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On the trail of viruses: understanding virus research

Worksheet C: Primer design

How do I design a primer?

The primers must be selected so that they hybridise specifically. This means that they may only bind to the desired position on the DNA. It should be noted, that the human genome consists of approximately 3 billion base pairs. For a primer that consists of only three nucleotides, for example, there are 4^3 possible combinations. Statistically, there is a hybridisation possibility for a trinucleotide every $4^3 = 64$ base pairs. In relation to the human genome, a trinucleotide at $3 \times 10^9 / 64 = 4.6875 \times 10^7$ hybridisation sites. In addition, care should be taken to ensure that there are at least two bases at the 3' end that form three hydrogen bonds (G or C). In order to take all this into account so that a primer only binds at the desired sites, the primer can be designed to be as long as possible. Usually around 17 to 28 nucleotides are sufficient. When designing, researchers also make sure that the primers do not bind to each other or to themselves, so that they only bind to the desired site on the DNA.

Task 1

a) Determine the possible combinations and the number of possible binding sites for a primer with 8 and 17 nucleotides in the human genome. Explain the length from which a primer is specific.

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In order for a primer to bind to the DNA at all, it must be separated into two individual strands. This is possible by heating the DNA to a temperature of 95°C. Once two individual strands are present, the primer can bind. Here, too, there are aspects that should be considered. The ideal hybridisation temperature (annealing temperature, T_a) must be low enough to allow hybridisation between the primer and the DNA template and high enough to prevent the formation of mismatched hybrids. This temperature can be estimated by determining the melting temperature (Tm) of the hybrid of primer and DNA template. At this temperature, the fully paired hybrid dissociates ("melts"); at 1 to 2 °C below this temperature, only correct hybrids can usually form between primer and DNA template.

Formula for determining the T_m:

$T_m = 4 \cdot (G+C) + 2 \cdot (A+T)$ [°C]

Task 2

a) Determine the melting and annealing temperatures for the following primer sequences.

Primer sequence (5´à 3`)	Calculation	T_m [°C]	T_a [°C]
TCCTCGGCGTCTACTACCACAA			
CCTGGATGGAGTCCGGCGT			
ATCGTCCGCGAGCCCGAG			

b) The T_a that is used in an actual experiment with these primers is 65°C. Explain why it is higher than at least two of the three temperatures you calculated?

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