Task II. Explaining the Polymerase Chain Reaction:

*Model answer:*

PCR stands for " Polymerase Chain Reaction" and denotes a method in science used for amplification of a target DNA sequence ( template). It applies the same enzyme, the DNA polymerase, for copying a DNA strand as found in nature. In repeating cycles, three steps are run which include, apart from the actual replication process, the separation of double strands and attachment of primers to the single strands. These steps are usually catalyzed by other enzymes in nature. In PCR, however, it is realized by shifting the temperature in each step for which a " thermocycler" is used. Proteins are heat-sensitive and will denaturate during normal PCR. A heat resistant polymerase, named 'Taq-Polymerase', is therefore required. It keeps its native state at even high temperature since it is found in ancient bacteria which are adapted to hot water springs.

As the DNA double-strand comprises two single strands which are tightly attached to each other through binding of complementary bases, the single strands must be separated first, in a step called 'melting'. Hydrogen bonds between bases constitute the attachment of both strands: the base pair adenine and thmine form two bonds, guanine and cytosine form three. With increasing temperature atoms vibrate faster, thus causing these noncovalent bonds to break apart ( at a temperature of 94°C-96 °C dependent on the proportion of the two base pairs).

In the second step, the temperature is lowered in order to allow 'primers' to bind to their complementary sequence on the template strands. This step is called 'annealing' and occurs at 65°-68°C. It is necessary since DNA-polymerase can only extend a preexisting DNA strand. Primers are short polynucleotides, complementary to the beginning and end region of the target sequence. The two primers attach to the 3'- ends of both single strands, thereby enabling the polymerase to bind.

In the third step, Taq Polymerase synthesizes the complementary strand starting from the primer and moving towards 5'- direction. Its optimal temperature to catalyze is 72°C. During this process of "elongation", the Taq Polymerase provides an open binding side for nucleotides to align. Nucleotides comprise a base, a sugar and a triple phosphate group. After complementary base pairing occured, pyrophosphate is released what drives this reaction forward (an exergonic reaction). The Taq -Polymerase has a low error rate because of its ability to proofread and replace erroneous nucleotides.

After completing all three steps, the cycle is repeated several times again. In each cycle the number of template strands is doubled, letting the number of template strands grow exponentially.