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On the trail of viruses: understanding virus research

Solutions worksheet C: Primer design

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.

Primer with 8 nucleotides:

4^8 = 65.536 possible combinations and $(3 \times 10^9) \div 4^8 \approx 45.776$ binding sites

Primer with 17 nucleotides:

$4^{17} \approx 1.7 \cdot 10^{10}$ possible combinations and $(3 \times 10^9) \div 4^{17} \approx 0.17$ binding sites

A primer is specific to the human genome if it contains at least 16 nucleotides. Shorter primers have more than one possible binding site:

15 nucleotides: $(3 \times 10^9) \div 4^{15} \approx 2.8$ binding sites

16 nucleotides: $(3 \times 10^9) \div 4^{16} \approx 0.7$ binding sites

Note: In purely mathematical terms, a value of 16 nucleotides results in only one binding site. However, to be on the safe side, in most cases a primer with 18 or more nucleotides is selected.

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	4 · (3+9) + 2 · (5+5)	68	66
CCTGGATGGAGTCCGGCGT	4 · (8+5) + 2 · (2+4)	64	62
ATCGTCCGCGAGCCCGAG	4 · (6+7) + 2 · (3+2)	62	60

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?

The melting temperature of a primer indicates when 50% of the primers are in their single-stranded form, i.e., all hydrogen bonds are broken. Conversely, this means that 50% are in double-stranded form and cannot be used to bind to the DNA.

The closer the temperature is to the melting temperature, the better the primers hybridise with the DNA.

In addition to being calculated, this temperature is also determined experimentally, as not only the composition of the primer plays a role, but also the length of the DNA to be amplified and the salt concentration of the PCR.

The higher the annealing temperature, the more specific the binding becomes. This is mainly due to physical reasons. Bindings between primer and DNA only occur where the sequence of primer nucleotides matches the sequence of DNA nucleotides to a very high degree. However, this can result in less product at the end. The lower the annealing temperature, the more non-specific the binding becomes. This results in more product at the end where not only the desired products are produced, but also others due to the non-specific binding. This can be recognised in the evaluation by the appearance of additional bands or less defined bands.

For the primer combinations used here, an annealing temperature of 65°C was determined experimentally. At this temperature, only the expected PCR fragments were present in sufficient quantities.

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