AMPLIQON
PCR ENZYMES & REAGENTS

FIDELITY

Fidelity depends on the polymerase, the buffer system that you use and the quality of your template DNA. Taq DNA Polymerase is quite precise when run at low-error conditions.

POLYMERASE-INDEPENDENT ERRORS

Polymerase-independent errors are caused by the DNA either because it has been damaged from the start (old DNA) or during the PCR. To avoid polymerase-independent errors the following tips could be useful:

- Add enough template DNA
- Run as few cycles as possible

Starting amount of DNA and cycle number above are interconnected. Because the lesser DNA at the beginning, the more cycles you have to run to obtain the same amount of the final product. With each additional amplification cycle the already existing errors will be copied and consequently doubled.

- Short DNA melting steps
- Low DNA melting temperatures

If DNA is exposed to high temperatures the DNA will be damaged and unwanted deamination of cytosine to uracil will occur. This results in a C-G to T-A mutation. To avoid this choose short denaturation time and if possible omit the initial denaturation step completely.

POLYMERASE-DEPENDENT ERRORS

To minimise polymerase-dependent errors you should choose conditions that promote a slow elongation rate. Because the slower the elongation rate of the polymerase, the more time is available to secure the incorporation of the correct nucleotides.

Conditions known to slow down polymerase extension rates are:

- Low enzyme concentrations
- Low dNTP concentrations
- Low Mg^{2+} concentrations

dNTP and Mg^{2+} concentrations are interconnected. High fidelity of Taq is obtained with equimolar concentrations of dNTPs and Mg^{2+}, e.g. 1 mM total dNTPs and 1 mM Mg^{2+}. Other substances in the reaction can consume Mg^{2+}, for example a chelator introduced with a DNA sample. Therefore, the optimal Mg^{2+} concentration for high fidelity is often a little higher than the theoretical values.

- Optimise cycling time

Unfortunately, high fidelity conditions are not the same as high yield conditions. To optimise yield with high fidelity conditions you should optimise your PCR cycling time. For that purpose use short DNA melting time and long annealing and elongation time.