Script for Polymerase Chain Reaction and Site Directed Mutagenesis

Introduction

Polymerase chain reaction, or PCR, is a powerful tool for amplifying a stretch of DNA. From as little as a single piece of
target DNA, you can make enough copies for sequencing, cloning or gel electrophoresis. This exploration will guide you
through a PCR amplification. A PCR reaction is typically carried out in a small tube placed in a machine called a
thermocycler. The thermocycler simply changes the temperature to selected values for defined periods of time according
to a program which you can repeat over and over again. The initial reaction mixture contains a target DNA, synthetic
oligonucleotides corresponding to two sequences in the target, DNA polymerase, and a mixture of the four
deoxyribonucleotides.

Cycle 1: Denaturation

Each cycle consists of three steps: denaturation, annealing, and extension. In the denaturation step, the temperature of
the reaction is raised to above 90 degrees C in order to break the hydrogen bonds between strands and produce
single-stranded DNA.

Cycle 1: Annealing

In the annealing step, the temperature is lowered in order to allow the oligonucleotide to anneal to the target DNA using
complementary base pairing. The exact temperature needed for the annealing step is a function of the length and
sequence of the oligonucleotide.

Cycle 1: Extension

In the extension step of the cycle, the annealed oligonucleotide is used as the primer for DNA synthesis. As you should
recall, all DNA polymerases synthesize in a 5-prime to 3-prime direction starting from double stranded DNA. The
extension step makes the complimentary DNA of the template sequence. The polymerases used for PCR differ from
normal DNA polymerases in that they are able to survive the extreme temperatures without being inactivated due to
denaturation. Typically they are cloned from bacteria which live in high temperature environments such as hydrothermal

vents.

Cycle 2: Denaturation

In subsequent cycles, the denaturation, annealing and extension steps are repeated over and over again. At the start of
the second cycle there are now twice as many strands of DNA to act as templates. Half of them end with one of the
synthetic oligonucleotides in the reaction mixture.

Cycle 2: Annealing

In the second cycle and beyond, the previously synthesized DNA can act as a template as well. However, unlike the
original template DNA with overhanging nucleotides of random lengths, the newly synthesized strands have defined
ends determined by where the oligonucleotides bind.

Cycle 2: Extension

With the completion of extension in the second cycle there is now four times as much template DNA as we started with.
Although the original template is still present, the majority of the DNA in the reaction has been newly synthesized.
Strands which used previously synthesized strands as templates are of defined length, because the polymerase ran out
of template when it reached the end of the oligonucleotide. The length of these strands is the distance between the two
sequences in the original target DNA corresponding to the sequence of the oligonucleotides.

Cycle 3

As the reaction finishes the third cycle, there are now 8-times as many DNA strands as when the reaction started.
Rapidly the original template is becoming a smaller and smaller percentage of the total template available, and more
and more strands have defined length.

Cycle 20 - 50

The PCR reaction is typically continued through 20 to 50 cycles. Each cycle the amount of DNA doubles. Quickly, virtually
all the DNA in the reaction is of defined length with the synthetic oligonucleotide on the 5-prime end.

PCR: Point Mutation

Oligonucleotides put into the reaction don't have to match the template exactly. They can still anneal with a small number
of point mutations where the base pairs are not complementary to the template.

PCR: Site Directed Mutagenisis

The oligonucleotides with the mutation are incorporated into the newly synthesized DNA. When they in turn become the
templates for synthesis, the resulting duplex DNA has the mutation in both strands, Because of the amplification power
of the PCR process, the final reaction contains almost all mutated sequences. This is a very useful method for making
changes in the sequence of DNA. If the mutated DNA encodes a protein, then this same technique can be used to make
genes which encode proteins with mutated sequences