

Module 7: PCR & ELISA

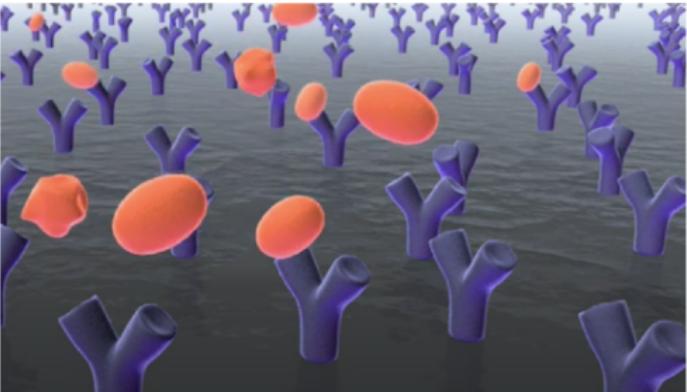
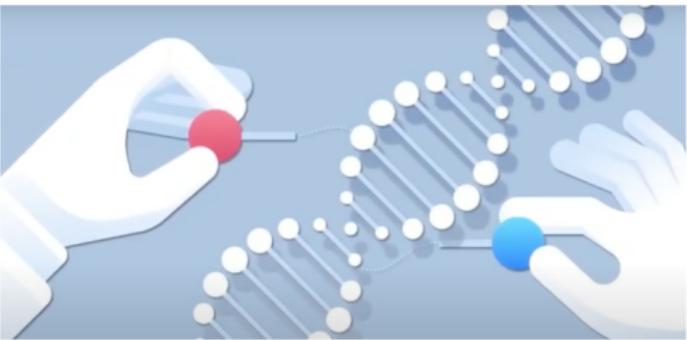
BMES Cell Team

Winter 2021



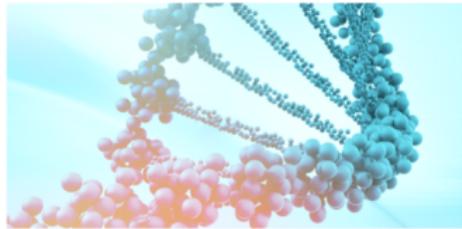
Outline

- PCR
 - Purpose
 - Procedure
 - Virtual Walkthrough
- ELISA
 - Purpose
 - Procedure
 - Virtual Walkthrough

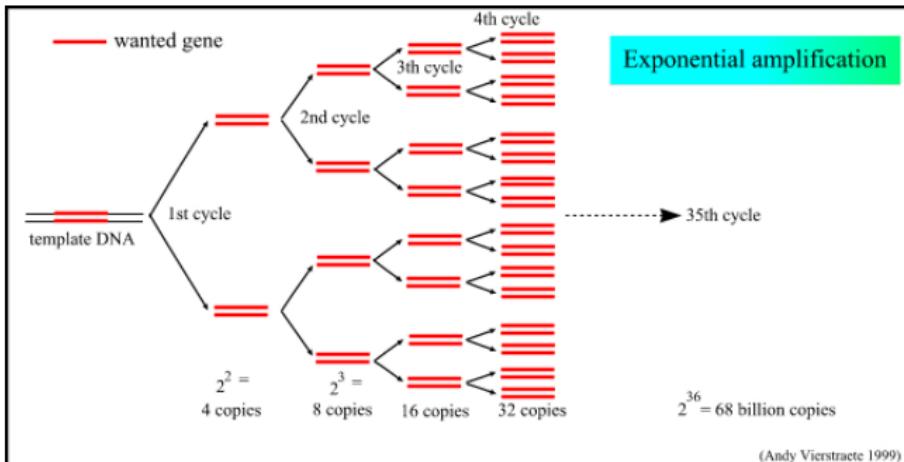


What is PCR?

- **Definition:** Polymerase Chain Reaction (**PCR**) is a method of copying small, targeted sequences of genetic material.
- Developed by Dr. Karry Mullis in 1983
 - Published 1985 article describing PCR for identifying hemoglobin mutation responsible for sickle-cell anemia
- Mullis awarded 1993 Nobel Prize in Chemistry for his invention of the PCR technique
- Roche bought the rights for PCR in 1991
 - Used PCR for molecular diagnostics



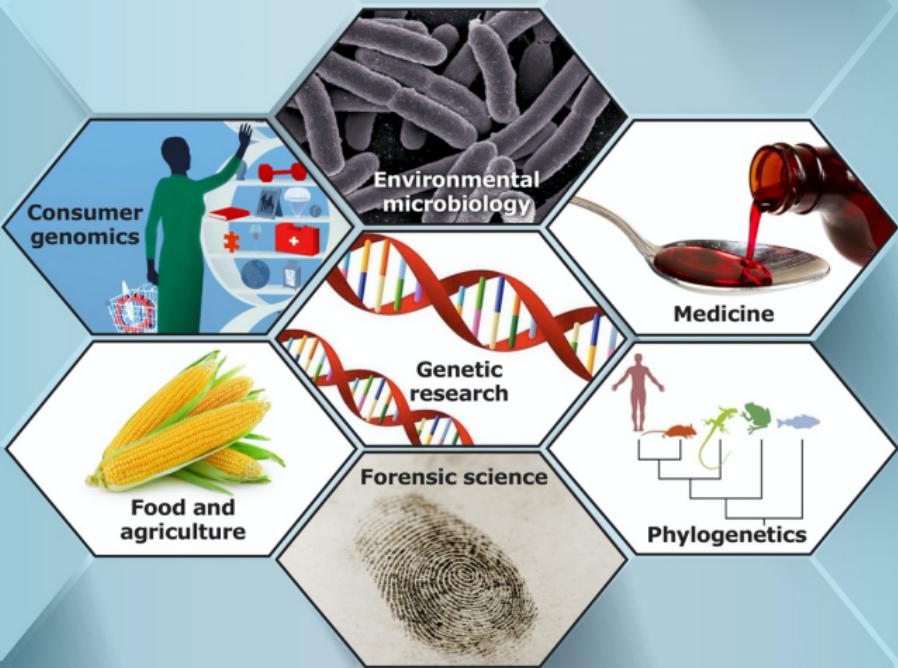
Why do we do PCR?



- DNA analysis only yields accurate data if the quantity of DNA is above the instrument's limit of detection
 - Requires amplification of harvested DNA sample
- Before PCR, this amplification process was indirect and laborious
 - PCR provided a faster way to get many more sample copies

Why do we do PCR?

WHAT IS PCR USED FOR?



Why do we do PCR?



Associated Companies

- EMLabs (mold)
- US Micro Solutions (fungus, bacteria)

- PCR is commonly used to detect the DNA of microorganisms in the environment
- Examples:
 - Coliforms in fresh water supplies
 - *Bacillus anthracis* spores in soil
 - Viruses in groundwater
 - MRSA in households of infected patients

Why do we do PCR?



Associated Companies

- SummerBio (UCLA COVID testing)
- Roche
- Fisher Sci

- PCR is commonly used to amplify DNA for medical diagnosis and disease study
- Examples:
 - Diagnosing SARS-CoV-2 infection
 - Diagnosing *N. gonorrhoeae* infection
 - Studying BRCA1 activity in breast cancer
 - Detection of Down's Syndrome

Why do we do PCR?



Associated Companies

- Centogene
- Novogene

- PCR is commonly used to amplify DNA for medical diagnosis and disease study
- Examples:
 - Testing for the Tay-Sachs gene
 - Examining changes in astronaut DNA

Why do we do PCR?



Associated Companies

- 23andMe
- Ancestry
- Examples:
 - At home genetic testing kits
 - Genetic mapping to find relatives

Why do we do PCR?



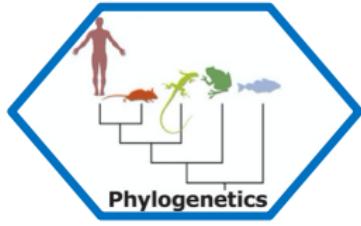
Food and agriculture

Associated Companies

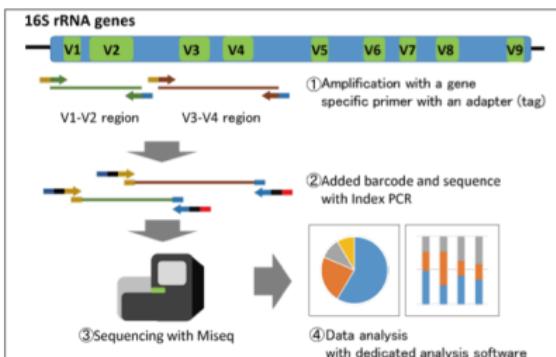
- Bio-Rad
- Thermo Fisher

- PCR is commonly used to test the quality of food products and amplify plant DNA for genetic modification
- Examples:
 - Detecting adulteration of meat
 - Identifying GMOs
 - Testing food samples for bacterial contamination

Why do we do PCR?



- PCR is commonly used to amplify DNA from ancient remains and determine phylogenetic relationships
- Examples:



- Understanding genetic relationships of different bacteria and plant species

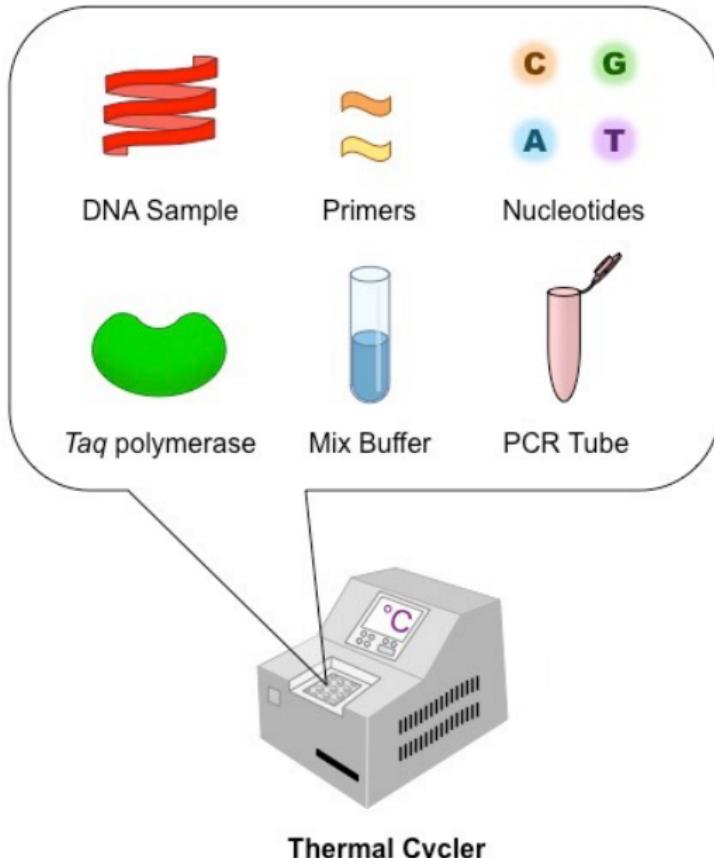
Why do we do PCR?



Companies You May Know

- CHANGE
- THESE
- TO
- SOMETHING
- LATER
- PLEASE
- ANYA

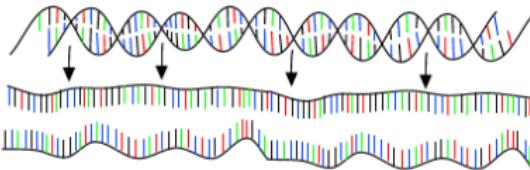
PCR Procedure: Required Components



PCR Procedure: The Three Main Steps

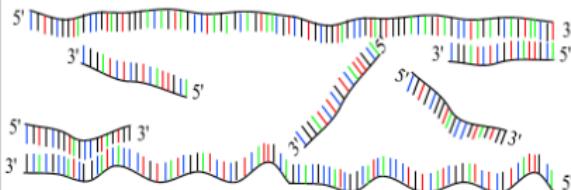
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

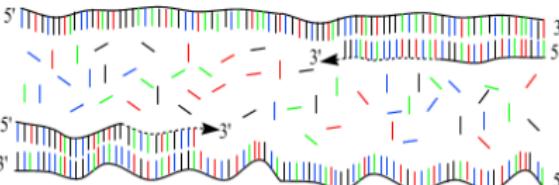
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse
primers !!!



Step 3 : extension

2 minutes 72 °C
only dNTP's

(Andy Vierstraete 1999)

PCR Procedure: Video



Oxford Walkthrough

<https://www.youtube.com/watch?v=jFl6HmcGw9Q>

Virtual Lab

<https://www.youtube.com/watch?app=desktop&v=G4sEhNKoPT8>

Benefits and Limitations of PCR

Benefits

- Exponential Amplification
- Fast
- Relatively Simple
- High Sensitive
- Companies manufacture ready-to-use PCR kits
- “Gold standard” in modern research

Limitations

- Contamination can manipulate results
- Conducted at 3 different temperatures
- Requires denaturation of DNA sample
- Requires prior known sequence to design primers

Alternatives to PCR

Recombinase Polymerase Amplification (RPA)

Mechanism

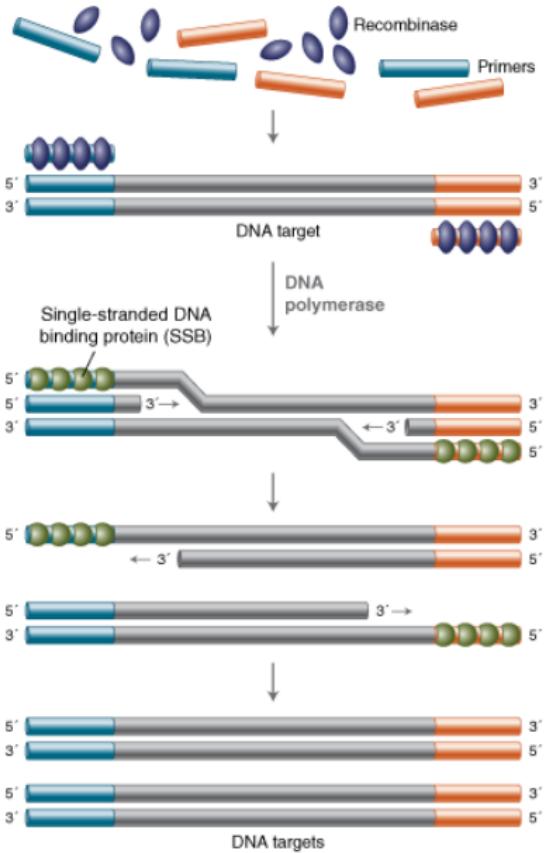
- Expansion from oligonucleotide primers

Benefits

- Isothermal
- Exponential Amplification
- Single Tube

Limitations

- Less established than PCR
- Kits not widely available



Alternatives to PCR

Fluorescence In Situ Hybridization (RNA-FISH)

Mechanism

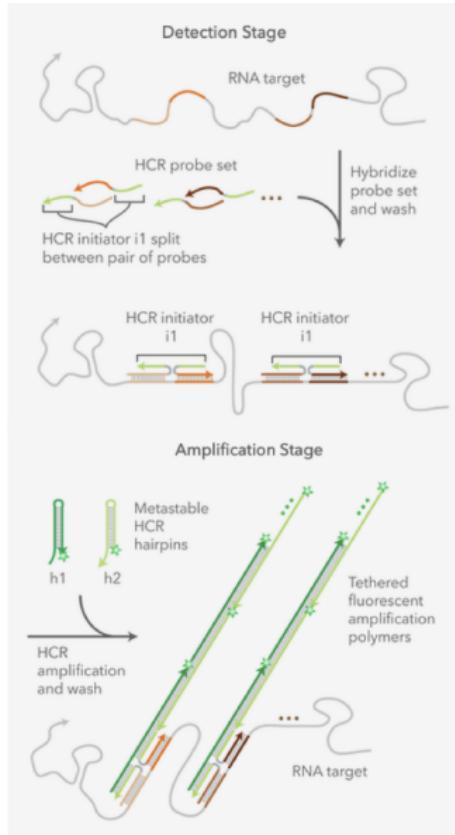
- Binding of DNA to a substrate → fluorescent DNA nanostructures

Benefits

- No enzymes
- Isothermal
- One step

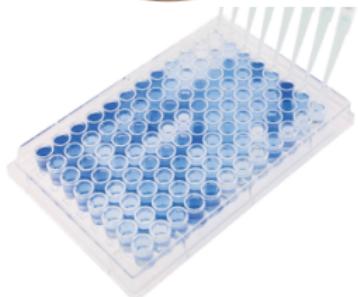
Limitations

- Linear Amplification → less sensitivity to analytes



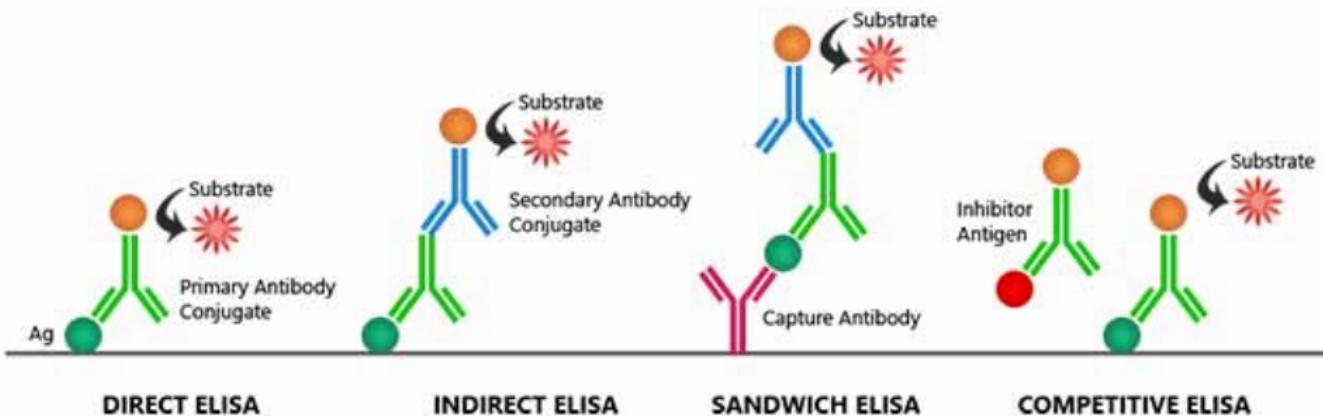
What is ELISA?

- **Definition:** Enzyme-linked immunosorbent assay (**ELISA**) is a method of ---.
- Developed by Eva Engvall and Peter Perlmann
 - Published their first paper in 1971
 - Engvall went on to apply ELISA to parasitology, microbiology, and oncology

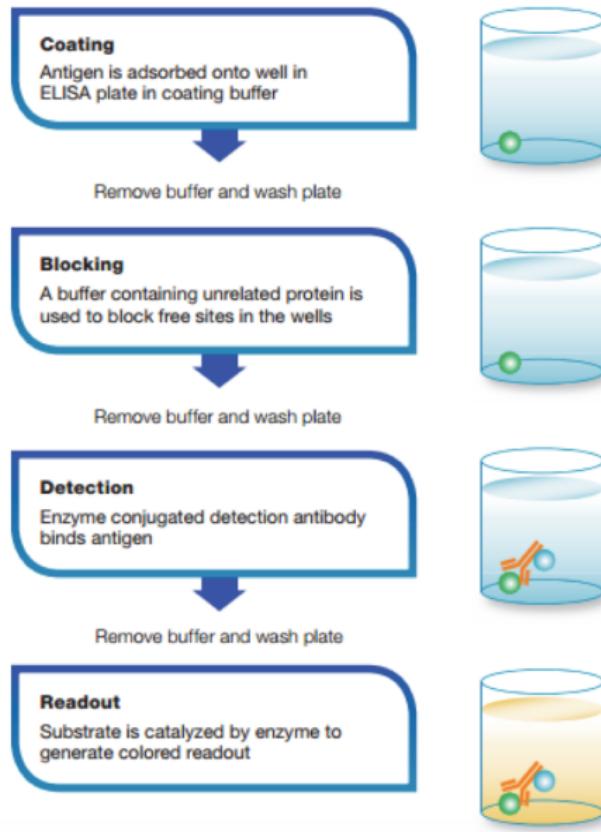


Why do we do ELISA?

ELISA Procedure: Required Components



ELISA Procedure: The Four Main Steps



ELISA Procedure: Video



Edvotek Walkthrough

https://www.youtube.com/watch?v=zR_xlV5v_f4&t=166s

Virtual Lab

<https://www.youtube.com/watch?v=pmdoA8Xiviw&t=45s>