PCR troubleshooting

Symptoms	Possible causes	Remarks/Remedie
Low yield or no PCR product	**Insufficient number of PCR cycles**	**Replace the PCR vials and run an extra 5 cycles**
	DNA-template degenerated	**Check DNA quality by electrophoresis**
	Thermocycler programme is not correct	**Check temperatures and cycle time**
	Temperature is too low in some areas of the thermocycler	**Start a controle PCR at several positions in the thermocyler**
	Thermocycler is open	**The lid must be closed to obtain a better heating and cooling regulation**
	Inhibitors present, which slow down the PCR	**Reduce the volume of sample in the reactionmix; carry out another ethanol precipitation with the samples**
	Missing reaction component	**Check the reaction components and carry out a new PCR**
	Unsuitable reaction conditions	**Decrease annealing temperature and/or increase elongation time for long amplicons**
	Problems with mineral oil	**Cover the reaction with high quality, nuclease-free mineral oil. DON'T use autoclaved mineral oil**
	PCR vials not autoclaved	**Autoclaving PCR vials prevents contamination, while inhibitors can not interfer with the reaction**
	Badly defined primers	**Primers should not be self-complementary or each others complement. Try longer primers.**
	Incorrect primer specificity	**Check whether primers are complementary with the right DNA strands**
	Primer concentration too low	**Check primer concentration and increase concentration if necessary**
	No optimal reaction conditions	**Optimize Mg^{2+} concentration, annealing temperature and elongation time. Always vortex PCR buffer. Primers should be present in equal concentrations**
	Nucleotides degenerated	**Store nucleotides in frozen batches,thaw quickly and keep on ice. Prevent frequent freeze/thaw cycles**
	Target is not present in DNA template	**Rewrite the experiment or try another region in the DNA template**
Several, non-specific amplification products	**Sub-optimal reaction conditions**	**Optimize $MgSO_4$/$MgCl_2$ concentration, annealing temperature, elongation time and number of cycles to prevent non-specific priming. Keep the reactions on ice, when all reagents are mixed**
	Badly defined primers	**Primers should not be self-complementary or each others complement, esp. near the 3'-end. Try longer primer and prevent 3 successive G's or C's at the 3'-end**

	Primer concentration too high	**Check primer concentration and decrease concentration, if necessary**
	Contamination with other template	**Use sterilized pipets and pipettips. Work in separate rooms when pre and post amplifying. Wear latex gloves**
	Several targets present within the same template	**Develop new primers with a higher specificity to the distinctive DNA sequence**

Troubleshooting for PCR and multiplex PCR

Questions	Solutions
(More) longer unspecific products. What can I do?	**Decrease annealing time Increase annealing temperature Decrease extenstion time Decrease extension temperature to 62-68˚C Increase KCl (buffer) concentration to 1.2x-2x, but keep $MgCl_2$ concentration at 1.5-2 mM Increase $MgCl_2$ concentration up to 3-4.5 mM but keep dNTP concentration constant Take less primer Take less DNA template Take less Taq polymerase If none of the above works: check the primer for repetitive sequences (BLAST align the sequence with the databases) and change the primer(s) Combine some/all of the above**
(More) shorter unspecific products. What can I do?	**Increase annealing temperature Increase annealing time Increase extension time Increase extension temperature to 74-78˚C Decrease KCl (buffer) concentration to 0.7-0.8x, but keep $MgCl_2$ concentra-tion at 1.5-2 mM Increase MgCl2 concentration up to 3-4.5 mM but keep dNTP concentration constant Take less primer Take less DNA template Take less Taq polymerase If none of the above works: check the primer for repetitive sequences (BLAST align the sequence with the databases) and change the primer(s) Combine some/all of the above**
Reaction was working before, but now I can't get any product.	**Make sure all PCR ingredients are taken in the reaction (buffer, template, Taq, dNTPs, etc.) Change the dNTP solution (very sensitive to cycles of thawing and freezing, especially in multiplex PCR). If you just bought new primers, check for their reliability (bad primer synthesis?) Increase primer amount Increase template amount Decrease annealing temperature by 6-10˚C and check if you get any product. If you don't, check all your PCR ingredients. If you do get products (including unspecific ones) reaction conditions as described abaove. Combine some/all of the above.**

My PCR product is weak. Is there a way to increase the yield?	Gradually decrease the annealing temperature to the lowest possible. Increase the amount of PCR primer Increase the amount of DNA template Increase the amount of Taq polymerase Change buffer (KCl) concentration (higher if product is lower than 1000 bp or lower if product is higher than 1000 bp) Add adjuvants. Best, use BSA (0.1 to 0.8 µg/µl final concentration). You can also try 5% (v/v, final concentration) DMSO or glycerol. Check primer sequences for mismatches and/or increase the primer length by 5 nucleotides Combine some/all of the above.
My two primers have very different melting temperatures (Tm) but I cannot change their locus. What can I do to improve PCR amplification?	An easy solution is to increase the length of the primer with low Tm. If you need to keep the size of the product constant, add a few bases at the 3' end. If size is not a concern, add a few bases to either the 3' or the 5' end of that primer.