Why do I have non-specific bands when I run my gel?

Causes	Trouble-shooting measures
Template concentration is inappropriate	Use appropriate template concentrations. For a 50 µl PCR reaction recommended concentrations are: human genomic DNA= 0.1-1 µg; *E.coli* genomic DNA = 10-100ng; lphage DNA= 0.5-2.5 ng; plasmid DNA 10-100 ng
Damaged template DNA	Minimize damage to template DNA by avoiding vortexing, heat treatment, strong UV, shearing or ultra sonication
Denaturation time is too short	Optimize the denaturation time in increments of 5 seconds
Denaturation temperature is too low	Optimize the temperature in increments of 0.5°C
Annealing temperature is too low	Raise the temperature in increments of 2°C
Extension time is too short	Lengthen the extension time in increments of 1 minute
Cycle number is too high	Reduce the number of cycles in decrements of 2 cycles
Primer design is not appropriate to amplify the target sequence	Design primers with high specificity to the target DNA
Primer concentration is too high	Decrease the primer concentration in decrements of 0.1 µM
Non-specific annealing of primers due to room temperature set up	Use Hot Start DNA polymerase
Contaminating DNA in reaction	Decontaminate work area and pipette. Use a dedicated pipette for PCR only. Use aerosol barrier tips and wear gloves
Mg^{2+} concentration inappropriate	Optimize Mg^{2+} concentration in 0.5 mM increments (for Mg^{2+} free buffer)
Template contains high GC region or high secondary structure	Use *LA Taq*™ with GC buffer or try addition of an enhancing reagent (see page 5)

What causes no or poor amplification yield?

Causes	Trouble-shooting measures
Enzyme concentration is too low	Increase the enzyme amount in increments of 0.5 U
Denaturation time is too short	Lengthen the denaturation in increments of 5 seconds
Denaturation temperature is low	Raise the temperature in increments of 0.5°C
Extension time is too short	Increase the extension time in increments of 1 minute
Cycle number is too low	Increase the number of cycles in increments of 2 cycles
Template concentration is too low	Increase the template amount in increments of 20% of the previously used amount
Template degraded/dirty	Reclean the DNA using ETOH precipitation, examine template quality via gel electrophoresis, Re-prepare template, if necessary
Enzyme inactive	Use fresh enzyme
dNTP's degraded	Use fresh dNTP's; store frozen aliquots and avoid freeze thaws
Primers not matched	Rethink and resynthesize the primers
Annealing temperature is too high	Lower the temperature in decrements of 2°C
Annealing time is too short	Increase annealing time incrementally
Problem with thermocycler operation or program	Run positive control with every reaction
Mg^{2+} concentration inappropriate	Optimize Mg^{2+} concentration in 0.5 mM increments (for Mg^{2+} free buffer)
Template contains high GC region or high secondary structure	Use *LA Taq*™ with GC buffer or try addition of an enhancing reagent (see page 5)

What might cause diffuse smearing within the lane on a gel?

Causes	Trouble-shooting measures
Concentration of primers is too high	Reduce the primer amount in decrements of 0.1 μM
Primers are not well designed for the target sequence	Increase the specificity of the primers by changing the complimentary region of the template, within 20 - 30 bases
Enzyme concentration is too high	Reduce the enzyme amount in decrements of 0.5 U
Cycle number is too high	Reduce the number of cycles in decrements of 2 cycles
Annealing temperature is too low	Raise the temperature in increments of 2°C
Non-specific annealing of primers due to room temperature set up	Use Hot Start DNA Polymerase
Extension time is inappropriate	Set time to 0.5 – 1 min./kb
Denaturation is not complete	Optimize denaturation conditions by extending the time in increments of 5 sec; raising the temperature in increments of 0.5°C; or adding an enhancing reagent (page 5)
Template concentration is too high	Reduce the template amount in decrements of 20% of the previously used amount
Mg^{2+} concentration inappropriate	Optimize Mg^{2+} concentration in 0.5 mM increments (for Mg^{2+} free buffer)
Contaminating DNA in reaction	Decontaminate work area and pipette. Use a dedicated pipette for PCR only. Use aerosol resistant tips and wear gloves