Polymerase chain reaction

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- **PCR is the in vitro enzymatic synthesis and amplification of specific DNA sequences**
- **Can amplify one molecule of DNA into billions of copies in a few hours**

WHAT IS *TAQ* POLYMERASE?

Taq DNA polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus.* It is commonly used to amplify DNA fragments in PCR. The enzyme is in a recombinant form, expressed in *E. coli*. It is able to withstand repeated heating to 95 °C (as is demanded by the PCR technique) without significant loss of activity. It has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity. Each lot of *Taq* DNA Polymerase is tested for PCR amplification and double-stranded sequencing. The enzyme is supplied at 5 units/μL and comes with an optimized 10x reaction buffer.

The Basic Protocol of PCR The first step of PCR simply entails mixing the template DNA, two appropriate oligonucleotide primers, DNA polymerase, deoxyribo - nucleoside triphosphates (dNTPs) and a buffer. Once assembled, the mixture is cycled many times, usually 30 times through temperatures that permit denaturation, annealing and synthesis. The product is then displayed on an appropriate gel and examined for yield and specificity 20. Hence, the basic PCR protocol may be outlined as follows:

1. Optimize MgCl2 concentration.
2. Prepare a 10X amplification buffer containing MgCl2 at 10-fold the optimal concentration.

3. Test the optimised 10X amplification buffer by preparing a reaction cocktail for a single PCR. If PCR is successful, prepare more reaction cocktail for storage at –200C.

4. Add 68µl 15µg/ml template DNA and 9µl of H2O.

5. Add 1µl 2.5U/µl Taq DNA polymerase.

6. Overlay the reaction mixtures with 100µl mineral oil to prevent evaporation

7. Heat samples for 90secs at 940C (in a bath or an automated thermal cycler) to denature the DNA.

8. Incubate at 550C for 2 min to anneal.

9. Incubate the sample at 720C for 3 min to extend.

10. Repeat steps 7 to 9 for another 29 cycles.

11. Electrophorese 10µl from each sample on an agarose non-denaturing polyacrylamide gel.

12. Stain the gels with ethidium bromide.

13. Examine the stained gel.

Most examples of applications of PCR in scientific research may be summarized as follows:

1- Direct sequencing of in vitro amplified DNA.

2-. Engineering DNA to meet specific needs.

3- Detection of mutation.

4- Detection of gene

5- Specific amplification of a DNA specie

6-. Geometric amplification of unknown DNA sequence through inverse PCR

7-. Analysis of DNA sequences in individual gametes.

8- Evolutionary analysis.

Primer Selection There is no set of rules that will ensure the synthesis of an effective primer pair. Yet more than anything else, it is the primers that determine the success or failure of an amplification reaction. However, the chances of success may be increased by

1- Selecting primers with random base distribution and with a GC content similar to that of the fragment being amplified.

2- Avoid sequences with significant secondary structure (the use of a computer programme is strongly recommended for this).

3- Reducing incidence “Primer dimmer” by avoiding primer complementarity.

Primer dimmer is an amplification artifact, usually a double stranded fragment whose length is very close to the sum of the two primers, and appears to occur when one primer is extended by the polymerase over the other primer. Most primers will be between 20 and 30 bases in length and the optimal amount to use in amplification will vary. Sequences not complementary to the template can be added to the end of the primer. These exogenous sequences become incorporated into the double stranded PCR product and provide a means of introducing restriction sites or regulatory elements.

PCR Progrram

OPA-01=5¯-CAGGCCCTTC-3¯

OPH-04=5¯-GGAAGTCGCC -3¯

OPH-05=5¯-AGTCGTCCCC - 3¯

Stag	Step		Time	Cycle
1	1	Denaturation 94č	1 min	1
2	1	Denaturation 94č	1 min	40
	2	Annealing 37 č	1.5 min	
	3	Extension 72č	2 min	
3	1	Extension 72č	10 min	1