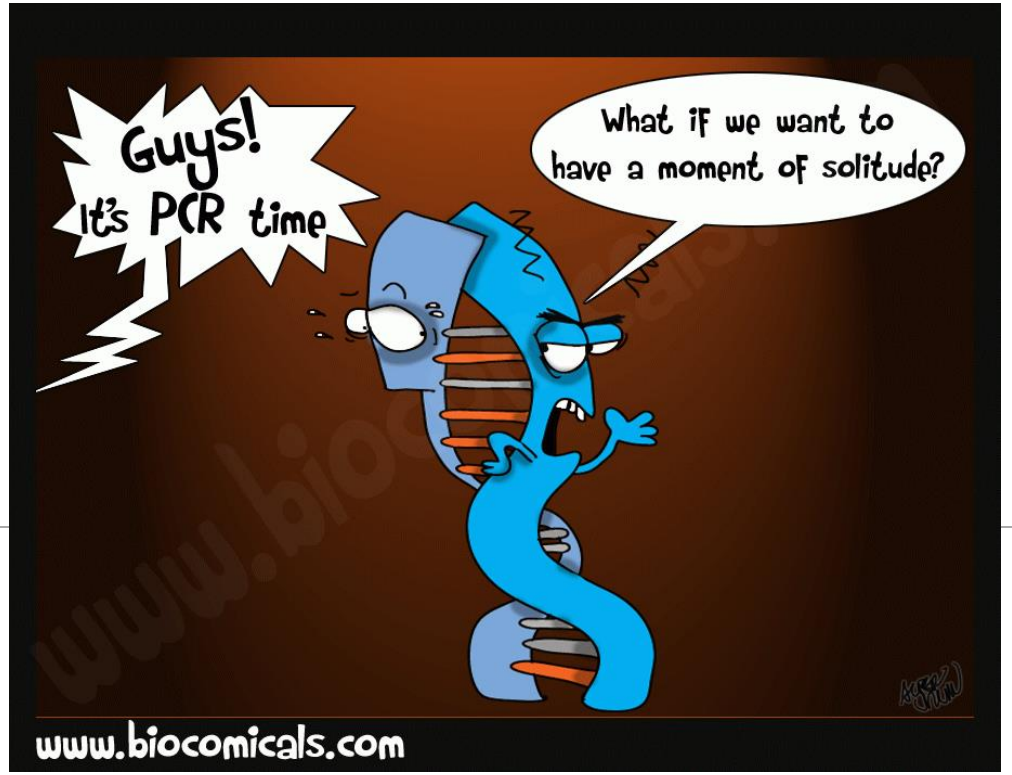
POLYMERASE CHAIN REACTION



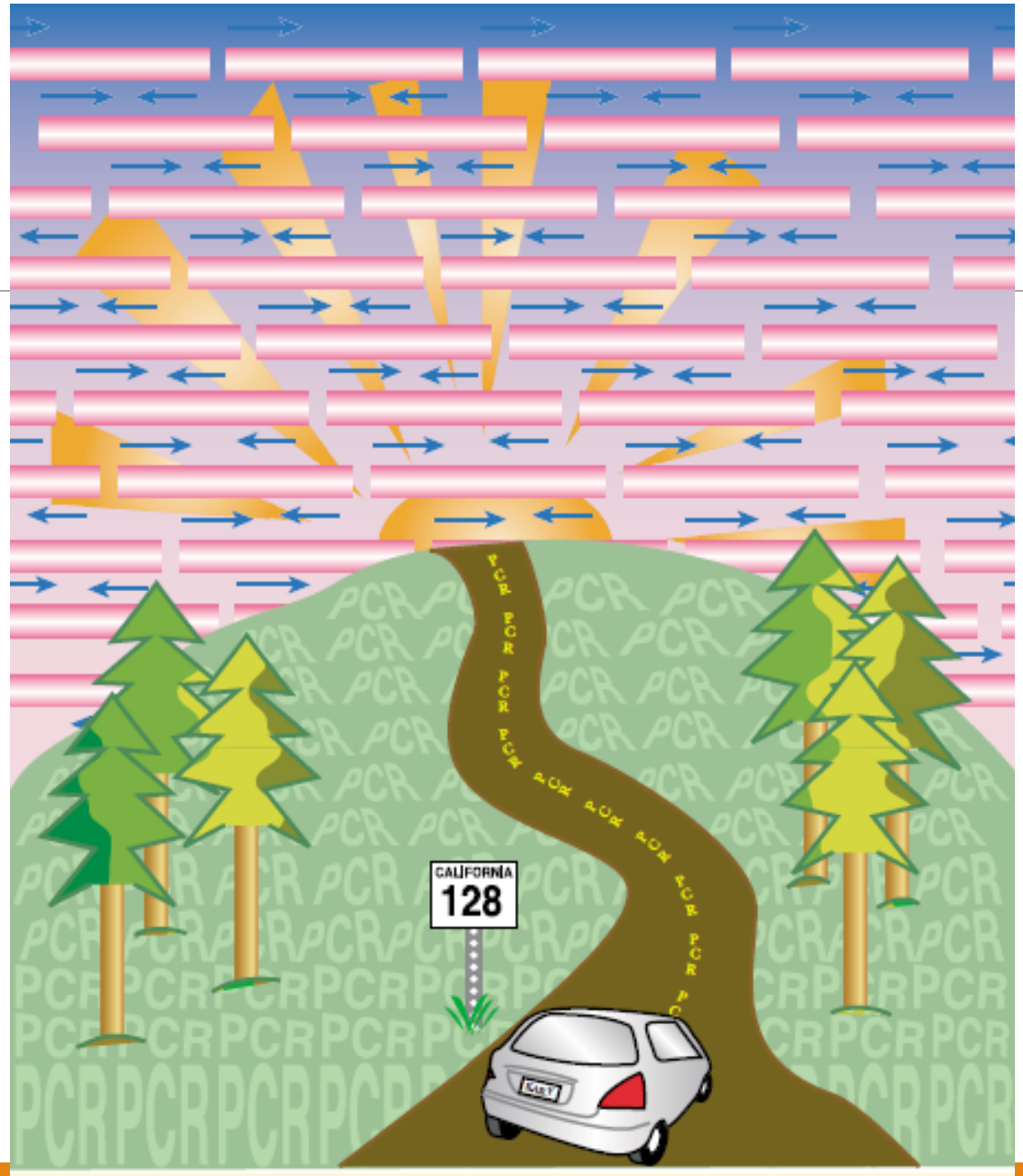
Presented by:

SNEHA U

Introduction

Amplification of a DNA sequence by repeated cycles of strand separation and replication

PCR was invented in 1983 by Kary Mullis & he received the **Nobel Prize** in Chemistry in 1993, for his invention.



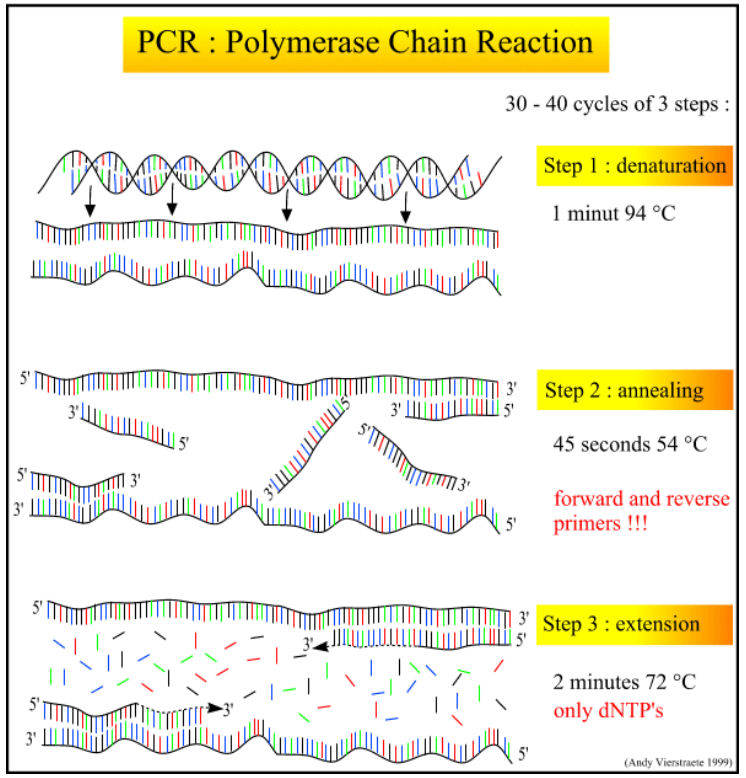
Kary Mullis Sees PCR in a Vision

PCR

- The **polymerase chain reaction** (**PCR**) is a scientific technique 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.

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

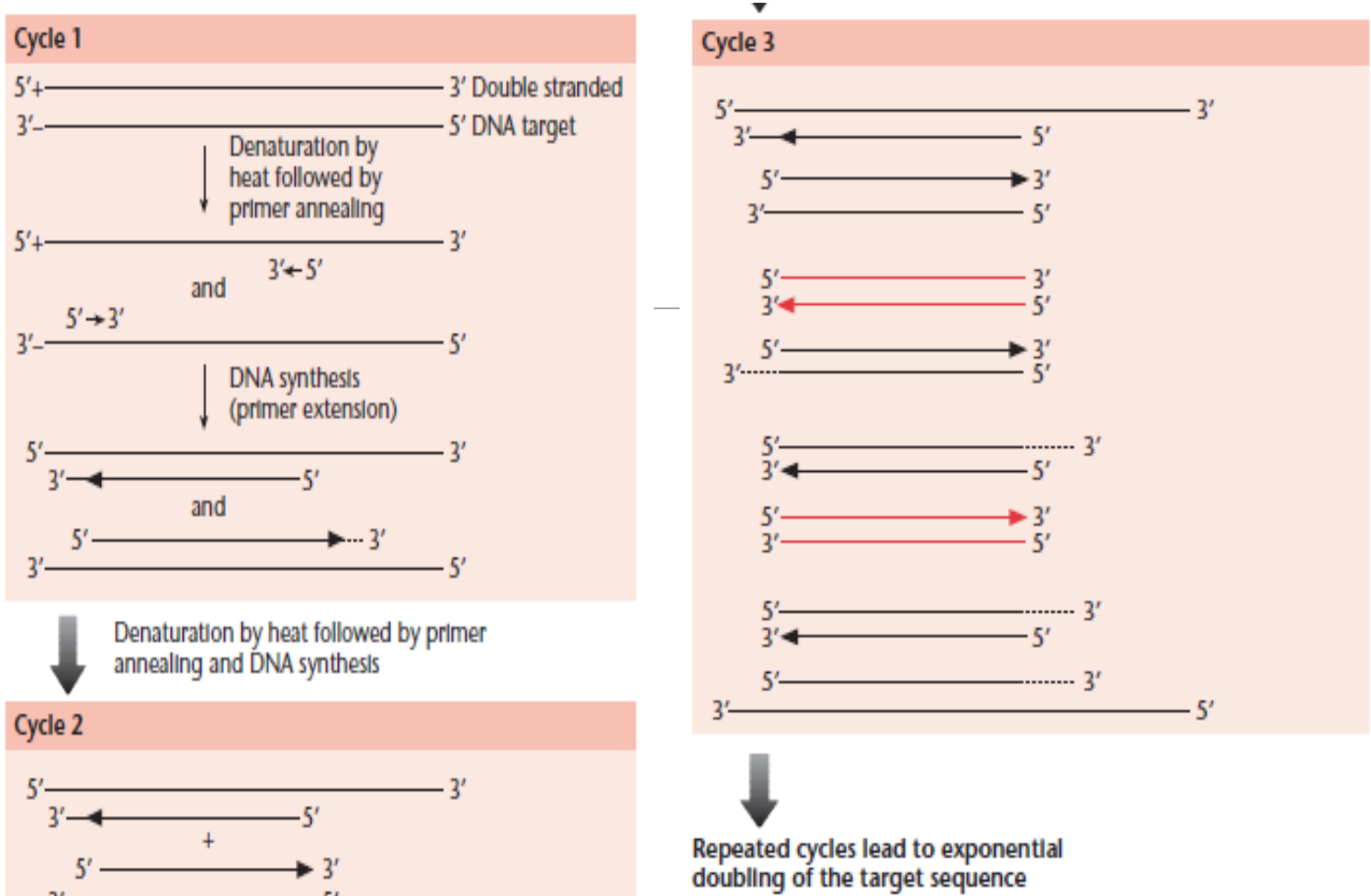
- DNA template
- Forward and reverse primers
- Taq polymerase
- Deoxynucleoside triphosphates
- Buffer solution
- Mg 2+ ions



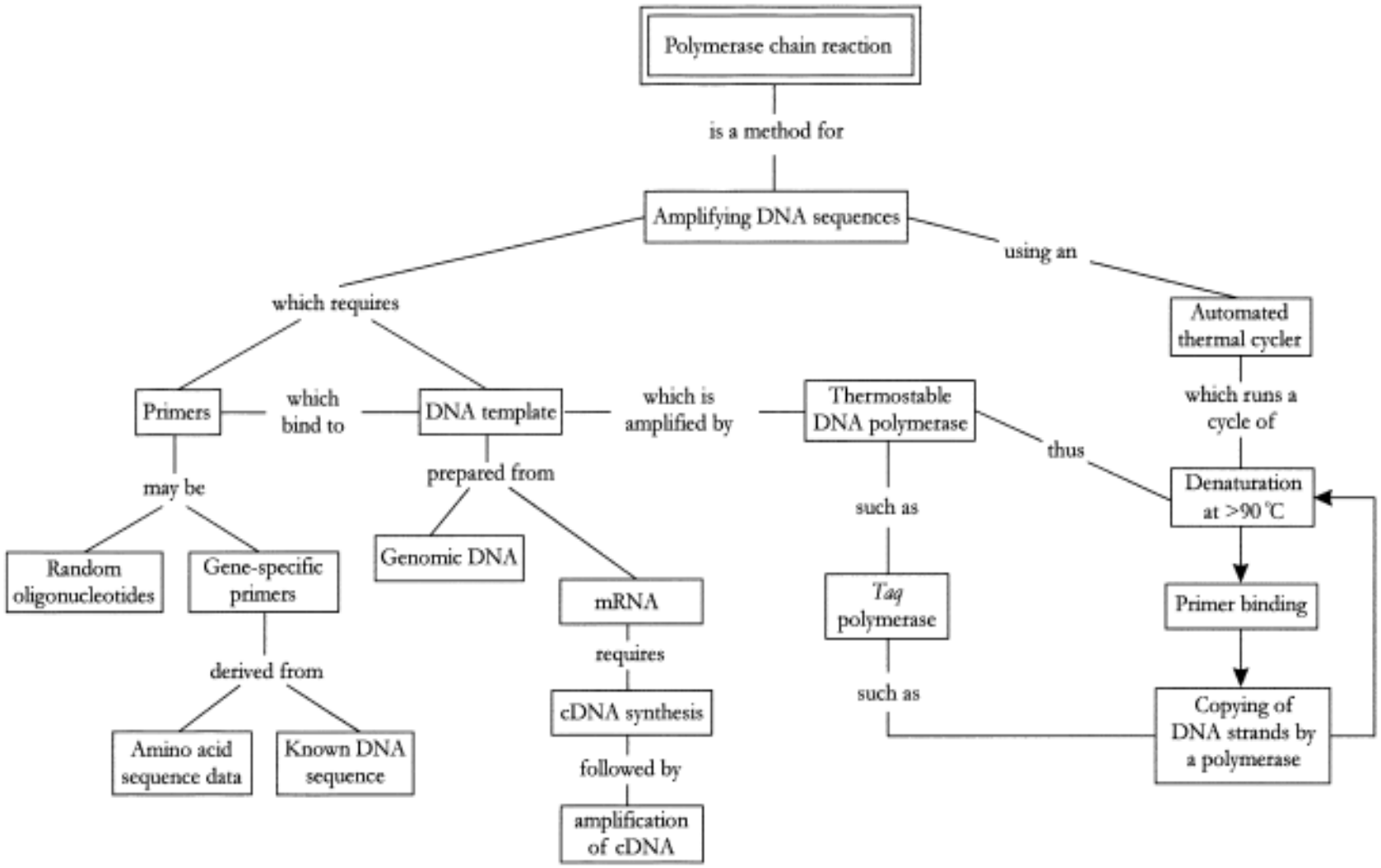
STEPS

Three basic steps which are in common in all types of PCR:

- **Thermal denaturation :**
  - DNAs are denatured mostly by temperature at about 94˚C & single stranded DNAs are generated.
- **Primer annealing :**
  - Primers are attached to ssDNA with their complementary regions.
- **Extension or polymerization :**
  - This is done by a temperature resistance polymerase named **T*aq* polymerase** which is extracted from ***Thermus aquaticus*.**

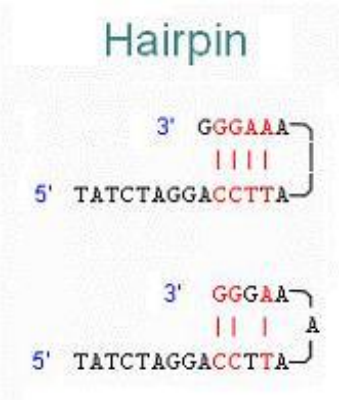

Cycle 1
5′+ 3′ Double stranded
3′– 5′ DNA target
Denaturation by heat followed by primer annealing
5′+ 3′
3′←5′
and
5′→3′
3′– 5′
DNA synthesis (primer extension)
5′ 3′
3′ 5′
and
5′ 3′
3′ 5′
Denaturation by heat followed by primer annealing and DNA synthesis
Cycle 2
5′ 3′
3′ 5′
+
5′ 3′
3′ 5′
+
5′ 3′
3′ 5′
+
5′ 3′
3′ 5′
Denaturation by heat followed by primer annealing and DNA synthesis
Cycle 3
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
5′ 3′
3′ 5′
Repeated cycles lead to exponential doubling of the target sequence

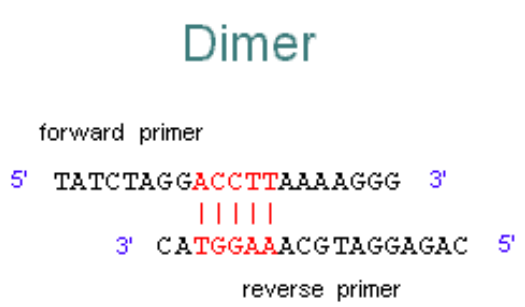
SUMMARY


Polymerase chain reaction
is a method for
Amplifying DNA sequences
using an
which requires
Automated thermal cycler
Primers
which bind to
DNA template
which is amplified by
Thermostable DNA polymerase
thus
which runs a cycle of
may be
prepared from
such as
Denaturation at >90 °C
Random oligonucleotides
Gene-specific primers
Genomic DNA
mRNA
Taq polymerase
Primer binding
derived from
requires
such as
cDNA synthesis
Copying of DNA strands by a polymerase
Amino acid sequence data
Known DNA sequence
followed by
amplification of cDNA

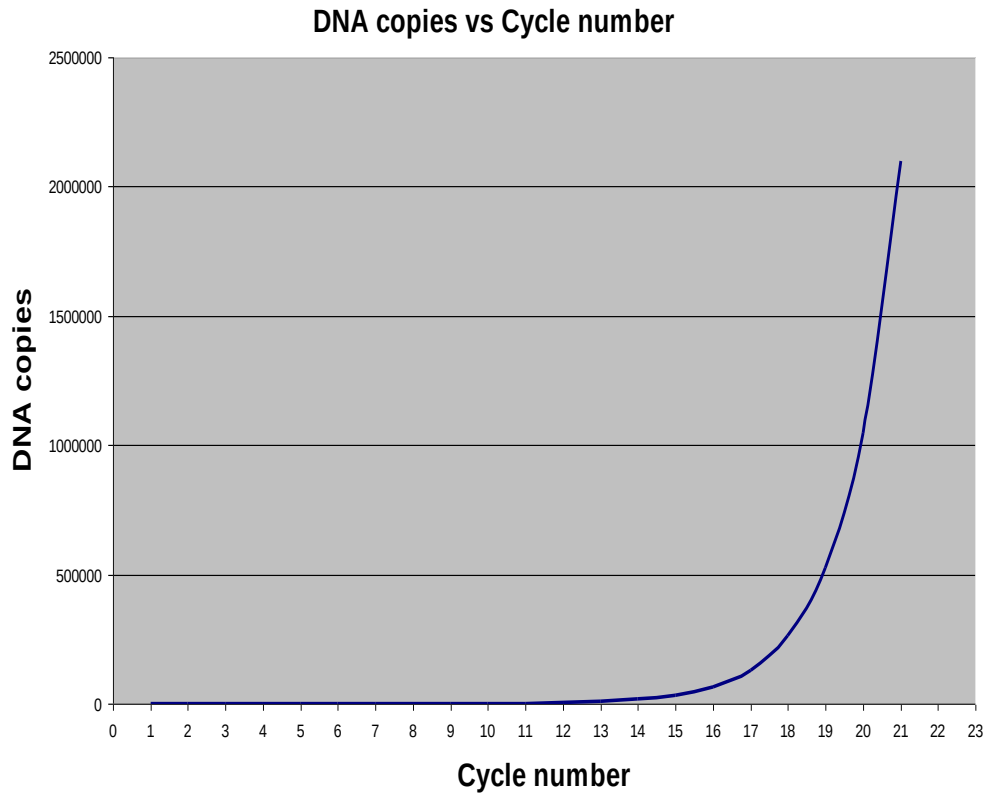
Good Primer's Characteristics

- A melting temperature (Tm) in the range of 52ºC to 65ºC
- Absence of dimerization capability
- Absence of significant hairpin formation (>3 bp)
- Primers should be 17 to 30 ntds in length
- There shall be one and only one target site in the template DNA where the primer binds.

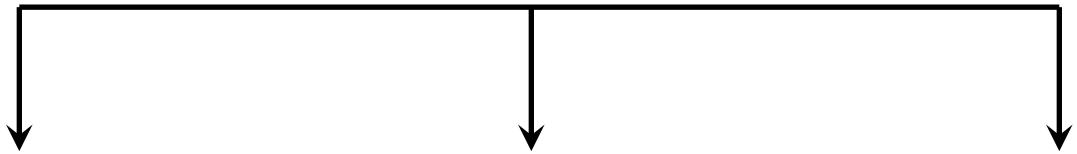




Number of PCR cycles (n)	Number of double-stranded copies of original DNA (2^n)
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
20	1,048,576
30	1,073,741,824



Applications of PCR



Molecular Identification

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- DNA fingerprinting
- Classification of organisms
- Genotyping
- Pre-natal diagnosis
- Mutation screening
- Drug discovery
- Genetic matching
- Detection of pathogens

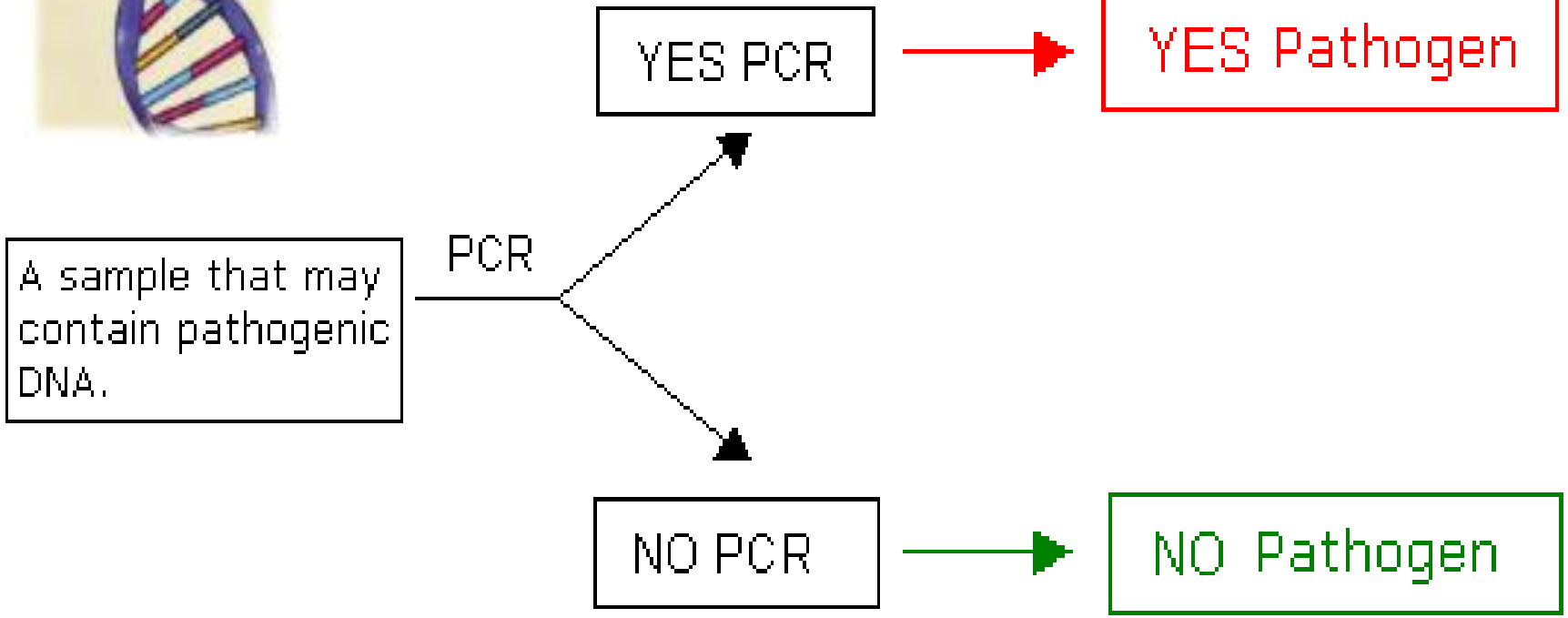
Sequencing

- Bioinformatics
- Genomic cloning
- Human Genome Project

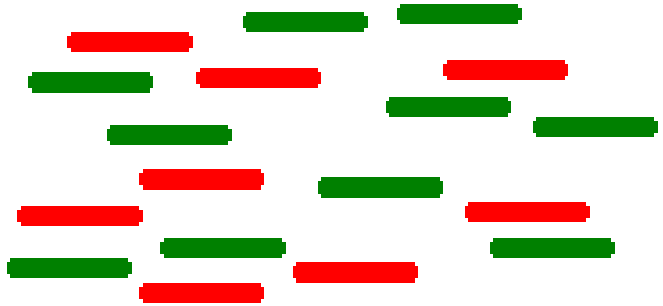
Genetic Engineering

- Site-directed mutagenesis
- Gene expression studies

Detection Of Pathogens



Detection of Unknown Mutations



Whole Genomic DNA

PCR

Desired DNA fragments that may contain a mutation in huge numbers.

Classification of Organisms

1) Relating to each other

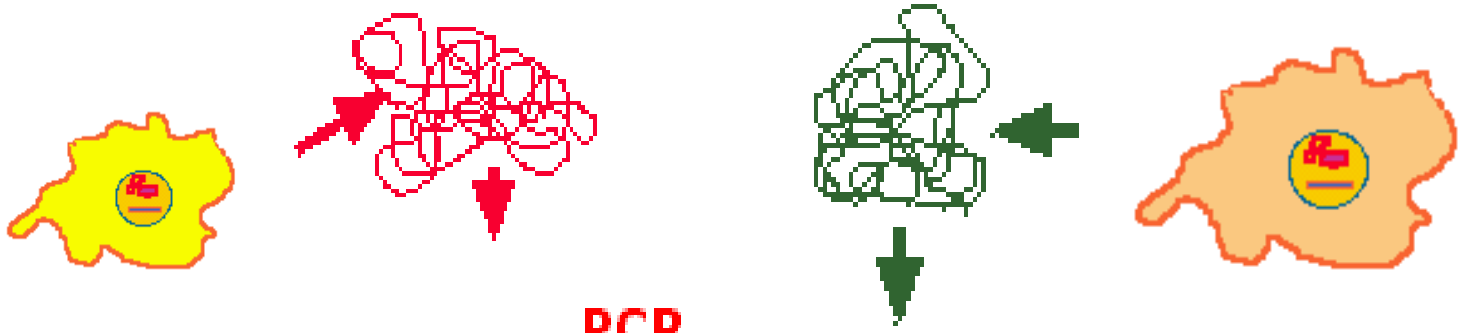
2) Similarities

3) Differences

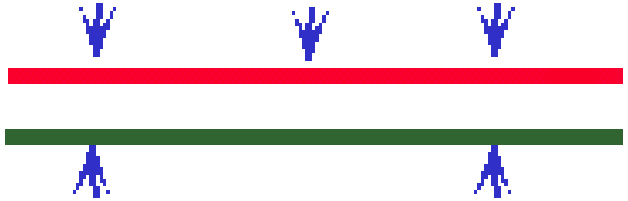
* Fossils
* Trace amounts
* Small organisms

} Insufficient data

! DNA !

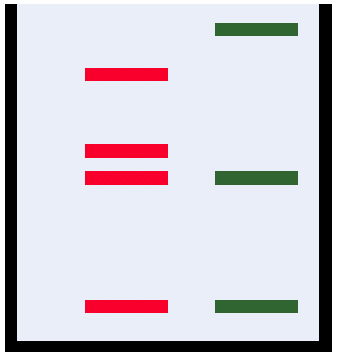


PCR



Specific PCR products are cut with restriction enzymes.

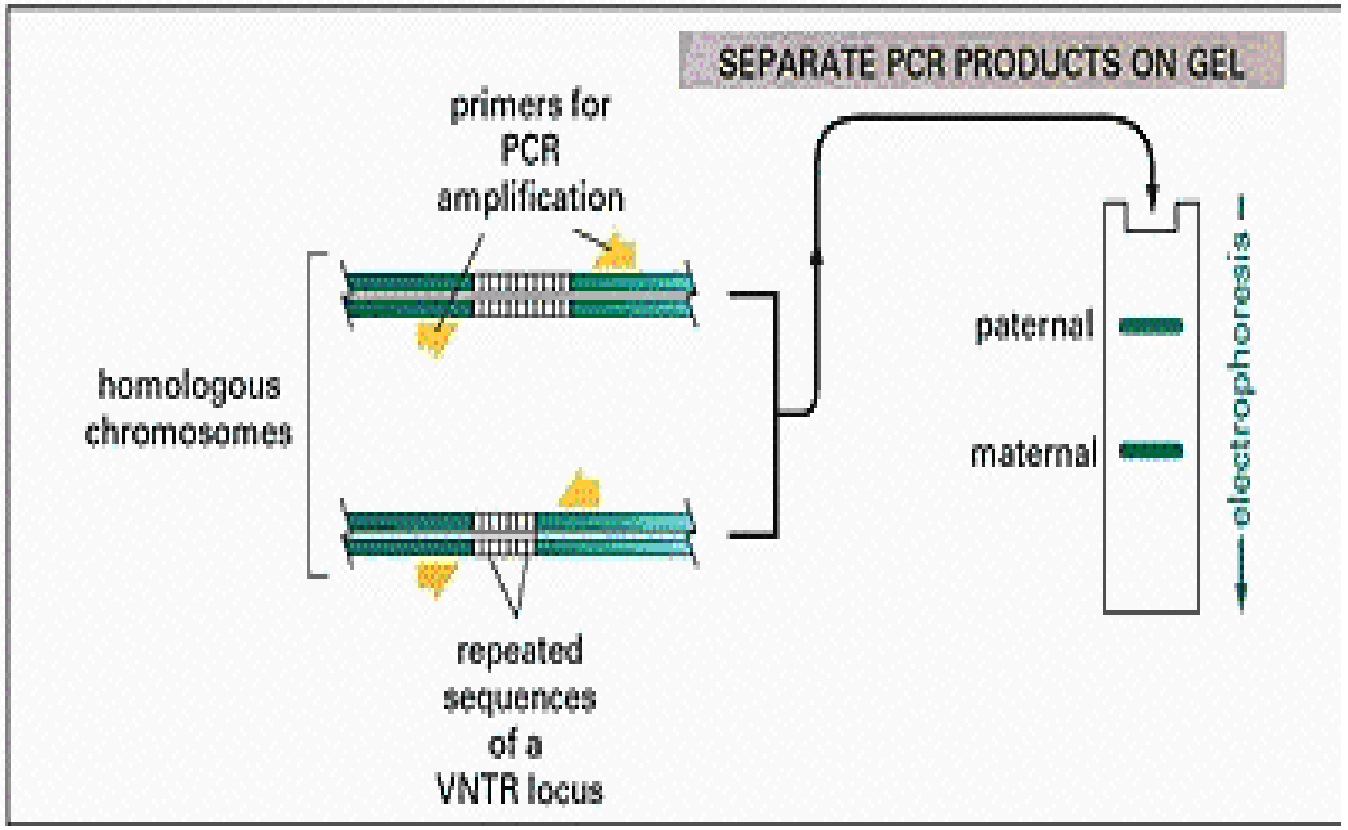
DNA FRAGMENTS

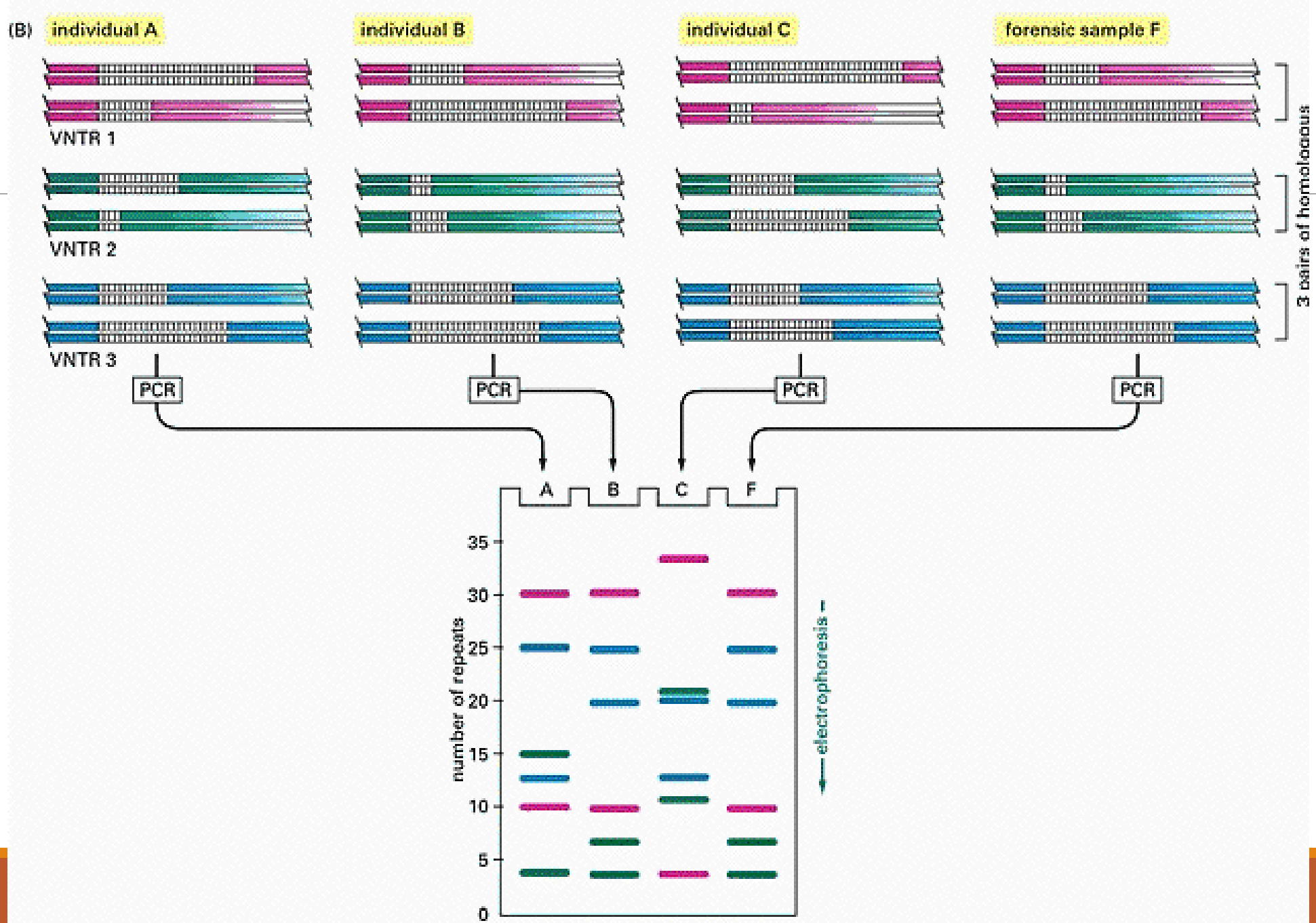


SEPARATE FRAGMENTS ON THE BASIS OF THEIR SIZE

PCR can also be used in forensic testing.

The DNA sequences used are of short repeating patterns called VNTR (variable number of tandem repeat), which can range from 4 to 40 nucleotides in different individuals




(B)
individual A
individual B
individual C
forensic sample F
VNTR 1
VNTR 2
VNTR 3
3 pairs of homologous
PCR
PCR
PCR
PCR
A
B
C
F
35
30
25
20
15
10
5
0
number of repeats
electrophoresis

TYPES OF PCR

- *Inverse PCR*
- *Nested PCR*
- *Real time quantitative PCR*
- *RACE PCR*
- *Reverse transcriptase PCR*
- *Ligation mediated PCR*
- *Methylation specific PCR*
- *Hot start PCR*
- *Asymmetric PCR*
- *Allele specific PCR*
- *Assembly PCR*
- *Overlap extension PCR*
- *Solid phase PCR*
- *RAPD*
- *Intersequence specific PCR*
- *Helicase dependent amplification*
- *Multiplex PCR*
- *Touchdown PCR*

Thank you!