PCR Troubleshooting

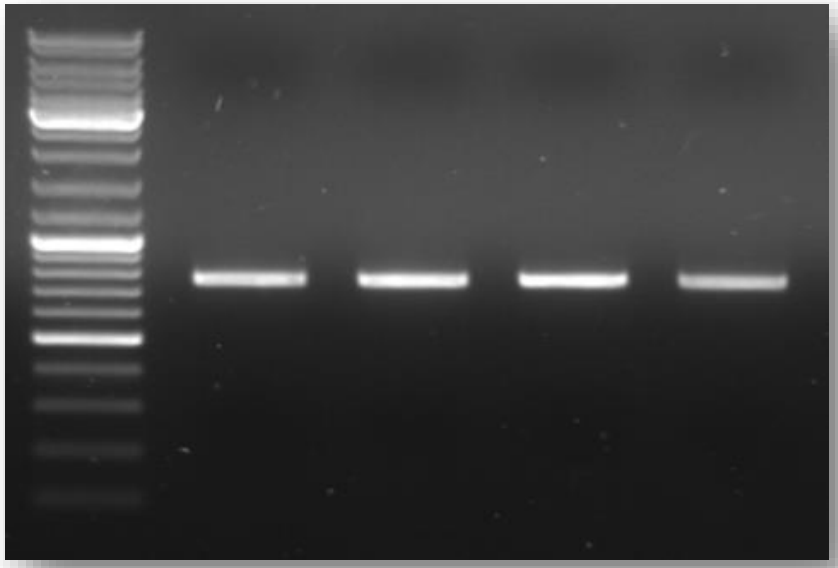
If you don't have a good PCR product

PCR troubleshooting is used to…

- Increase primer specificity
- Increase quantity of PCR product
- Increase quality of PCR product

Factors Influencing PCR Success

- Quantity and quality of DNA
- Length of the DNA fragment to be amplified
- Primer specificity
- PCR Reaction Components
- PCR Program



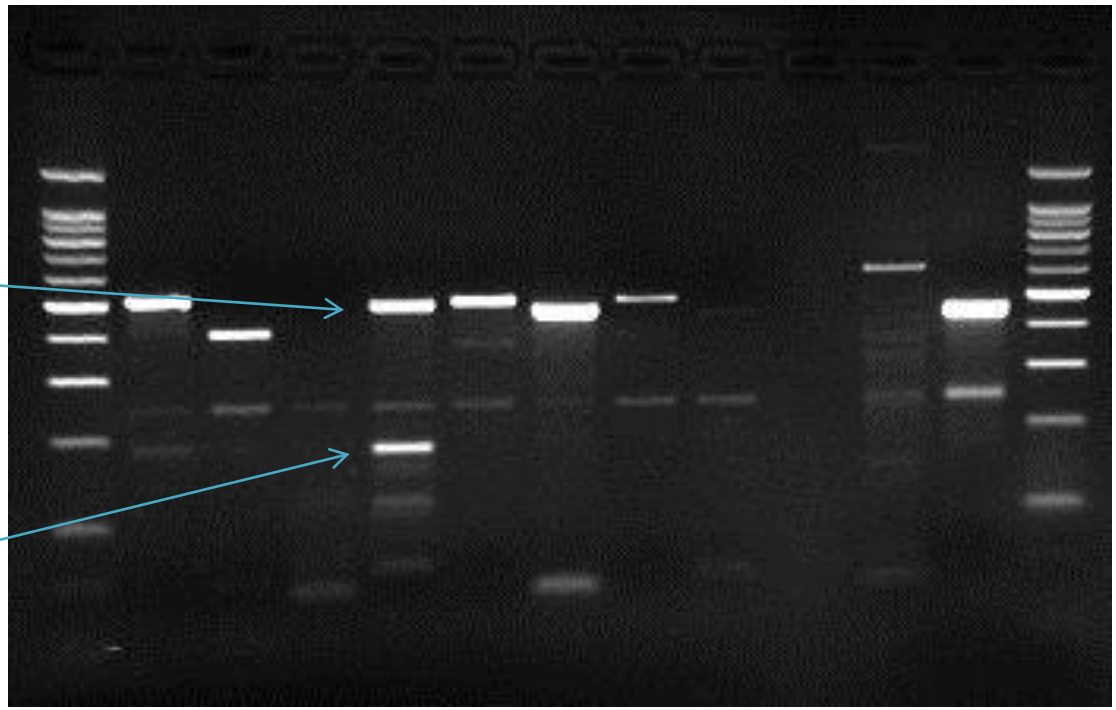
(I) Poor or no amplification of bands

- Loss of PCR Components
- Thermocycler efficiency
- Electrophoresis
- Cycles numbers
- Primer Design
- Quantity and quality of template
- PCR program
- PCR component efficiency

(I) Poor or no amplification of bands

Problems	Solution
Problem with thermocycler, set-up,reagents	Run positive control
Enzyme concentration low	⇑ Concentration
Annealing temp too low	Optimize by gradient PCR
Extension time too short	⇑ Time for longer products
Cycle number too low	⇑ Cycle number until 35-40
Primer design not appropriate	⇑ Specificity
Primer concentration too high	Optimize by titration
$MgCl_2$ concentration too low	Optimize by titration
Non-specific priming	⇑ Specificity, Hot Start
GC-rich template, ⇑ 2° structure	PCR additives

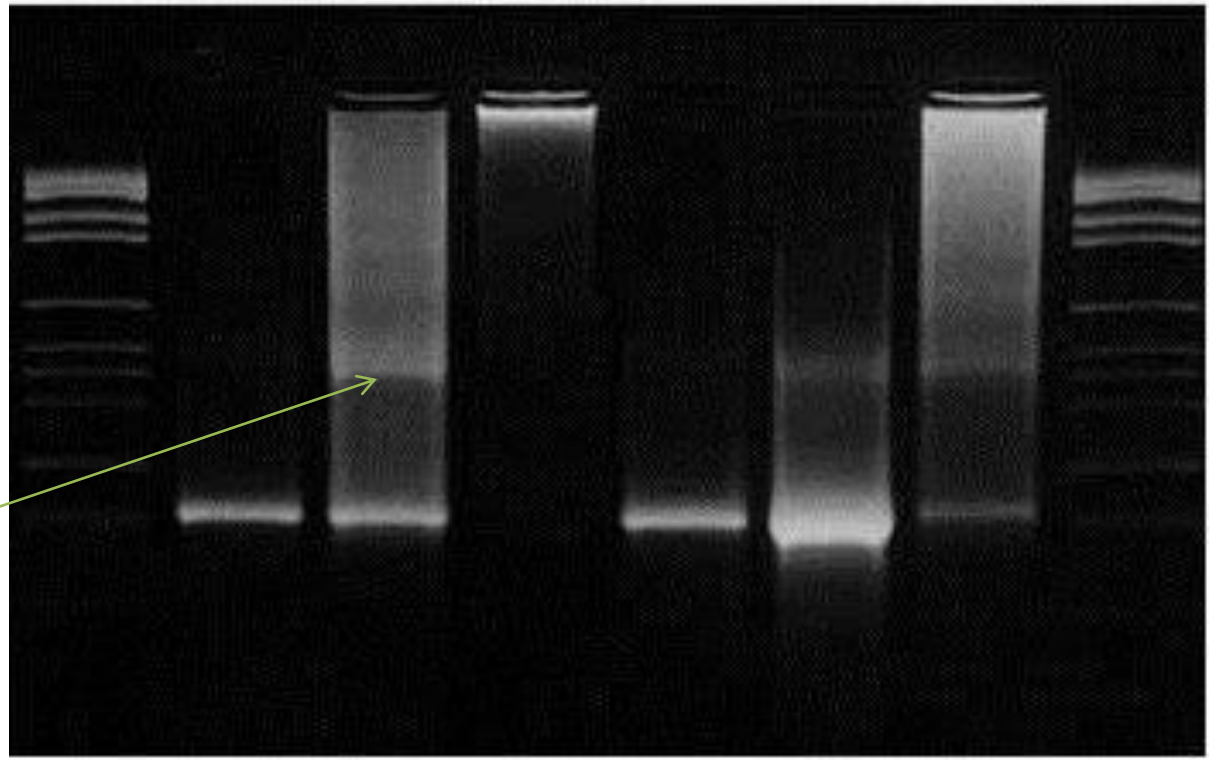
(II)- Non-specific bands on your gel



(II)- Non-specific bands on your gel

Problems	Solution
Set-up, reagents	Run negative control
Template concentration inappropriate	Optimize Concentration
Annealing temp too low	Optimize by gradient PCR
Extension time too short	⇑ Time for longer products
Cycle number too high	⇓ Cycle number
Primer design not appropriate	⇑ Specificity
Primer concentration too high	Optimize by titration
$MgCl_2$ concentration too high	Optimize by titration
Non-specific priming	⇑ Specificity, Hot Start
Contaminating DNA	Decontaminate work area, wear gloves, reagents, UV treat plastics

(III) Diffuse smearing on your gel



Smearing

(III) Diffuse smearing on your gel

Problems	Solution
Template concentration inappropriate	Optimize Concentration
Taq concentration too high	Optimize by titration
Cycle number too high	⇓ Cycle number
Primer design not appropriate	⇑ Specificity
Primer concentration too high	Optimize by titration
$MgCl_2$ concentration too high	Optimize by titration
Non-specific priming	Use of Hot Start
Contaminating DNA	Decontaminate work area, wear gloves, reagents
GC-rich template, ⇑ 2° structure	PCR additives

Cures for miss-priming

- "Cheap" fixes
  - Set up reactions on ice

- Hot-start PCR –holding one or more of the PCR components until the first heat denaturation
  - Manually - delay adding polymerase
  - Wax beads
  - Polymerase antibodies

- Touch-down PCR – set stringency of initial annealing temperature high, incrementally lower with continued cycling

- PCR additives
  - 0.5% Tween 20
  - 5% polyethylene glycol 400
  - betaine
  - DMSO

Common PCR additives

BSA (usually at 0.1 to 0.8 µg/µL final concentration)

Stabilize *Taq* polymerase & overcome PCR inhibitors

DMSO (usually at 2-5% v/v, inhibitory at ≤ 10% v/v)

Denaturant - good at keeping GC rich template/primer strands from forming secondary structures.

Glycerol (usually at 5-10% v/v)

Increases apparent concentration of primer/template mix, and often increases PCR efficiency at high temperatures.

Non-ionic detergents (Triton X, Tween 20 or Nonidet P-40) (0.1–1%)

Stabilize *Taq* polymerase & suppress formation of 2º structure

I'm sure you can make it...