Annealing time and temperature

Annealing time

An annealing time of 30-45 seconds is commonly used in PCR reactions. Increase in annealing time up to 2-3 minutes did not appreciably influence the outcome of the PCR reactions. However, as the polymerase has some reduced activity between 45 and 65°C (interval in which most annealing temperature are chosen), longer annealing times may increase the likelihood of unspecific amplification products (data not shown).

Annealing temperature (see also figure 18, page 08)

Annealing temperature is one of the most important parameters that need adjustment in the PCR reaction. Moreover, the flexibility of this parameter allows optimization of the reaction in the presence of variable amounts of other ingredients (especially template DNA). For example, the PCR product depicted in Fig. 22 could be amplified easily at annealing temperatures of 55°C in the presence of 1-100 ng genomic DNA template. Below this limit, there was no detectable PCR product on agarose gels (this primer pair amplifies a polymorphic locus, explaining the two bands seen on non-denaturing agarose gels). It was observed that the specific product can be detected again, even in the presence of very low DNA template concentrations, if the annealing temperature is also decreased. In the reactions depicted in figure 22, the DNA template amount was decreased to 3.1 pg (which is about half the DNA content of a diploid human cell). Remarkably, only one allele was preferentially amplified when the template DNA was approximately 6.6 pg. To achieve these results, reaction was performed at 45°C annealing temperature (a 10 degrees drop from usual). No unspecific products are seen. However, if the same reaction is performed in the presence of a higher amount of DNA template, the low annealing temperature results in the appearance of many unspecific secondary products. Thus, it appears that by decreasing the amount of DNA template, the number of potentially unspecific sites is also decreased, making possible the drop in annealing temperature.

Fig. 22. PCR amplification of a polymorphic locus in the presence of decreasing, low amounts of genomic template DNA and at an annealing temperature 10°C lower than normal.

Lanes A-F show slight variation in the amount of product, when vials with identical reaction mixture were placed in different position in the metal block of a thermocycler. Amount of template was 800pg/reaction.
Polymorphisms and annealing temperature

Annealing temperature is important in finding and documenting polymorphisms. Slight mismatches, (even 1 base-pair mutations) in one of sequences bound by the two primers used to amplify a DNA locus, can be detected by slight variations in annealing temperature and/or by multiplex PCR. In Fig. 23 such a polymorphism on human Y chromosome is detected in a few DNA samples by amplifying that locus along with other ones using multiplex mixture C (see also Fig. 1). In Fig. 24, same polymorphism is detected by performing PCR reaction only with the specific primer pair, but increasing the stringency of the annealing temperature.

![Fig. 23. Single-locus PCR on 7 different template DNAs with a primer pair amplifying a polymorphic locus (yellow). Multiplex PCR of the same templates when the primer pair is part of mixture C. Reactions were performed in the same cycling conditions (annealing at 54 °C). The slight mismatch in primer binding (polymorphism) is detected only in the multiplex reaction by the lack of the amplification product (magenta arrows).](http://www.info.med.yale.edu/genetics/ward/tavi/p09.html)

![Fig. 24. Same primer mismatch described above can be detected by single-locus PCR reactions after increasing the stringency of the annealing temperature. Samples 3 and 4 show a decrease of product at 61 °C annealing temperature but have a “normal” appearance at 59 °C annealing temperature (magenta arrows).](http://www.info.med.yale.edu/genetics/ward/tavi/p09.html)