# 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<br>▪ Adjust $MgCl_2$ concentration as advised in product data sheet |
| | Poor template quality | ▪ Test DNA using gel electrophoresis before and after addition of $MgCl_2$.<br>▪ 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<br>▪ Recheck the PCR program<br>▪ 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<br>▪ Set PCR reaction up on ice. |
| | Too low annealing temperature | ▪ Increase annealing temperature<br>▪ 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<br>▪ Increase length of primers<br>▪ Avoid GC-rich 3' ends |
| | Contamination with non-template DNA | ▪ Always use filer tips, PCR grade water.<br>▪ 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 AccuPOL DNA Polymerase with Ammonium Buffer |
| | Template DNA has been damaged | ▪ Prepare a new DNA template<br>▪ Limit the exposure of template DNA to UV<br>▪ Lower initial heating time |
| | Suboptimal reaction conditions | ▪ Decrease extension time<br>▪ Decrease $MgCl_2$ concentration<br>▪ Lower the amount of cycles |
| | Problems with nucleotide composition | ▪ Make a fresh solution of nucleotide mix |