Experiment (7): PCR Troubleshooting

Aim:

- Be familiar with common PCR difficulties.
- PCR troubleshooting.

Introduction:

PCR troubleshooting is a collection of techniques that alter PCR reactions in order to achieve optimum PCR results. Even with the simplest PCR reaction things can go wrong, so you need to have a good checklist of ideas for PCR troubleshooting and rectifying the problem. Fixing the error is done by changing the parameters (PCR components and thermal cycling condition) that discussed in the previous lab.

Common Issues in PCR:

Common issues of PCR is usually fall in the three following categories:

1. No or low amplification (no band or faint band).
2. Non-specific band or primer dimer.
3. Incorrect product size.
4. Smeared Bands.

The possible causes of each error are listed below, depending on the error, PCR troubleshooting could performed.

1. No or low amplification (no band or faint band):

Causes related to cycling condition	Causes related to PCR components
Too Few cycles were used.	No enough template was in the reaction.
Extension time was too short.	Primer concentration was too low.
Incorrect annealing temperature.	Impure primers, dNTPs, or water.
Denaturation temperature was too low	PCR product has high GC content.
	Primers were designed or synthesized incorrectly.
	No enough Mg^{2+}.

2. Non-specific band or primer dimer:

Causes related to cycling condition	Causes related to PCR components
Annealing temperature was too low.	Too much primer was added.
Too many cycles were used.	Too much Mg2+ was added.
Extension time was too long.	Primers were designed or synthesized incorrectly.
	Impure primers, dNTPs, or water.

To identify primer dimers, it always has a very low molecular weight (less than 100 bp usually).

3. Incorrect product size:

To have a single band, however it is not the same size of your target.

Causes related to cycling condition	Causes related to PCR components
Incorrect annealing temperature	Mispriming.
	Improper Mg2+ concentration.
	Impure primers, dNTPs, or water.
	Primers were designed or synthesized incorrectly.

4. Smeared Bands:

Causes related to cycling condition	Causes related to PCR components
Too many cycles were used.	Too much template was added.
	Impure primers, dNTPs, or water.
	Template contained an exonuclease or was degraded.

Common PCR additive reagents:

Additive reagents may yield results when all else fails. Understanding the reagents and what they are used for is critical in determining which reagents may be most effective in the acquisition of the desired PCR product. The following is a list of some of the common additives and the purpose of them. (2)

1. Additives that benefit GC Rich templates:

1. **1-10% DMSO (Dimethylsulfoxid):**

In PCR experiments in which the template DNA is particularly GC rich (GC content >60%), adding DMSO may enhance the reaction by disrupting base pairing and effectively lowering the Tm. (2)

2. **Q-solution:**

Q-Solution will often enable or improve PCR systems that have a high degree of secondary structure or that are GC-rich by changing the melting behaviour of the DNA. In addition, Q-Solution increases PCR specificity in certain primer–template systems. (3)

3. **PCRx Enhancer:**

For problematic and/or GC-rich templates, the PCRx enhancer system offers higher primer specificity, broader magnesium concentration optima, broader annealing temperature optima and improved thermostabilization of Taq DNA polymerase. (4)

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

1. **400 ng/µl BSA (Bovine serum albumin).**
2. **Non-ionic detergents:** Ex: 0.1 to 1% Triton X. (2)

References:

1. http://www.bio-rad.com/en-sa/applications-technologies/pcr-troubleshooting
2. Lorenz TC. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. J Vis Exp,2012;63:e3998.
3. Taq PCR Handbook from qiagen.
4. PCRx Enhancer System handbook from invitrogen.