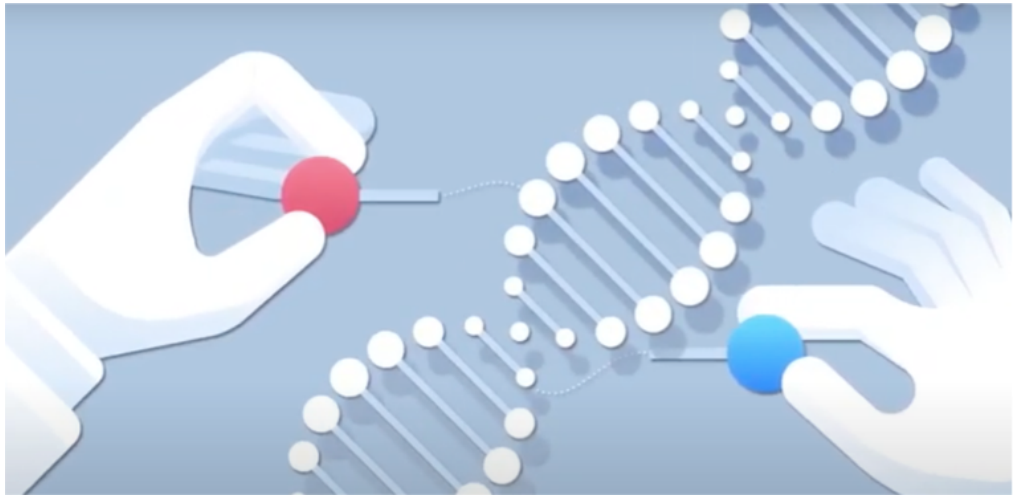
Module 7: PCR & ELISA

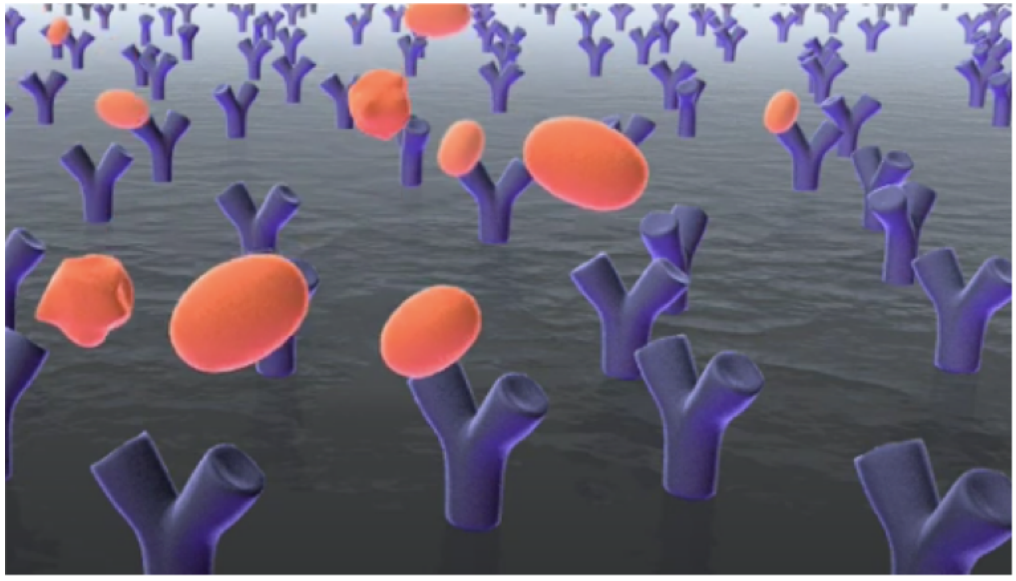
BMES Cell Team

Winter 2021

Outline

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





https://www.youtube.com/watch?app=desktop&v=G4sFhNKoPT8 https://www.youtube.com/watch?v=6Ue1Hd3dvaQ

PCR

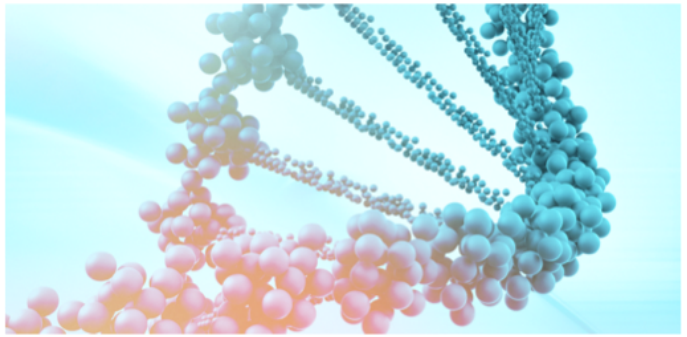
BMES Cell Team

Winter 2021

What is PCR?

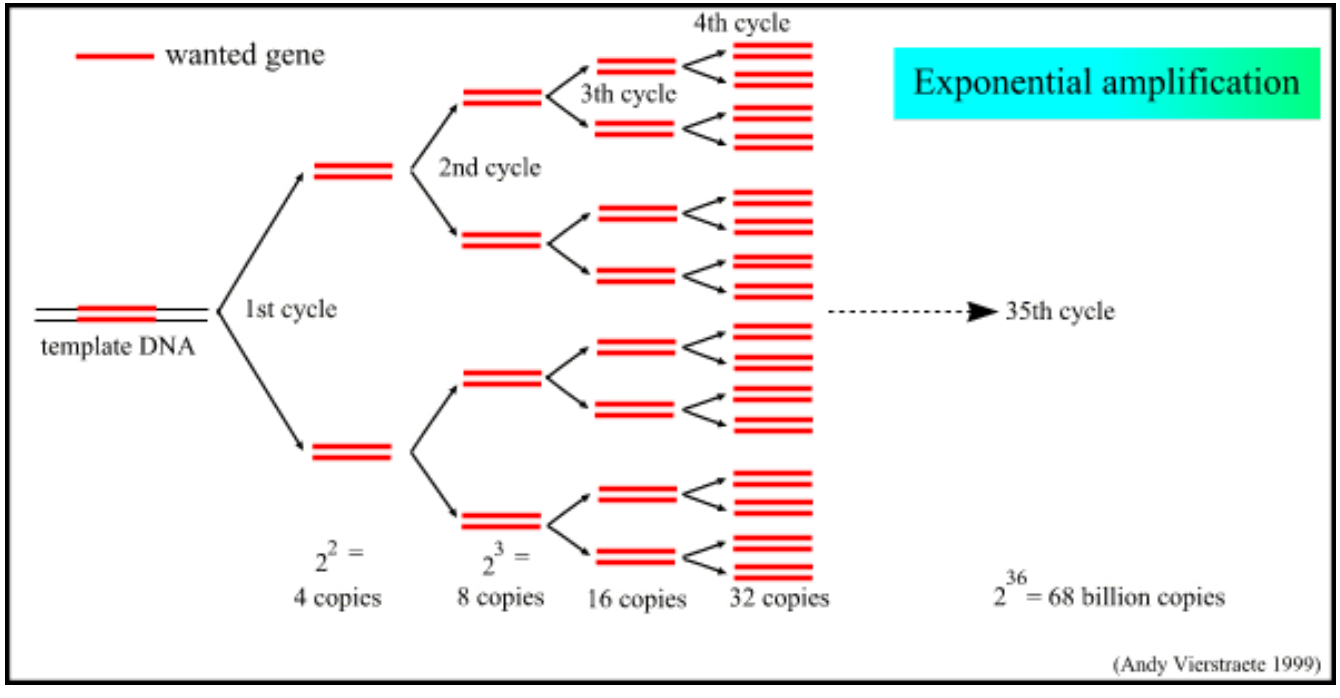
- **Definition:** Polymerase Chain Reaction (PCR) is a method of copying small, targeted sequences of genetic material.

- 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



https://diagnostics.roche.com/global/en/article-listing/advances-in-pcr-roche-innovations.html https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet

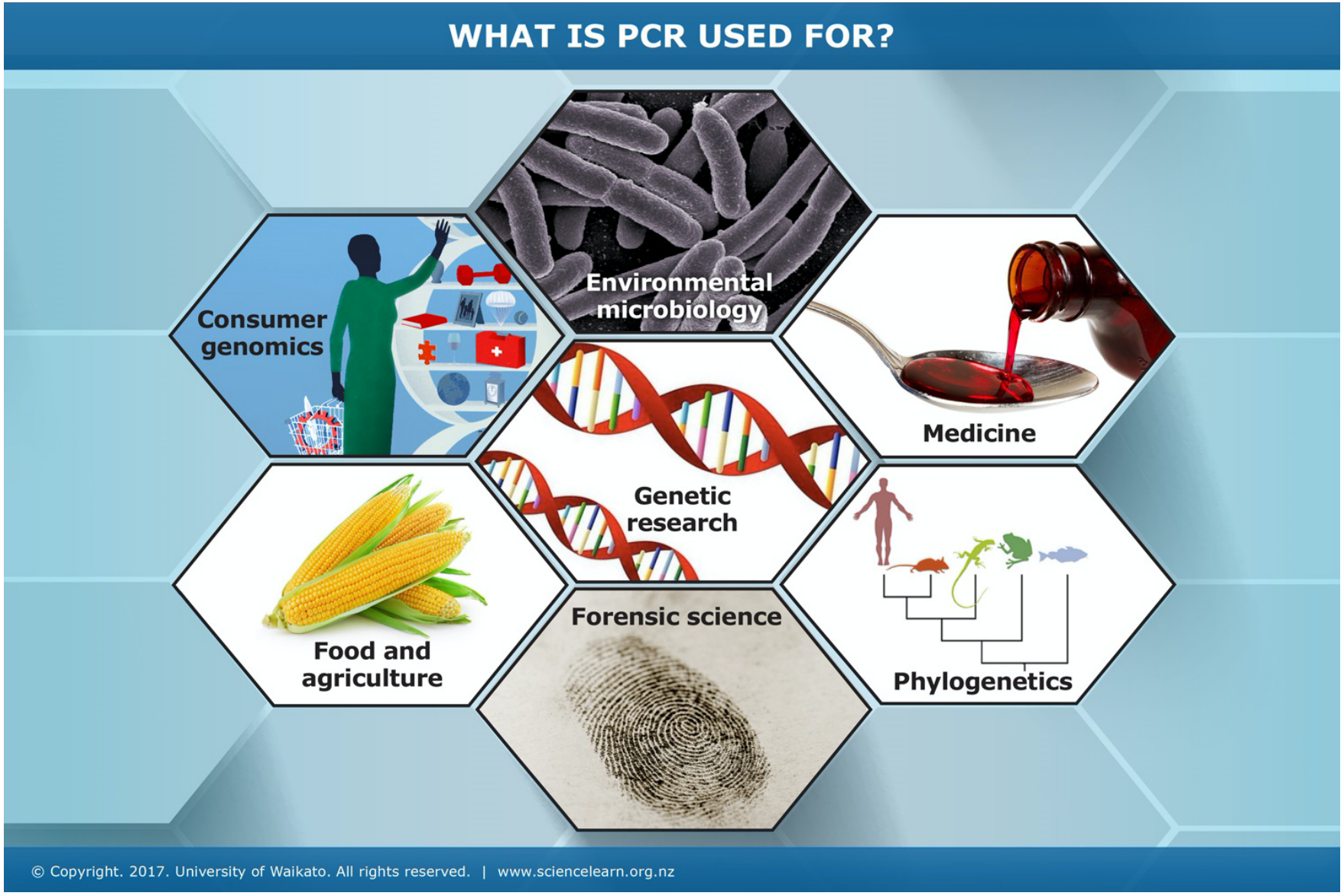
Why do we do PCR?



- 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

https://www.nature.com/scitable/topicpage/the-biotechnology-revolution-pcr-and-the-use-553/

Why do we do PCR?



https://www.sciencelearn.org.nz/image_maps/35-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 microorganisms in the environment

- Examples:
  - Coliforms in fresh water supplies
  - *Bacillus anthracis* spores in soil
  - Viruses in groundwater
  - MRSA in households of infected patients

https://jamanetwork.com/searchresults?author=Lauren+N.+Singh&q=Lauren+N.+Singhhttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC91045/ https://academic.oup.com/femsec/article/23/2/159/481990 https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

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. gonorrheae* infection
  - Studying BRCA1 activity in breast cancer
  - Detection of Down's Syndrome

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4391122/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3768498/ https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

Why do we do PCR?

- 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

Associated Companies

- Centogene
- Novogene

https://www.genesinspace.org/news/blog/using-pcr-your-genes-space-proposal/ https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

Why do we do PCR?

Associated Companies

- 23andMe
- Ancestry

- PCR is commonly used to amplify DNA for consumers interested in their own genetics

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

https://www.genesinspace.org/news/blog/using-pcr-your-genes-space-proposal/ https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

Why do we do PCR?

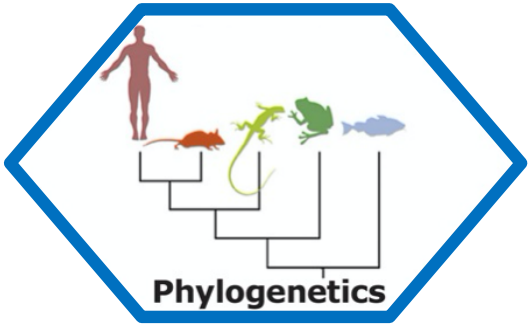
Associated Companies

- Bio-Rad
- Thermo Fisher

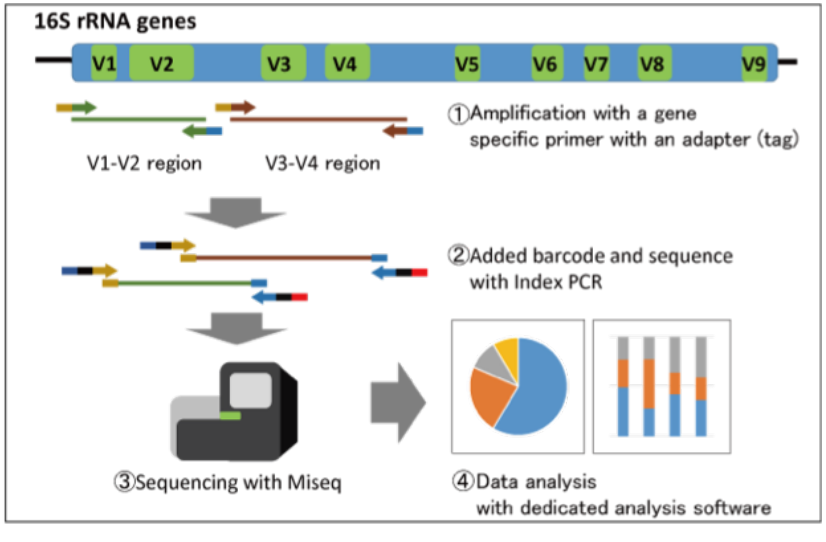
- PCR is commonly used to test the quality of food products and amplify plant DNA for genetic modification
- Examples:
    - Detecting adulteration of meat
    - Identifying GMOs
    - Testing food samples for bacterial contamination

https://www.btci.org/k-12-programs/biotechnology-field-trips/pcr-to-detect-genetically-modified-organisms/
https://www.tandfonline.com/doi/full/10.1080/10942912.2017.1369103

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

https://academic.oup.com/femsle/article/153/1/191/485464

Why do we do PCR?

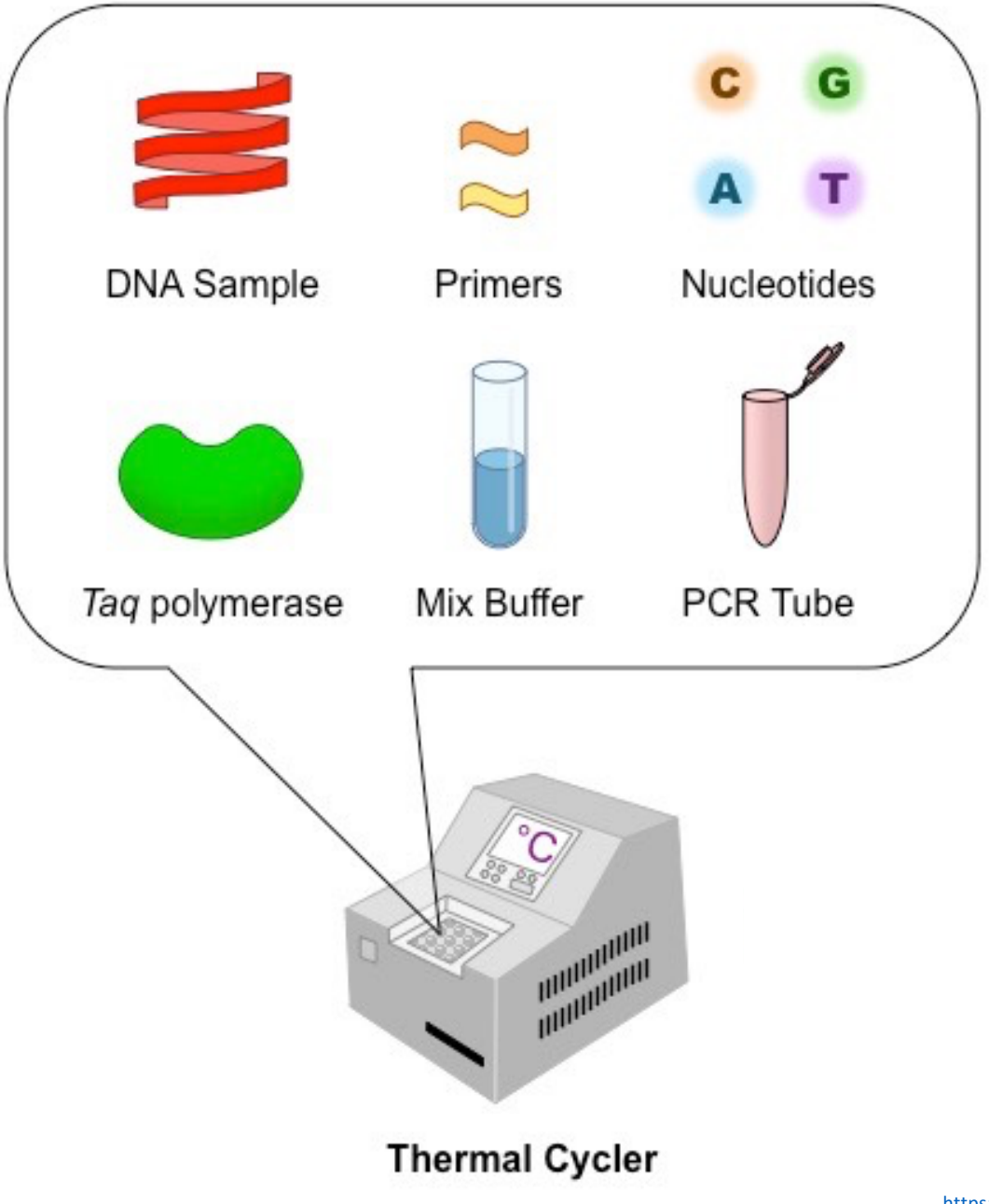
Associated Companies

- GEDMatch
- miniPCR
- QIAGEN

- PCR is commonly used to amplify DNA from crime scenes and identify perpetrators
- Examples:
    - Genetic evidence used to identify the Golden State Killer

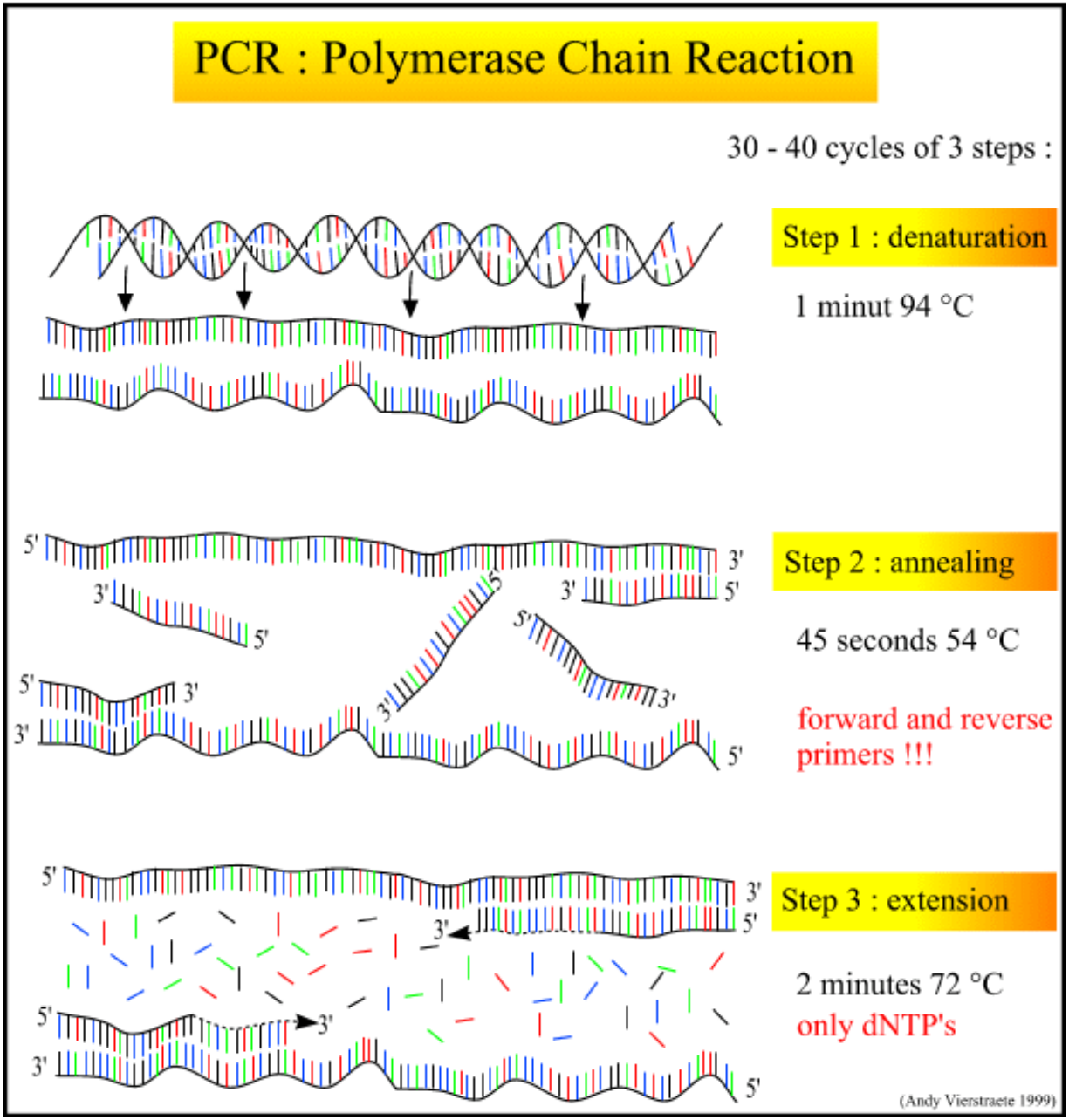
https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

PCR Procedure: Required Components



https://www.goldbio.com/goldbios-pcr-overview#PCR

PCR Procedure: The Three Main Steps



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PCR Procedure: Video

Oxford Walkthrough

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

Virtual Lab

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

https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

Benefits and Limitations of PCR

Benefits

- Exponential Amplification
- Fast
- Relatively Simple
- Highly 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

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4102308/

Alternatives to PCR

Recombinase Polymerase Amplification (RPA)

Mechanism

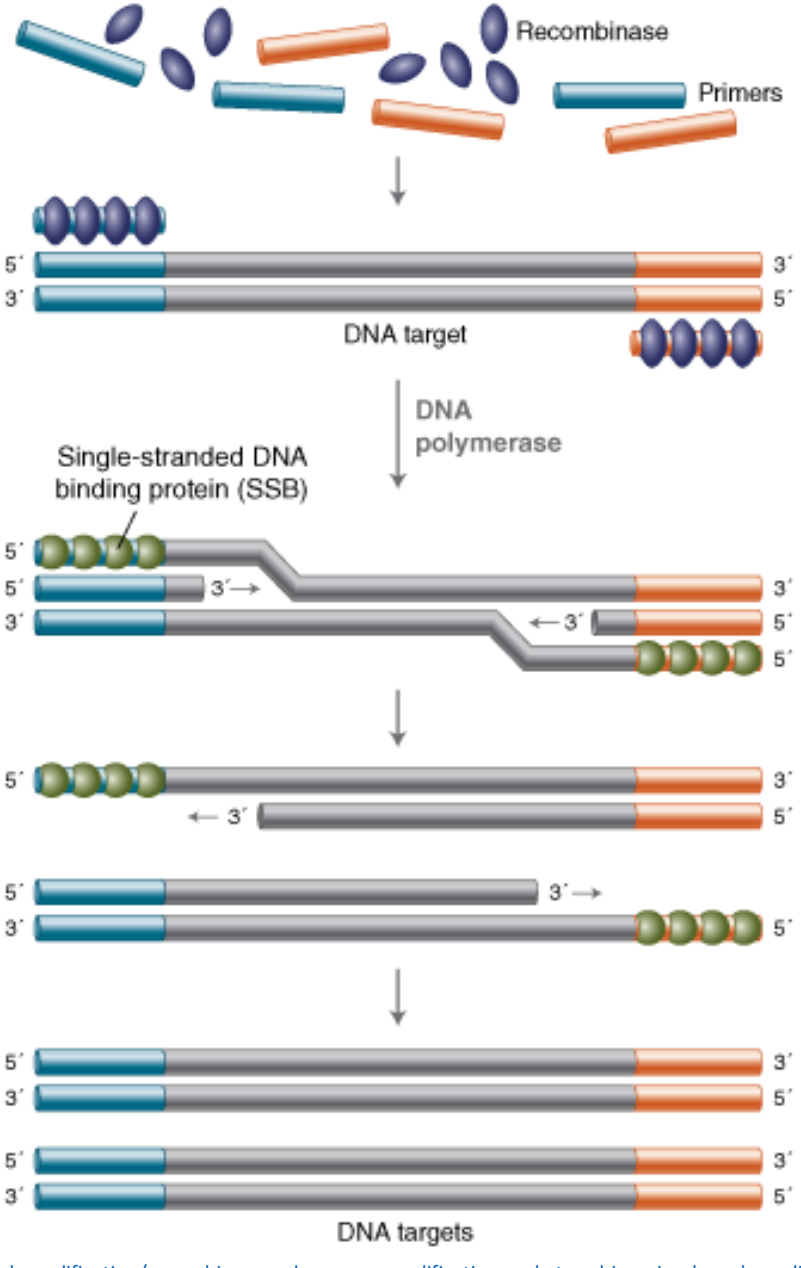
- Expansion from oligonucleotide primers

Benefits

- Isothermal
- Exponential Amplification
- Single Tube

Limitations

- Less established than PCR
- Kits not widely available



https://www.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/recombinase-polymerase-amplification-and-strand-invasion-based-amplification

Alternatives to PCR

Fluorescence In Situ Hybridization (RNA-FISH)

Mechanism

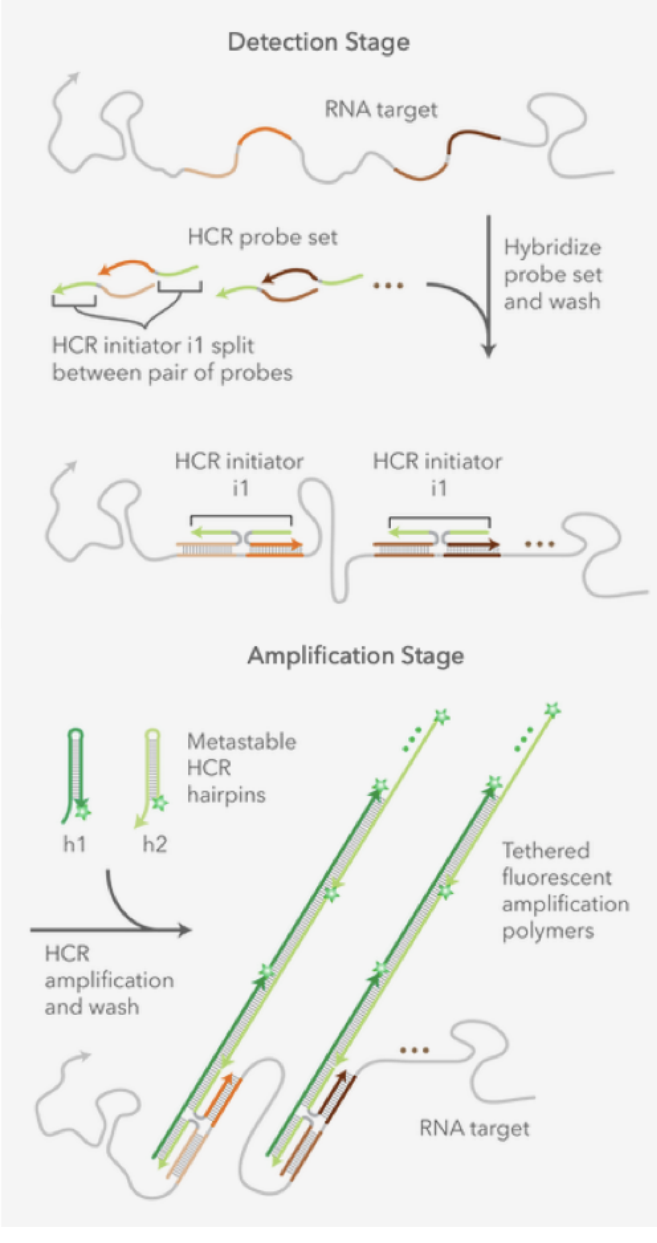
- Binding of DNA to a substrate → fluorescent DNA nanostructures

Benefits

- No enzymes
- Isothermal
- One step

Limitations

- Linear Amplification → less sensitivity to analytes



https://www.nature.com/articles/nmeth1204-186a#Fig1 https://www.molecularinstruments.com/protocols

ELISA

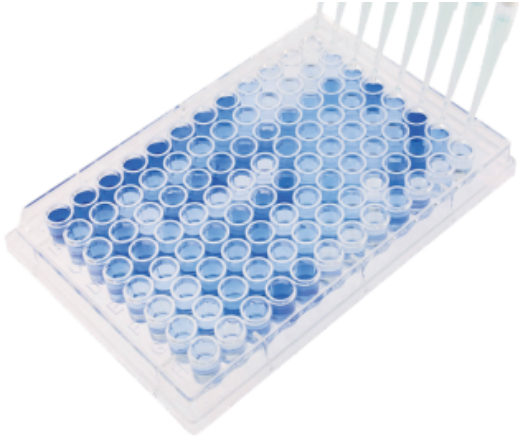
BMES Cell Team

Winter 2021

What is ELISA?

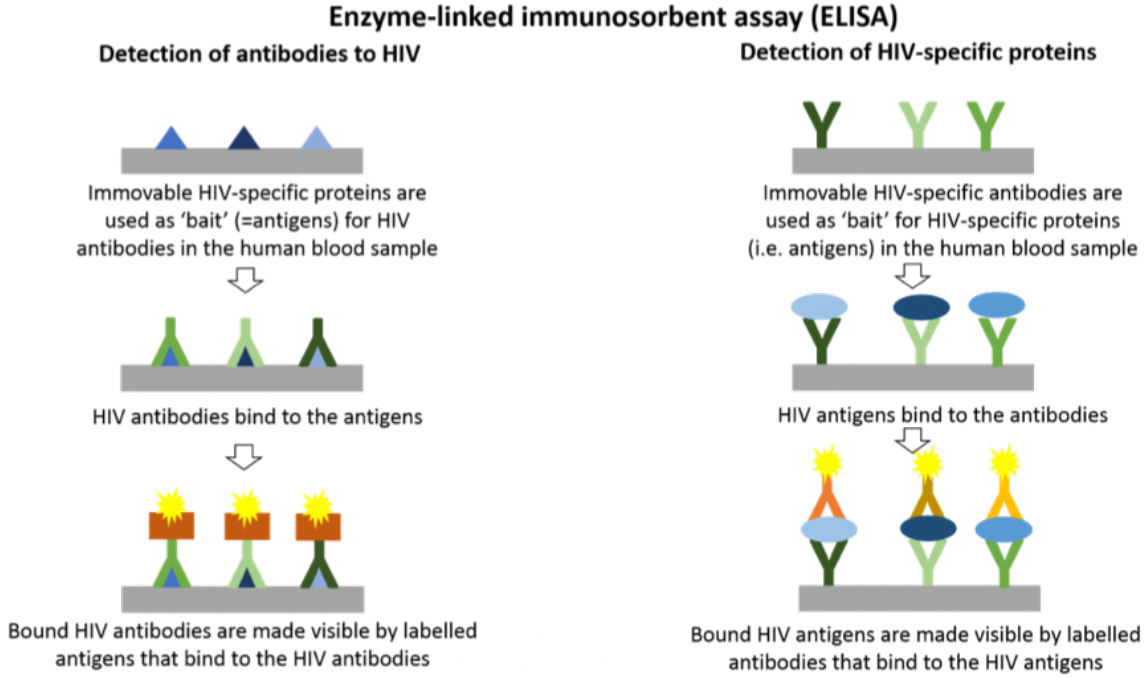
- **Definition:** Enzyme-linked immunosorbent assay (ELISA) is a method of detecting specific antibodies and antigens.

- Developed by Eva Engvall and Peter Perlmann
  - Published their first paper in 1971
  - Engvall went on to apply ELISA to parasitology, microbiology, and oncology
- Currently used to test for antibodies, food allergens, and disease antigens



https://diagnostics.roche.com/global/en/article-listing/advances-in-pcr-roche-innovations.html, https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet

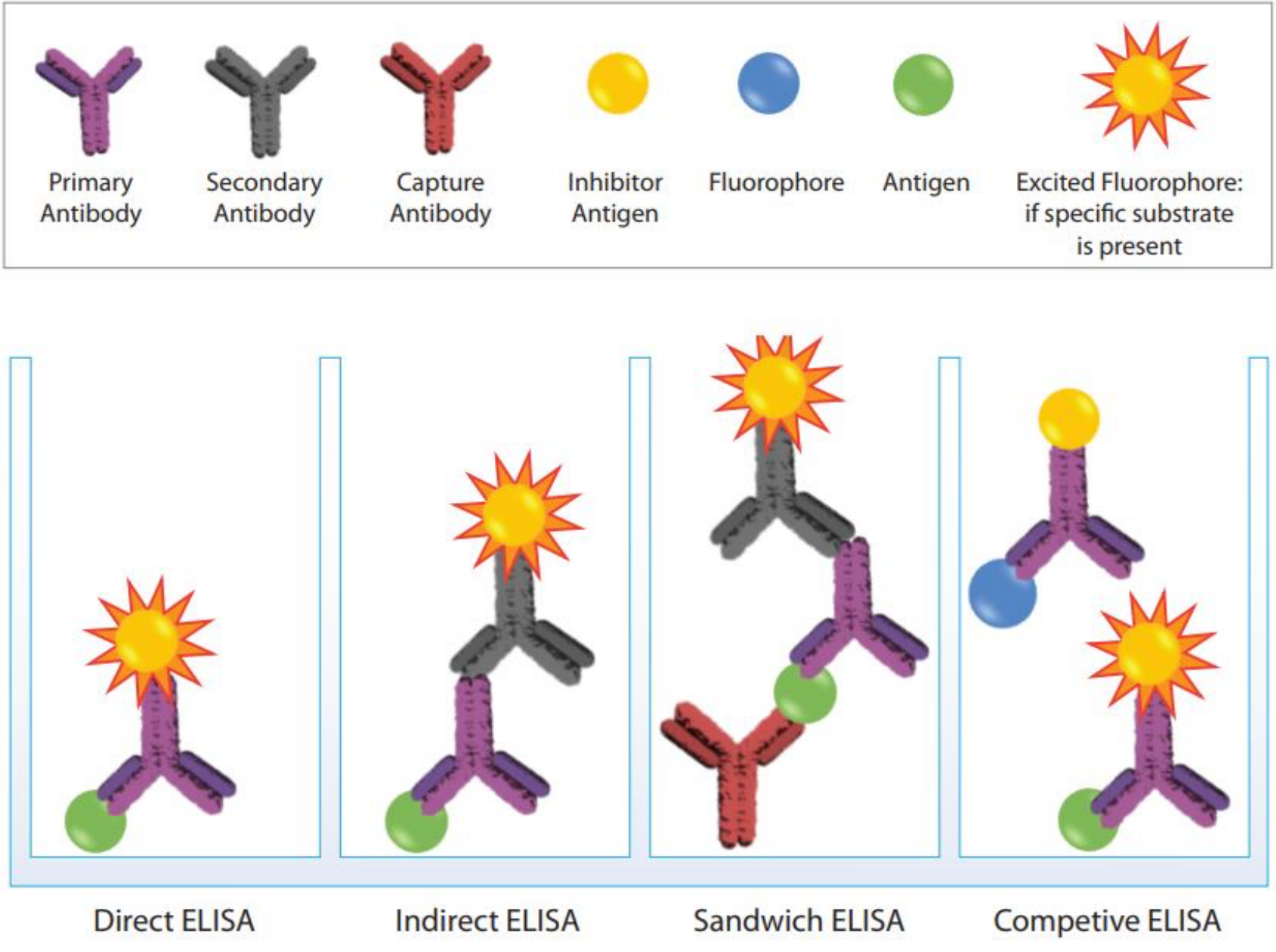
Why do we do ELISA?



- The ELISA system can be utilized to detect many different types of proteins in biological samples
- ELISA is key to detecting:
    - Pregnancy
    - HIV infection

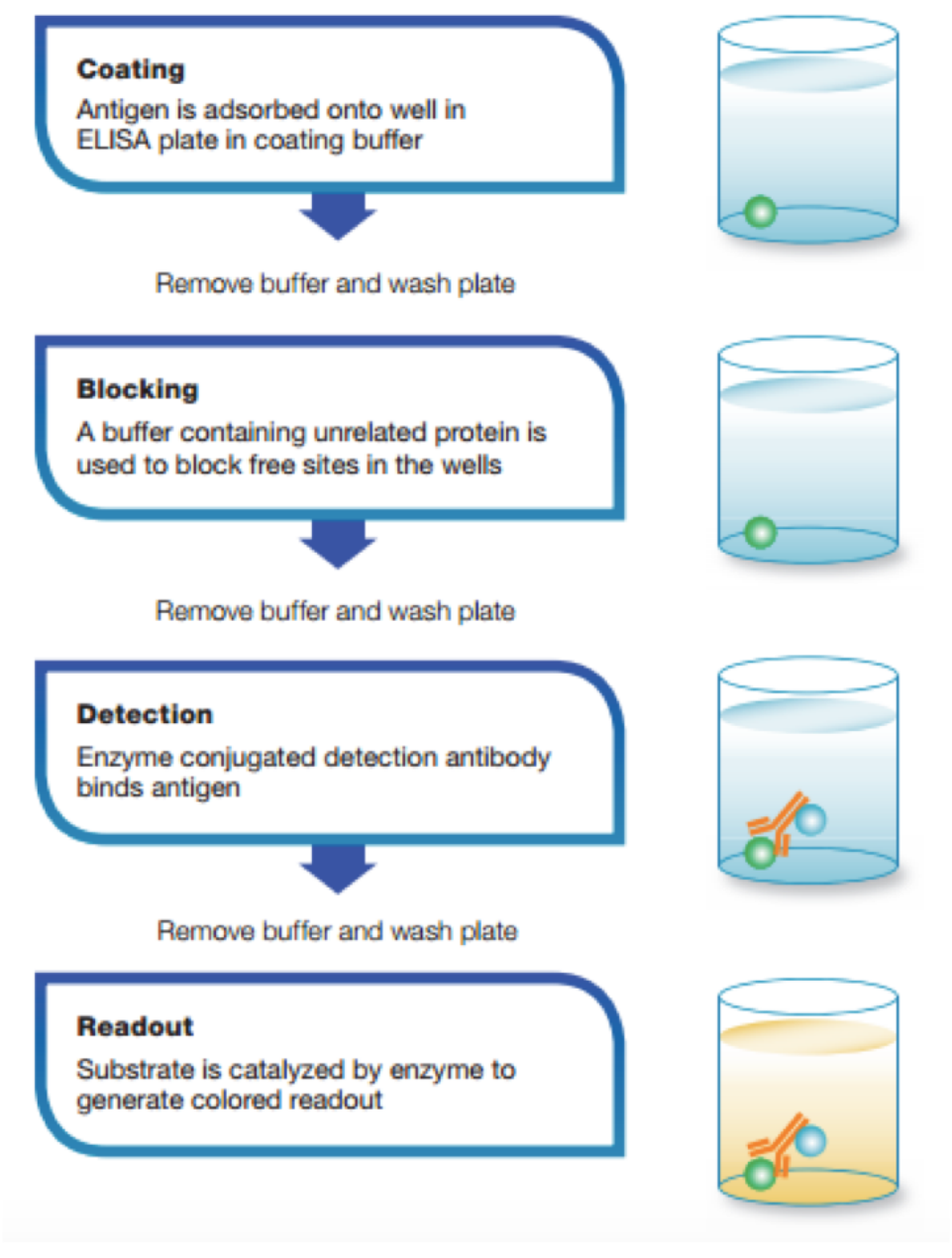
https://diagnostics.roche.com/global/en/article-listing/advances-in-pcr-roche-innovations.html, https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet

ELISA Procedure: Required Components



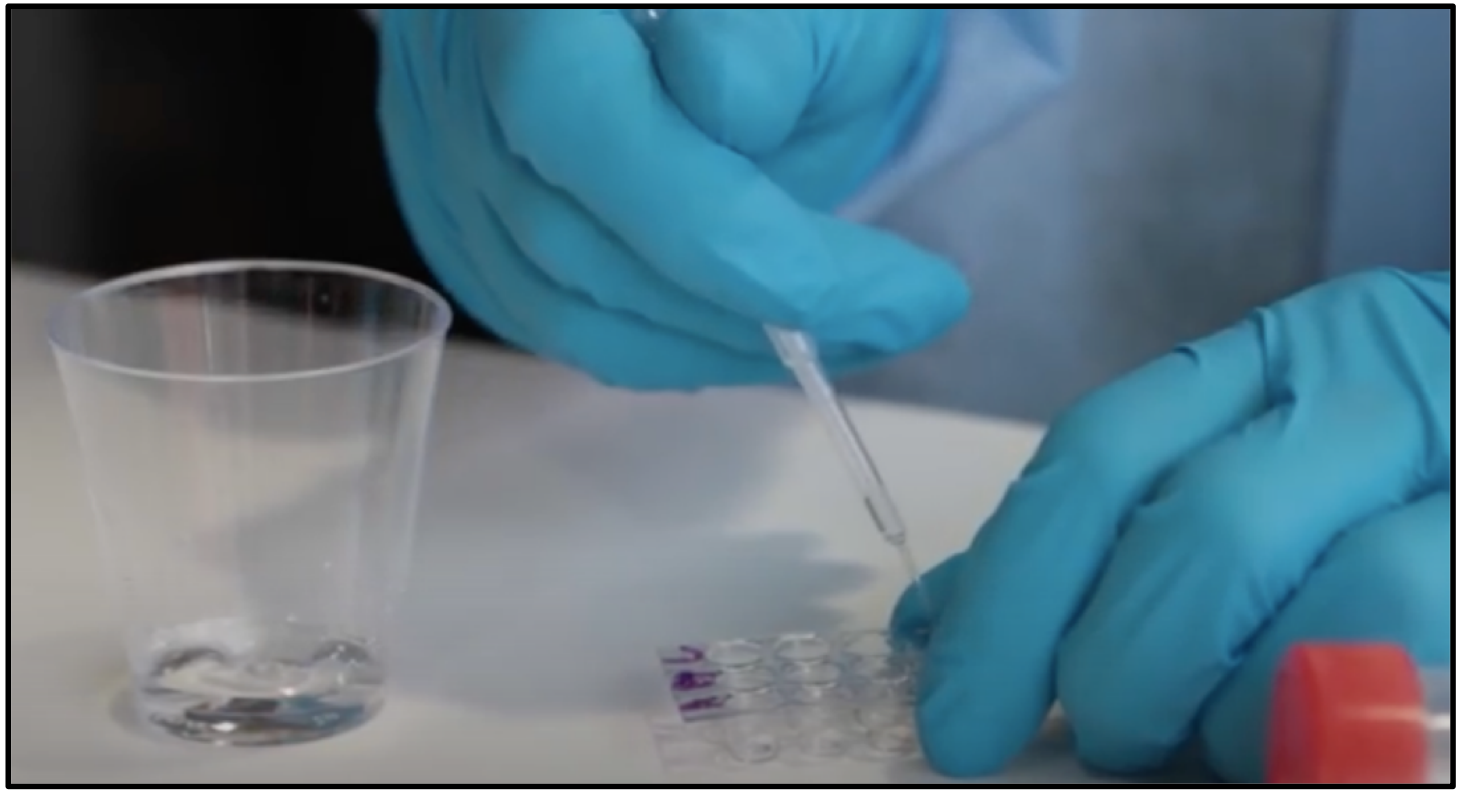
https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

ELISA Procedure: The Four Main Steps



https://www.bio-rad-antibodies.com/elisa-procedure.html?JSESSIONID_STERLING=DD702E1D5C1BA0C72B19169A64741892.ecommerce1&evCntryLang=US-en&cntry=US&thirdPartyCookieEnabled=true

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

https://www.sciencelearn.org.nz/image_maps/35-what-is-pcr-used-for

Benefits and Limitations of ELISA

Benefits

- Selective
- Relatively Simple
- Highly Sensitive
- Safe
- Cheap
- Companies manufacture ready-to-use ELISA kits

Limitations

- Antibodies require refrigerated transport and storage
- Will yield false results if there is not sufficient blocking
- High probability of a false positive or false negative

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5775980/

Alternatives to ELISA

Radioimmunoassay (RIA)

Mechanism

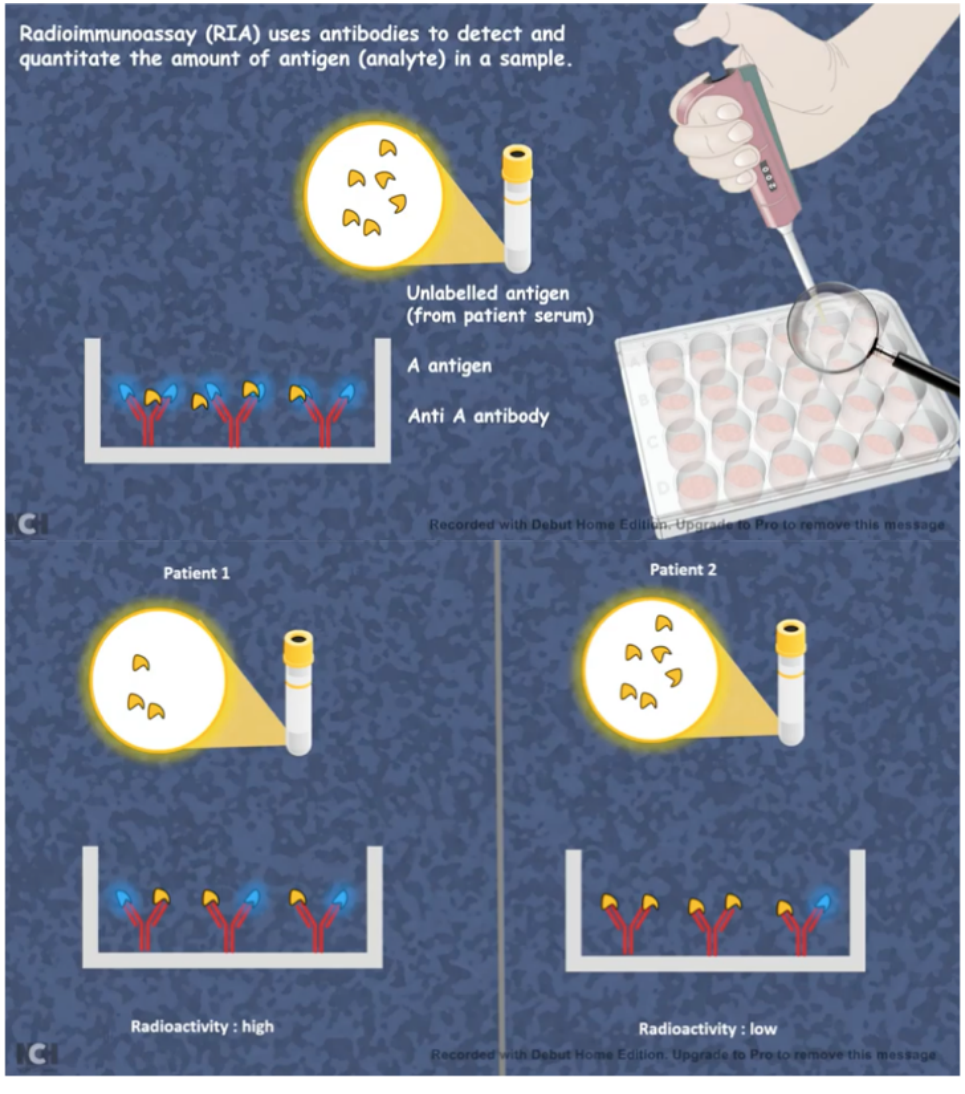
- Radioactive and non-radioactive antigens compete for antibody binding sites

Benefits

- Highly Sensitive
- Quantitative Measurement

Limitations

- Uses Radioactive Materials (Hazardous)



https://www.youtube.com/watch?v=JtbcqkiFf7w

Alternatives to ELISA

Multiplex Assay (Luminex Beads)

Mechanism

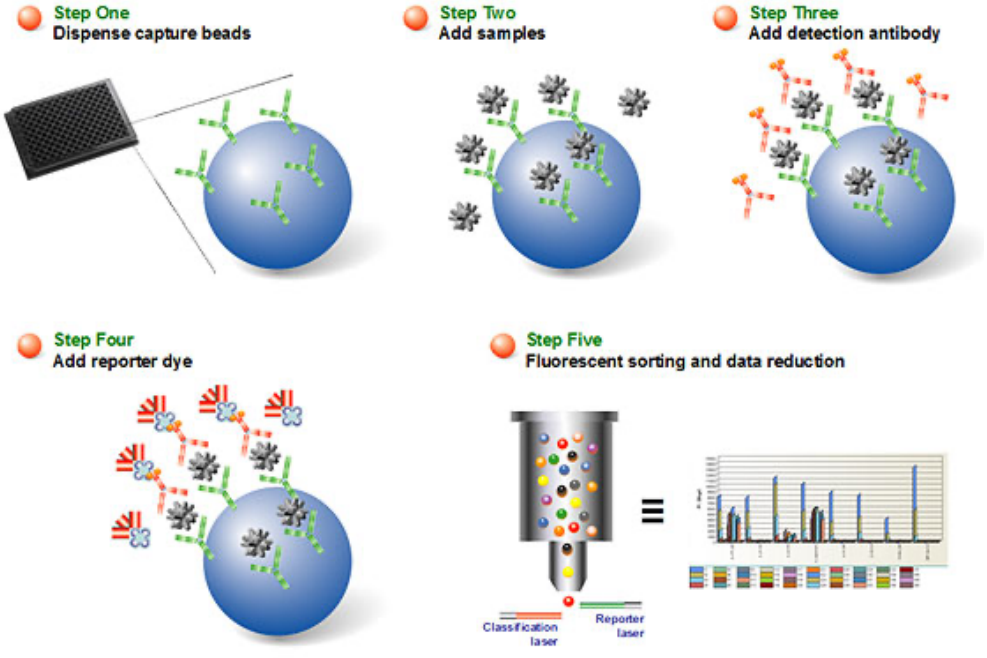
- Immunoassay that uses beads to bind the antibody

Benefits

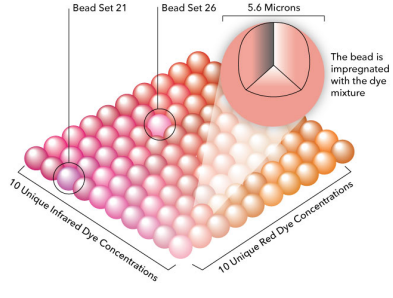
- Multiple analytes at the same time
- Measure analytes concentration of different orders of magnitude

Limitations

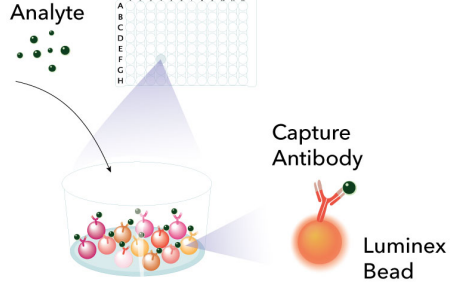
- Less established than PCR



Luminex Bead Spectrum



Luminex Assay Principle



https://blog.gyrosproteintechnologies.com/spinblog/multiplex-assays-advantages-disadvantages