Genetics screening of thalassemia HBB genes

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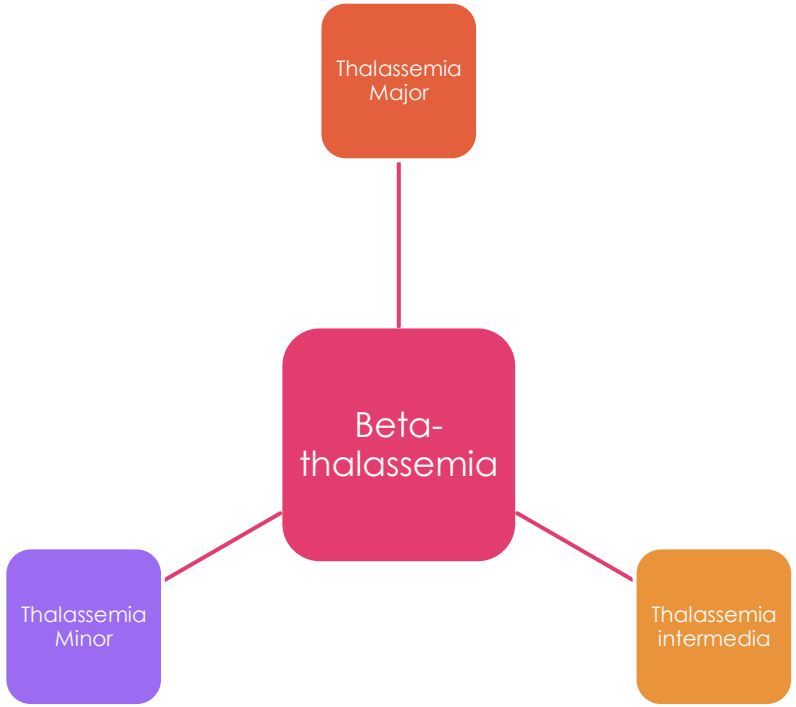
Introduction

- The term thalassemia is gotten from the Greek, Thalassa (ocean) and haima (blood).

- Beta-thalassemia conditions are a gathering of genetic blood issues portrayed by diminished or missing beta globin chain blend, bringing about diminished Hb in red platelets (RBC), diminished RBC creation and iron deficiency.(1)



Types of beta thalassemia (2)

Thalassemia Minor or Thalassemia Trait.

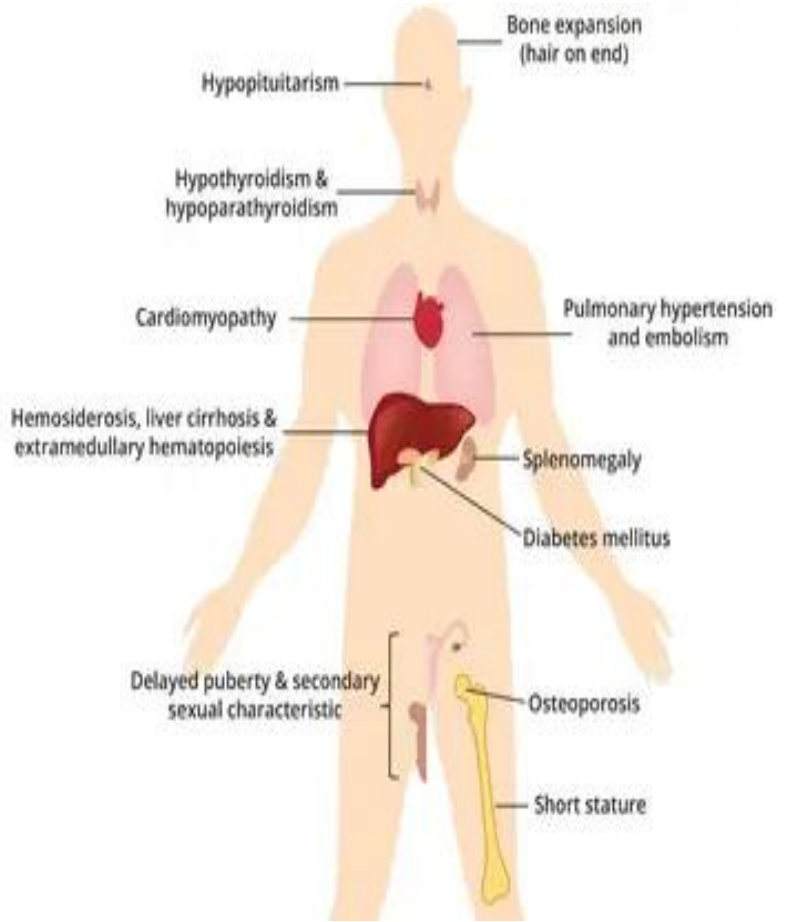
- the lack of beta protein is not great enough to cause problems in the normal functioning of the hemoglobin.
- A person with this condition simply carries the genetic **trait** for thalassemia and will usually experience no health problems other than a possible mild anemia.

Thalassemia Intermedia.

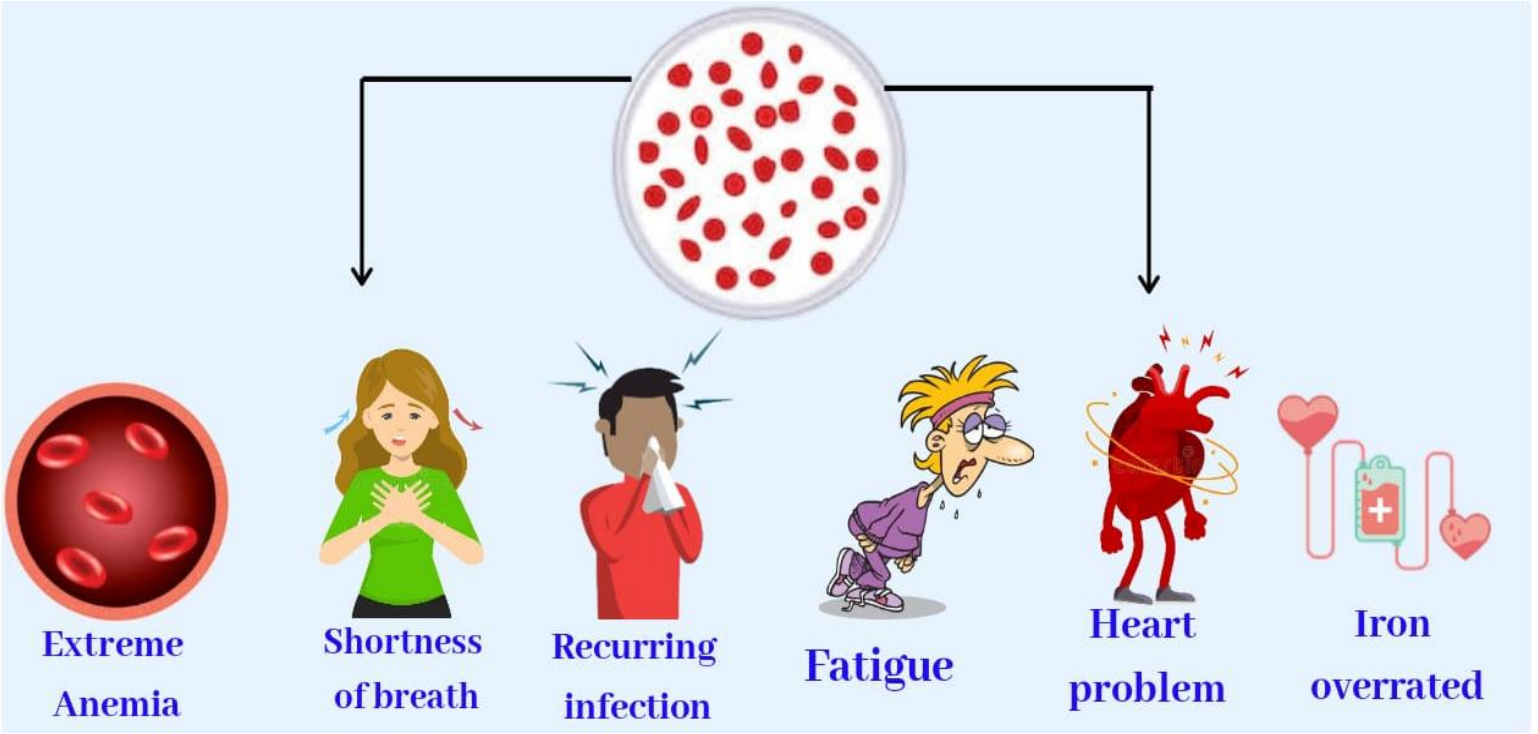
- lack of beta protein in the hemoglobin is great
  - bone deformities
  - enlargement of the spleen
  - patients with thalassemia intermedia need blood transfusions to improve their quality of life, but not in order to survive.

Thalassemia Major or Cooley's Anemia.

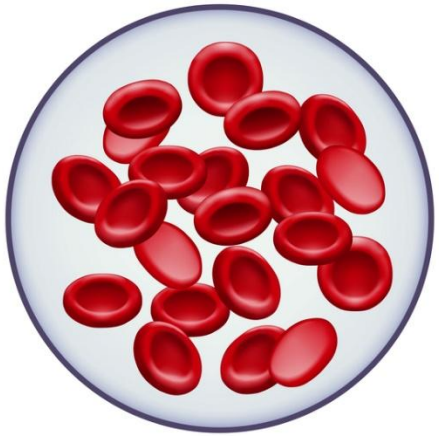
- This is the most severe form of beta thalassemia
- complete lack of beta protein in the hemoglobin causes a life-threatening anemia
- requires regular blood transfusions and extensive ongoing medical care.
- lead to iron-overload which must be treated with chelation therapy



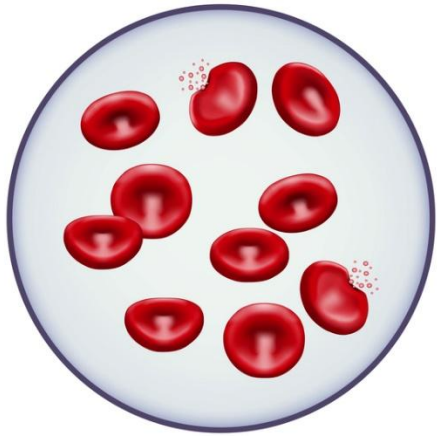
Symptoms of thalassemia (3)



Causes of thalassemia (4)

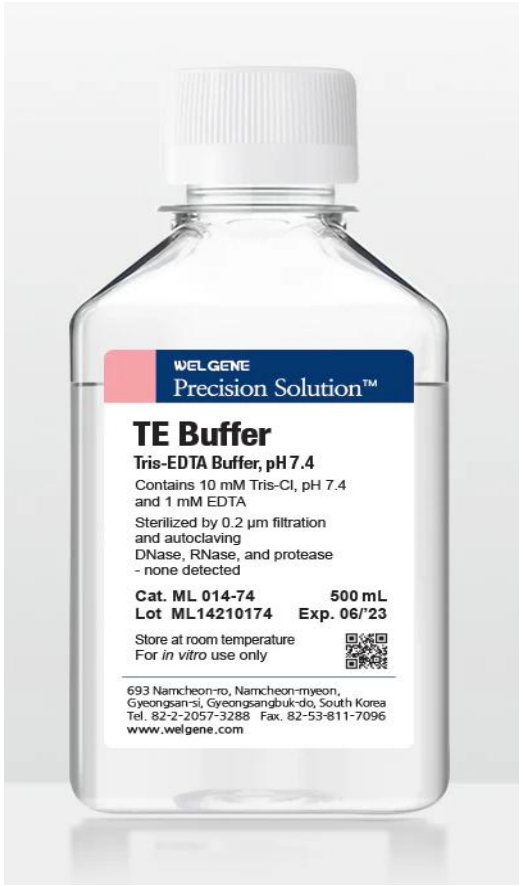


Normal



Thalassemia

METHODOLOGY



1.Sample collection

2. DNA Extraction

3. Add chilled TE buffer 1ml

6. Centrifugation 15 minutes

5. Discard the supernatant

4. Added 900µL chilled TE buffer and shaking

7. Centrifuge again for 15 min. at room temperature

8. Added 800µL TE buffer and shaked by hand

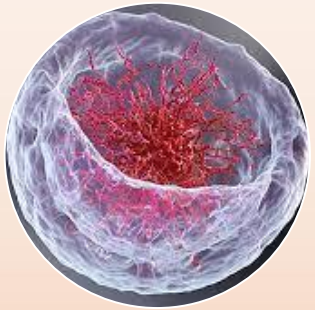
9. Again spin 13000rpm for 15 minutes

METHODOLOGY: DIGESTION PROCESS

After that discard the supernatant and I have added 200µL TEN buffer,20µL SDS and 10µL proteinase K solution. Again, re-suspended pallet by shaking and vortex mixing by vortex machine

After that process incubate the mixture overnight at 56 degrees centigrade for digestion.

METHODOLOGY: PHASE SEPARATION



1. Use organic method for DNA extraction.

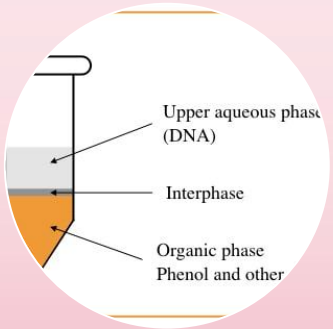
Use PCI for DNA extraction.

2. After the complete digestion add 300µL of phenol-chloroform-isoamyl alcohol solution and mix gently

3. Use centrifuge machine for spin at 13000rpm for 10 minutes .

3 layers observed

METHODOLOGY: PHASE SEPARATION



4. Carefully take upper aqueous layer because that layer containing DNA. Take up with 1ml pipette and transfer to a new labeled 1.5ml centrifuge tube.

5. Added equal volume of chilled isopropanol and invert the tube gently till DNA is visible

5. Addition of isopropanol spin the sample in centrifuge machine at 1300rpm for 1 minutes at room temperature. After spin shows again a little pellet. After that process I discard supernatant carefully.

METHODOLOGY: PHASE SEPARATION

7. Added 200ml absolute ethanol and vortex that sample for 15 sec

8. Use centrifuge machine for spin at 8000rpm for 1 minute

9. Spin again small pellet are formed

Preparation of Agarose Gel (03%)

7. Add 200ml 75% ethanol and vortex sample for 15 sec.

8.Addition of ethanol again. Centrifuge for spin at 8000rpm for 1 minute

9. Dry the sample overnight. Add 100 µL sterile distilled water to dissolve the DNA pellet.

10. Incubate the DNA at 72C for 30 minutes. After that whole process store the DNA at -20C.

Preparation of Agarose Gel (03%)

Agarose 0.3gm

Reagents

TAE buffer(1X) 30ml

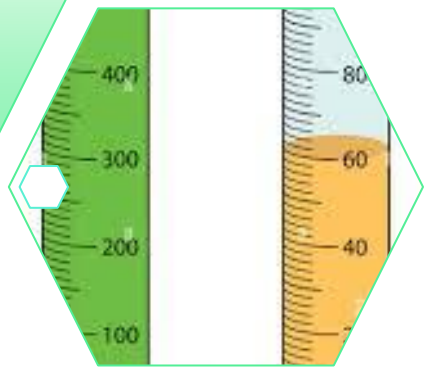
Ethidium bromide 4µL

Preparation of Agarose Gel (03%)

Equipment

Conical flask

Measuring cylinder



Gel caster with combs

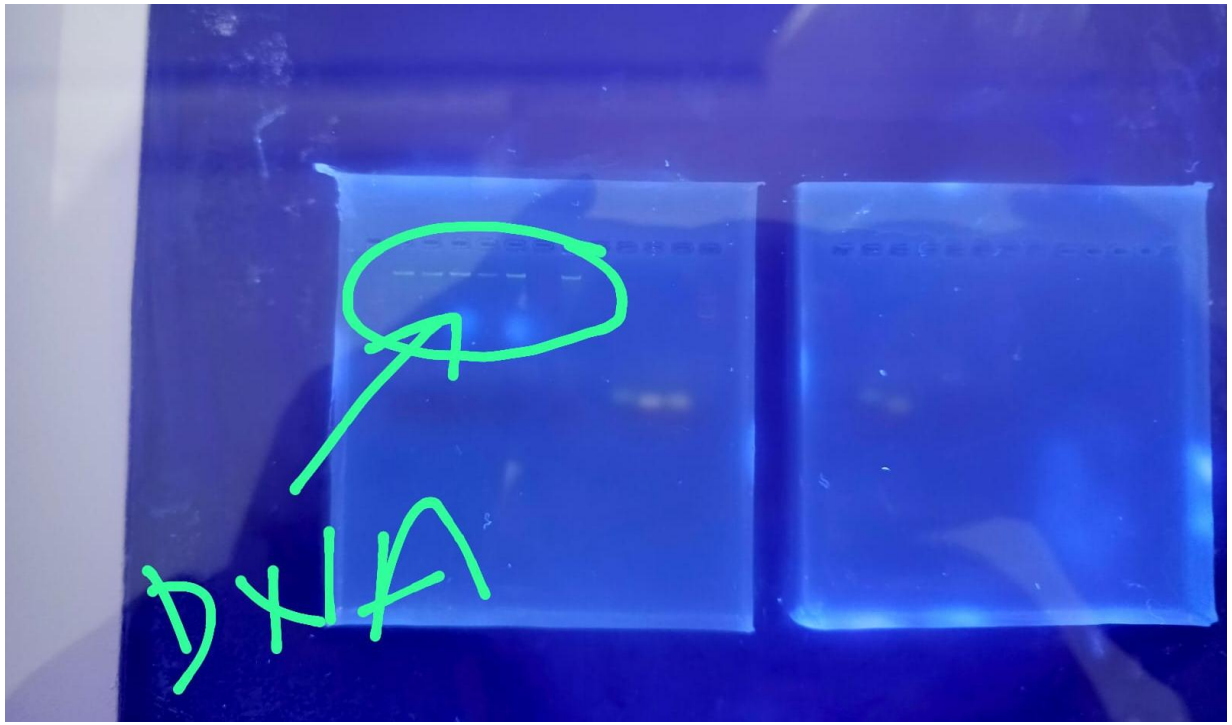
Preparation of Agarose Gel (1%): SAMPLE LOADING

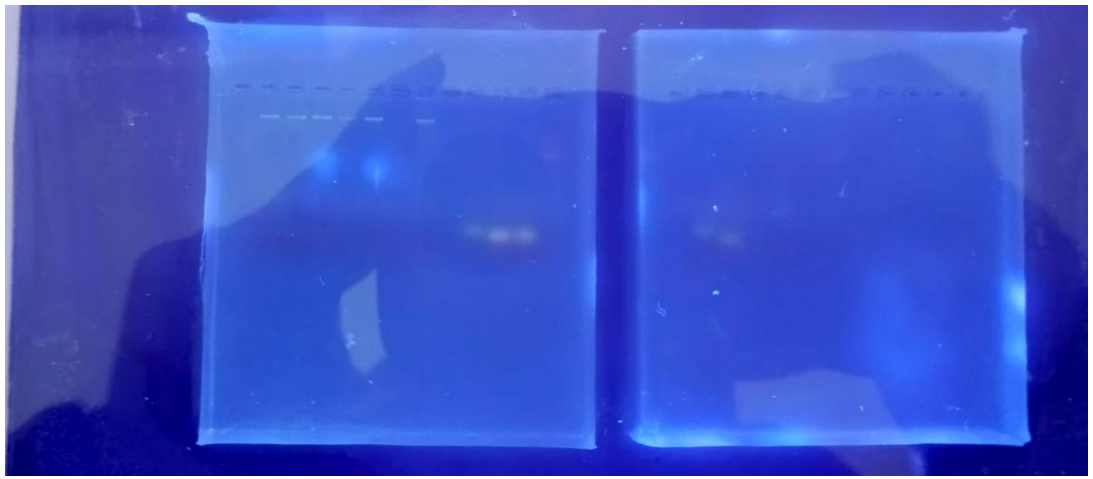
ITake 0.3gm agarose and 30ml of TAE buffer (1x) and heat solution for 90sec until boiled. cool solution then add ethidium bromide and mix it then load solution in gel caster. after gel solidified transfer in the gel tank.

After that load the sample in wells. Take 5µL DNA sample and 1.5µL red dye mixed it with carefully and load in the wells.

Set the apparatus with 110 voltage for 30 minutes and after that on supply. After the appropriate time I off supply and take gel and observed the DNA under UV light with the help of UV transilluminator

Under the observation of UV light, I observe DNA bands.





Primers

Primer ID	Sequence 5'----3'	Length	Tm	GC%	Product
F_HBB_Ex1	agggttggccaatctactcc	20	60	55	535
R_HBB_Ex1	CACTCAGTGTGGCAAAGGTG	20	60	55	
F_HBB_Ex1a	gccatctattgcttACATTTGC	22	59	40	484

agggttggccaatctactccaggagcagggagggcaggagccagggctgggcataaaagtcagggcagagccatctattgcttACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGgttggtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcatgtggagacagagaagactcttgggtttctgataggcactgactctctctgcctattggtctattttcccaccccttagGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTG

PCR AMPLIFICTION: REAGENTS REQUIRED

- Taq DNA polymerase, dNTPs, $MgCl_2$ and PCR buffer
- PCR master mixer
- PCR primer
- PCR grade water
- DNA template
- DNA polymerase

PCR AMPLIFICTION: EQUIPMENT

Thermal cycler with analysis software

Vortex mixture

Microcentrifuge

Pipettes

PCR safety cabinet

PCR tubes

PCR AMPLIFICTION: PROCEDURE

- First of all, label the PCR tubes for sample place in PCR tubes racks and after that prepare PCR reaction mixture. A generalized recipe of PCR is given below;

DNA template(1x)	3µL
DNA polymerase	0.5µL
dNTPs	2µL
Buffer	2.5µL
$MgCl_2$	2.5µL
ddH_2O	12.5µL

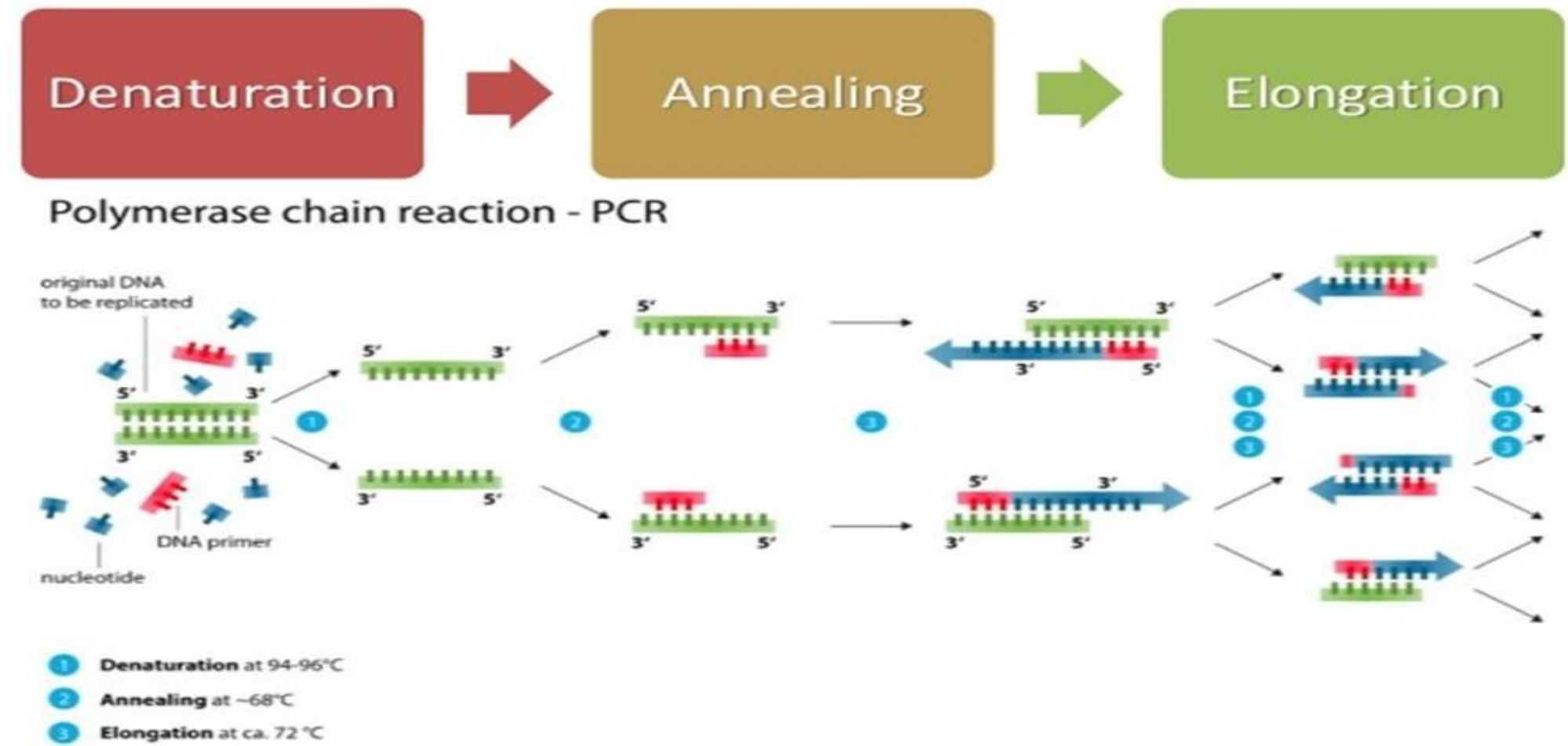
PCR AMPLIFICTION: PROCEDURE

- After the addition of all these components that mixture is called master mixer. Now after that I added master mixture in label PCR tubes and added forward primer and reverse primer 1µL in PCR tubes and after that I mix the reagent by gentle vortex by short spin.

- After the short spin open PCR machine software and I added in run parameters. Such as user ID etc. And also set stages or step cycling protocol for PCR.

PCR: STAGES

Denaturation → Annealing → Elongation

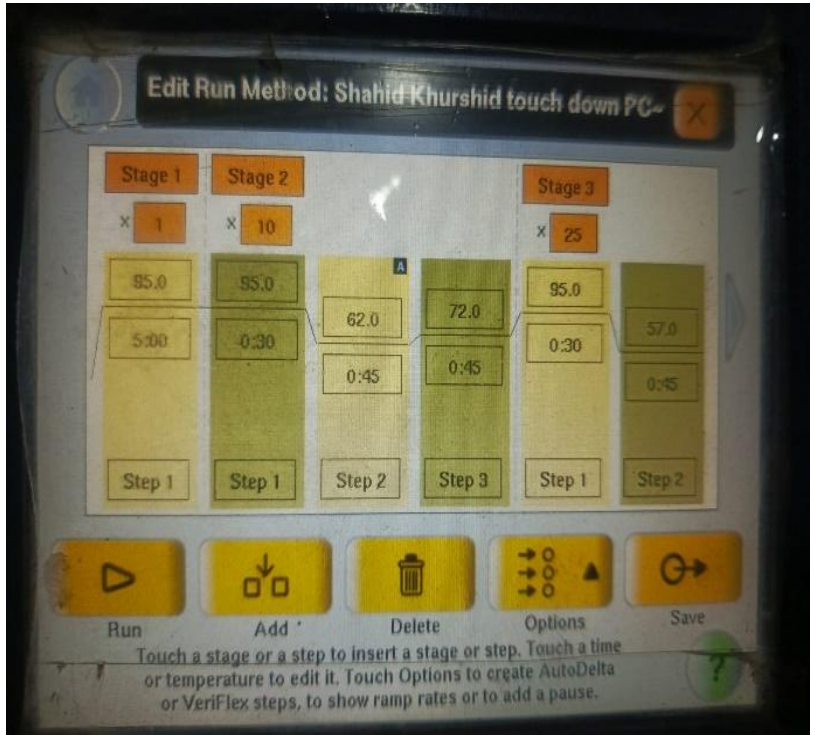


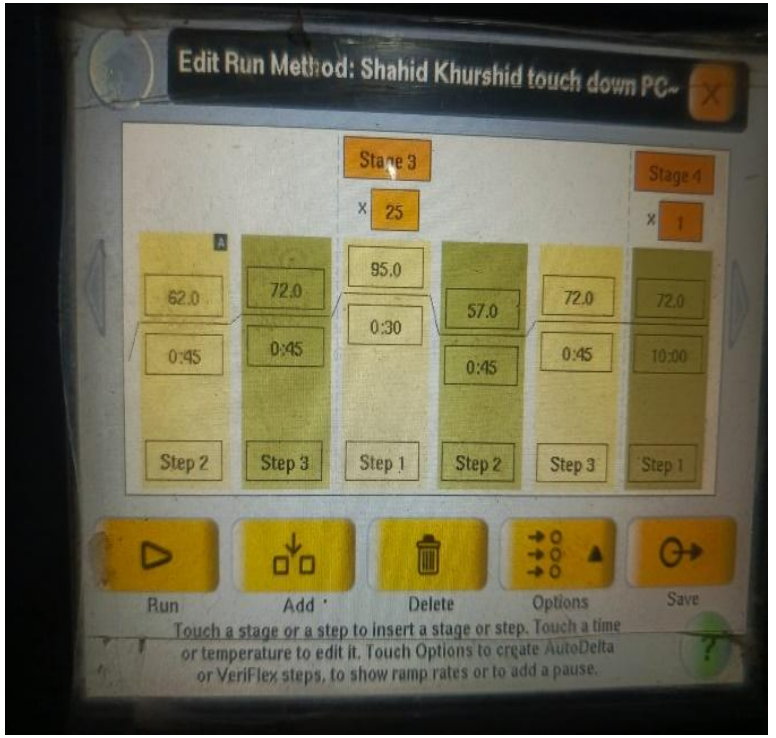
PCR AMPLIFICTION: PROCEDURE

- For initial denaturation I set 95C for 5 minutes. After that second stage is called denaturation for this, I set 94C for 30 second. After denaturation start annealing process for this, I set 60C for 40second. After annealing start extension cycle in these stages I set 72C for 01minute. After that start last cycle that is called final extension at 72C for 10 minutes. For whole process I set 30cycle for completion of PCR reaction.

- After that process place the sample tubes in thermal cycler and close the lid and know run the program. After the completion of all cycle and appropriate time I removed the tubes from thermal cycler and proceed agarose gel electrophoresis.

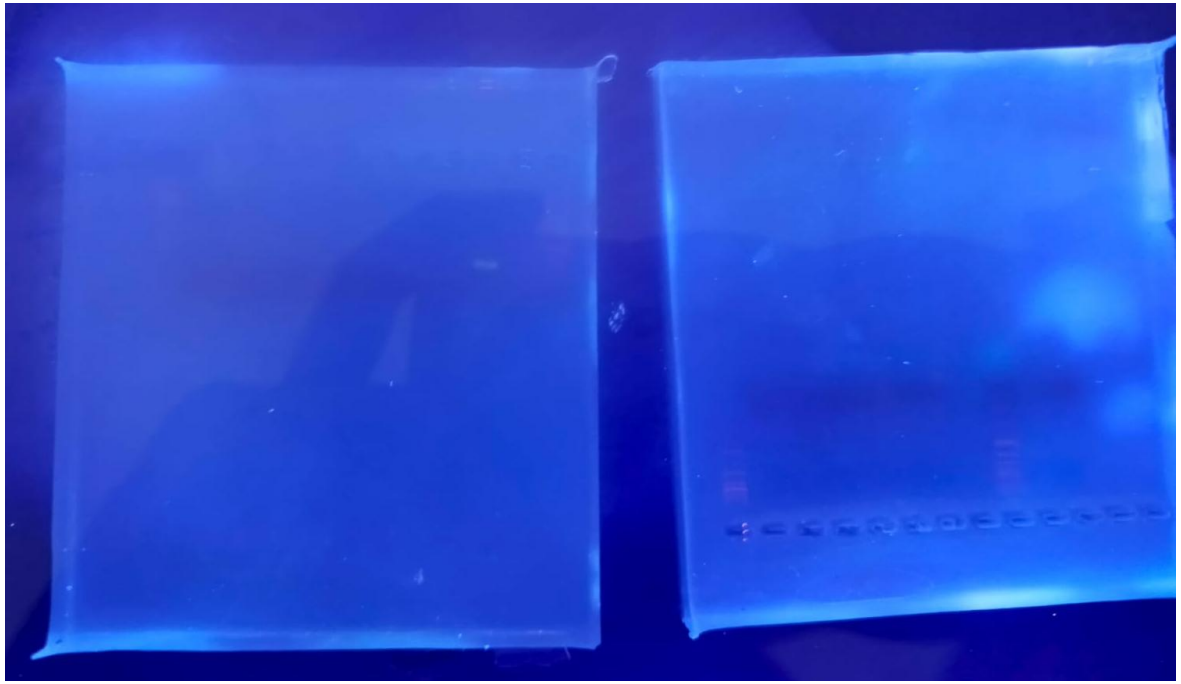
PCR AMPLIFICTION: PROCEDURE





Results

- DNA visible in DNA extraction.
- No result was found in PCR product under UV trans illuminator.



Sequencing result

- TTGTTTTTGTCTCGACGCGAACTGASTGAWCAACACCWTGRTGCCCTGAC
  TCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTG
  GATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGA
  CAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACT
  CTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCC
  ACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTGAGTCCTTT
  GGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGC
  TCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGG
  ACAACCTCAAGGGCACCTTTGCCACACTGAGTGAAG


991_HBB.ab1
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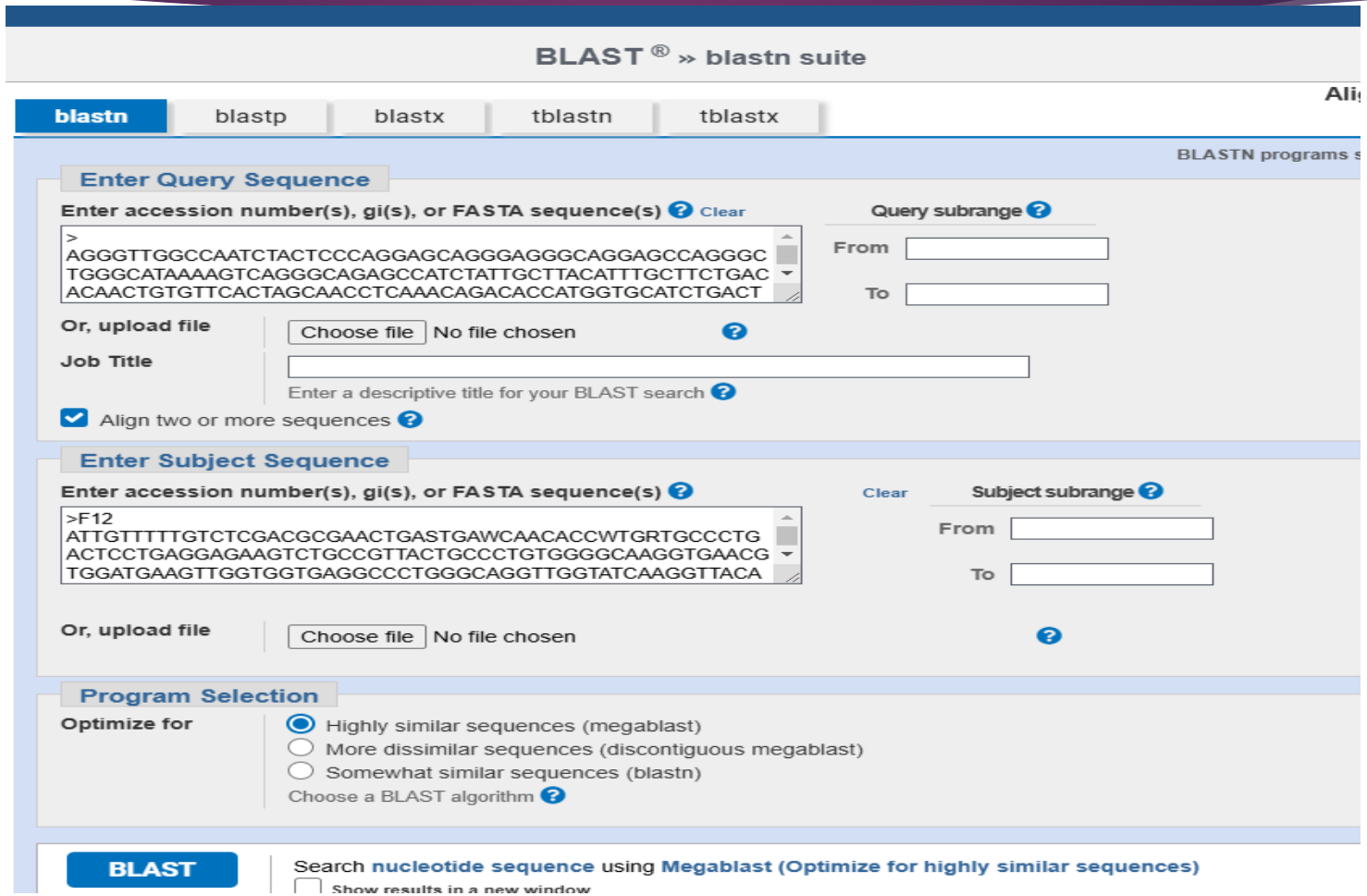
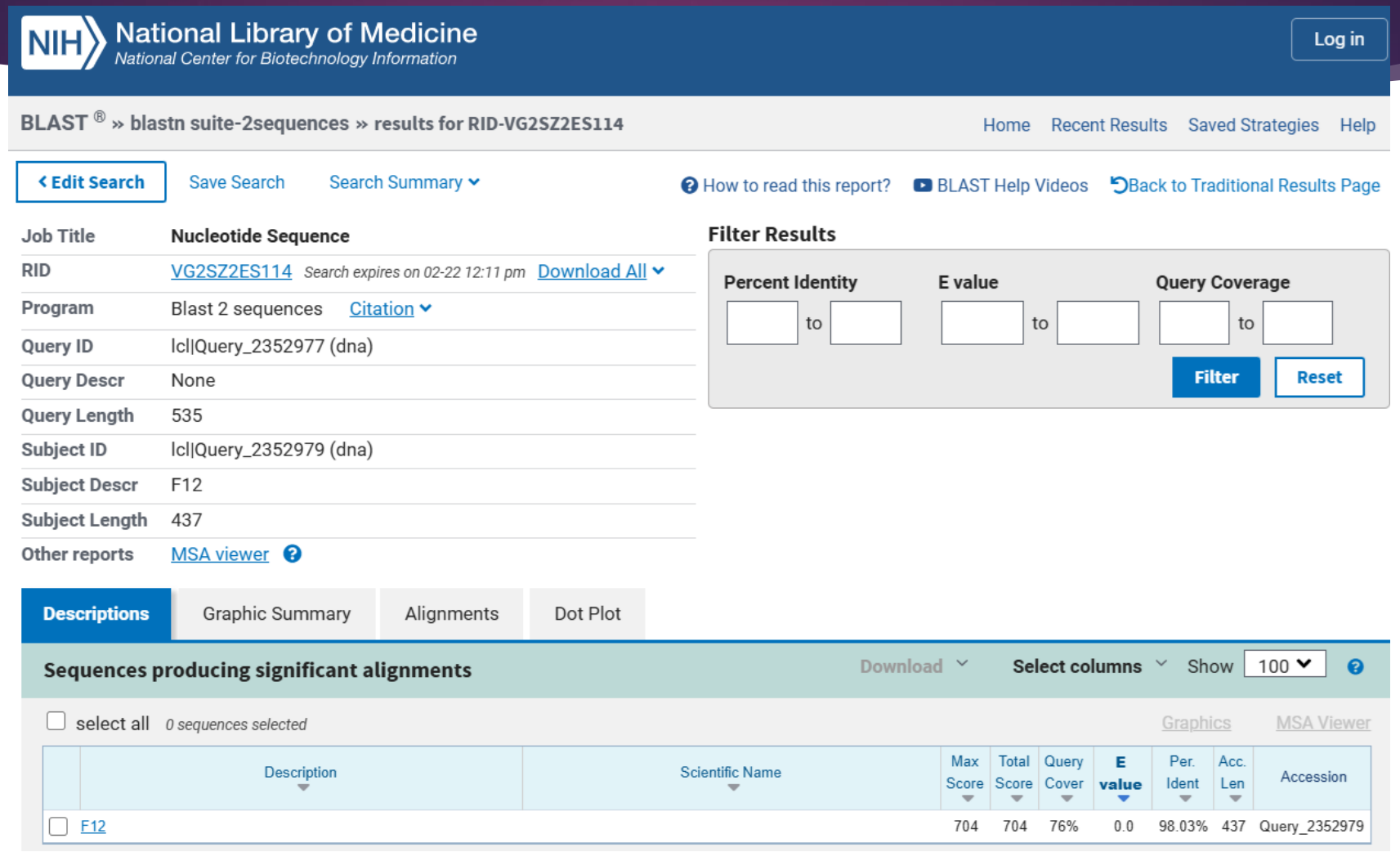
60
70
80
90
100
110
G G A G A A G T C T G C C G T T A C T G C C C T G T G G G G C A A G G T G A A C G T G G A T G A A G T T G

NCBI BLAST Analysis



NCBI BLAST Analysis



Sequence alignment analysis

```
Alignment statistics for match #1
Score    Expect  Identities      Gaps    Strand
704 bits(381)   0.0     398/406(98%)    5/406(1%)       Plus/Plus
Query  130  ACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGG  189
            ||||| || |||  ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  34   ACACCWTGRTGC-CCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGG  92

Query  190  TGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACA  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93   TGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACA  152

Query  250  GGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTG  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  153  GGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTG  212

Query  310  ATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTC  369
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  213  ATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTC  272

Query  370  TACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTT  429
            ||||||||||||||||||    ||||||||||||||||||||||||||||||||||||||
Sbjct  273  TACCCTTGGACCCAGAGG----TTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTT  328

Query  430  ATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGC  489
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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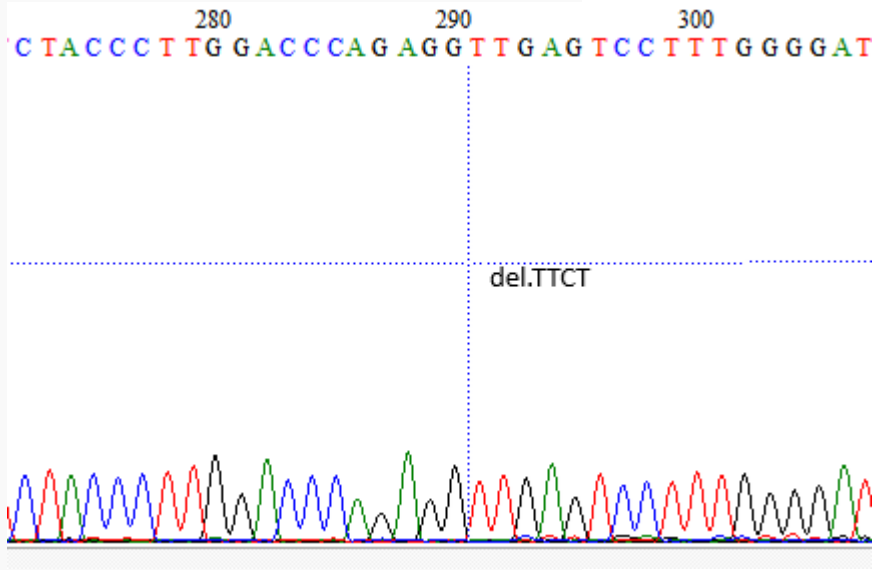

del.TTCT in the 2nd exonic region

Conclusion

- Sequence analysis of HBB gene showed TTCT deletion resulting the frameshift which caused disease thalassemia in this family.

Reference

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Thank
You