PCR Techniques

* **The** **polymerase chain reaction** (**PCR**) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
* Developed in 1983 by Kary Mullis
* PCR is used to amplify a specific region of a DNA strand (the DNA target).

**Materials:**

A basic PCR set up requires several components and reagents. These components include:

1. DNA template that contains the DNA region (target) to be amplified.
2. Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
3. Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.
4. Deoxynucleoside triphosphates (dNTPs; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.
5. Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
6. Divalent cations, magnesium or manganese ions; generally Mg2+ is used, but Mn2+ can be utilized for PCR-mediated DNA mutagenesis, as higher Mn2+ concentration increases the error rate during DNA synthesis
7. Monovalent cation potassium ions.

**Procedure**

1. PCR consists of a series of 20-40 repeated temperature cycles, with each cycle consisting of 2-3 discrete temperature steps.
2. The cycling is often preceded by a single temperature step (called *hold*) at a high temperature (>90°C),
3. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters.
4. **Initialization step:**

Temperature 94–96 °C,

Time required 1–9 minutes.

It is only required for DNA polymerases that require heat activation by hot-start PCR.

1. **Denaturation step:**

First regular cycling event

Temperature 94–98 °C

Time required 20–30 seconds.

It causes melting of the DNA template by disrupting the hydrogen bonds, yielding single-stranded DNA molecules.

1. **Annealing step:**

Temperature 50–65 °C

Time required 20–40 seconds

Allowing annealing/binding of the primers to the single-stranded DNA template.

The polymerase binds to the primer-template hybrid and begins DNA synthesis.

1. **Extension/elongation step:**

Temperature 72 °C (depending on the DNA polymerase used)

Taq polymerase has its optimum activity temperature at 75–80 °C,

DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs.

Notice:

* The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified
* Under optimum conditions a thousand bases per minute can be amplified, if there are no limitations due to limiting substrates or reagents

**This video going to explain the thermo cycler of PCR technique.**

<http://www.youtube.com/watch?v=ZmqqRPISg0g&feature=player_detailpage>

<http://www.youtube.com/watch?v=DkT6XHWne6E>

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