

OPTIMIZATION OF PCR (from Fermentas Life Sciences)

PCR has several qualitative characteristics such as specificity, efficiency (yield of product), sensitivity and fidelity (error rate).

First of all it is very important to evaluate the main characteristics for each PCR case. For the detection of rare copy sequences, genetic analysis or forensic testing the sensitivity and specificity of PCR are very important. In the case of preparative PCR for synthesis of probes for hybridization or for sequencing, the specificity and a high yield of a product are high important. For synthesis of DNA fragments, specificity of reaction and fidelity (error rate) are of greater importance.

If the product obtained under standard conditions does not satisfy you should undergo changing the components of the mix and reaction conditions that have an impact on most PCR characteristics. Values in parentheses are the recommended final concentration ranges and should not be exceeded in most cases.

Sensitivity of PCR one can increase by:

- * increasing concentration of primers (0.2-1 μ M final concentration);
- * increasing concentration of Taq DNA polymerase (0.25-0.5 units);
- * increasing number of cycles;
- * increasing/decreasing annealing temperature;
- * increasing/decreasing annealing and extending time.

Specificity of PCR one can increase by:

- * decreasing concentration of Taq DNA polymerase;
- * reducing annealing time;
- * reducing extending time;
- * increasing annealing temperature;
- * decreasing number of cycles;
- * using "Hot Start" technique.

The yield of PCR products is improved by increasing:

- * dNTP concentration (0.1-0.5 mM final concentration);
- * Mg^{2+} concentration (1-5 mM final concentration);
- * Taq DNA polymerase concentration;
- * annealing time;
- * extending time;
- * number of cycles.

Fidelity of PCR one can increase by:

- * decreasing dNTP concentration;
- * decreasing Taq DNA polymerase concentration;
- * decreasing Mg²⁺ concentration;
- * minimizing reaction time at high temperatures;
- decreasing number of cycles;
- lowering pH in a reaction mixture.

Other additions that may improve results:

- DMSO, up to 10%
- Detergent (Triton X-100, Tween 20) up to 1%