

Chapter 6

Primer Designing for Polymerase Chain Reaction

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The most significant factor for successful PCR is the design of primers. The primer sequence determines several things such as the length of the product, its melting temperature and ultimately the yield. A poorly designed primer can result in little or no product due to non-specific amplification or primer-dimer formation, which can become competitive enough to suppress product formation. This note is provided to give rules that should be taken into consideration, when designing primers for PCR.

Primer Selection

Several variables must be taken into account when designing PCR primers. Among the most critical are:

a) Primer Length

Since both specificity and the temperature and time of annealing are at least partly dependent on primer length, this parameter is most critical for successful PCR. In general, oligonucleotides between 18 and 24 bases are extremely sequence specific, provided that the annealing temperature is optimal. Primer length is also proportional to annealing efficiency: in general, the longer the primer, more inefficient the annealing. With fewer templates primed at each step, this can result in a significant decrease in amplified product. The primers should not be too short, however, unless the application specifically calls for it. As discussed below, the goal should be to design a primer with an annealing temperature of at least 50°C.