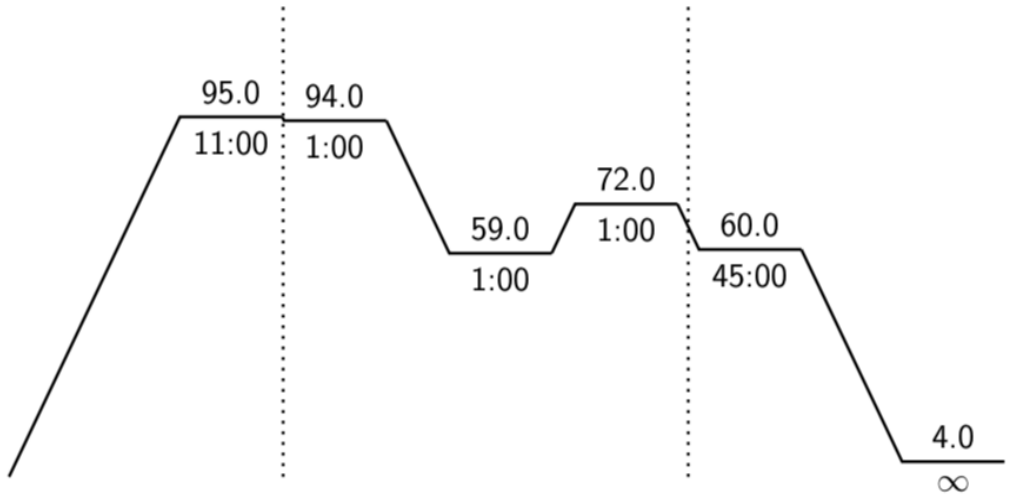
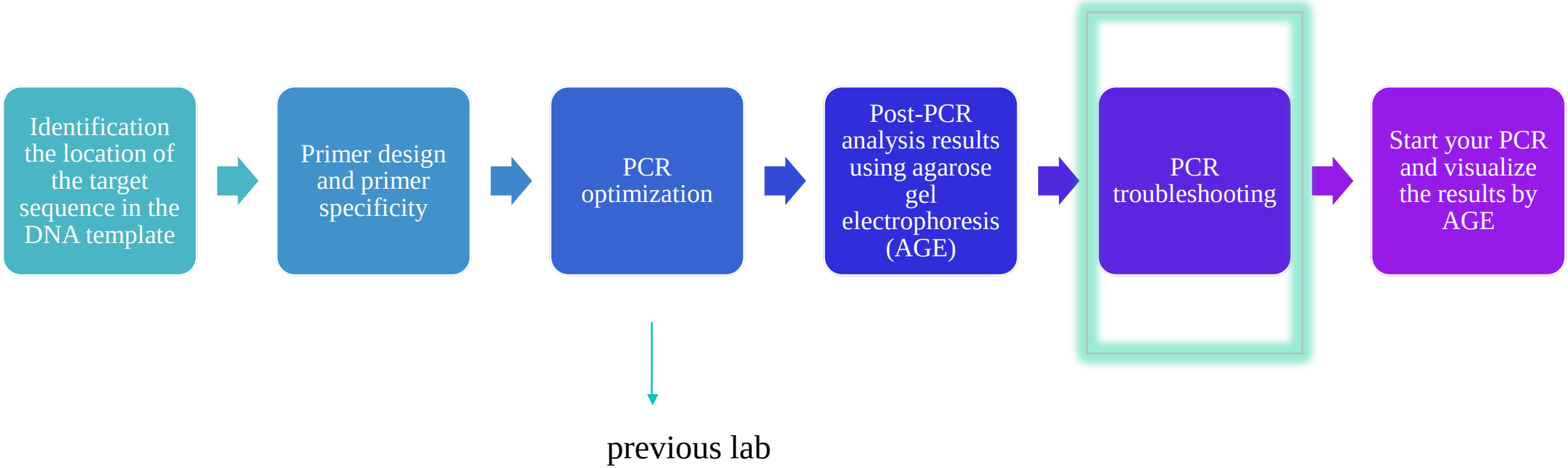
PCR Troubleshooting

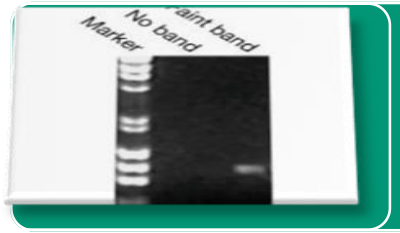
BCH361- Practical



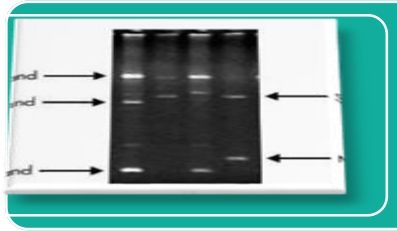
Performing PCR steps :



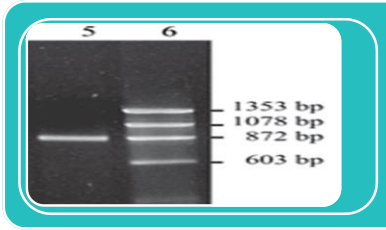
Common Issues in PCR:



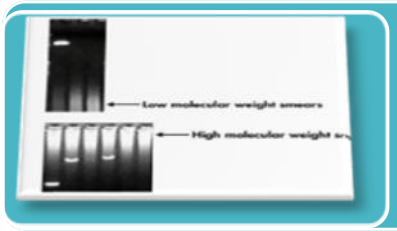
Low or no amplification



Non-specific band or primer dimer



Incorrect product size

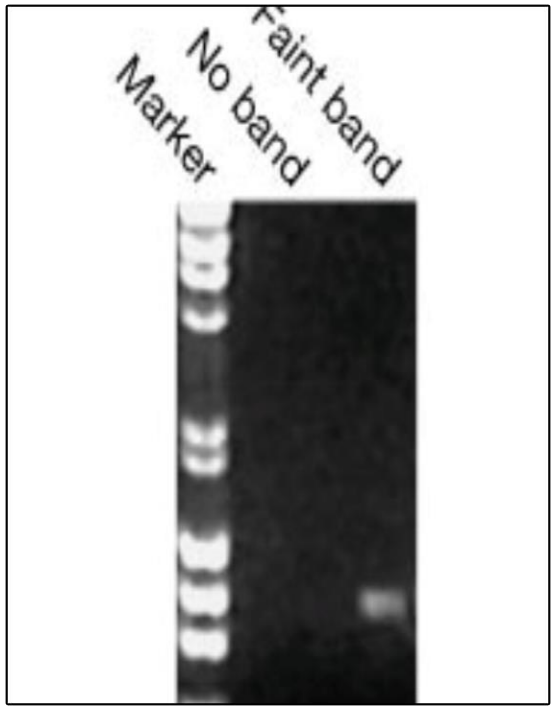


Smeared Bands

1- No Band or Faint Band:

Causes Related to Cycling Times and Temp.

- Too Few cycles were used.
- Extension time was too short.
- **Incorrect annealing temperature.**
- Denaturation temperature was too low.



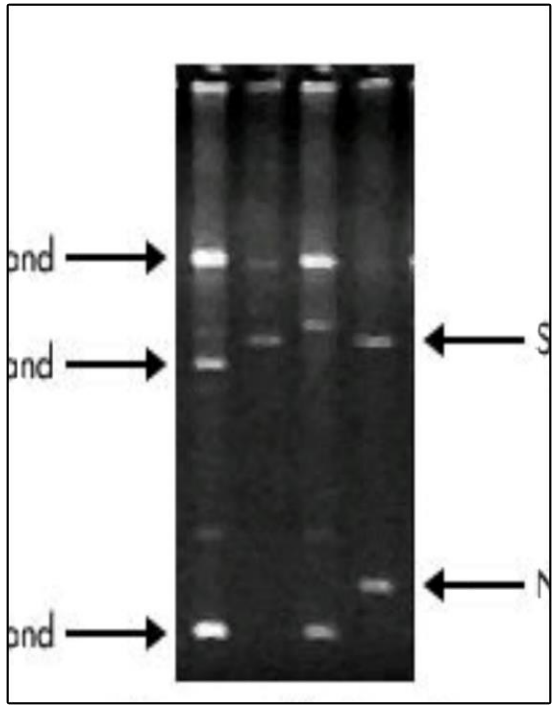
Causes Related to PCR Components

- **No enough template was in the reaction.**
- Primer concentration was too low.
- Impure primers, dNTPs, or water
- **PCR product has high GC content.**
- Primers were designed or synthesized incorrectly.
- **No enough Mg^{2+}.**

2- Nonspecific Bands or Primer Dimer:

Causes Related to Cycling Times and Temp.

- **Annealing temperature was too low.**
- Too many cycles were used.
- Extension time was too long.



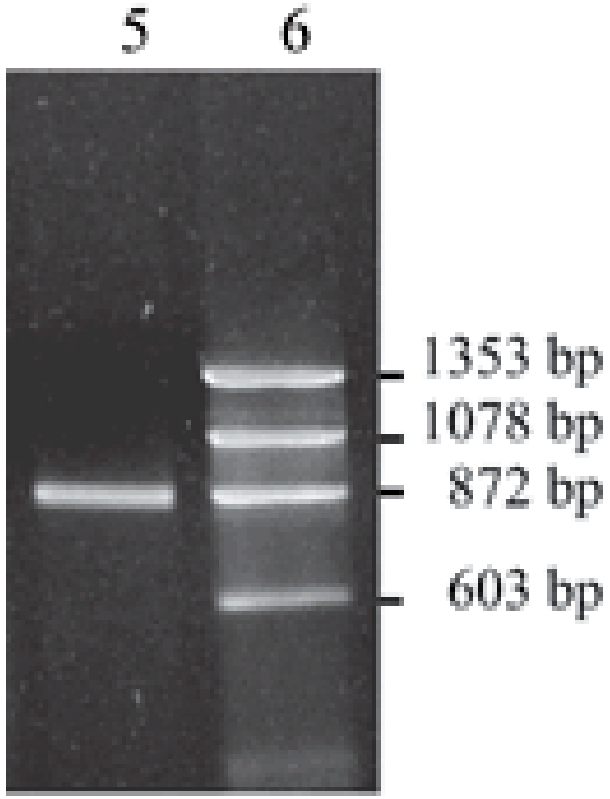
Causes Related to PCR Components

- **Too much primer was added.**
- **Too much Mg2+ was added.**
- Primers were designed or synthesized incorrectly by user or manufacturer.

3- Incorrect PCR product size:

Causes Related to Cycling Times and Temp.

- **Incorrect annealing temperature.**



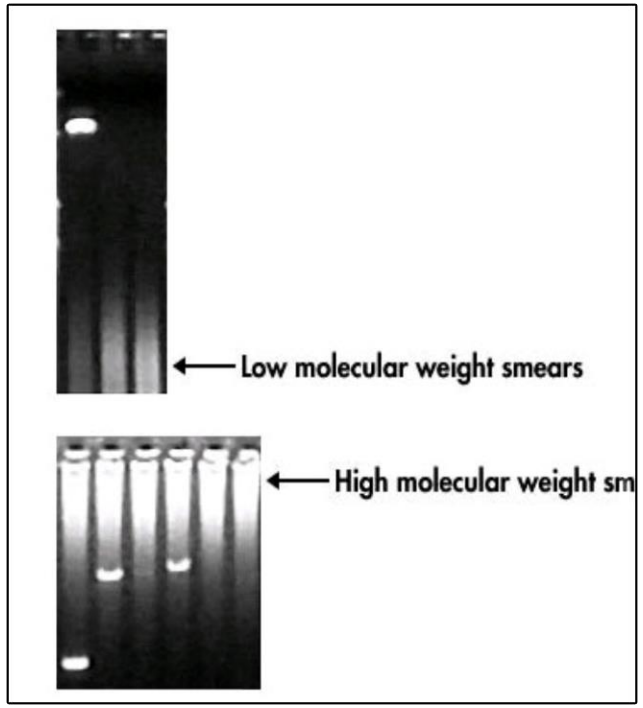
Causes Related to PCR Components

- Mispriming.
- **Improper Mg2+ concentration.**
- Impure primers, dNTPs, or water
- Primers were designed or synthesized incorrectly by user or manufacturer.

4- Smeared Band:

Causes Related to Cycling Times and Temp.

- Too many cycles were used.



Causes Related to PCR Components

- **Too much template was added.**
- Impure primers, dNTPs, or water.
- **Template contained an exonuclease or was degraded.**

Common PCR additive reagents:

1. Additives that benefit GC Rich templates:

➔1-10% DMSO (Dimethylsulfoxid):

- GC rich (GC content >60%).
- Lowering the Tm.
- Distrusting the base pairing.

➔Q solution:

- High degree of secondary structure.
- GC-rich.
- Increases PCR specificity.

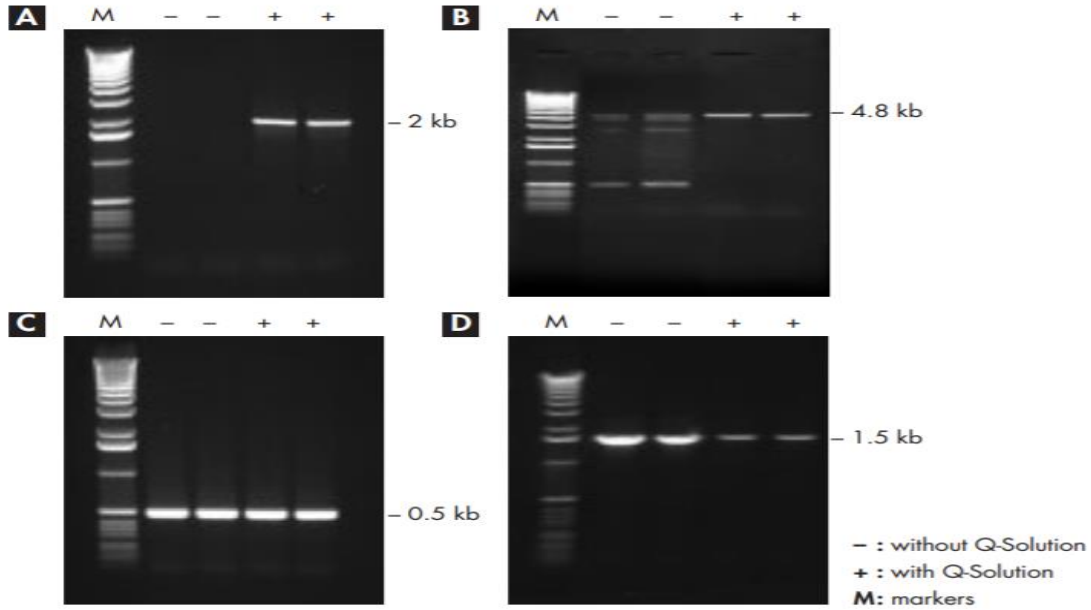
➔PCRx Enhancer:

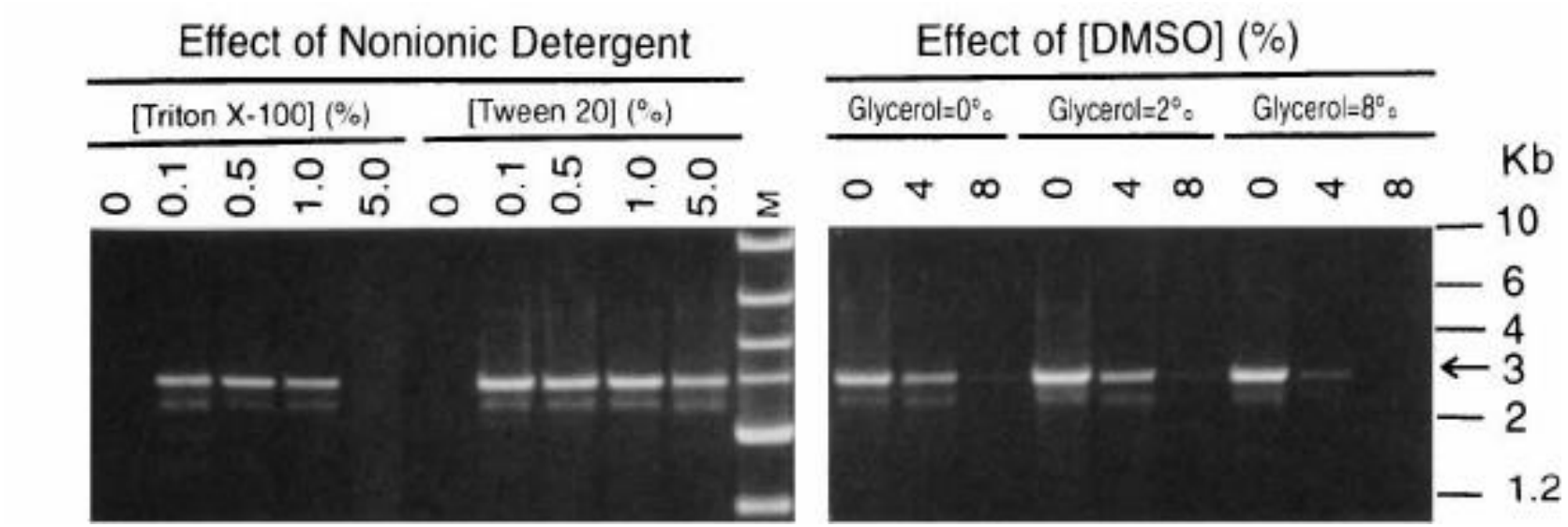
- For problematic and/or GC-rich templates.
- Higher primer specificity, broader magnesium concentration optima, broader annealing temperature optima.

2. Additives that help PCR in the presence of inhibitors:

➔400 ng/µl BSA (Bovine serum albumin).

➔Non-ionic detergents: Ex: 0.1 to 1% Triton X.


A
M – – + +
– 2 kb
B
M – – + +
– 4.8 kb
C
M – – + +
– 0.5 kb
D
M – – + +
– 1.5 kb
– : without Q-Solution
+ : with Q-Solution
M: markers


Effect of Nonionic Detergent
[Triton X-100] (%)
[Tween 20] (%)
0 0.1 0.5 1.0 5.0 0 0.1 0.5 1.0 5.0 M
Effect of [DMSO] (%)
Glycerol=0% Glycerol=2% Glycerol=8%
0 4 8 0 4 8 0 4 8
Kb
10
6
4
3
2
1.2

Home Work:

- TERT is a gene code for telomerase, an enzyme that elongate the telomere and mutated in some cancers. Your aim is to amplify a region in its promoter using PCR technique.

1. Draw a flow chart that illustrate the steps of performing PCR .
2. Design a primer using primer3plus tool.
3. Check the specificity of the primer.