Molecular Genetic Testing for Heamoglobinopathies at PTPP

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DNA Lab Consultant, PTPP

PTPP Introduction

- Govt Funded Project for Prevention of Beta Thalassemia.
- Four Regional labs (36 District of Punjab)

1. Lahore Head Office at Sir Ganga Ram Hospital
2. Multan regional lab at Nishtar Hospital, Miultan
3. Rawalpindi Regional lab at Holy family Hospital
4. Bahawalpur Regional lab at Victoria Hospital

Laboratory Profile

- Two Lab Section

1. Hematology Section
   All four regions
2. DNA Section
   Head Office, SGR Hsp

Hematology lab

1. Pre/Post-marital Screening for Beta Thalassemia
2. General Screening for Beta Thalassemia
3. Family screening of Affected Childs

DNA Lab

1. Prenatal Diagnosis for Heamoglobinopathies.
   Beta Thalassemia, HbE, HbS, HbD Punjab

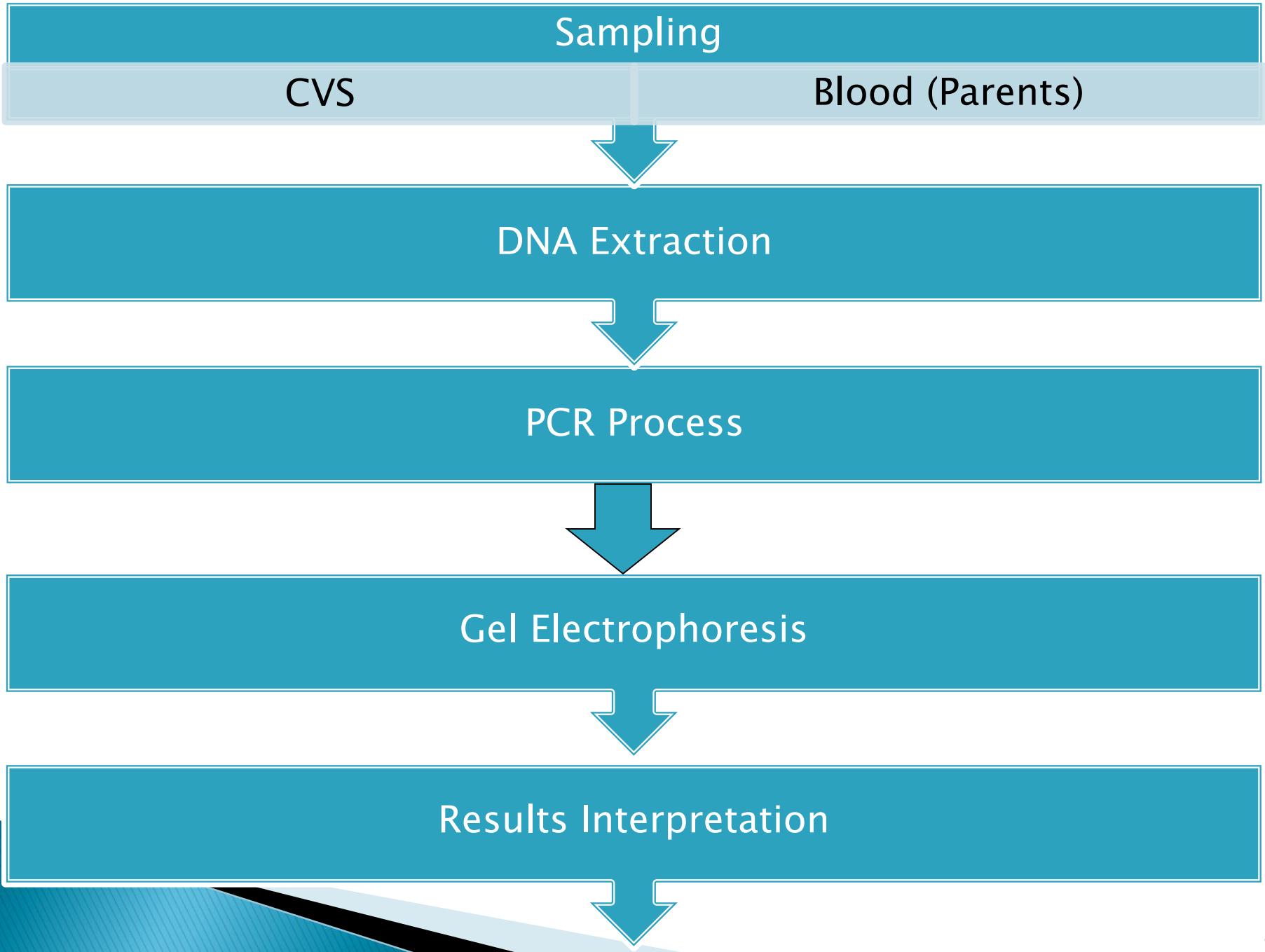
2. Diagnostic PCR for Heamoglobinopathiess
   Beta Thalasemia, HbE, HbS, HbD Punjab,
   XMN I Polymorphism,
   BCL11A Polymorphism,

Prenatal Testing

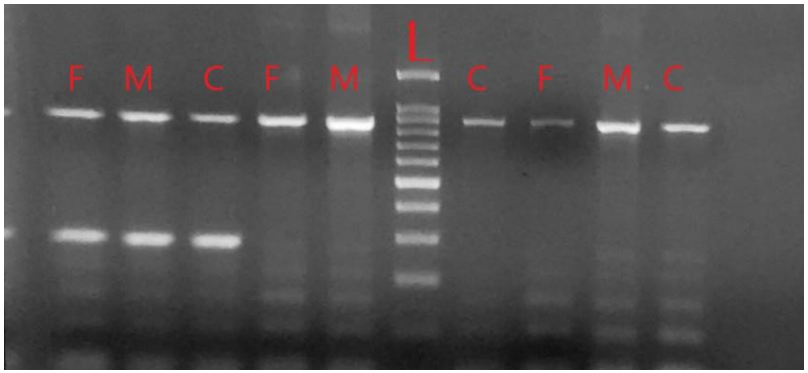
- Detection Methods
- ARMS (amplification-refractory mutation system)
- Real Time-HRM (High Resolution Melt Analysis)
- Real Time Probe

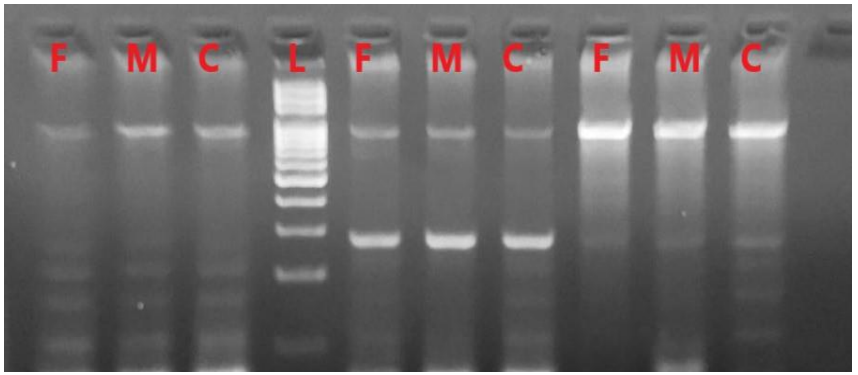
ARMS PCR

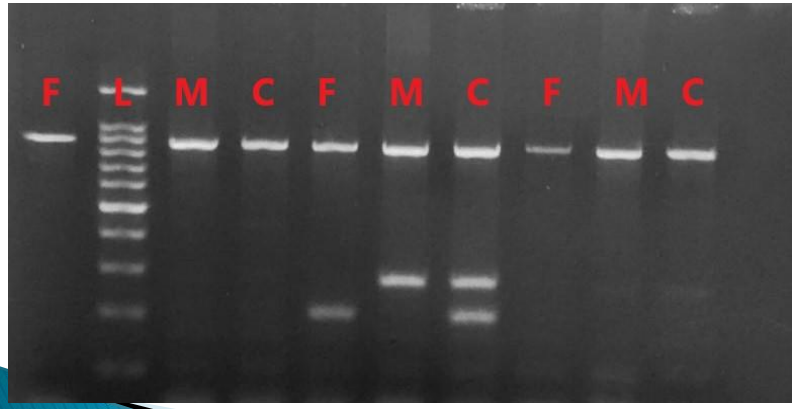
- Allele Specific PCR
- Primers designed for specific mutation amplification and detection
- PCR WORK FLOW CHART

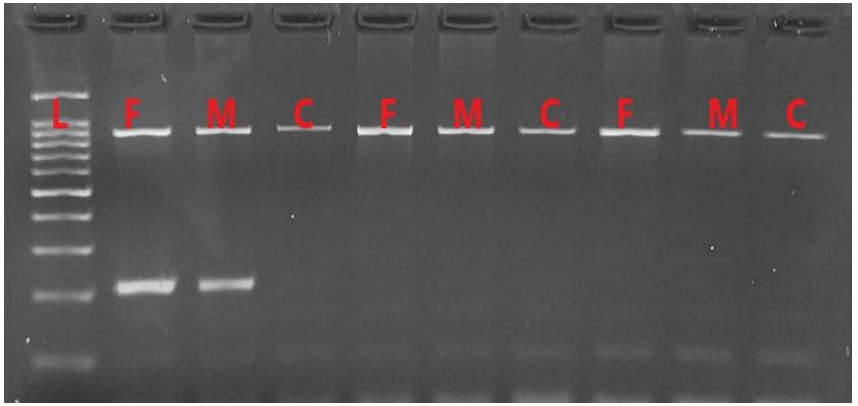

Sampling
CVS
Blood (Parents)
DNA Extraction
PCR Process
Gel Electrophoresis
Results Interpretation

Multiplex PCR (Mutation Identification)

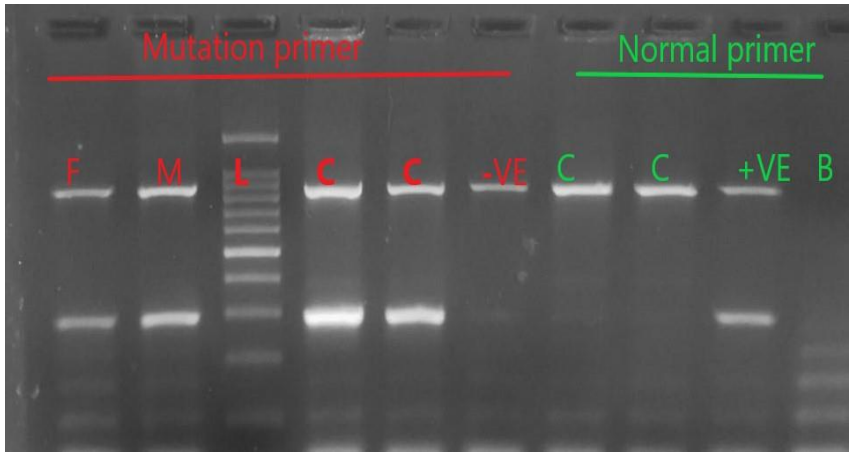


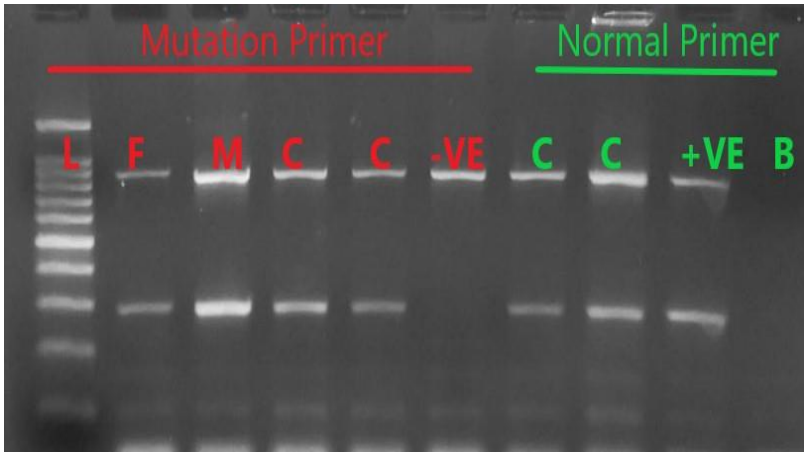


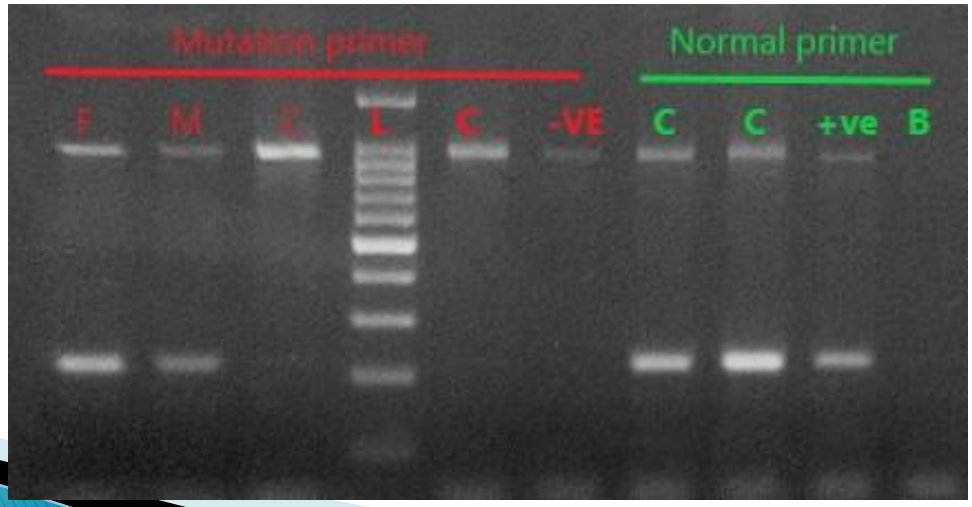




Mutation Specific ARMS PCR



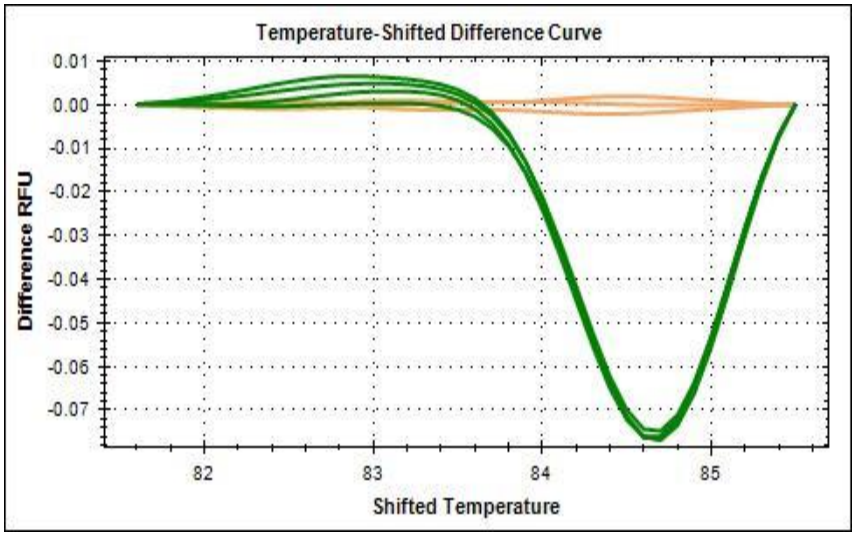


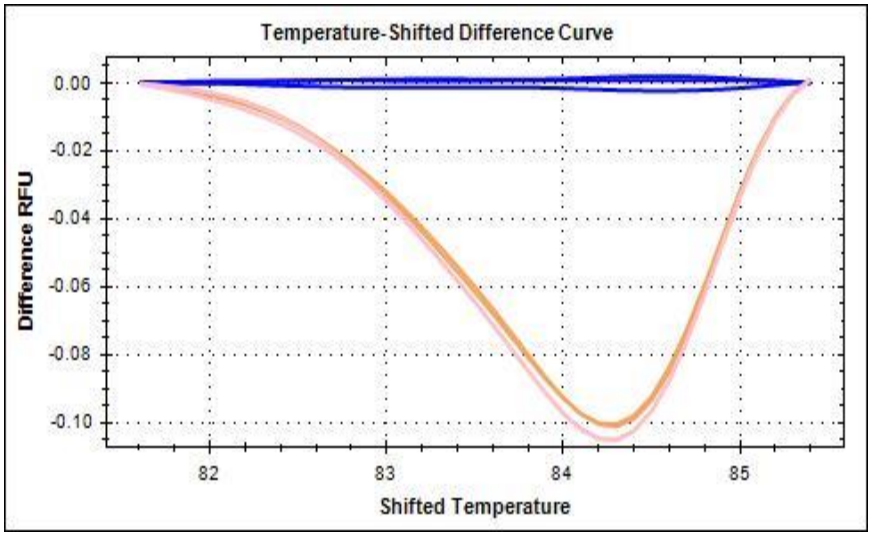


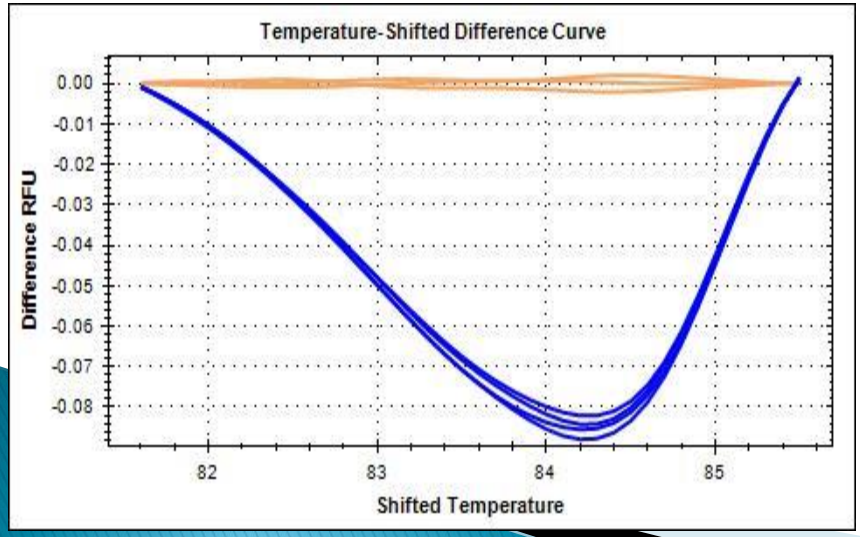
High Resolution Melt Curve Analysis

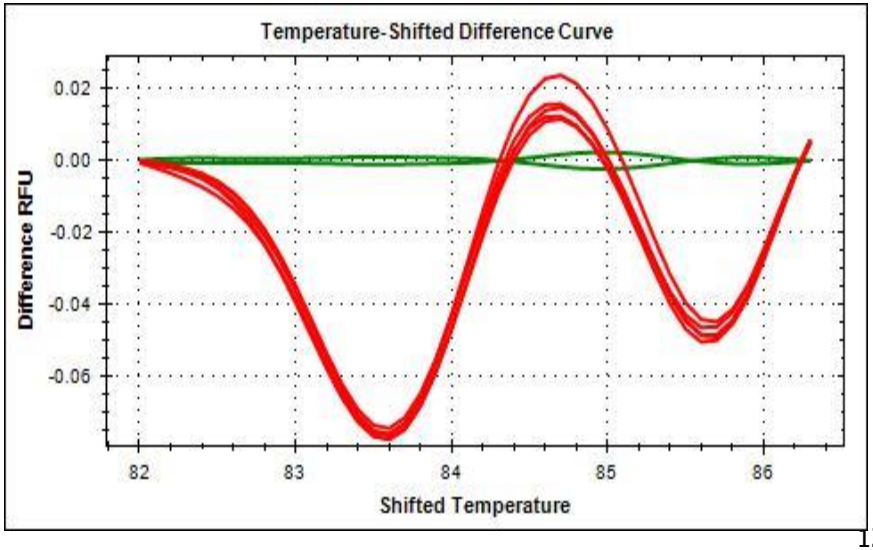
- Post-PCR analysis method used to identify variations in nucleic acid sequences.
- The method is based on detecting small differences in PCR melting curves.

HRM
Results (Beta Thal Trait samples)

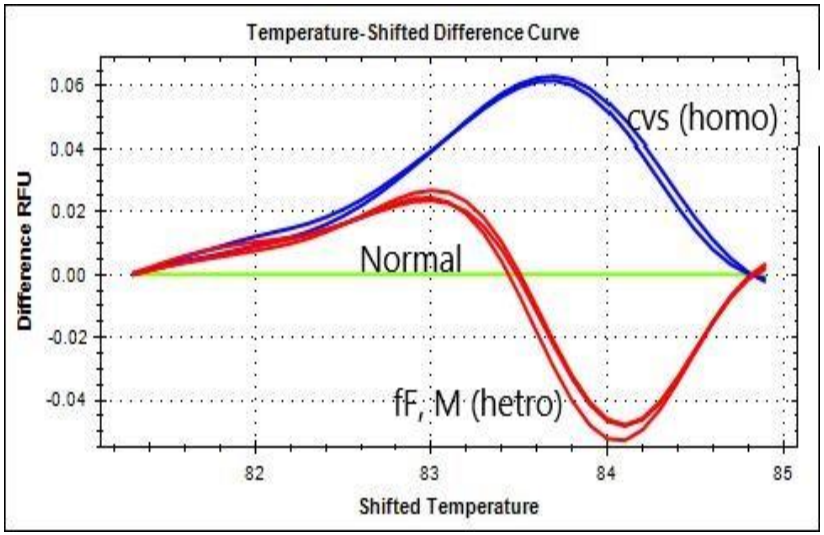


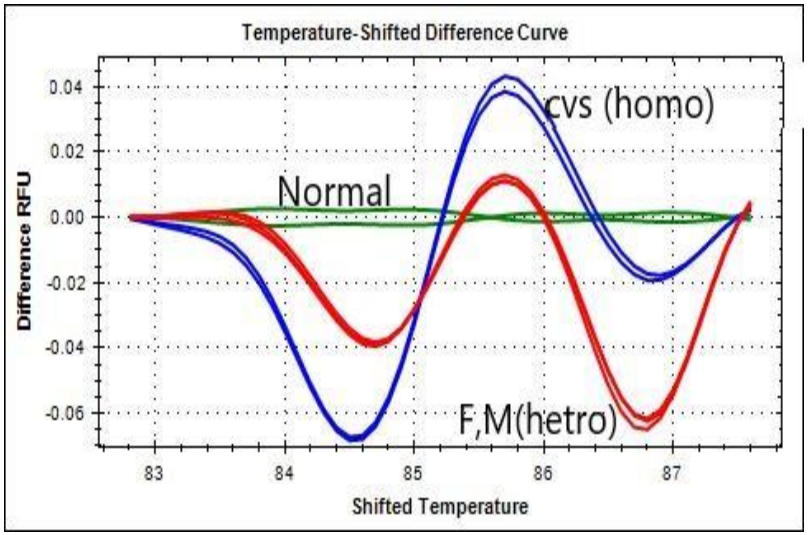


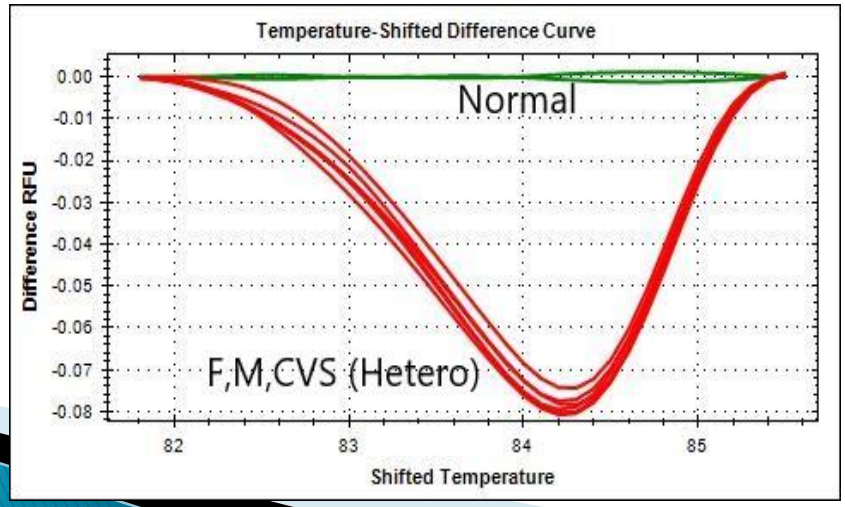




HRM
Prenatal Testing



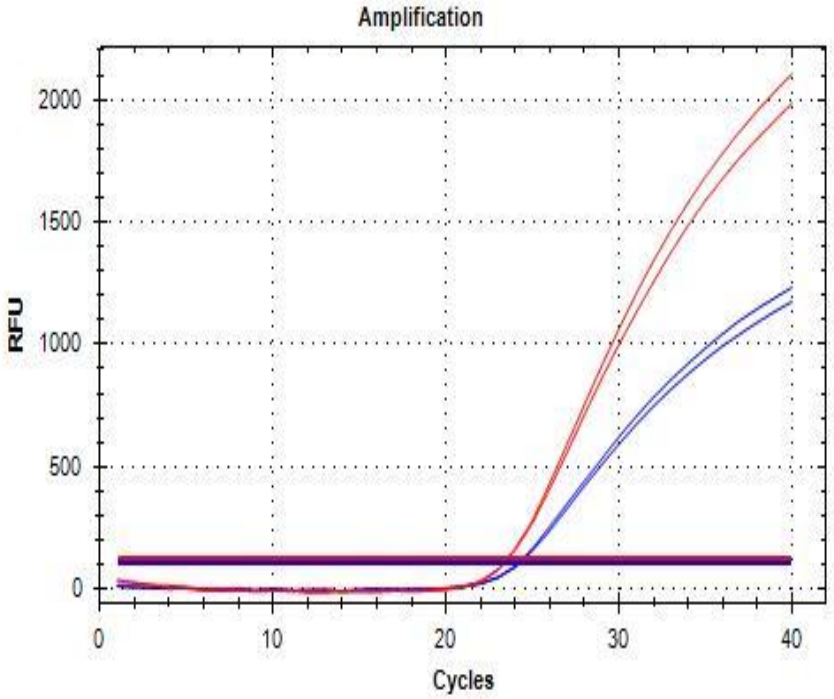


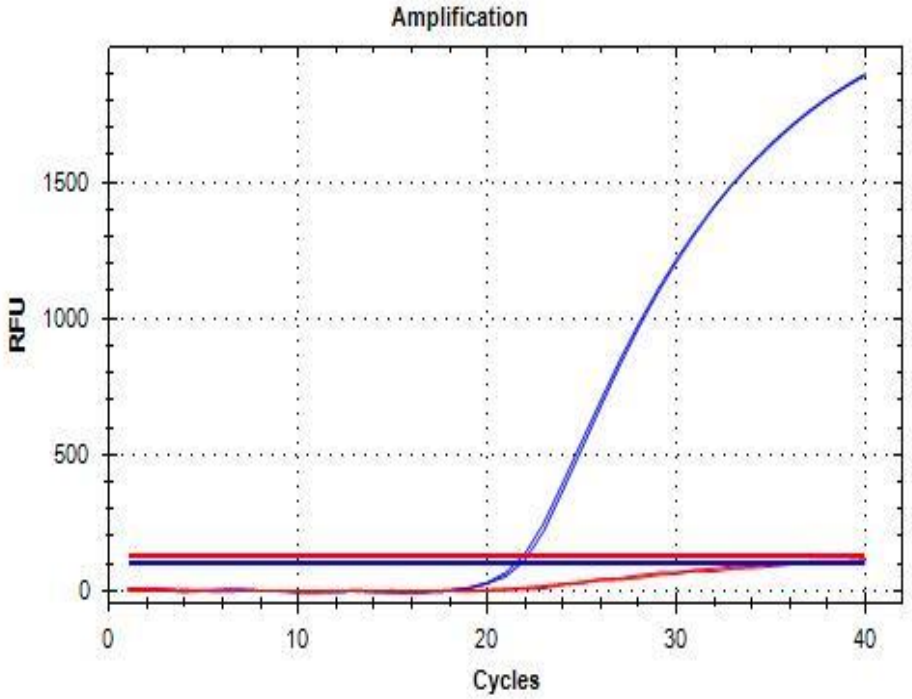


Real Time PCR-Probes

- Probes generates fluorescence signal from the hydrolysis by 5’ to 3’ exonuclease activity of Taq Polymerase.
- The hydrolysis separates fluorescein from a quenching dye and results a fluorescein signal measured by Real Time PCR.

IVS1-1 G-T:

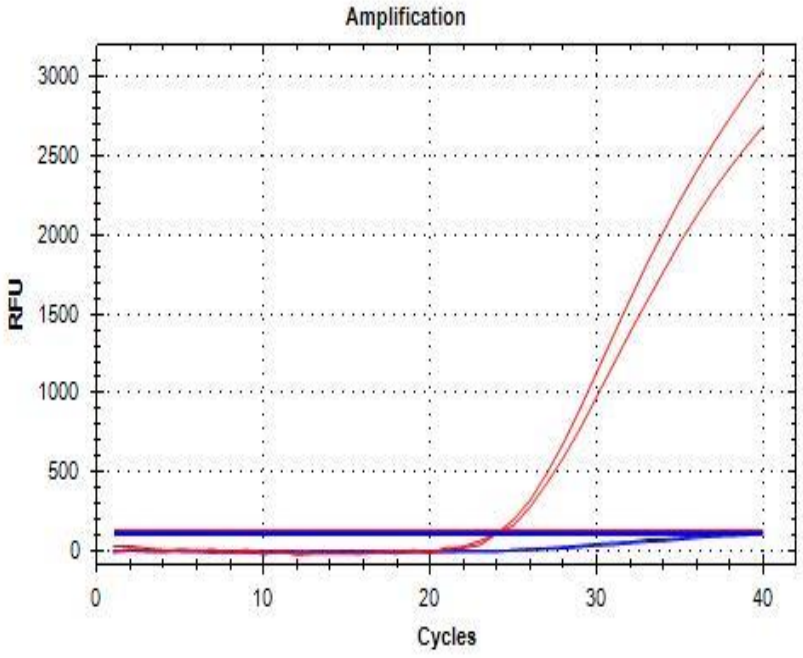




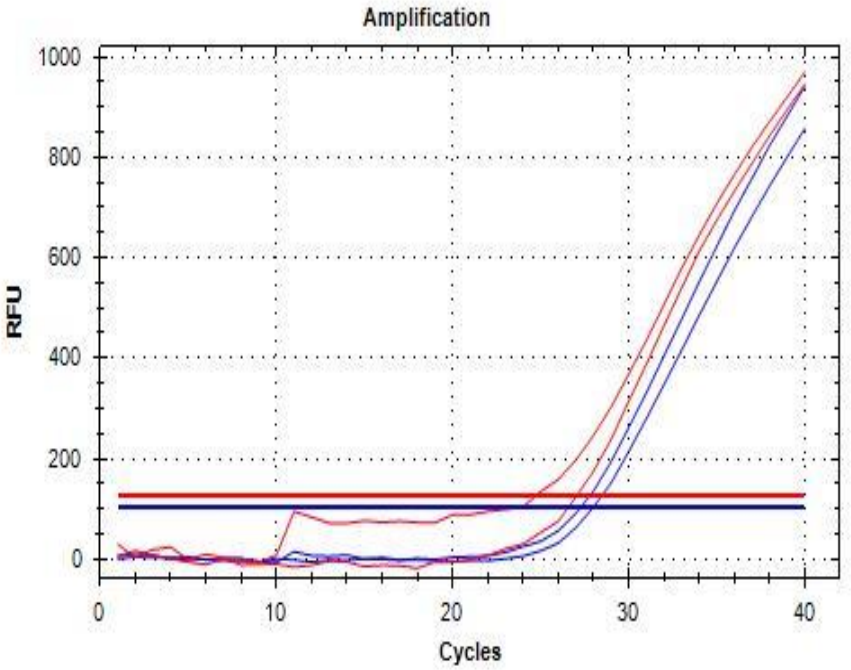
Heterozygous

Normal

Fr 4142 (-TCTT)

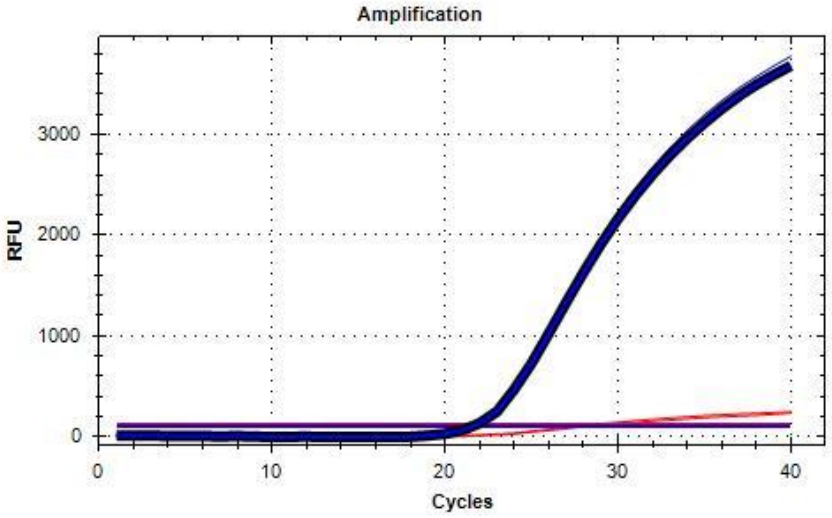


Normal

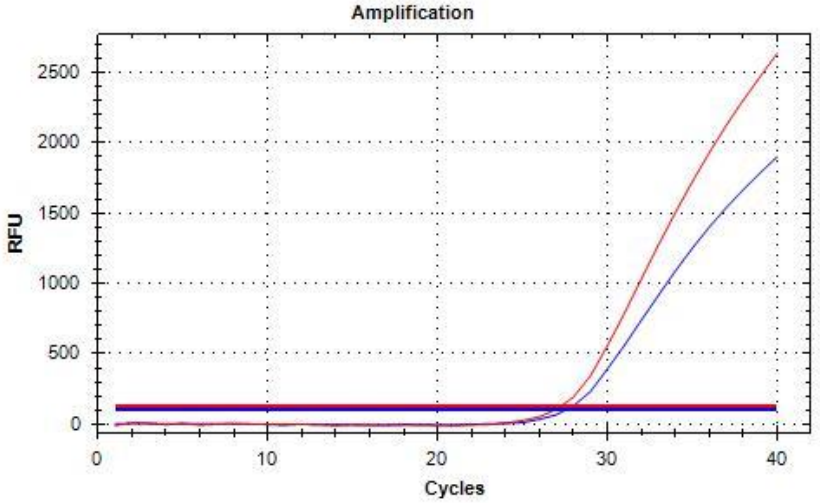


Heterozygous

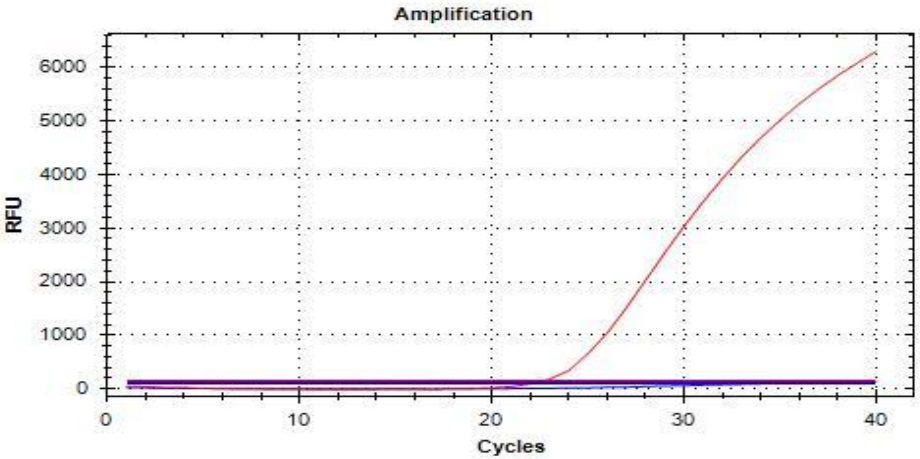
Hb-E



Normal



Heterozygous



Homozygous

VNTR Analysis of CVS tissue

- A variable number tandem repeat (**VNTR**) is a short nucleotide sequence organized as a tandem repeat.
- Number of repeats are different among individuals.
- To rule out maternal contamination in CVS tissue.

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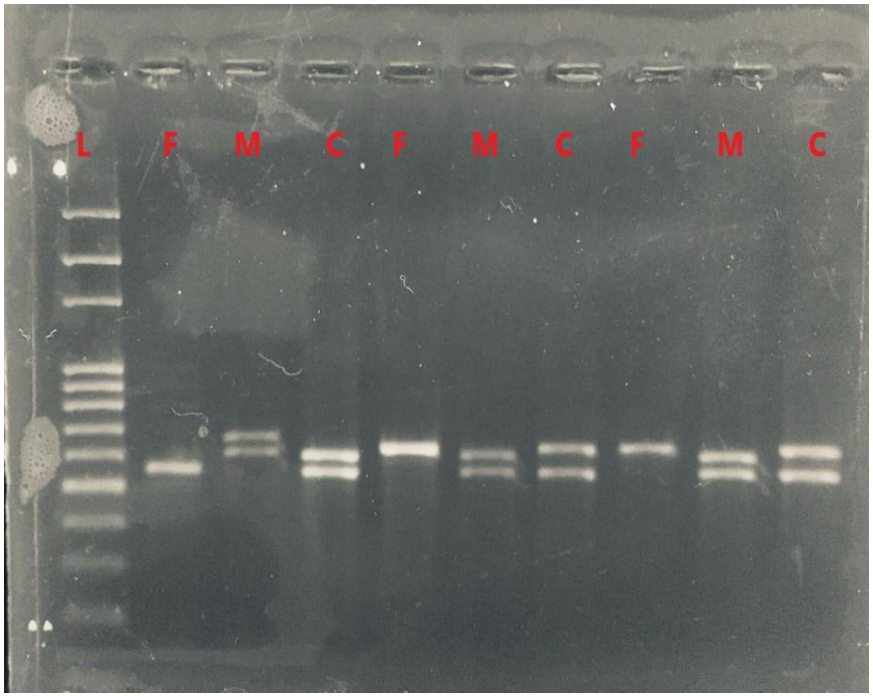
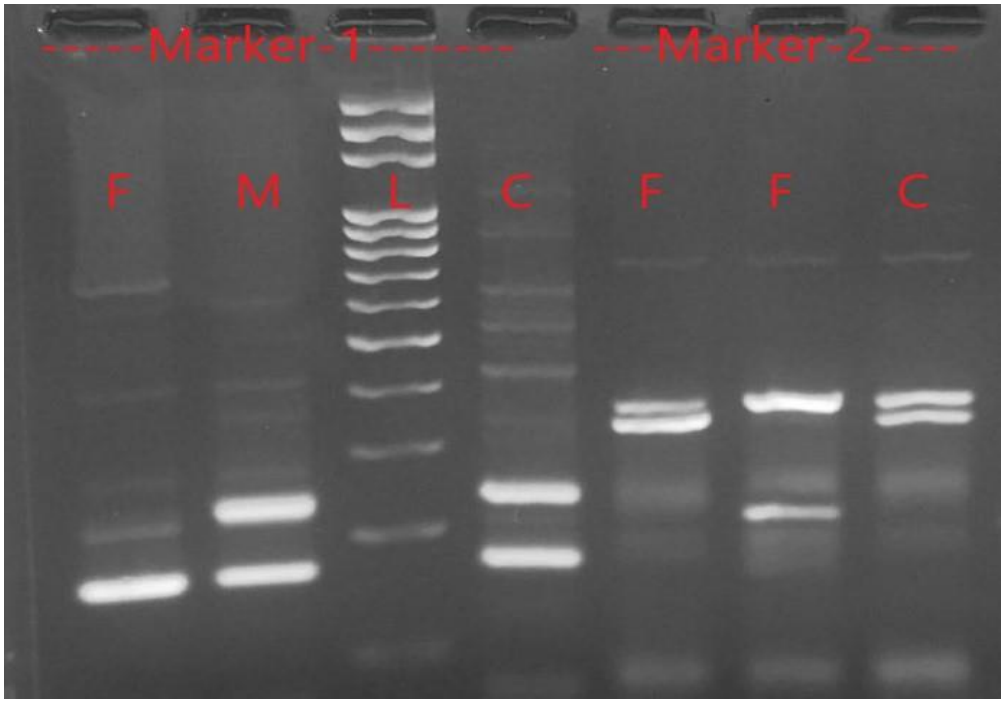
VNTR Analysis





Xmn-I and BCL11A Polymorphism

- Presence of these SNP increases the Blood transfusion gap in Beta Thalassemia patients.

Xmn-1 Polymorphism		**BCL11A**	
Wild type	= CC	Wild type	= AA
Heterozygous	= CT	Heterozygous	= AC
Homozygous	= TT	Homozygous	= CC

Ongoing Research

- GAP PCR for 3.7 and 4.2 Deletion
- HBS1L-MYB Polymorphism

Future Plans

- Direct Sequencing
- Cell Free DNA
- STR Analysis

THANK YOU