



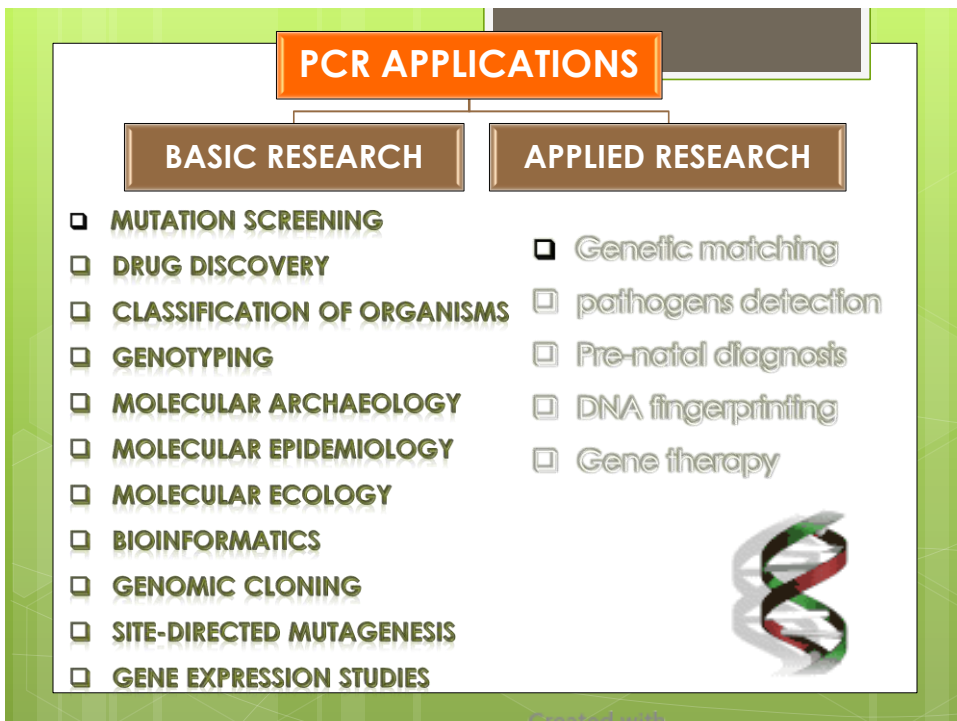

APPLICATIONS OF PCR

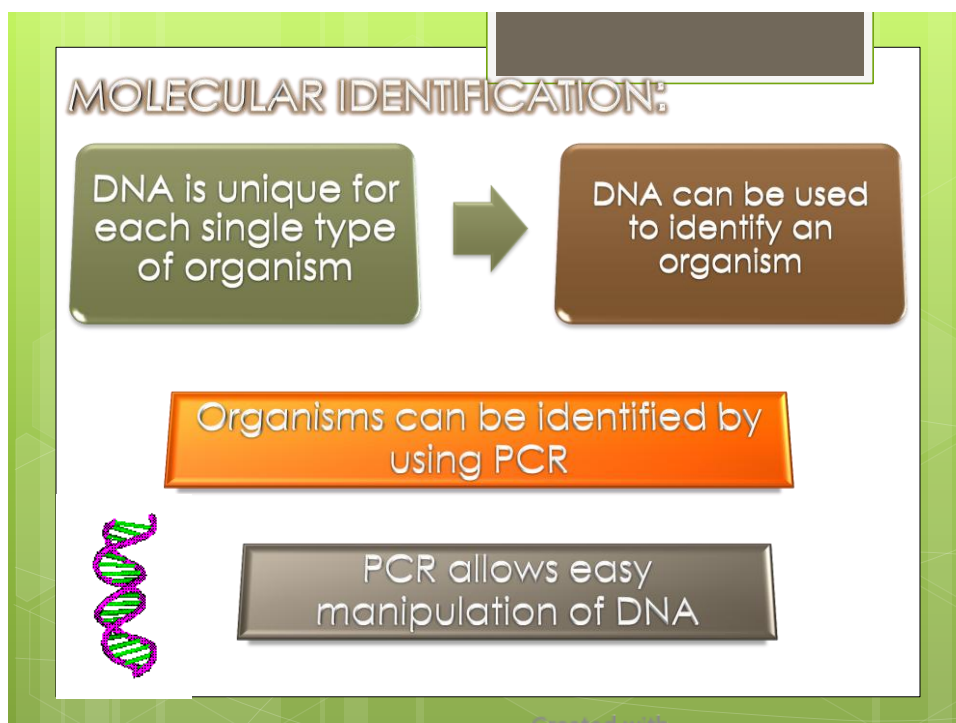
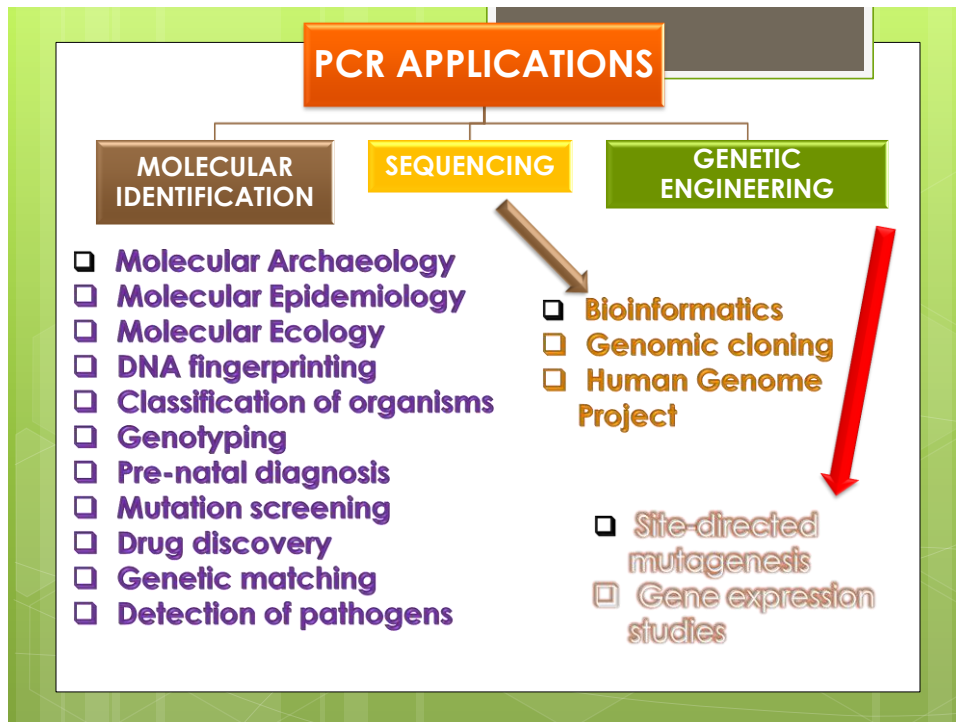
BY

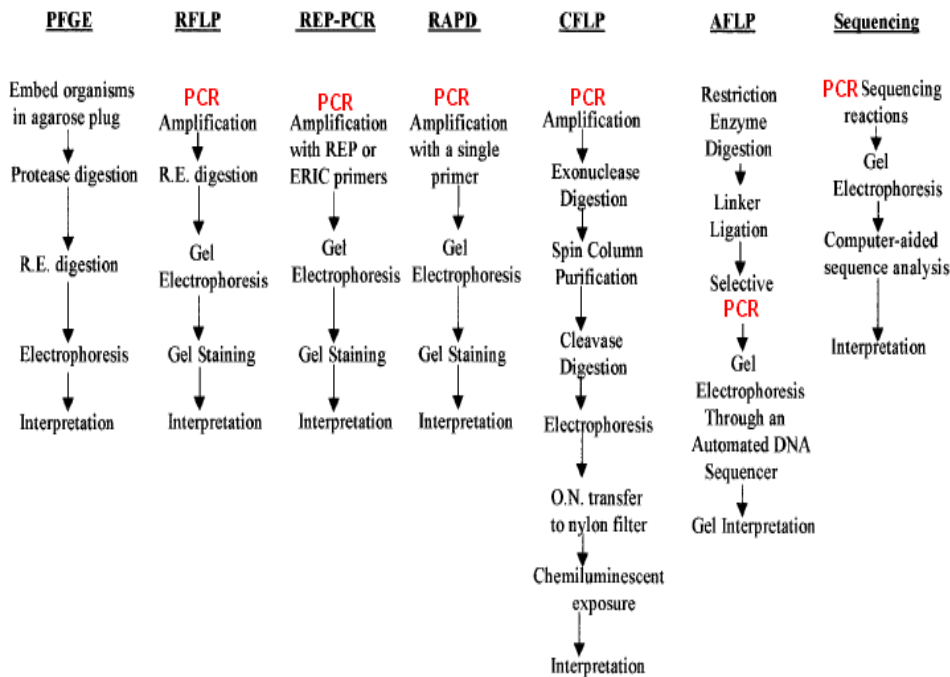
PROF. DR. ASMAA HUSSEIN

DIRECTOR OF THE
MOECLULAR BIOLOGY
RESEARCH UNIT (MBRU)

ASSIUT UNIVERSITY







Molecular Identification:

Detection of Unknown Mutations



Whole Genomic DNA

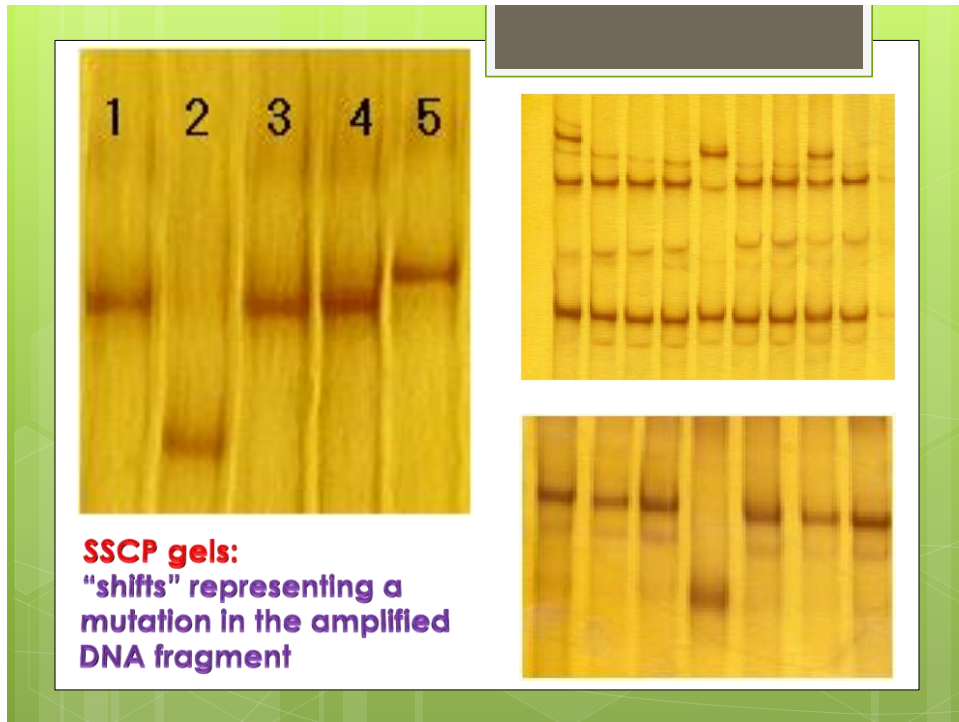
PCR

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

SSCP



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Molecular Identification:

Classification of Organisms

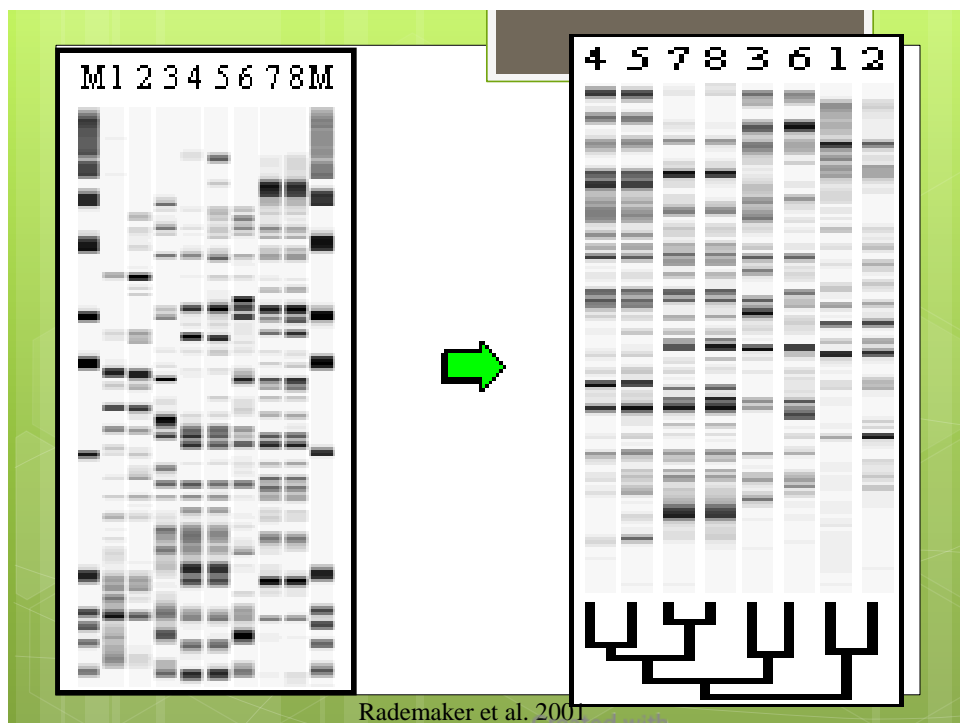
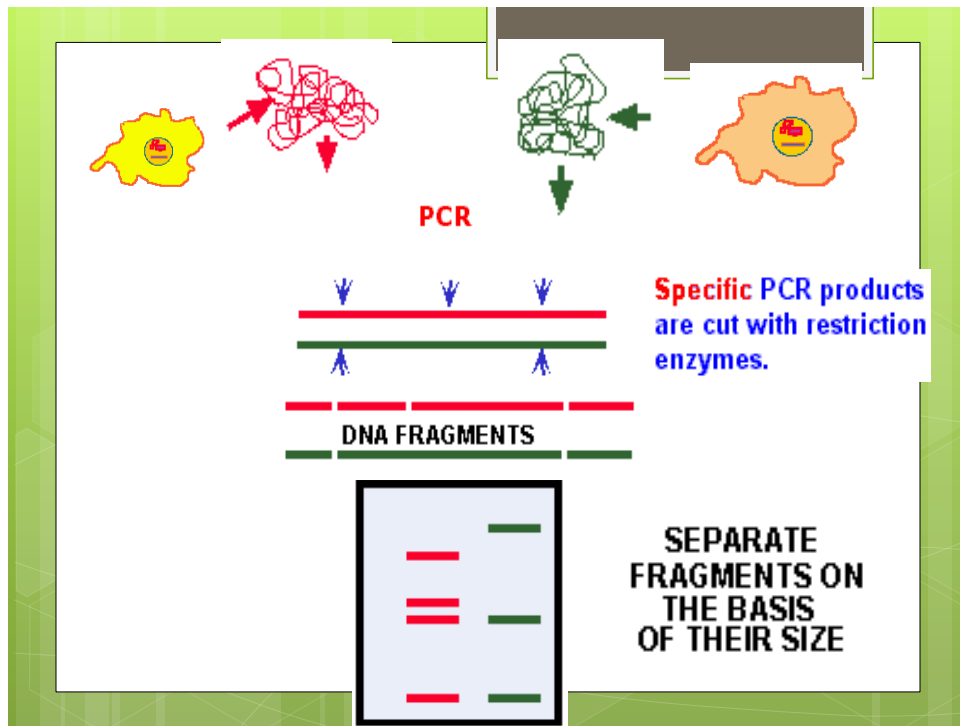
- 1) Relating to each other
- 2) Similarities
- 3) Differences

- * FOSSILS
- * TRACE AMOUNTS
- * SMALL ORGANISMS

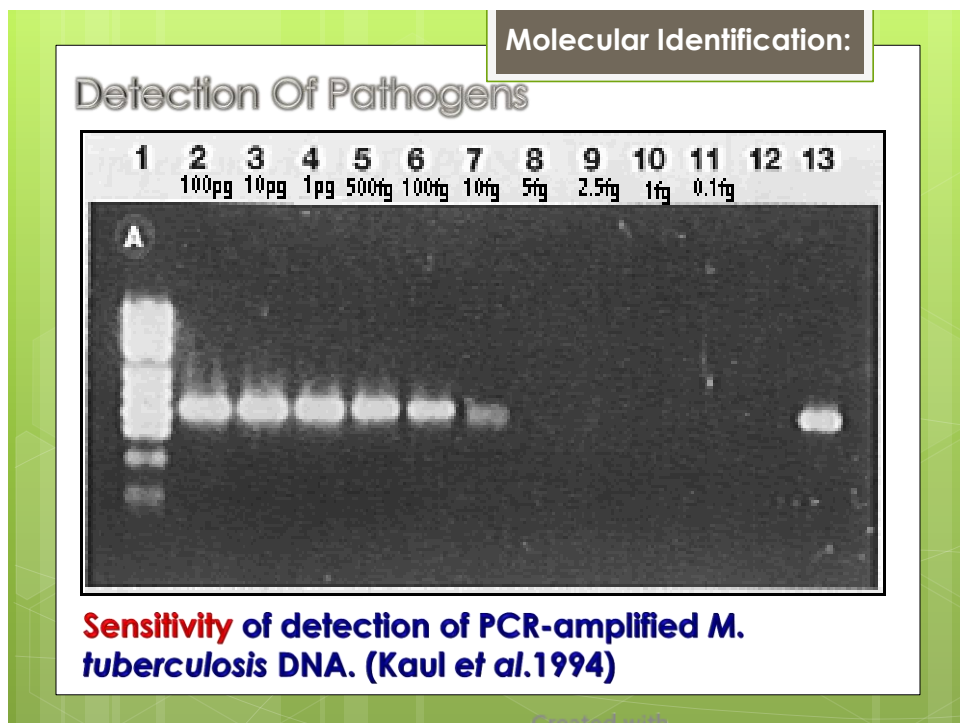
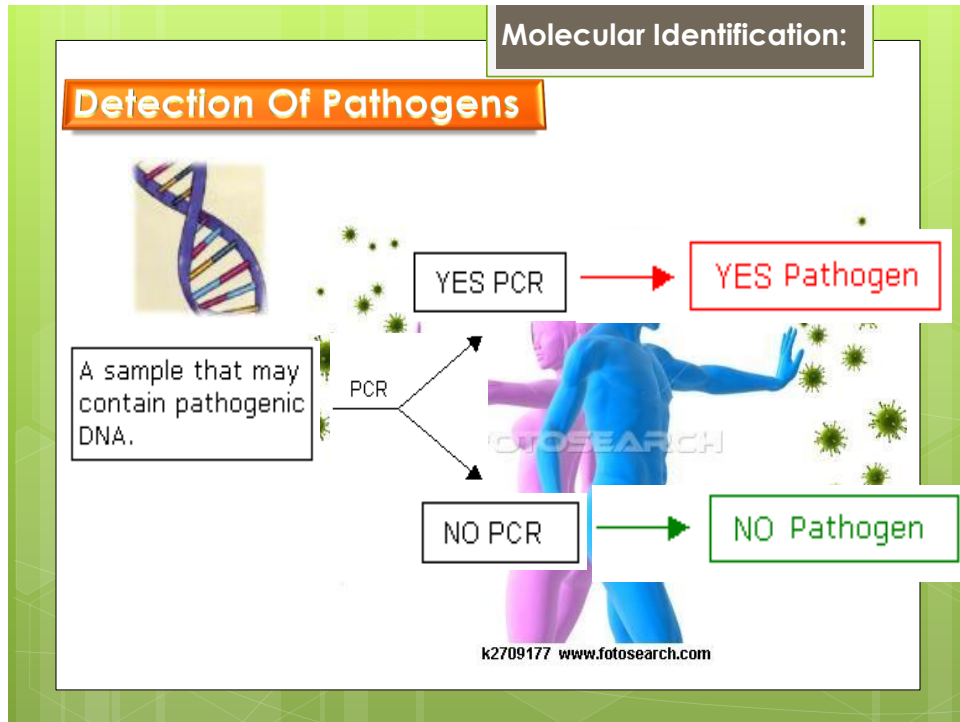
Insufficient data

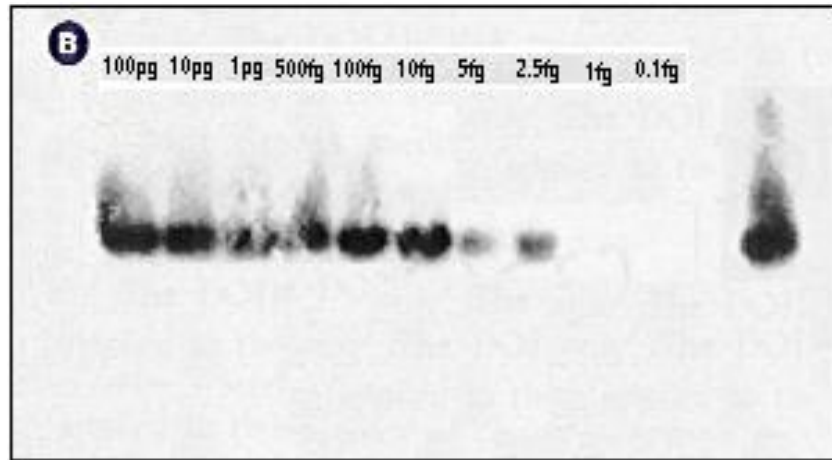
! DNA !





Rademaker et al. 2001

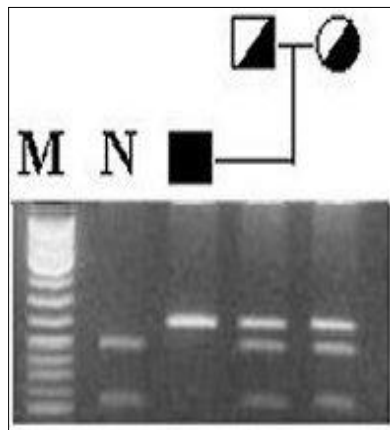




Sensitivity of detection of PCR-amplified *M. tuberculosis* DNA. (Kaul et al.1994)

Molecular Identification:

Prenatal Diagnosis



- Chorionic Villus
- Amniotic Fluid

← 644 bp
 ← 440 bp
 ← 204 bp

Molecular analysis of a family with an autosomal recessive disease

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SEQUENCING

Nucleotides (dNTP) are modified
(dideoxynucleotides = ddNTP)

NO polymerisation after a dideoxynucleotide!

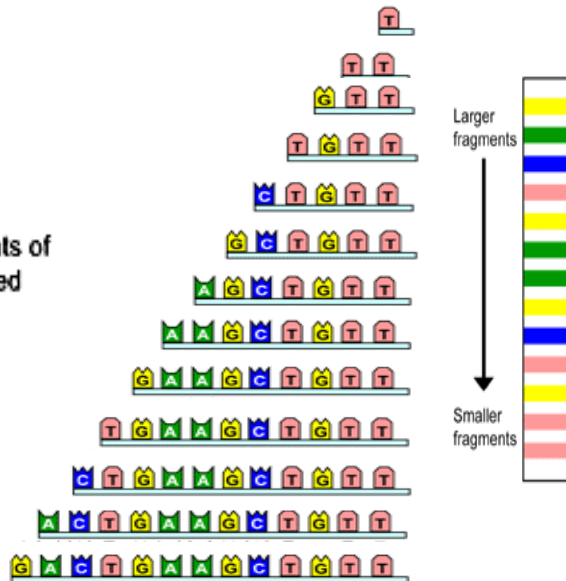


Fragments of DNA differing only by one nucleotide
are generated

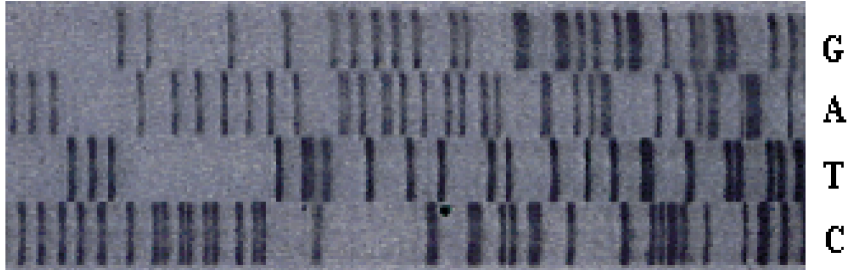
Nucleotides are either **Radioactive** or **fluorescent**

DNA Polymerase I
dATP
dGTP
dCTP
dTTP
plus limiting amounts of
fluorescently labelled

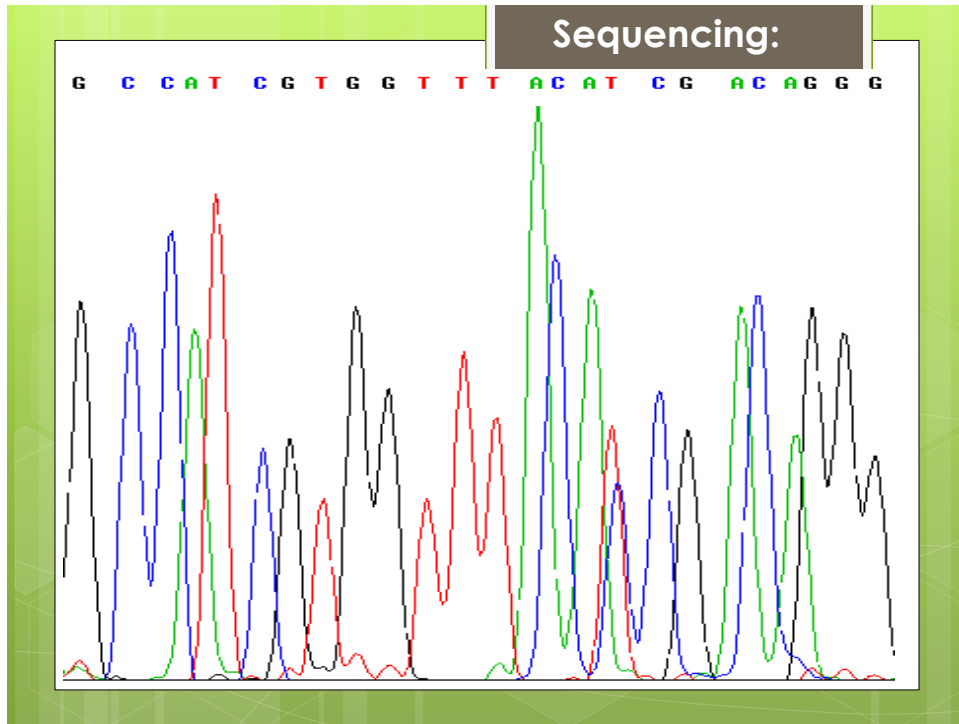
ddATP
ddGTP
ddCTP
ddTTP




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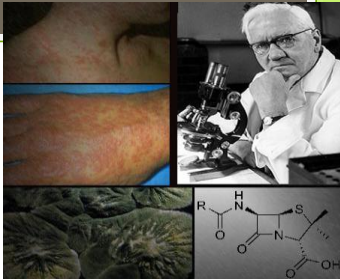
Sequencing:**Classical Sequencing Gel****Sequencing:****Reading Classical Sequencing Gels**

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1928



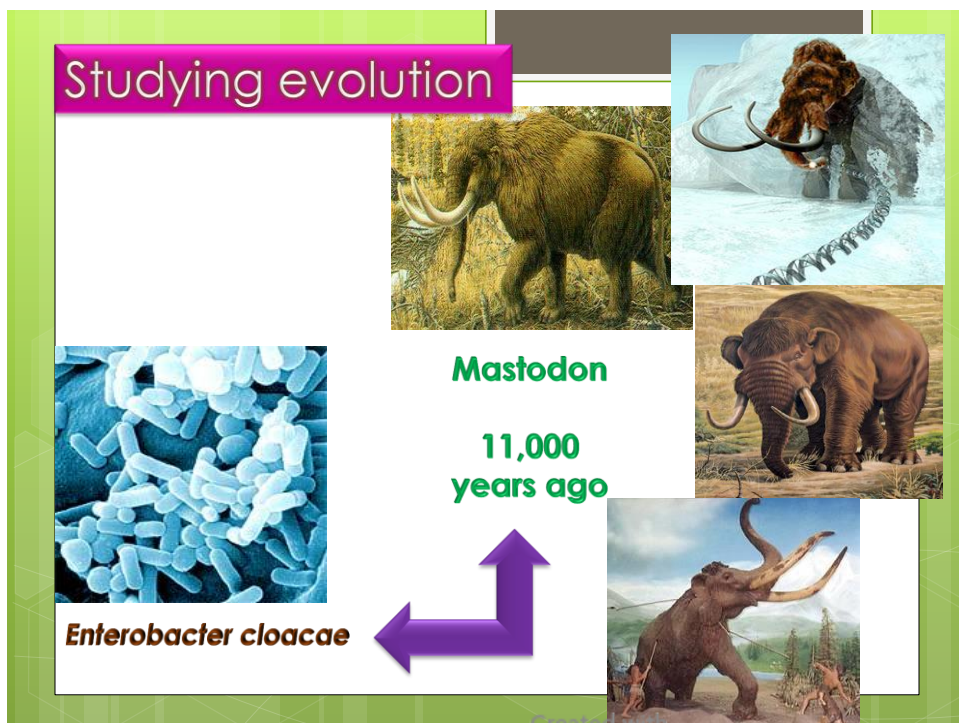
Alexander Fleming
Nobel Prize 1945

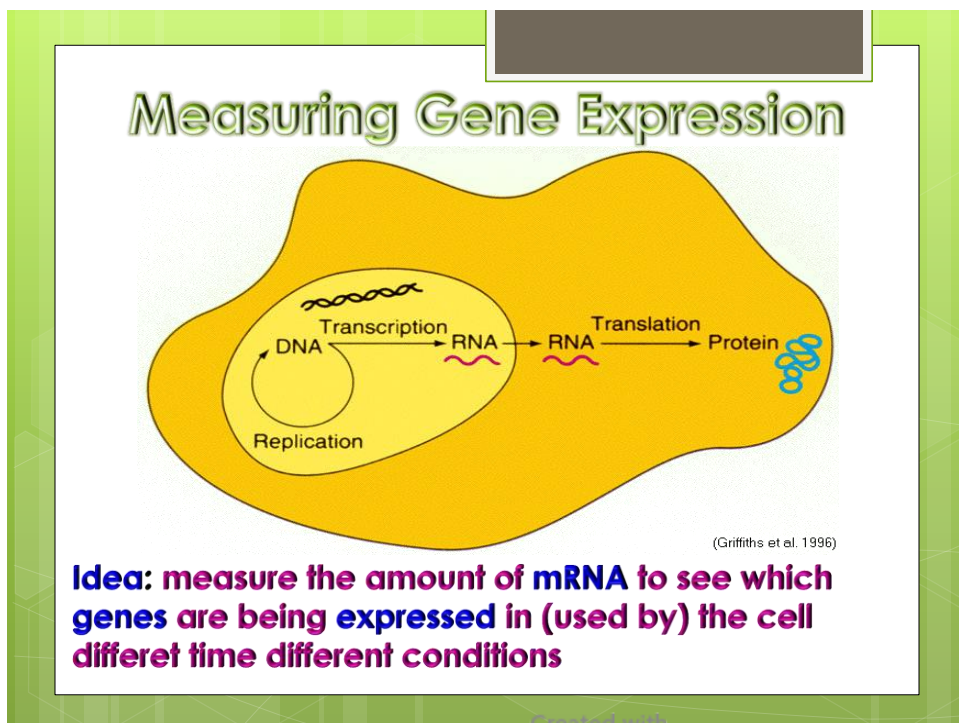
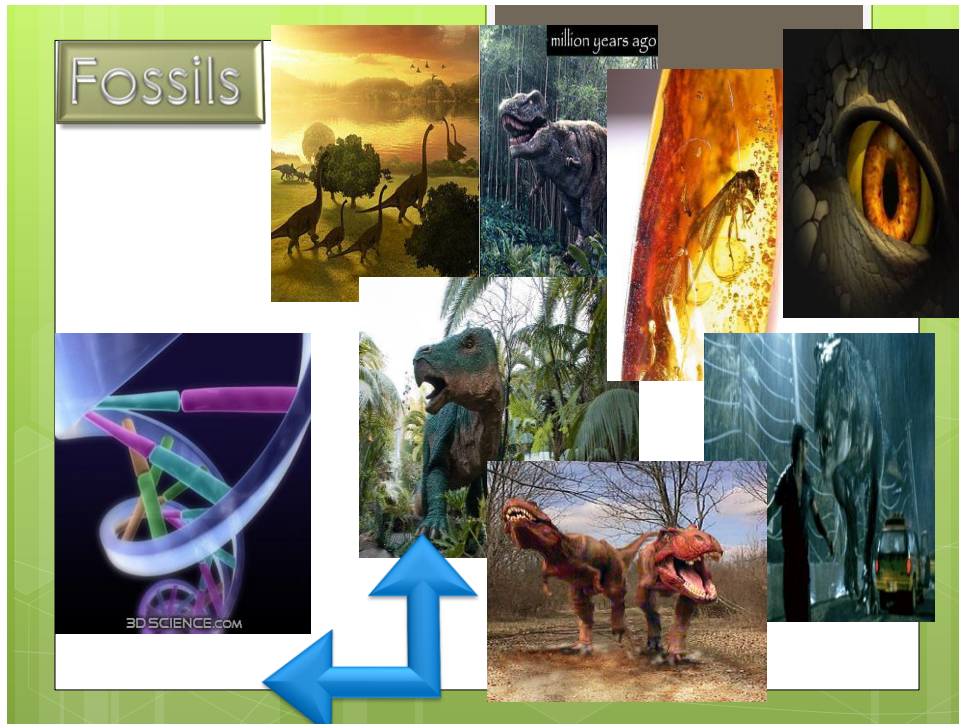



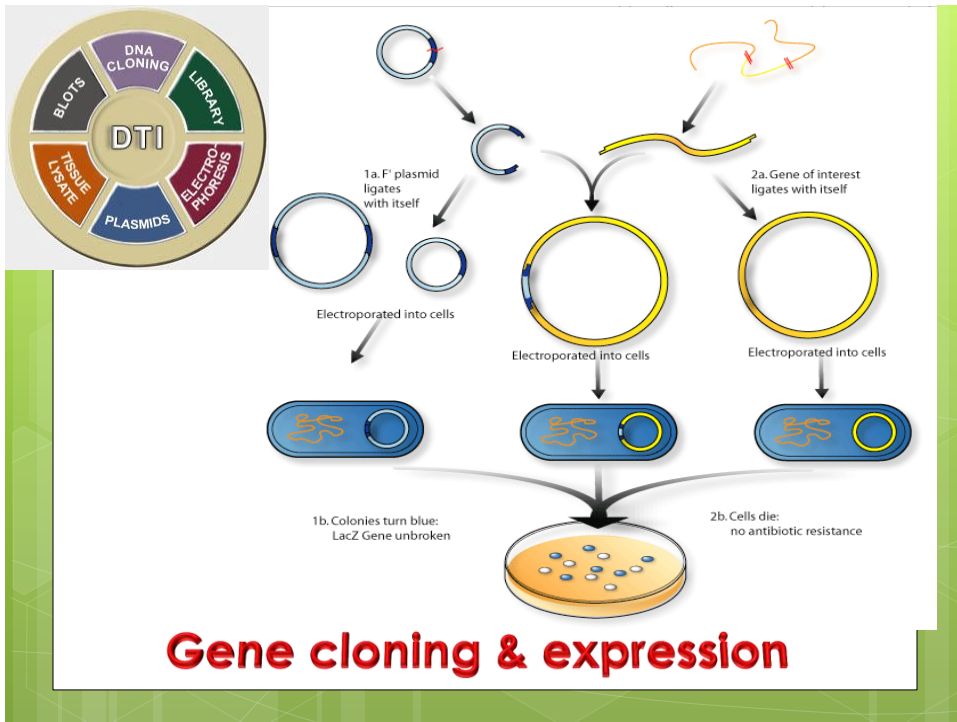
Ernst Boris Chain & Howard Florey
Nobel Prize 1945

THE WONDER DRUG

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Creating mutation

Random or specific

ACCATCGG**C**CTGCATCA
TGGTAGC**C**TGACGTAGTCAT
GGTAGCCTGACT----

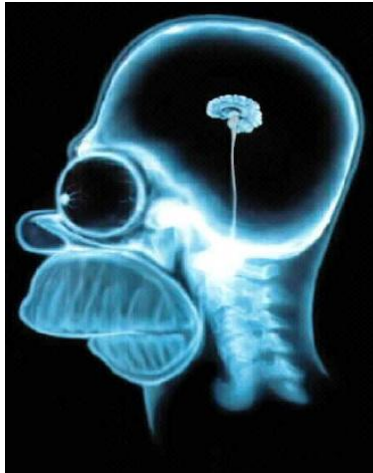
The collage includes a DNA double helix, a chick, a fly, and a beetle, representing different organisms that can be used to study mutations.



nitroPDF professional

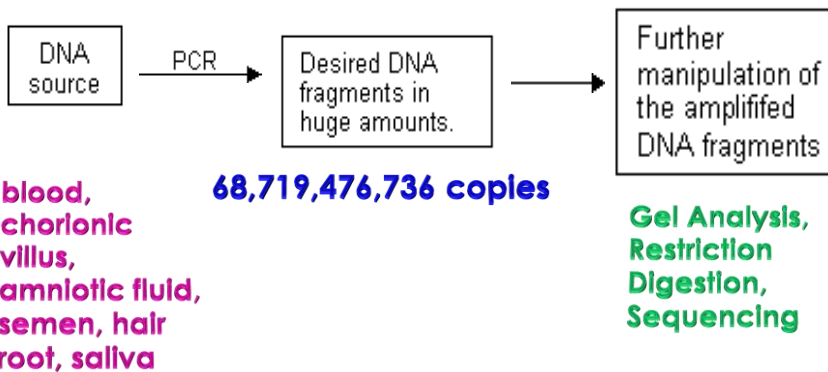
download the free trial online at nitropdf.com/professional

PCR is just a tool



How to use it,
is up to you

Summary



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Conclusion

THE SPEED & EASE OF USE, SENSITIVITY, SPECIFICITY & ROBUSTNESS OF PCR HAS REVOLUTIONISED MOLECULAR BIOLOGY & MADE PCR THE MOST WIDELY USED & POWERFUL TECHNIQUE WITH GREAT SPECTRUM OF RESEARCH & DIAGNOSTIC APPLICATIONS