First set of experiments

a) Putative *ga20ox* mutant: complementation experiment.

b) Rescuing dwarf mutants by application of GA:

1. +
2. -

PCR

Polymerase

Chain

Reaction

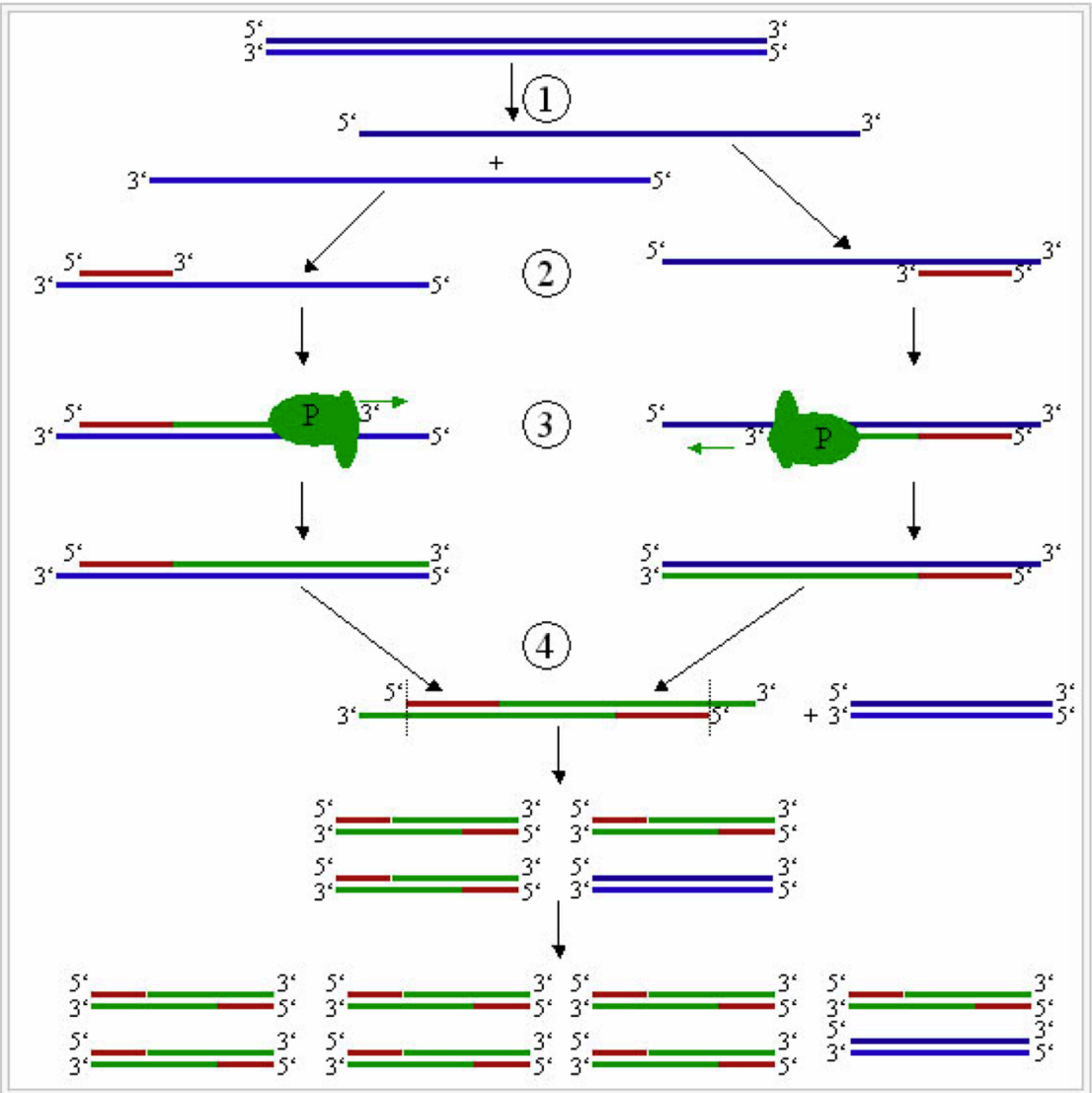


Figure 2: Schematic drawing of the PCR cycle. (1) Denaturing at 94-96°C. (2) Annealing at (eg) 68°C. (3) Elongation at 72°C (P=Polymerase). (4) The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycle, thus doubling the amount of DNA duplicated for each new cycle.

Kary Mullis developed the PCR method in 1983 and received the Nobel price for this in 1993.

A concept similar to that of PCR had been described before Mullis' work. Nobel Prize laureate H. Gobind Khorana and Kjell Kleppe, a Norwegian scientist, authored a paper seventeen years earlier describing a process they termed "repair replication" in the *Journal of Molecular Biology*.

Wikipedia

PCR allows the amplification of specific fragments of DNA:

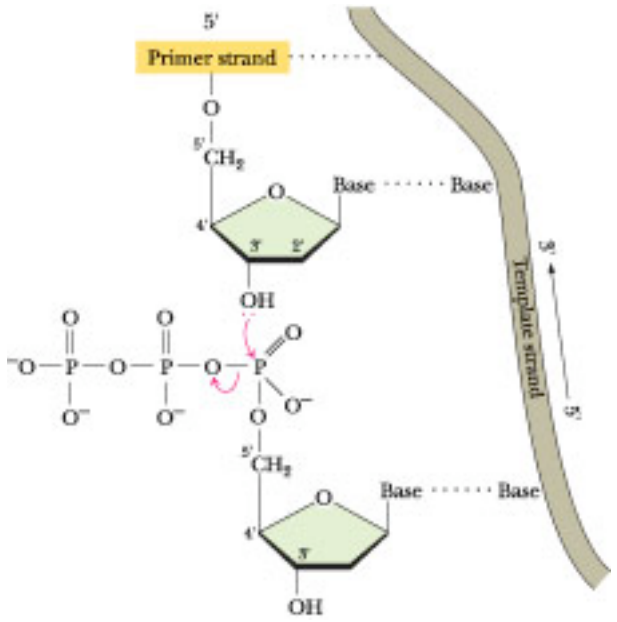
You need some sequence information of what you are going to amplify because primers are sequence specific.

PCR Movies

http://www.youtube.com/watch?v=j9Gu7iwBi4I
http://www.dnalc.org/resources/animations/pcr.html

DNA polymerization occurs from 5' to 3'

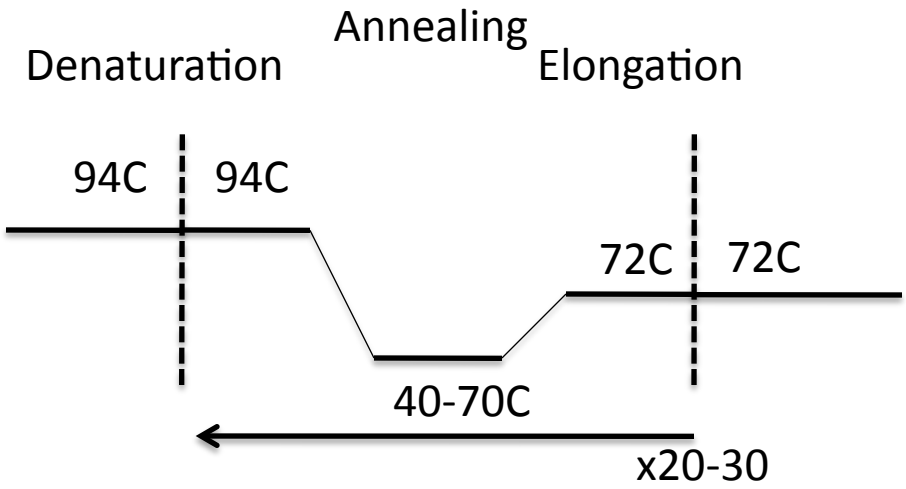
5'



3'

http://web.virginia.edu/Heidi/chapter30/chp30.htm

A typical PCR reaction has 3 steps



Reaction components:

1. Thermo stable DNA polymerase
(e.g. Taq polymerase from Thermus aquaticus)

2. Mg^{2+}
3. dNTPs
4. DNA Template
5. Primers

What are the factors affecting:

Annealing temperature

primer length, primer GC content, salt concentration

Elongation time

enzyme processivity, fragment length

(Taq polymerase has an extension rate of 35-100 nt/sec)

PCR problems

When would you get no product?

One or more components missing
Wrong annealing temperature
Elongation temperature is too short

When would you get the wrong product?

Unspecific hybridization of primers
Polymerase mistakes
DNA contamination

Uses of PCR

CLONING:

amplifying DNA fragments for further processing
mutating DNA fragments: addition of nucleotides
exchange of nucleotides
amplifying homologous DNA fragments

DETECTION:

quantifying DNA: real time PCR
mapping mutations: point mutations
insertions/deletions