PCR – Polymerase Chain Reaction

PCR is an extremely powerful technique used to amplify any specific piece of DNA of interest. The DNA of interest is selectively amplified out of the whole genome allowing scientists to look at that specific piece of DNA.

Advantages and Disadvantages:

One advantage of PCR is that it is very sensitive. The DNA of interest can be amplified with the DNA from just one cell. Thus, very small amounts of starting material can be used. Also, old or degraded DNA very often yields enough starting material to amplify the DNA of interest. The sensitivity of PCR is also its major disadvantage since very small amounts of contaminating DNA (from a different sample) can also be amplified.

How it works

Reactants

- Template (DNA sample from which your target sequence will be amplified)
- Primer (short single stranded DNA molecules specific for the ends of the piece of DNA you intend to amplify)
- dATP, dCTP, dGTP, dTTP
- DNA polymerase (polymerase from a thermophyllic bacteria is used so that extension can be done at high temperature, ex – Taq polymerase)
- Buffer (buffers, supplies magnesium and chloride)

PCR uses DNA polymerase, the enzyme that replicates DNA in living cells, to amplify the DNA. Thus PCR is a biologically based reaction. DNA amplification during PCR follows the same rules that DNA replication follows in vivo. Nucleotide bases are only added to a pre-existing 3'0H end (the primer). As occurs during DNA replication, the base inserted is complimentary to that found in the template strand. The specificity of the reaction is determined by the DNA sequences at the ends of the DNA to be amplified. Primers specific for these DNA sequences are made and used to prime repeated rounds of DNA synthesis.

There are three basic steps to the process (shown below). These three steps are performed by repeatedly cycling the tube through different temperatures.

1. Denaturation (94ºC)– separates the two strands of template DNA
2. Annealing (55ºC) – primers attach to the DNA
3. Extension (72ºC) – DNA polymerase synthesizes DNA starting at the primer.

These steps are repeated around 35 times.

Below is shown a PCR reaction of a portion of the following piece of DNA

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Cycle 1
Denaturation (94ºC) – strands separate

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’

3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Annealing (55ºC) – primers attach

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ GTCACG 5’

5’ GTCCAT 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Extension (72ºC)

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Cycle 2
Denaturation (94ºC) – strands separate

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’

3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’

3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Annealing (55ºC) – primers attach

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ GTCACG 5’

5’ GTCCAT 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ GTCACG 5’

5’ GTCCAT 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Cycle 2 (Cont)
Extension (72ºC)

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

Cycle 3
Denaturation and Annealing

5' ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3'
3'   GTCACG 5'

5' GTCCAT 3'
3' TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5'

5' GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3'
3'   GTCACG 5'

5' GTCCAT 3'
3' TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5'

5' GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3'
3'   GTCACG 5'

5' GTCCAT 3'
3' CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5'

5' GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3'
3'   GTCACG 5'

5' GTCCAT 3'
3' TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5'

Cycle 3
Extension

5’ ACTGGTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGCTGAGTGCTGA 3’
3’ CAGGTAATTAACCCCAGCATCAGTCCGGTCACG 5’

5’ GTCCATTAATTGGGGTCGTAGTCAGGCCAGTGC 3’
3’ TGACCAGGTAATTAACCCCAGCATCAGTCCGGTCACGACTCACGACT 5’

After 35 cycles there will be

2 x 35 = 70 long pieces of DNA (you don’t see these)

$2^{35} = 3.4 \times 10^{10}$ short piece of DNA (piece we are looking for)

The size of the amplified product is determined by gel electrophoresis.

Uses:

Genome mapping – specific variable regions of DNA are amplified and compared between individuals with a disease and those without it
DNA fingerprinting – again specific variable regions of DNA are amplified and compared to samples of interest (forensics, paternity)
Disease or general biology research – genes of interest can be amplified. Because PCR is so sensitive researchers can work with DNA from old fossils, or a very small tissue biopsy.s