



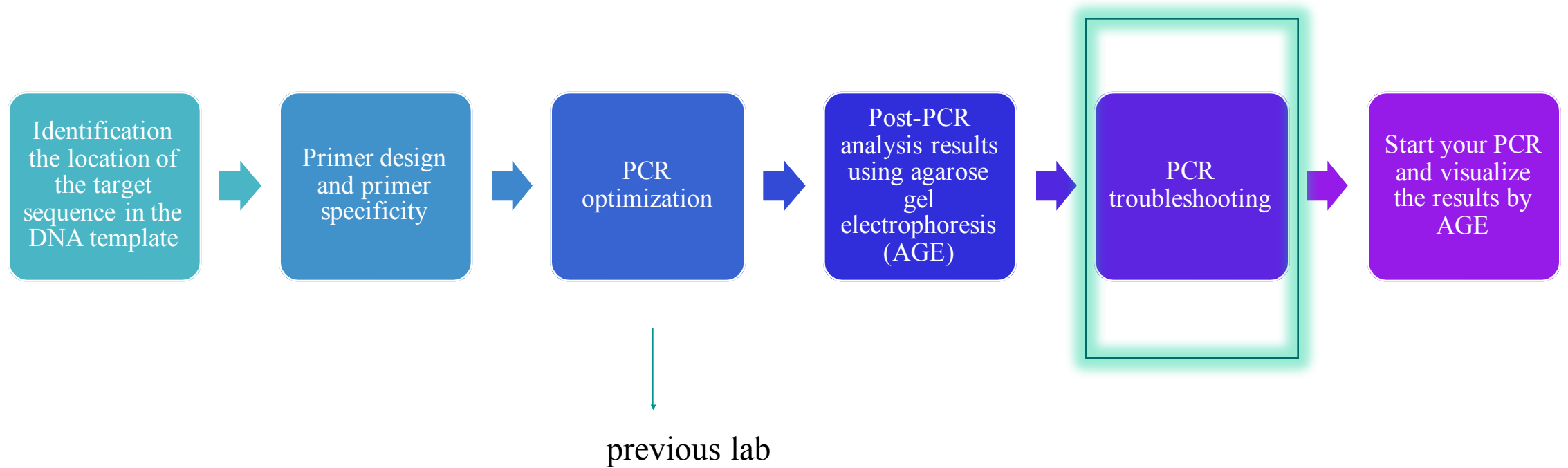
# PCR Troubleshooting

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# Polymerase Chain Reaction (PCR)=DNA Photocopier



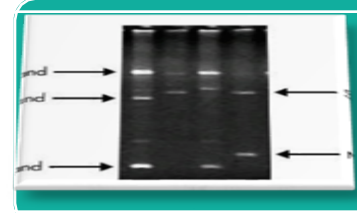
# Performing PCR steps:



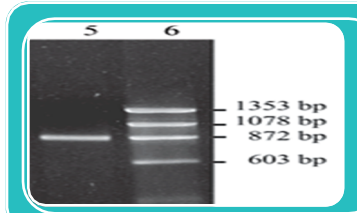
# Common Issues in PCR:



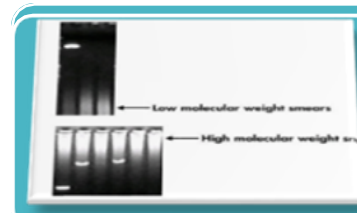
Low or no amplification



Non-specific band or primer dimer



Incorrect product size

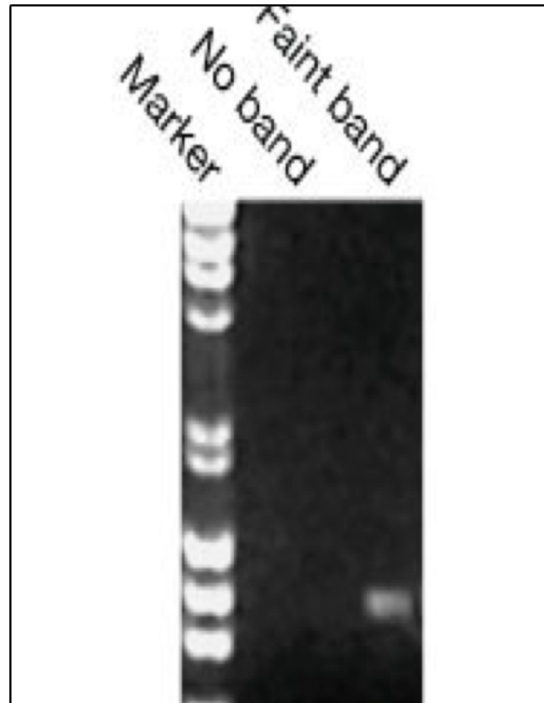


Smear Bands

# 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.



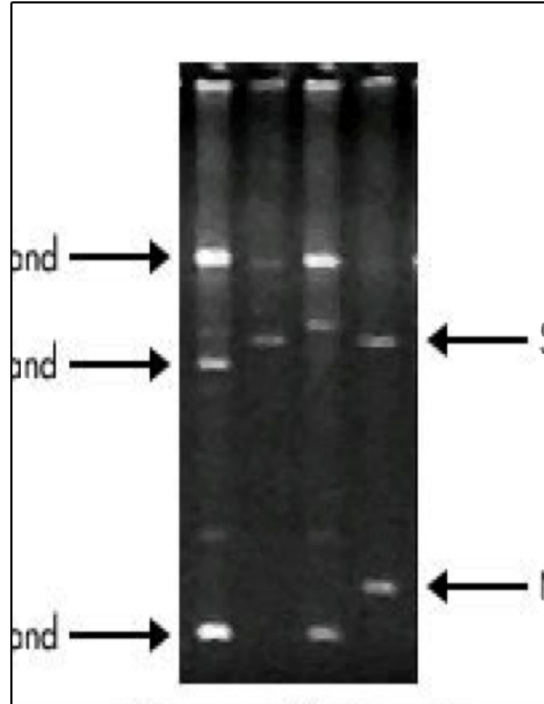
## Causes Related to PCR Components

- **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.
- **Not enough Mg<sup>2+</sup>.**

# 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.



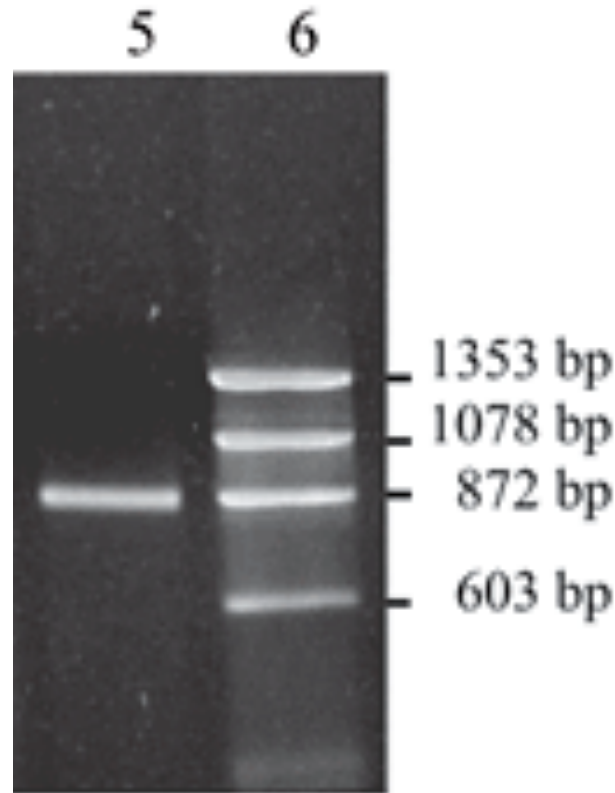
## Causes Related to PCR Components

- **Too much primer was added.**
- **Too much Mg<sup>2+</sup> 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.**



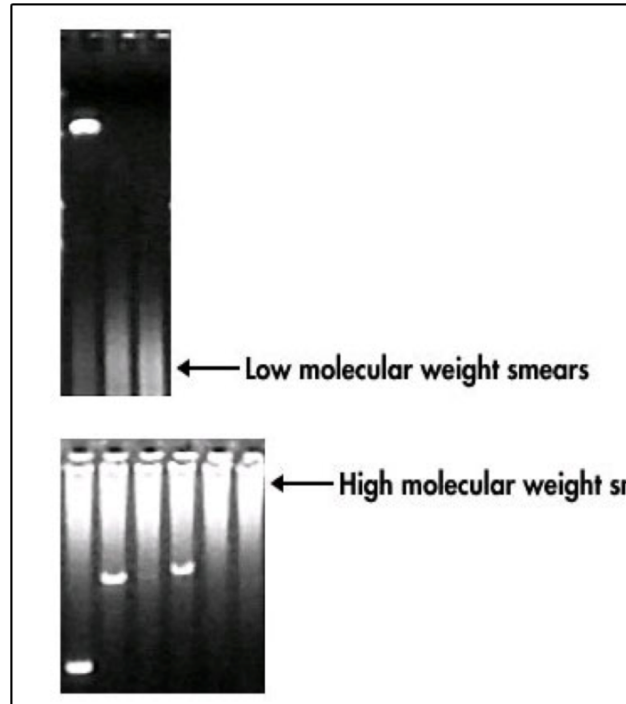
## Causes Related to PCR Components

- Mispriming.
- **Improper Mg<sup>2+</sup> concentration.**
- Impure primers, dNTPs, or water
- Primers were designed or synthesized incorrectly by user or manufacturer.

# 4- Smear 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 T<sub>m</sub>.

### →Q solution:

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

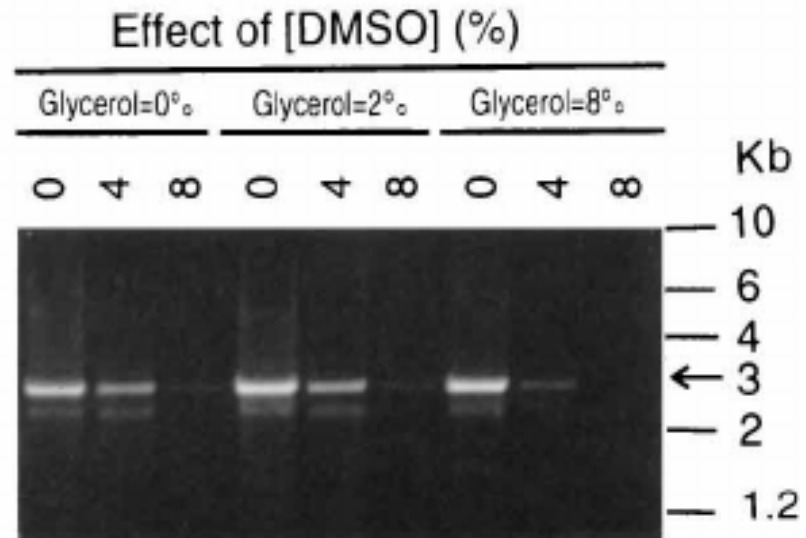
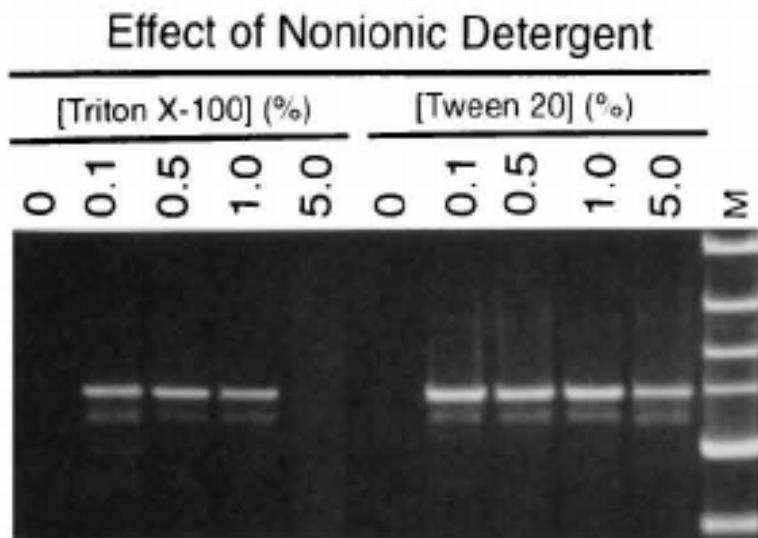
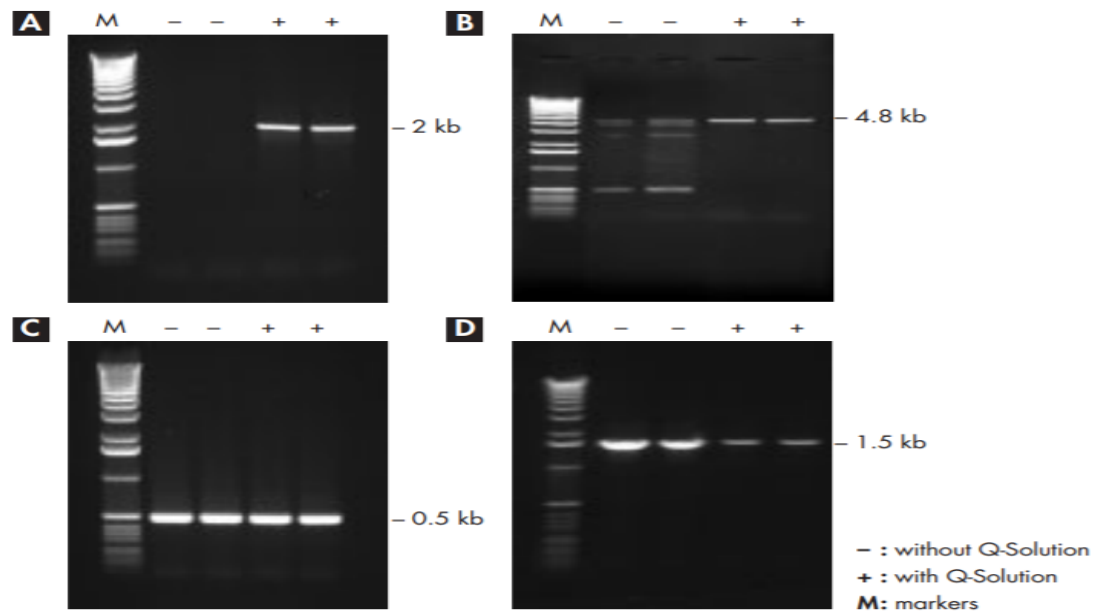
### →PCRx Enhancer:

- 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.





# 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.

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