TROUBLESHOOTING

The table below covers most problems and solutions hereof, experienced when handling PCR experiments.

To avoid spending a lot of time on optimisation of PCR setup we recommend the usage of Ampliqon Ammonium Buffer for most PCR applications. Ammonium Buffer is a very robust 10x PCR buffer, resulting in high yield of PCR products and minimises the need for optimisation of Mg^{2+} and/or annealing temperatures.

Observed problem	Possible cause	Solution
PCR product does not have the correct size	Contamination by nucleases	▪ Try again with fresh reagents
	Mispriming	▪ Test that primers do not have additional complementary regions within the template DNA
	Non optimal $MgCl_2$ concentration	▪ Adjust $MgCl_2$ concentration as advised in product data sheet.
	Non optimal annealing temperature	▪ Retest Tm values of primers
Absence of PCR product	Low primer specificity	▪ Verify that primers are complementary to the correct target sequence
	Too low primer concentration	▪ Adjust in the range 0.1 – 1 µM
	Suboptimal reaction conditions	▪ Optimise annealing by running a temperature gradient ▪ Adjust $MgCl_2$ concentration as advised in product data sheet
	Poor template quality	▪ Test DNA using gel electrophoresis before and after addition of $MgCl_2$. ▪ Check 260/280 ratio of DNA template
	Missing a reaction component	▪ Make a new PCR mix
	Inhibitors in the reaction	▪ Ensure that template DNA is purified or decrease sample volume.
	PCR run is non optimal	▪ Add more cycles ▪ Recheck the PCR program ▪ Recalibrate heating block
	Your template or target is complex	▪ For GC-rich sequences or other complex DNA targets optimize conditions using GC-rich Target kit.
Smears or multiple band on the gel	Premature replication	▪ Use TEMPase Hot Start DNA Polymerase instead ▪ Set PCR reaction up on ice.
	Too low annealing temperature	▪ Increase annealing temperature ▪ If not already using Ammonium Buffer, then shift to this buffer.
	Excess primers	▪ Adjust in the range 0.1 – 1 µM
	Non optimal $MgCl_2$ concentration	▪ Adjust $MgCl_2$ concentration as advised in product data sheet
	Non optimal primer design	▪ Ensure that primers are non-complementary ▪ Increase length of primers ▪ Avoid GC-rich 3' ends
	Contamination with non-template DNA	▪ Always use filer tips, PCR grade water. ▪ Use separate areas for PCR reaction setup, DNA preparation, PCR thermal cycling and gel electrophoresis
	Incorrect template concentration	▪ Adjust template concentration as advised in product data sheet.
Sequence errors	Low fidelity polymerase	▪ Use AQ97 High Fidelity DNA Polymerase
	Template DNA has been damaged	▪ Prepare a new DNA template ▪ Limit the exposure of template DNA to UV ▪ Lower initial heating time
	Suboptimal reaction conditions	▪ Decrease extension time ▪ Decrease $MgCl_2$ concentration ▪ Lower the amount of cycles
	Problems with nucleotide composition	▪ Make a fresh solution of nucleotide mix