UG – Research Experiments

Module 1 - PCR

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Overview

Objective - To Amplify a Gene with a SNP associated to a Human disease using PCR and its Extraction

Procedure -

1) Design best primers.
2) Find the Optimal annealing temp. for primers.
3) Perform a PCR on PCR to amplify the product.
4) Do Gel Extraction of the product and get the product concentration by nanodrop.
5) Send for Sequencing.

Telomerase Reverse Transcriptase – TERT Gene

- Telomerase reverse transcriptase (TERT) is the catalytic subunit of the **telomerase enzyme**, which plays a crucial role in maintaining the length of **telomeres** by addition of the telomere repeat TTAGGG.
- Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres.
- Deregulation of telomerase expression in somatic cells may be involved in oncogenesis. Studies in mouse suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks. [provided by RefSeq, Jul 2008]

Primers Design

Criterions:

- Primer length ~ 15-30 bp
- G-C content ~ 40 – 60%
- Melting Temp. ~ 55 - 60°C

- Avoid Di-nucleotide repeats or single base runs could lead to slipping or hairpin loop structures.
- Primers shouldn't be complimentary to each other, could lead to formation of primer dimers and hairpin loop structures.

Type: Homozygous

Chromosome Position: 5:1287079

SNP Site: GAGACCCG[C/T]CTGGTGCA

SNP Accession No.: rs2853677

Forward Primer (5'->3'): AGACTTTGGGGCAATACAGGG

Reverse Primer (5'->3'): AACAGACCCCAATCCCCCA

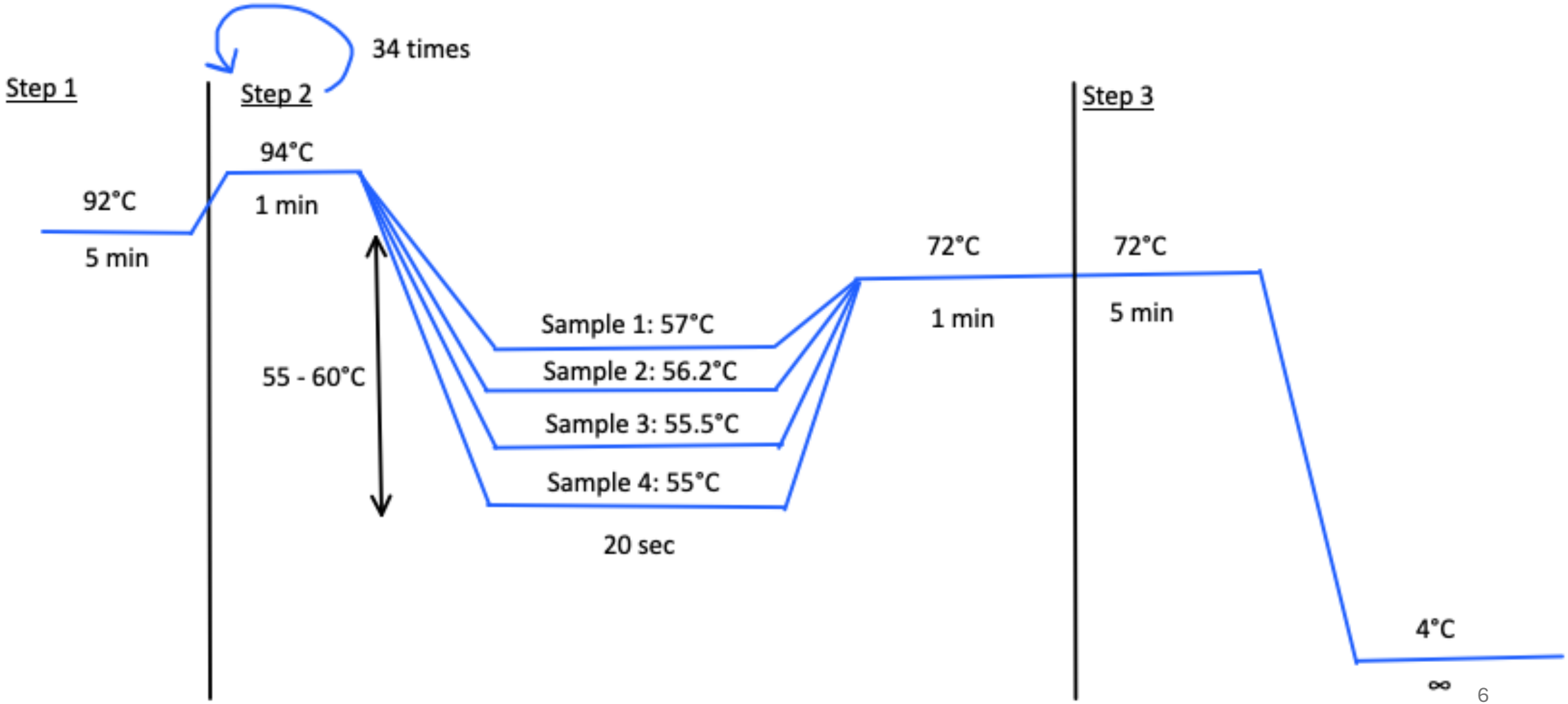
Product Length: 289

PP	Template Strand	Length	Tm	GC%
Forward	Plus	21	59.99	52.38
Reverse	Minus	19	60.47	57.89

Method

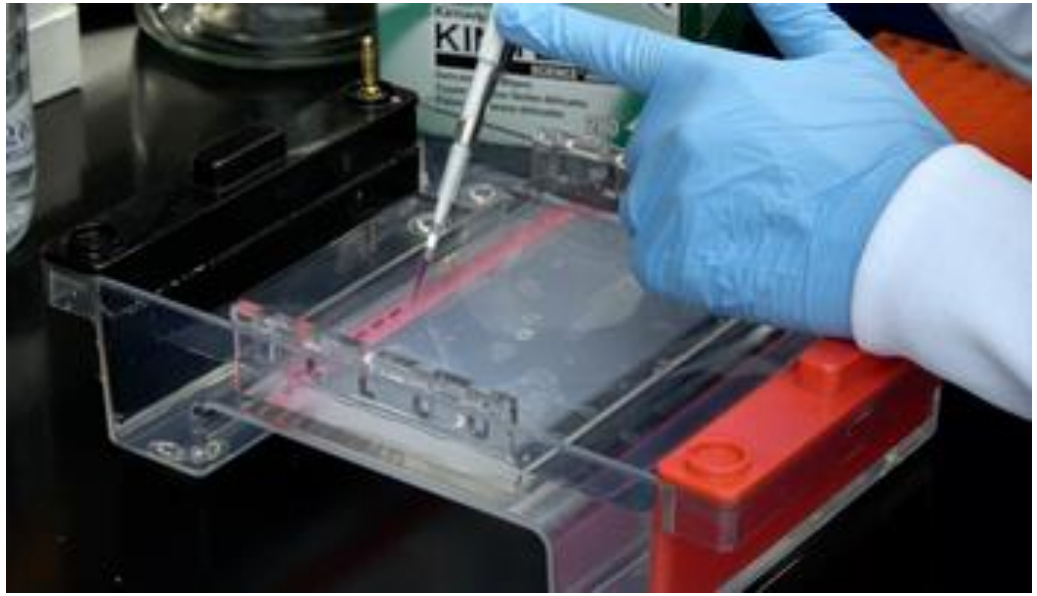
- Prepared 20µL working stock of the primers using NFW.
- Prepared Master mix
  - Template DNA - 1.5µL (HEK Cells)
  - Forward Primer - 1µL
  - Reverse Primer - 1µL
  - DNTPs - 2µL
  - Taq polymerase - 1µL
  - 10 × Buffer - 5µL
  - NFW – 38.5µL
- Performed PCR with 4 samples with a gradient of Annealing temp.

PCR – To find the optimal annealing temp.



Gel prep

- Prepared 1% agarose gel + 2µL EtBr (50ml)
- Took 8µL of 10µL from each sample and added 2µL of Loading Dye.
- Prepared Ladder (100bp) - 4µL Ladder + 4µL Loading Dye.
- Loaded the gel and ran at 120 V.



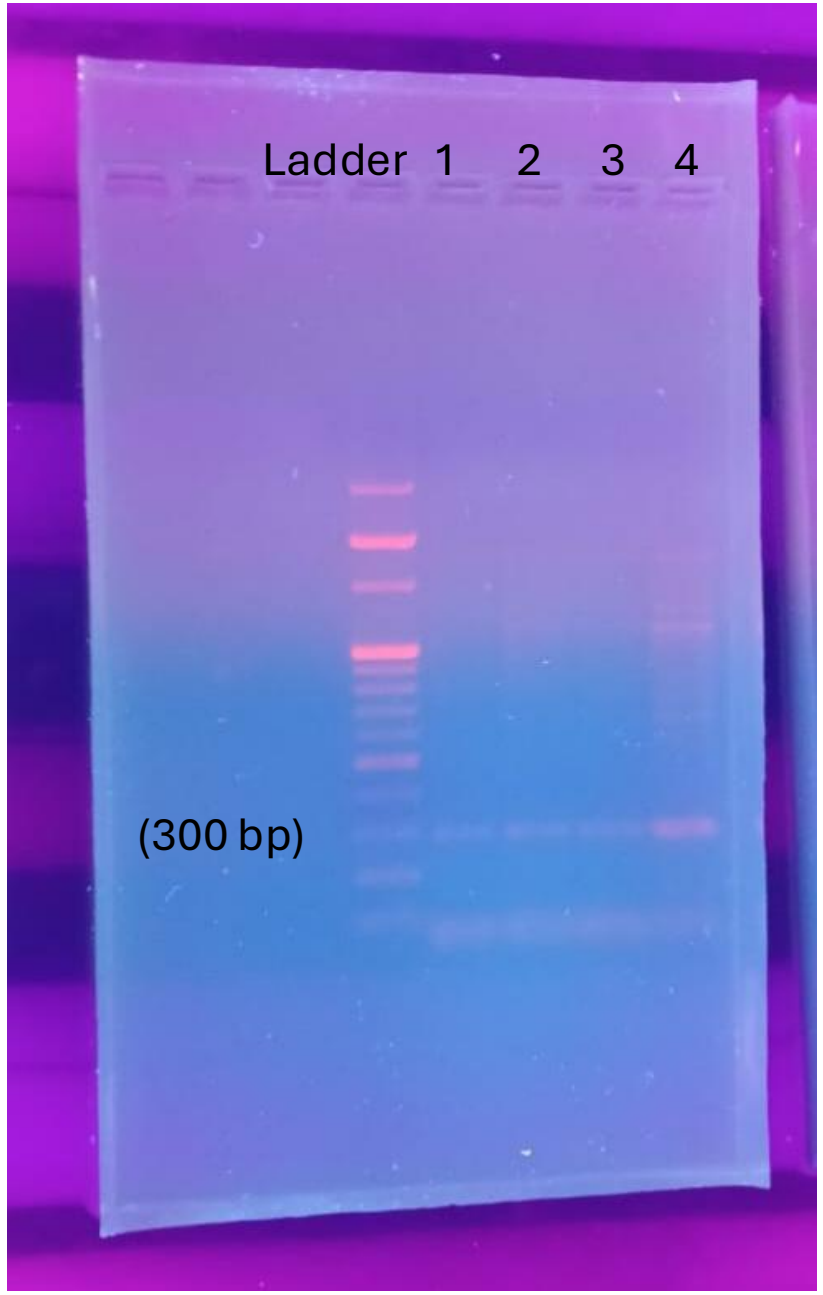


Fig 1. PCR results of all 4 samples.

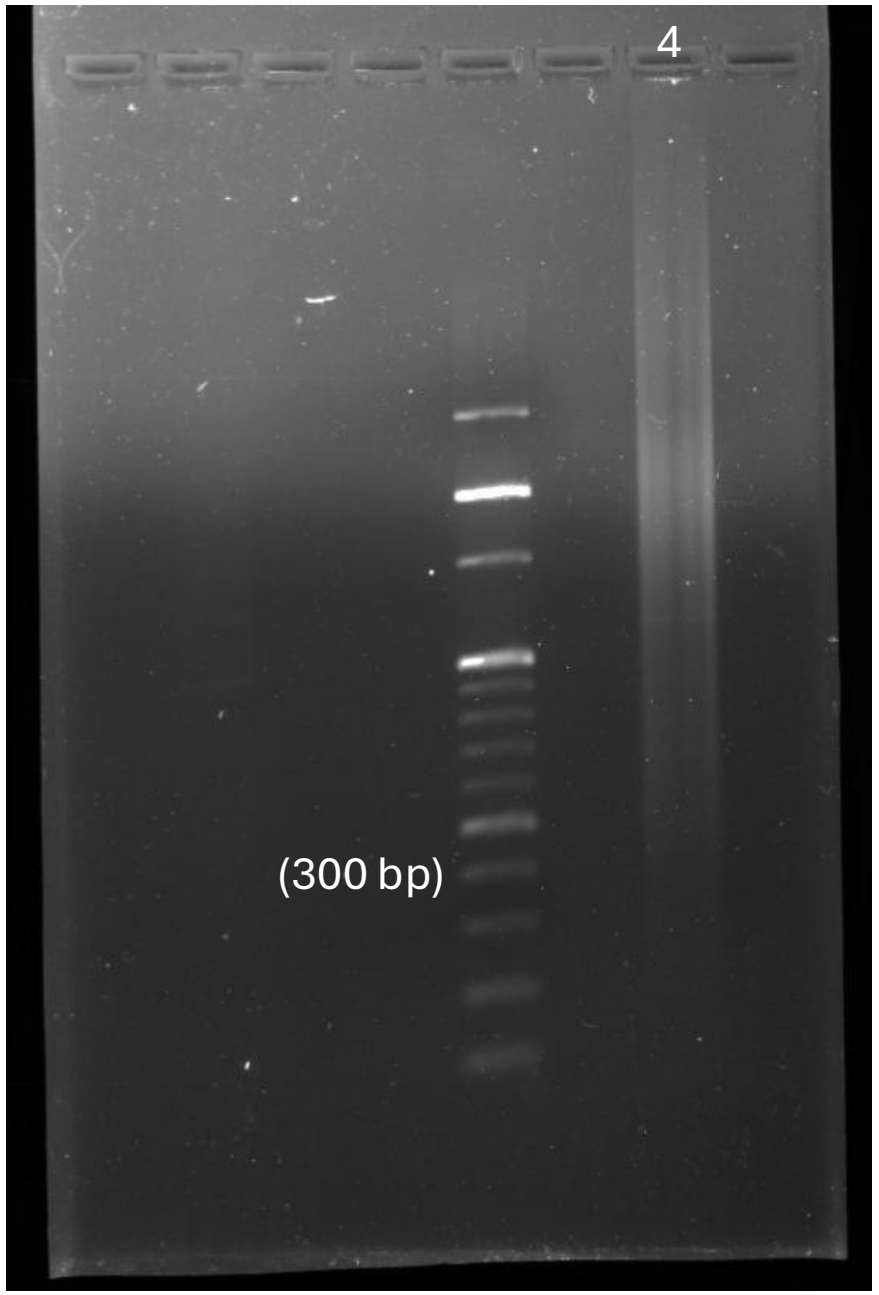
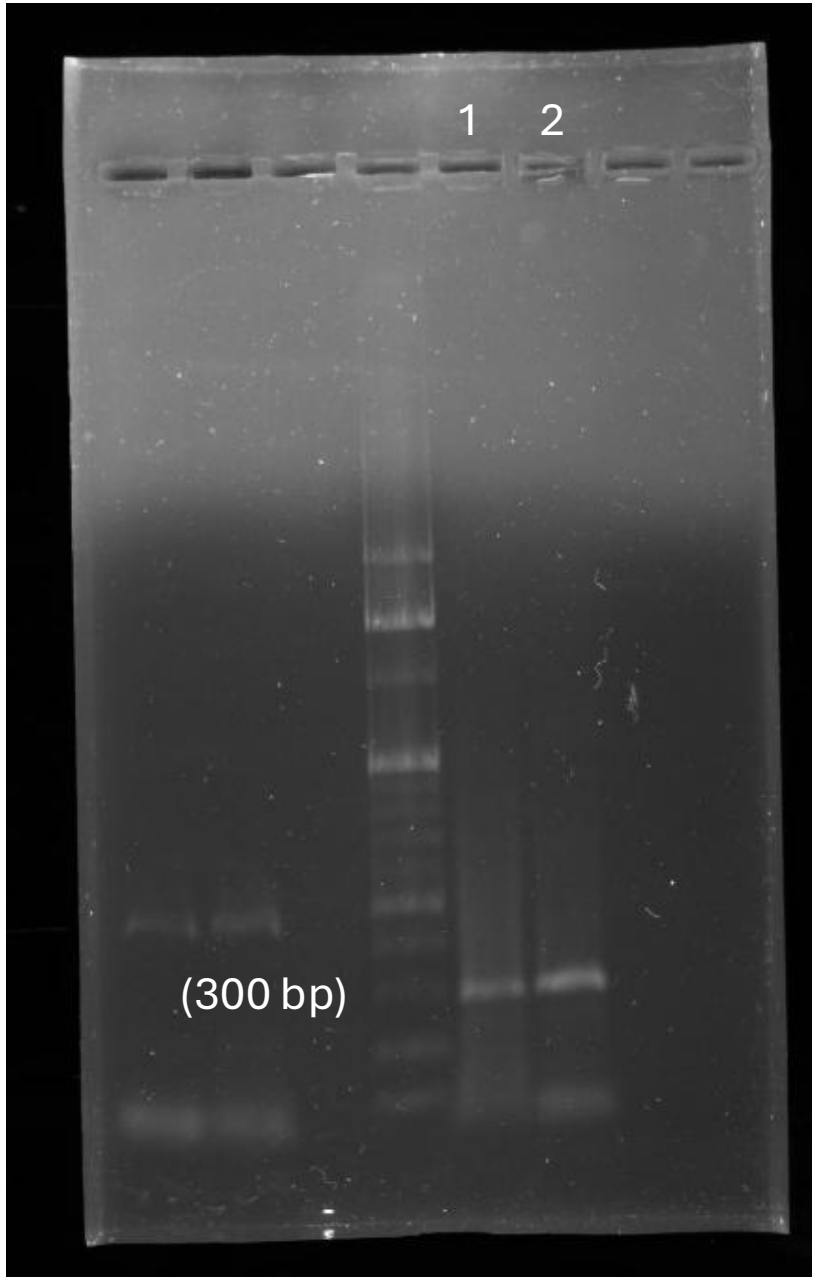


Fig 2. Performed PCR on PCR on Sample 4

1. Performed PCR on Sample 4 again from template DNA (55°C) (35µL - Preserved)
2. Performed PCR on PCR on 2µL of sample 2 at 56.2°C (35µL - Preserved)



Gel Extraction

- Used 70µL (35 + 35µL) and ran it on 1% agarose gel at 80V and cut the product out.
- Used QIAquick Gel extraction kit to extract the product

Procedure:

1) Weighed the product
2) Add buffer QG and incubate at 50°C
3) Add isopropanol (100µL per 100mg)
4) Put it in QIAquick column and centrifuge for 1min
5) Discard flow through and wash with 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min
6) Discard flow through and centrifuge for 1 min.
7) Elute DNA add 50 µl of Buffer EB to the center of the QIAquick membrane and centrifuge the column for 1 min in a clean 1.5 ml microcentrifuge tube.

Nanodrop

- To measure the concentration of the result.
  - Select your desired application (DNA, RNA, or protein).
  - Gently clean and buff the pedestal with a lint-free wipe.
  - Pipette 1-2 µL of your blanking solution (Water in my case).
  - Click “Measure blank.”
  - Lift the arm and wipe clean with a lint-free wipe.
  - Then Put the 1-2 µL of sample and click sample.

- Results :

DNA concentration: 36.8 ng/µL
A260 value: 0.737
A260/A280 ratio: 1.86
A260/A230 ratio: 0.26

DNA concentration: 16.7 ng/µL

A260 value: 0.737

A260/A280 ratio: 1.81

A260/A230 ratio: 0.27

So, the final **DNA concentration of combined sample is 26.75 ng/µL**.

Thank You!

Equipements used -

- NCBI Primer-BLAST – Primer designing
- HiMedia (100bp) – ladder
- QIAquick gel extraction kit - extraction
- Nanodrop