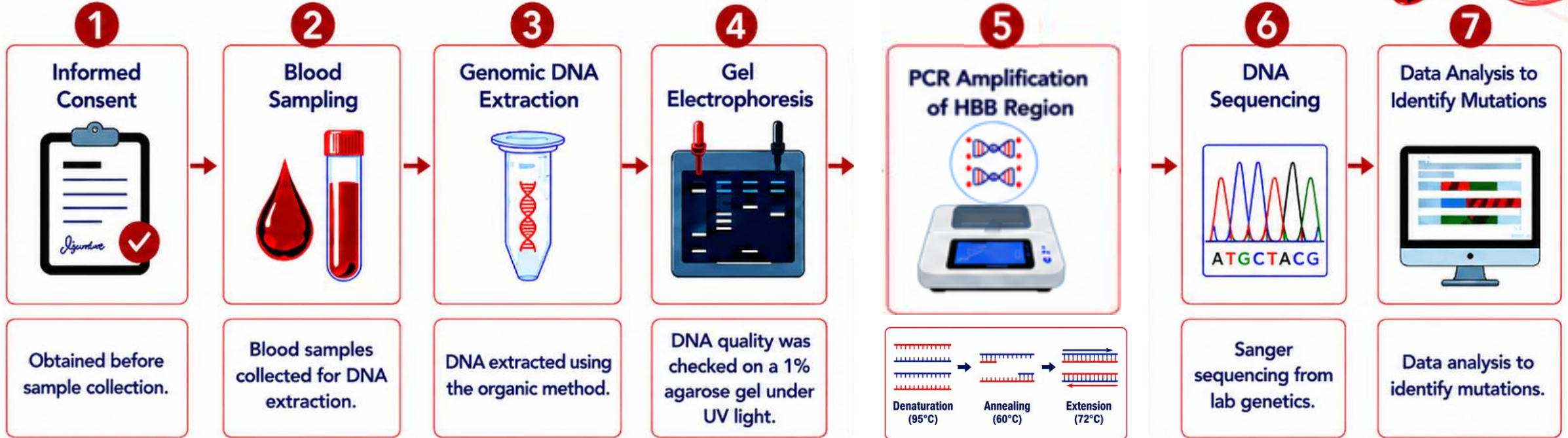
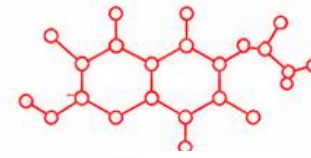


Heme group (one subunit)





Experimental Workflow

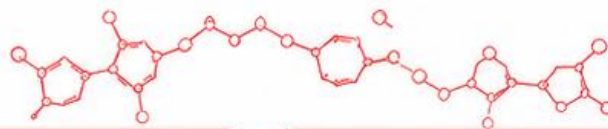


Primer Design					
Primer	Sequence (5'→3')	Length (nt)	Tm (°C)	GC%	Product Size (bp)
Forward Primer (F_HBB_Ex1)	agggttgccaatctactcc	20	54	55%	535
Reverse Primer (R_HBB_R1)	CACTCAGTGTGGCAAA GGTG	20	54	55%	535

PCR Reagents and Reaction Setup		
Component	Volume (μL)	Function
DNA template (1X)	3 μL	Template DNA
DNA polymerase	0.5 μL	Enzyme for DNA synthesis
dNTPs	2 μL	Nucleotide supply
10X Buffer	2.5 μL	Provides optimal pH and ions
MgCl ₂	2.5 μL	Cofactor for DNA polymerase
ddH ₂ O	12.5 μL	Adjusts final volume
PCR master mix and forward and reverse primer		



Results



1 DNA Extraction & Gel Electrophoresis



Extracted DNA



Gel Electrophoresis Setup



UV Gel Image (1% Agarose)

2 PCR Amplification



PCR Thermal Cycler Screen

3 Sanger Sequencing & BLAST Analysis



Sequencing read (HBB fragment)

TTGTTTTGTCTCGACGCGAACTGATTGACCAACACCTGTGCGCTGACTCCTG
AGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTT
GGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGTTACAAGACAGGTTTAAGG
AGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGAT
AGGCACTGACTCTCTCGCCTATTGGTCTATTTCCACCCCTAGGCTGCTGGTG
GTCTACCCCTTGACCCAGAGGTTGAGTCCTTTGGGGATCTGTCCACTCCTGATG
CTGTTATGGGCAACCAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCT
TTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACACTGA
GTGAAG

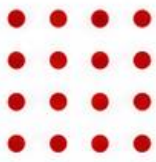
NCBI BLAST
confirmed alignment
with the HBB gene
region.

4 Key Finding — TTCT Deletion

Score	Expect	Identities	Gaps	Strand
706 bits(382)	0.0	398/405(98%)	4/405(0%)	Plus/Plus
Query 130	ACACCATGGTGTCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGT	189		
Sbjct 33	ACACCCGTGTTGCCCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGT	92		
Query 190	GAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAG	249		
Sbjct 93	GAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAG	152		
Query 250	GTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGA	309		
Sbjct 153	GTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGA	212		
Query 310	TAGGCACTGACTCTCTGCTATTGGTCTATTTCCACCCCTTAGGCTGCTGGTGGTCT	369		
Sbjct 213	TAGGCACTGACTCTCTGCTATTGGTCTATTTCCACCCCTTAGGTTCTTTGAGTCCTTTGGGGAT			
Query 370	ACCCTTGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGT			
Sbjct 273	ACCCTTGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGT			
Query 430	TGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCC	489		
Sbjct 329	TGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCC			
Query 490	TGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACACTGAGTG			
Sbjct 389	TGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACACTGAGTG	433		

TTCT deletion

This deletion is the main reason for absence of beta globin chain.



Limitations



1 Small Sample Size



2 Expensive Sequencing



3 Limited Funding



Learning Outcome

- Covered the molecular biology workflow from sampling to bioinformatic analysis.
- Built hands-on wet-lab experience for postgraduate study.



References

- Khaliq, S. (2022). Thalassemia in Pakistan. *Hemoglobin*, 46(1), 12–14.
- Galanello, R., & Origa, R. (2010). Beta-thalassemia. *Orphanet Journal of Rare Diseases*, 5, 1–15.
- Cao, A., & Galanello, R. (2010). Beta-thalassemia. *Genetics in Medicine*, 12(2), 61–76.

