Cleveland Clinic

Molecular Testing in Transfusion Medicine

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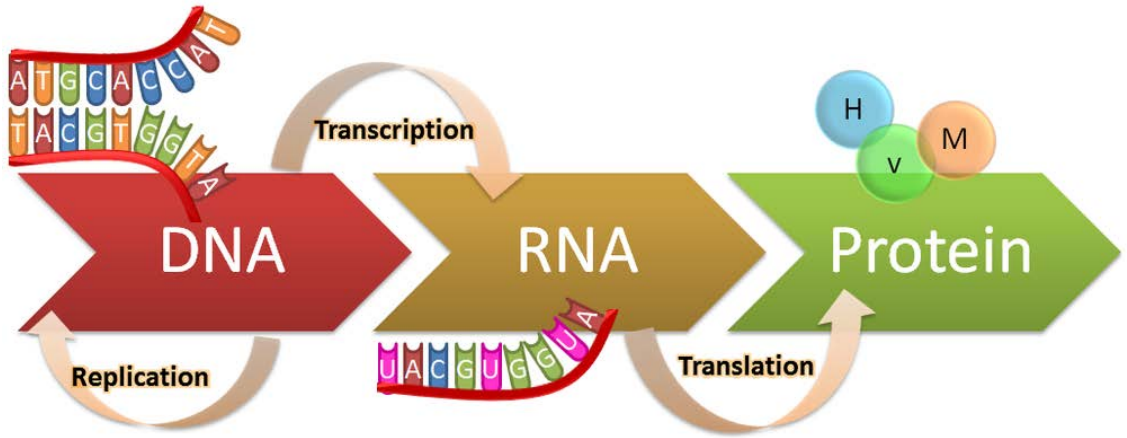
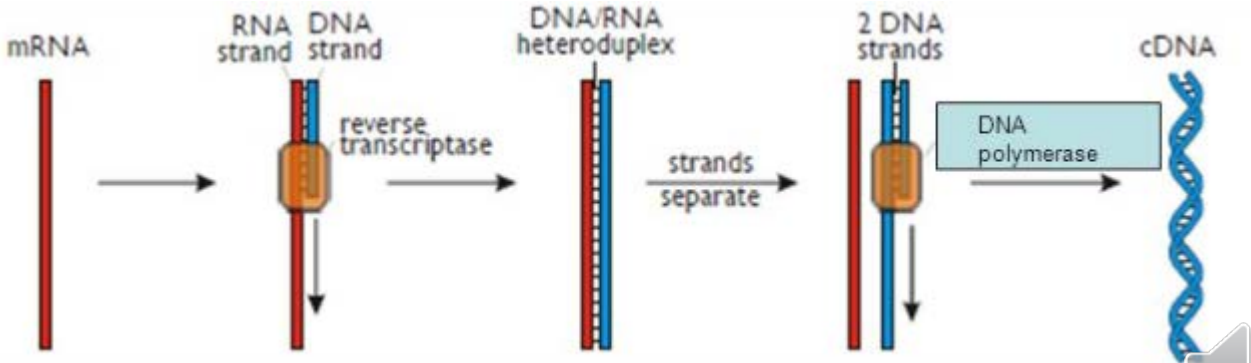
Use of Molecular Biology in Transfusion Medicine

- Molecular genetics
- Biotechnology
- Molecular diagnostics

Main concepts in Molecular Biology

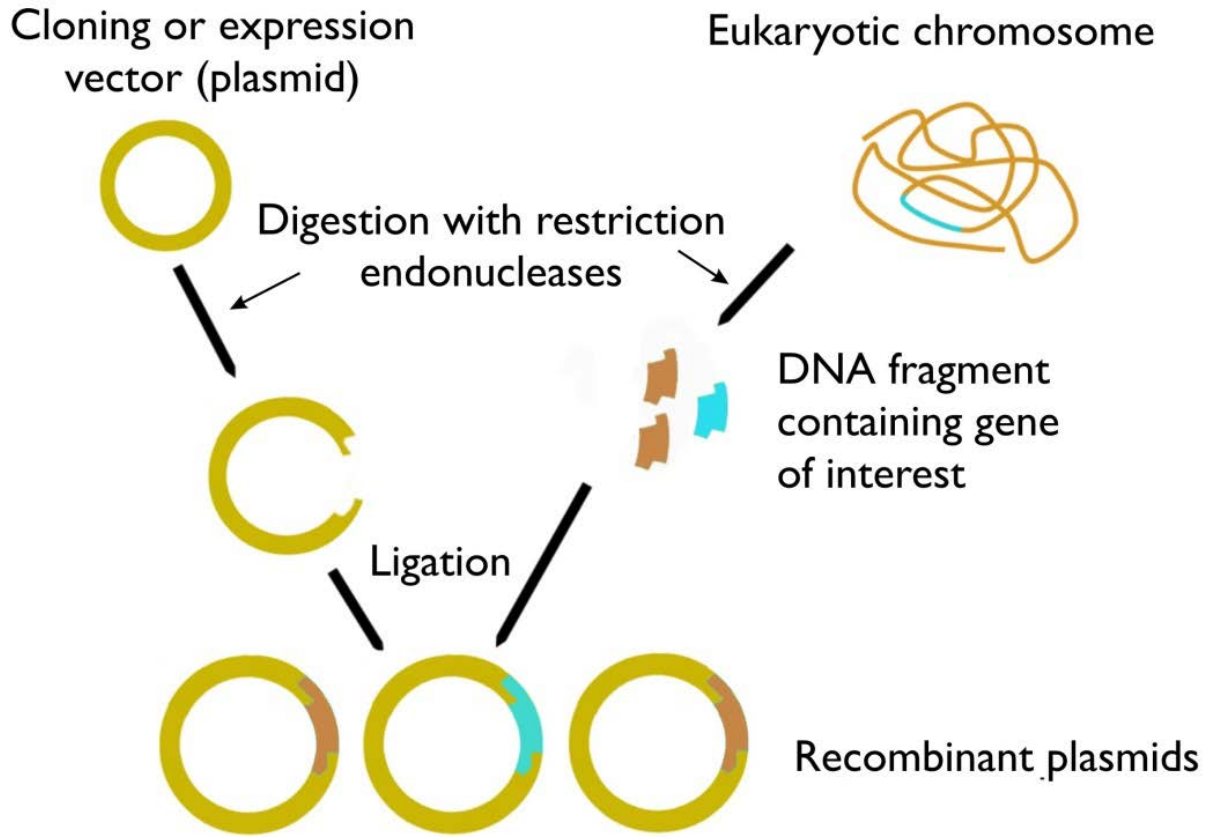
- Genetic material is DNA which is self-replicating
- DNA is transcribed to mRNA - serves as template for the synthesis of proteins
- Reverse transcription – Synthesis of DNA from RNA





Recombinant DNA

- DNA from one organism injected into a carrier DNA molecule or vector (plasmid, bacteriophage) which can then be introduced into another, simpler, host organism
- Used to quickly produce clones

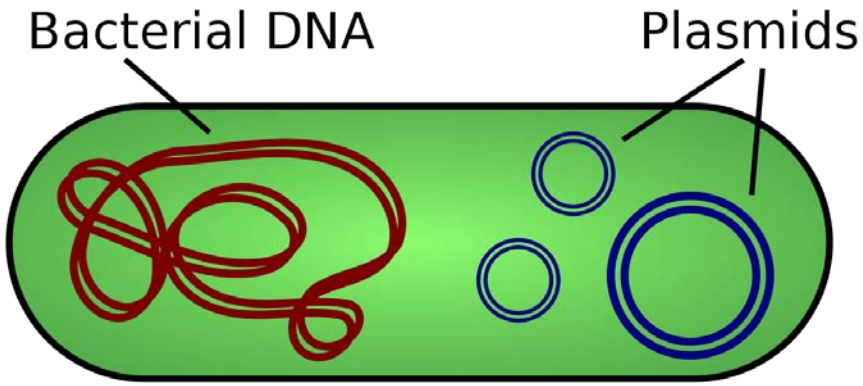


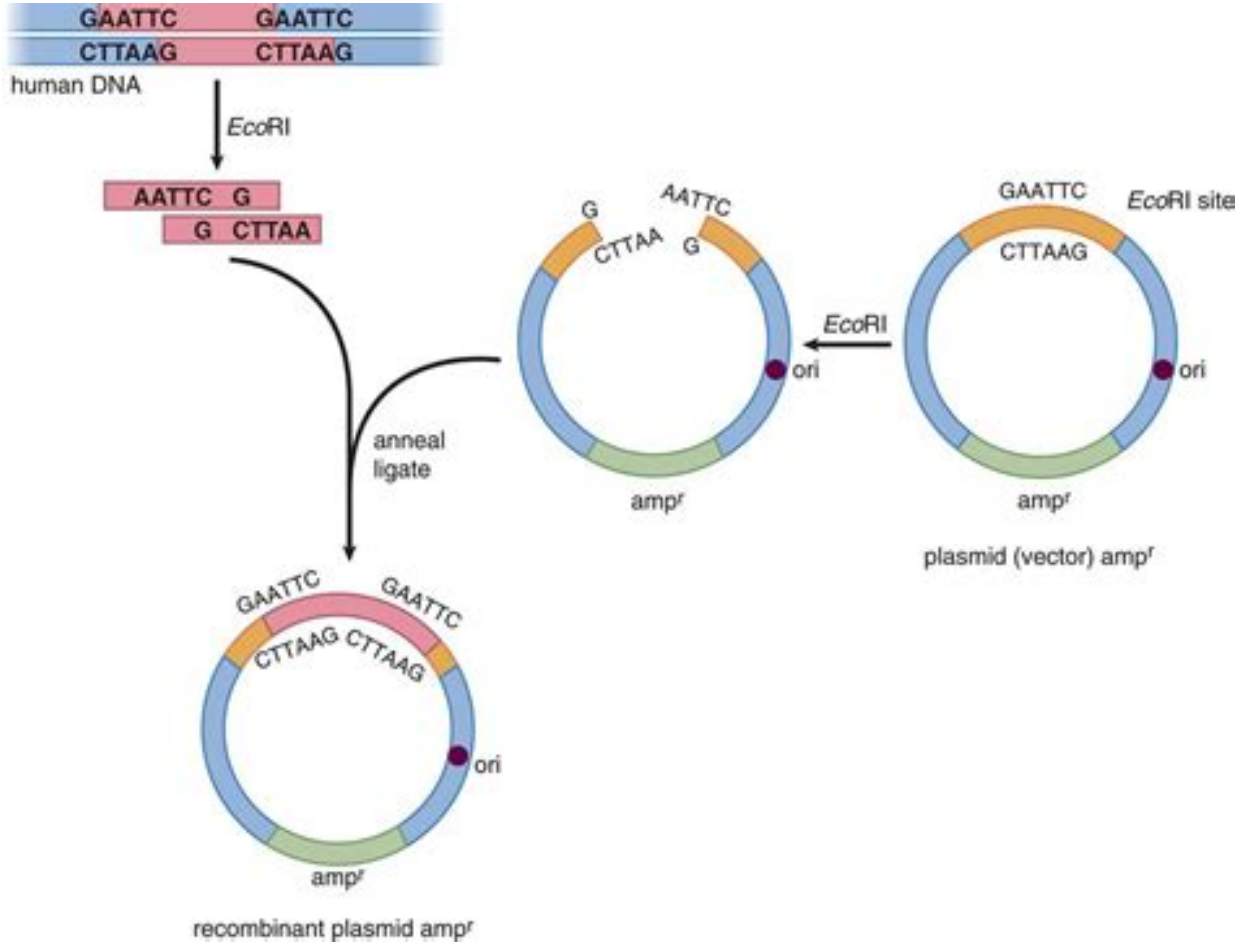
Tools for Molecular Cloning

- Vectors
- Gel electrophoresis
- Restriction endonucleases

Vectors

- DNA molecule of known nucleotide sequence that is used to carry a foreign DNA fragment into a host organism
- Plasmids used as main vector

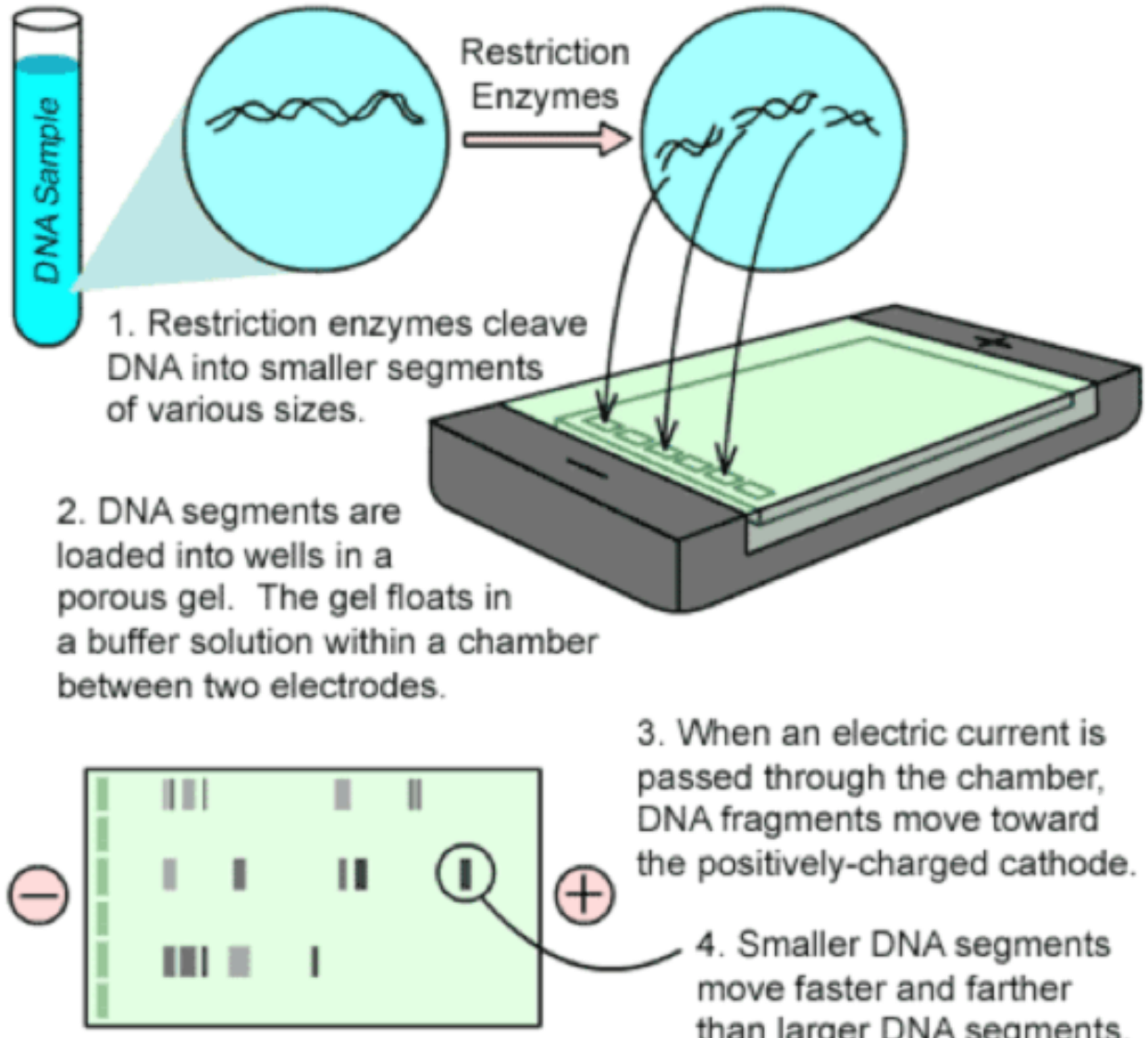




Gel Electrophoresis

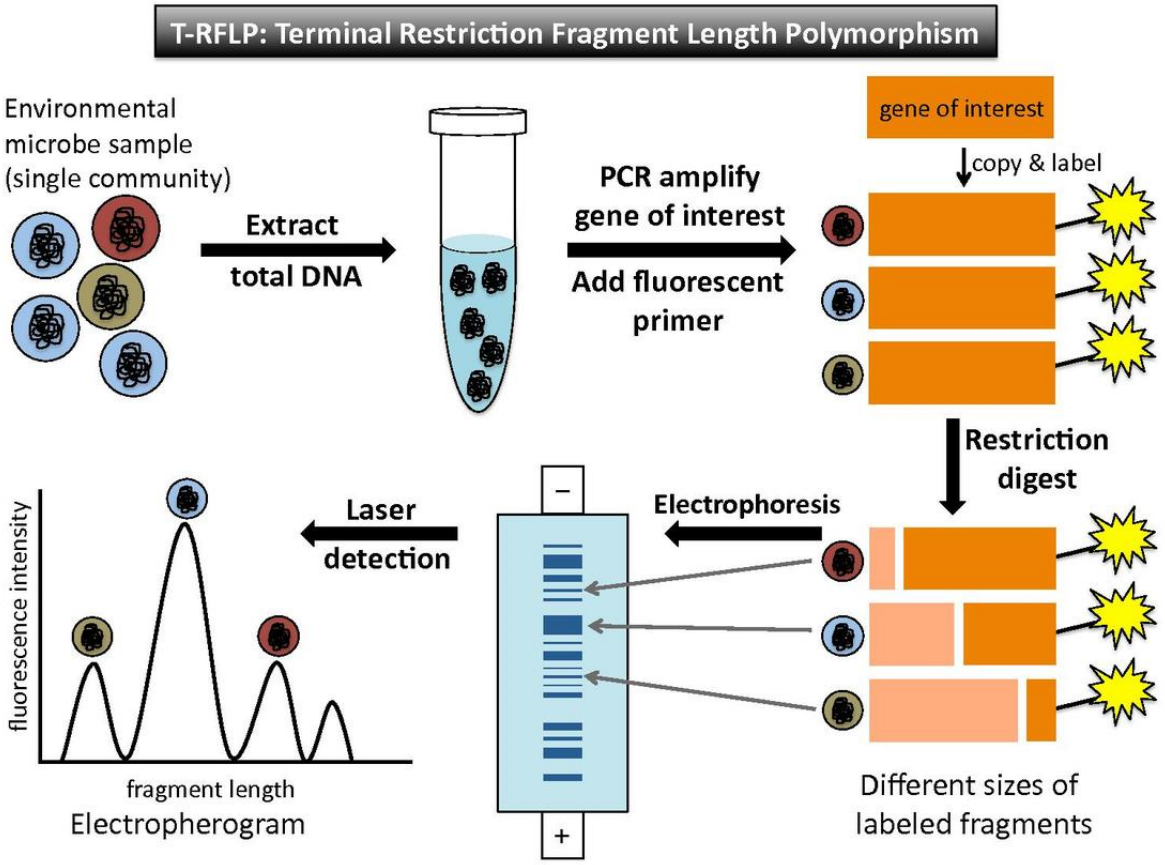
- Method used to separate DNA fragments
- Used to isolate and purify the vector and insert

Figure S-2: Gel Electrophoresis



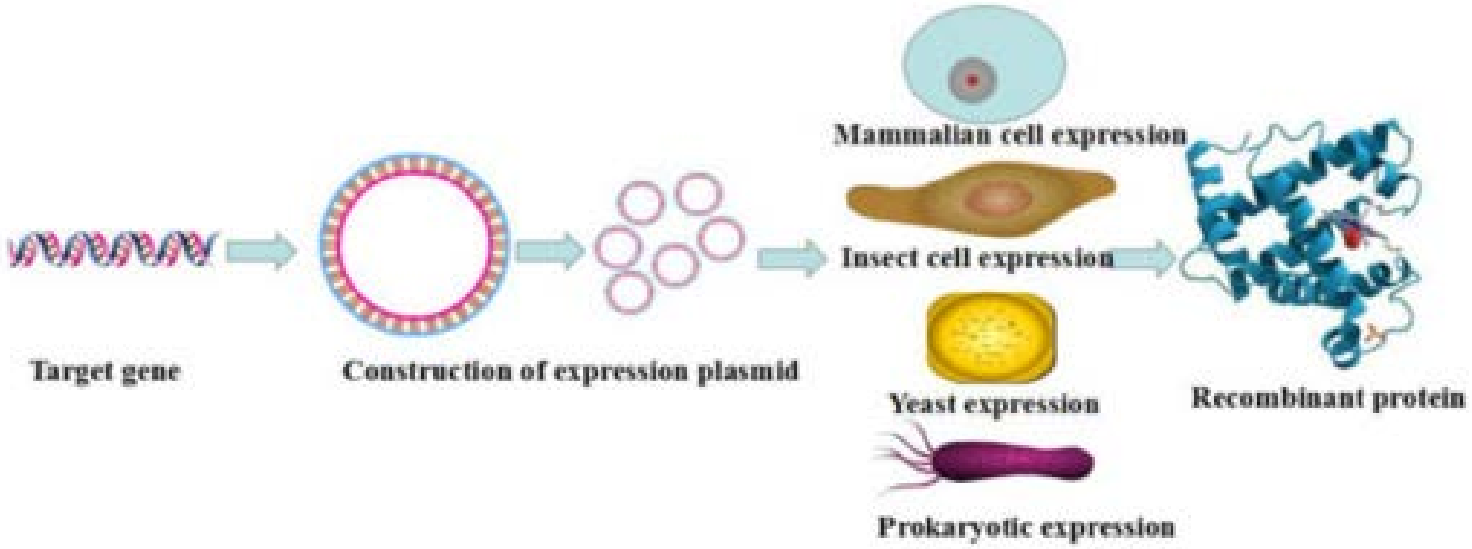
Restriction Endonucleases

- Enzymes that cleave DNA at specific sequences in a controllable and predictable fashion that can then be pasted into a new fragment with ligases
- Recognize 4-8 nucleotide long sequence
- Restriction enzyme mapping
  - Create restriction fragment length polymorphisms (RFLP) patterns
  - Fragments detected by gel electrophoresis



Recombinant Proteins

- Expression of cloned genes
- High cost and effort to isolate proteins with therapeutic functions from original source
- Clone genes to make recombinant proteins

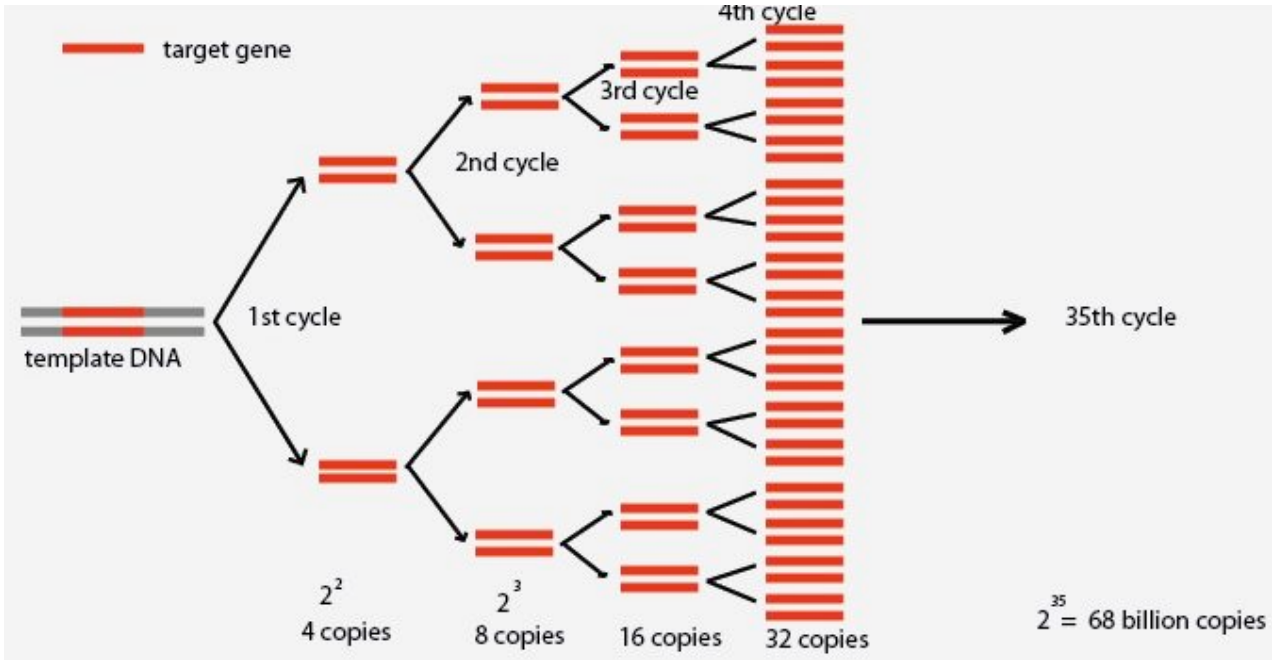


Recombinant Proteins in Transfusion Medicine:

- **Interferon-α** – treat hairy cell leukemia and hepatitis C and B
- **Hepatitis B vaccine**
- **Recombinant antihemophiliac factor**
- **Recombinant coagulation factor IX**
- **Granulocyte colony-stimulating factor (GCSF)-** increases production of hematopoietic stem cells (HSC) for stem cell transplant

Polymerase Chain Reaction (PCR)

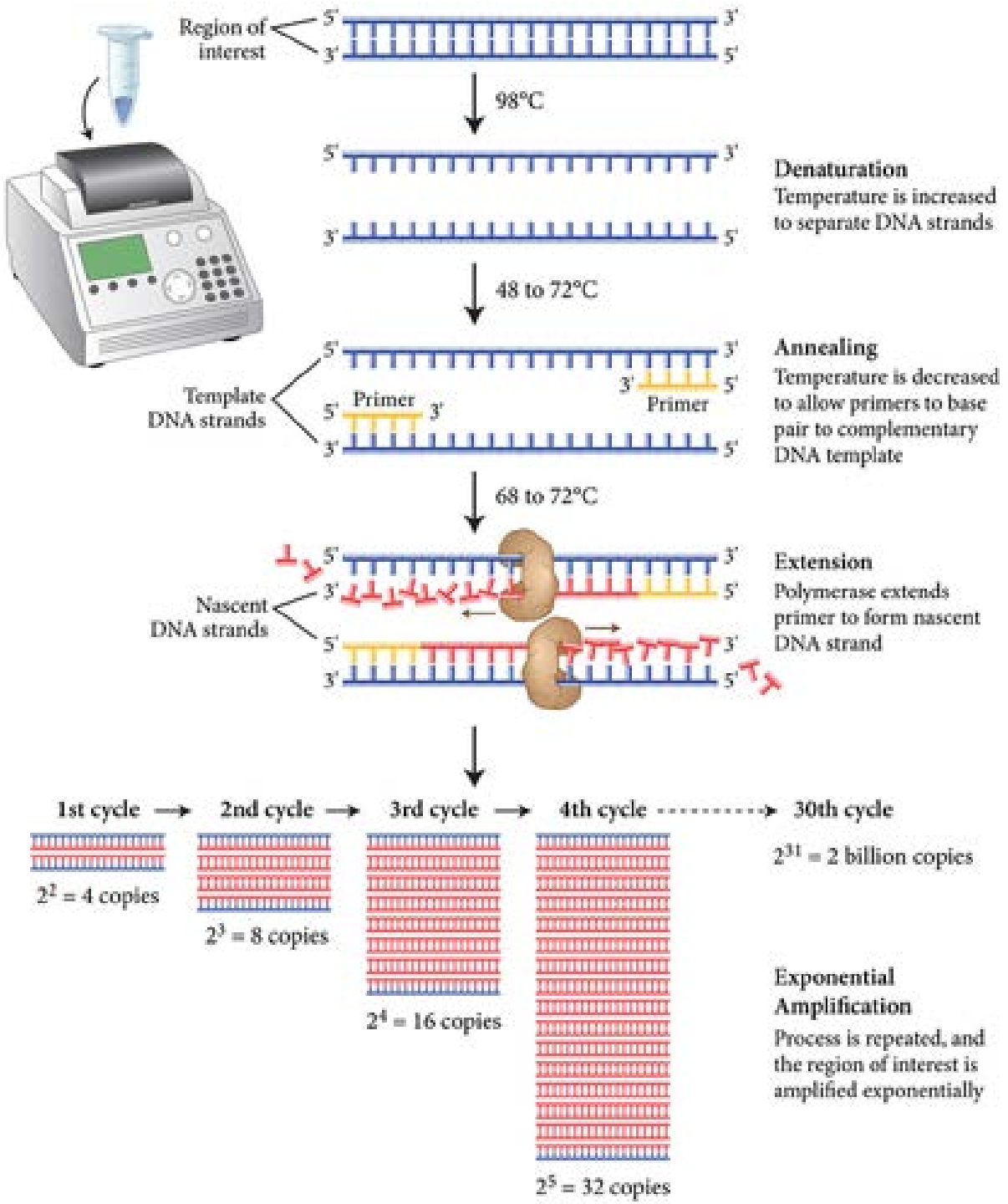
- Alternative to cloning for isolating large amounts of single DNA fragments or genes
- Completed in-vitro
- Amplify DNA segment several million times
- Done in test tube or microtiter plate



Steps in PCR

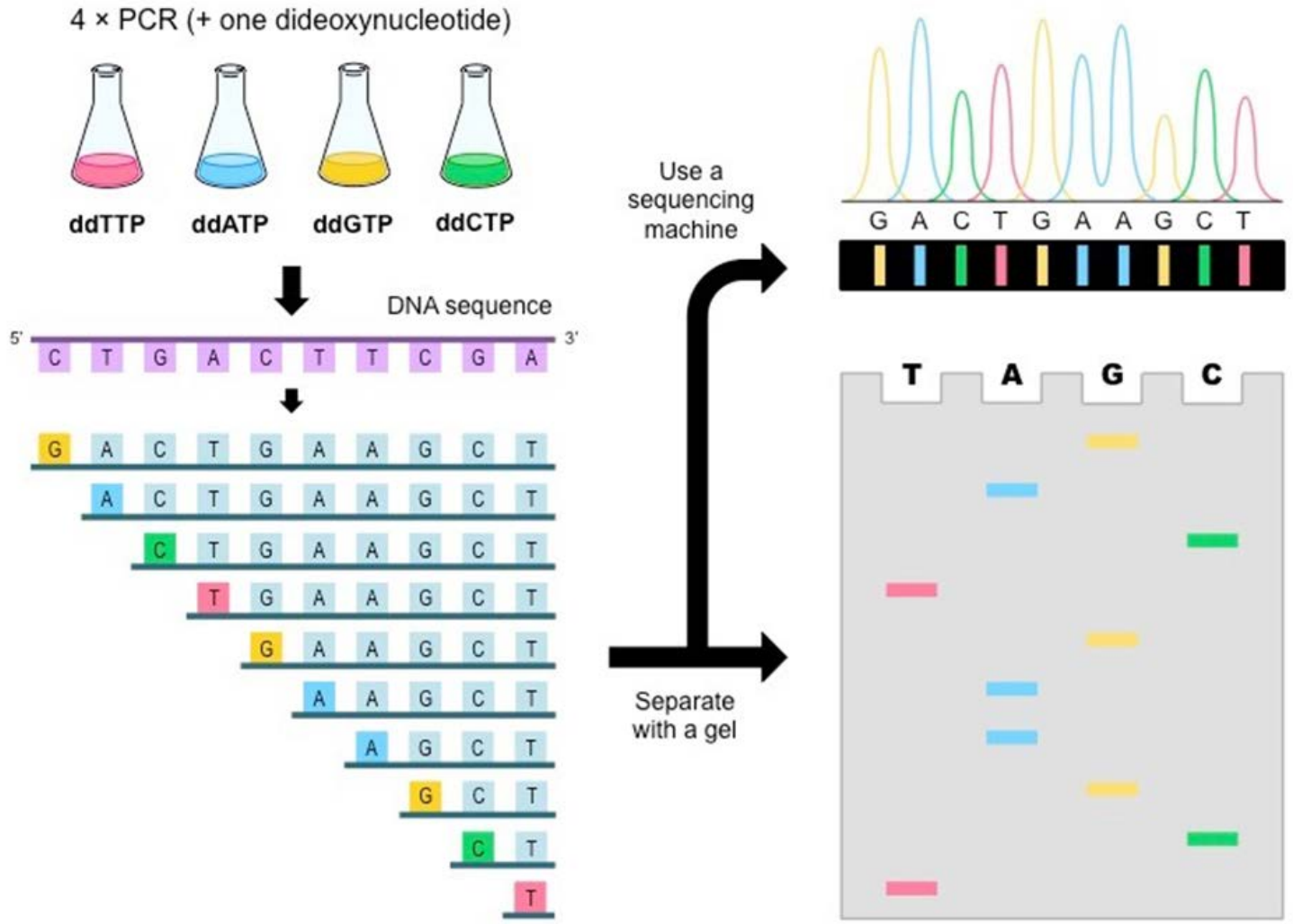
Include in test tube:

- Two primers for target region
- DNA polymerase
- 4 deoxyribonucleoside triphosphates (dNTPs)
- Magnesium (enzyme cofactor)



DNA Sequencing

- Determine nucleotide sequence either by molecular cloning or PCR
- Incorporate ddNTPs labeled with fluorescent dyes
- ddNTPs lack hydroxyl group- terminate chain
- Use the dye and gel to determine the sequence

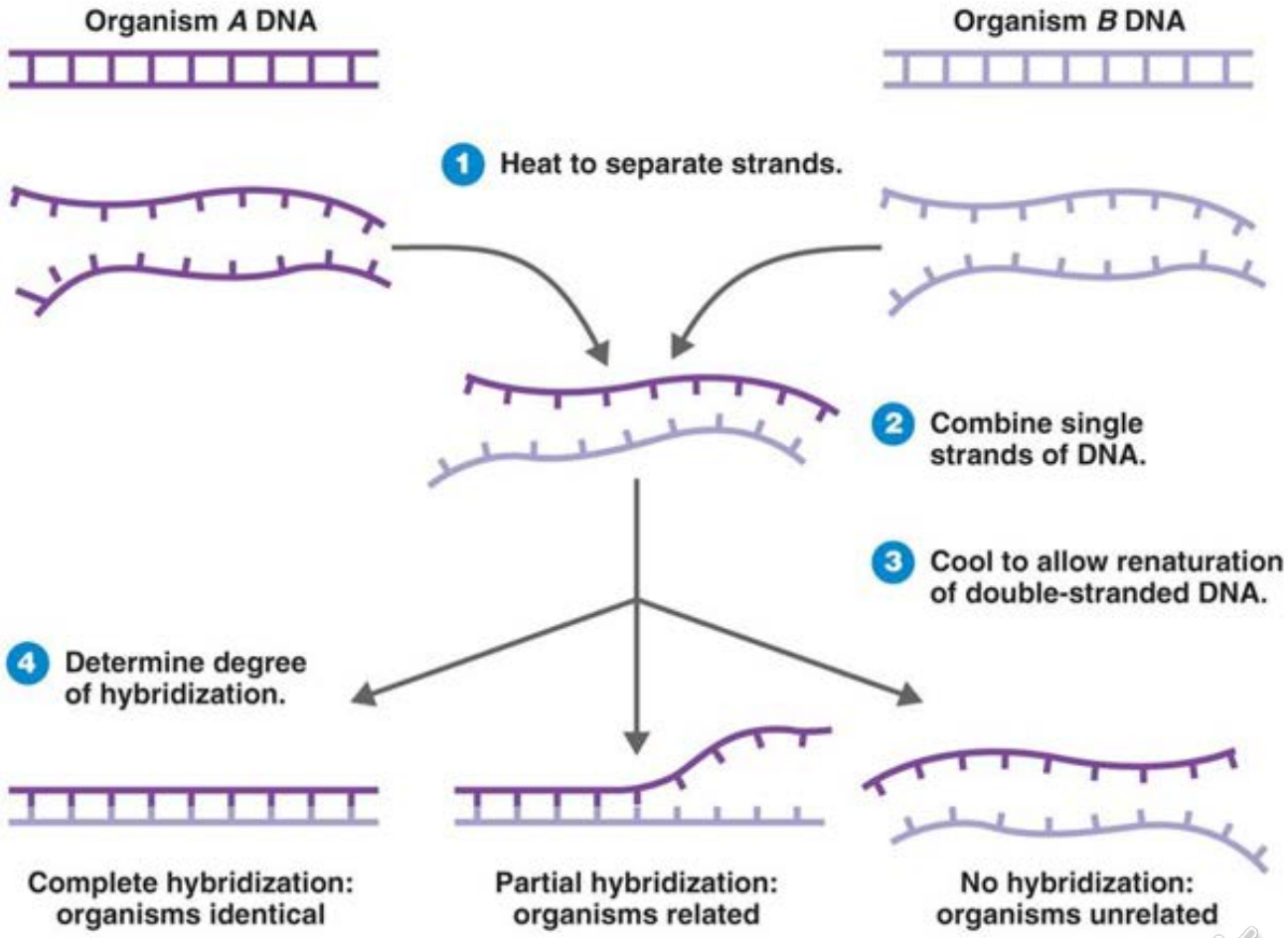


Detecting Nucleic Acids and Proteins

- Nucleic Acid Hybridization
- PCR based techniques
  - Real-Time PCR
  - Reverse Transcriptase PCR
  - Transcription Mediated Amplification
- Western Blot

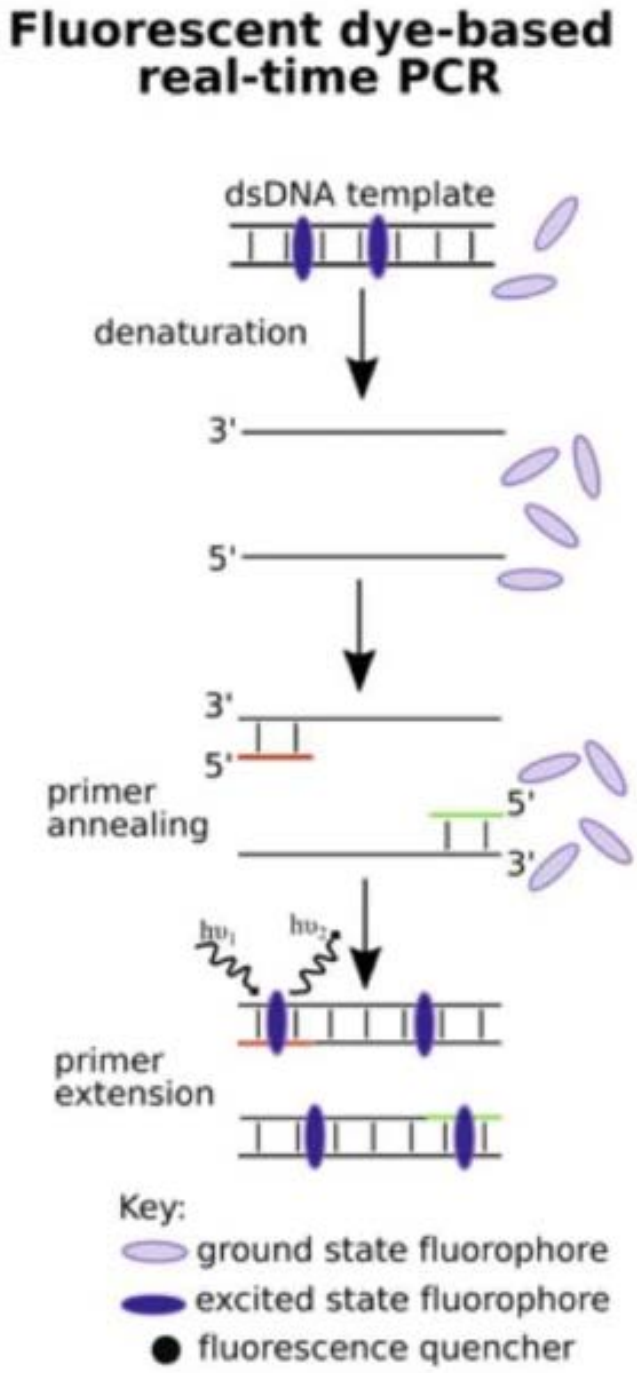
Nucleic Acid Hybridization

- Process of renaturation (of DNA or RNA)
- DNA or RNA sequences complementary to any purified DNA fragment can be detected due to labels:
  - Radioactive
  - Fluroescent
  - Chemiluminescent
- Labeled DNA is used as a probe
  - Detected with autoradiography or digital imaging
- Includes: Southern Blotting, Northern Blotting, DNA Microarrays, Fluorescent in Situ Hybridization (FISH)



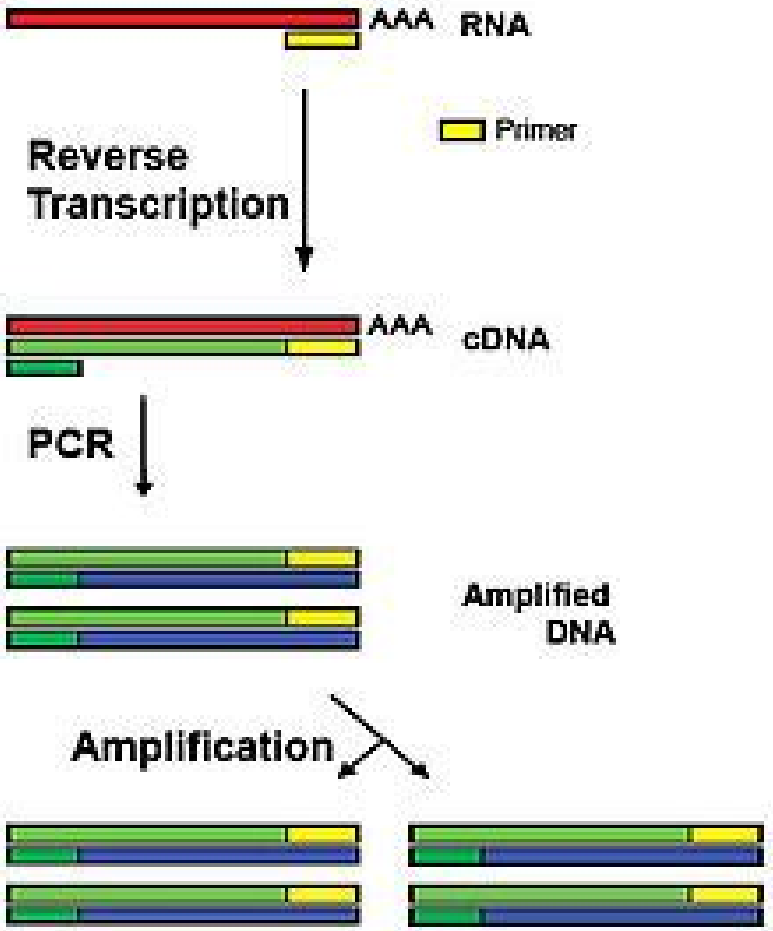
Real-Time PCR

- Product formed during each cycle of amplification is detected by fluorescence at the same time it is produced
- Mixture contains DNA probes that are complementary to region between the primers labeled with flurophores which emit fluorescent light when binding



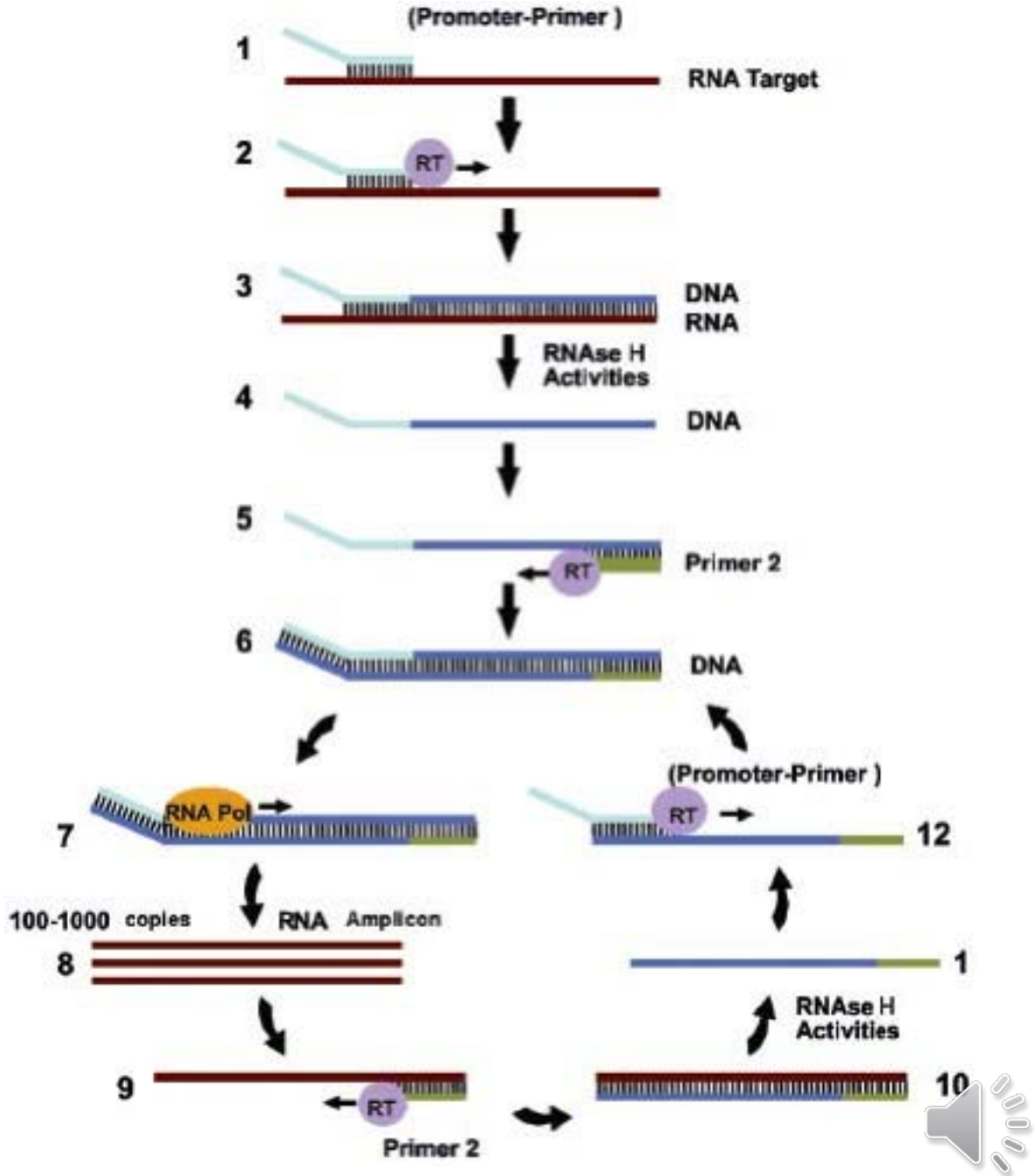
Reverse Transcriptase PCR

- Detects single copies of RNA
- Add step of cDNA synthesis prior to PCR (RT-PCR) amplification
- Early detection of transfusion-transmitted viruses
  - HIV
  - Hepatitis B
  - Hepatitis C



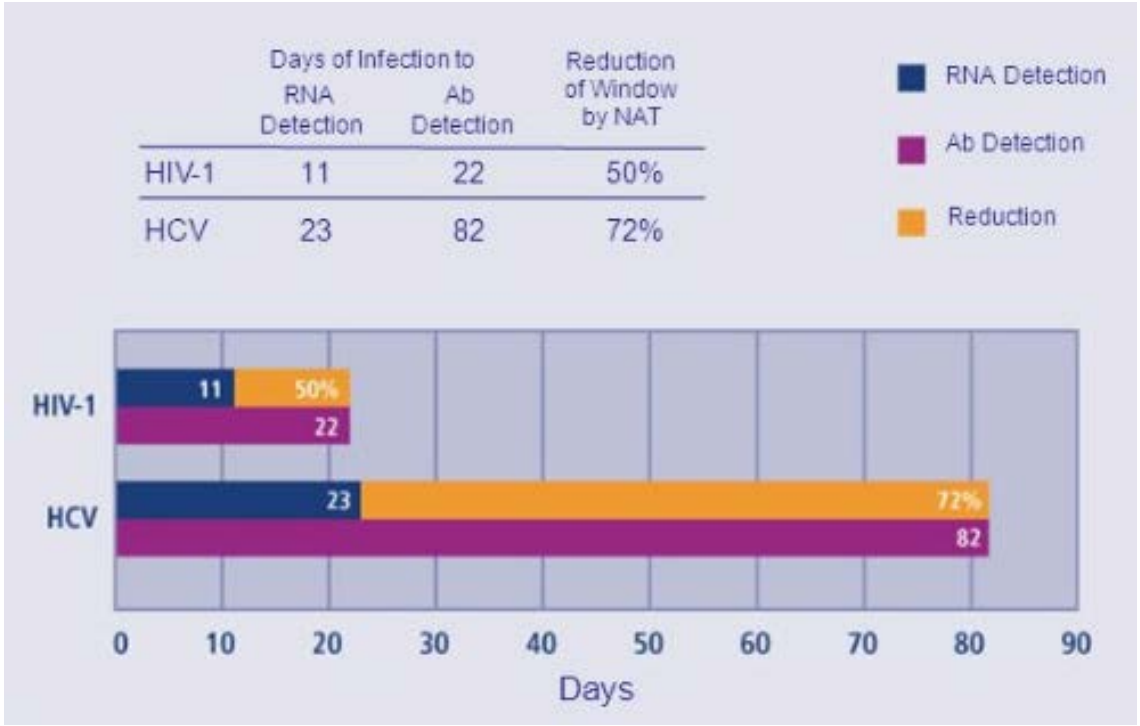
Transcription Mediated Amplification (TMA)

1. RNA is template
2. Reverse Transcriptase synthesizes RNA/DNA hybrid
3. RNAse H removes RNA from the hybrid
4. Only cDNA is left
5. Second primer binds to complete dsDNA
6. Complete dsDNA
7. RNA polymerase binds and transcribes to RNA
8. Many copies of RNA stand are made
9. Reverse Transcriptase converts back to DNA/RNA hybrid and begins the cycle again



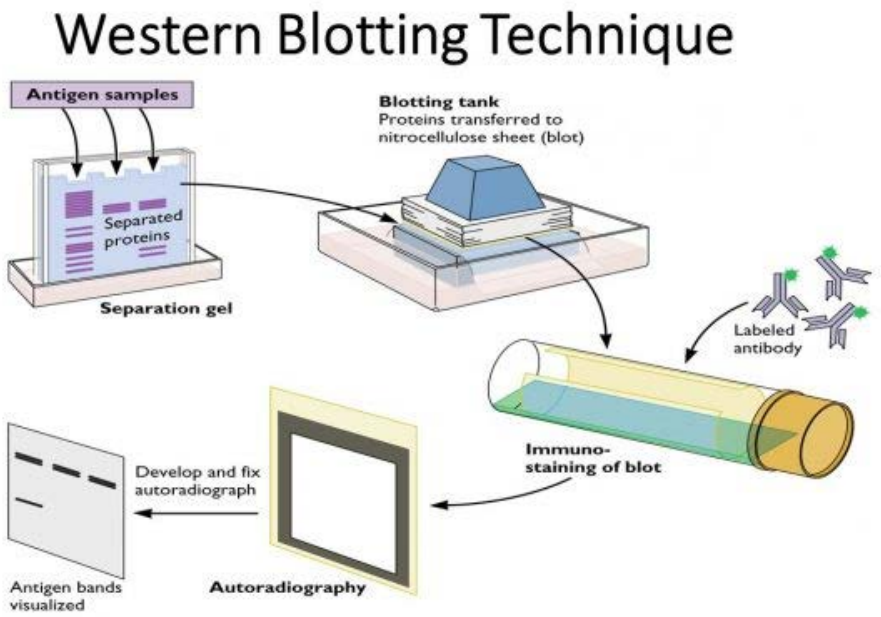
Transcription Mediated Amplification (TMA) Tests

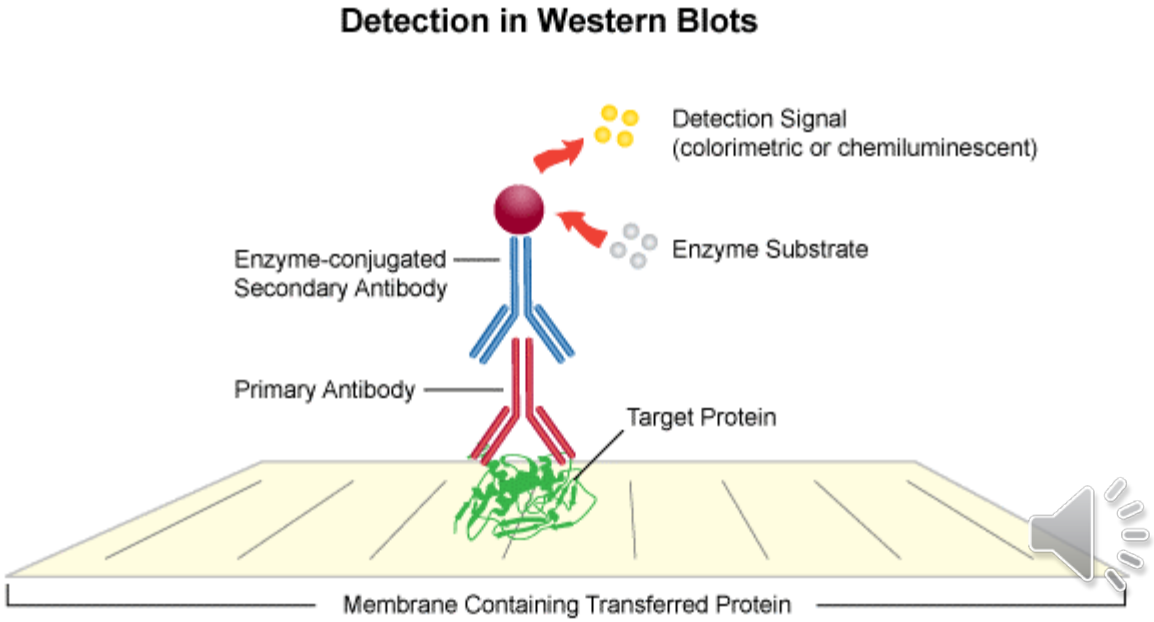
- Hybridization Protection Assay (HPA)
  - ssDNA probes have chemiluminescent labels
  - Form hybrids with amplified RNA
  - Light emitted when hybridized
  - Detect many pathogens with RNA present in the sample
- Nucleic Acid Testing (NAT)
  - Amplification through TMA and detection through HPA
  - Standard method in Blood Banking
  - Detect pathogens before immune response
  - Used for HIV, Hepatitis B and C, West Nile Virus



Western Blot

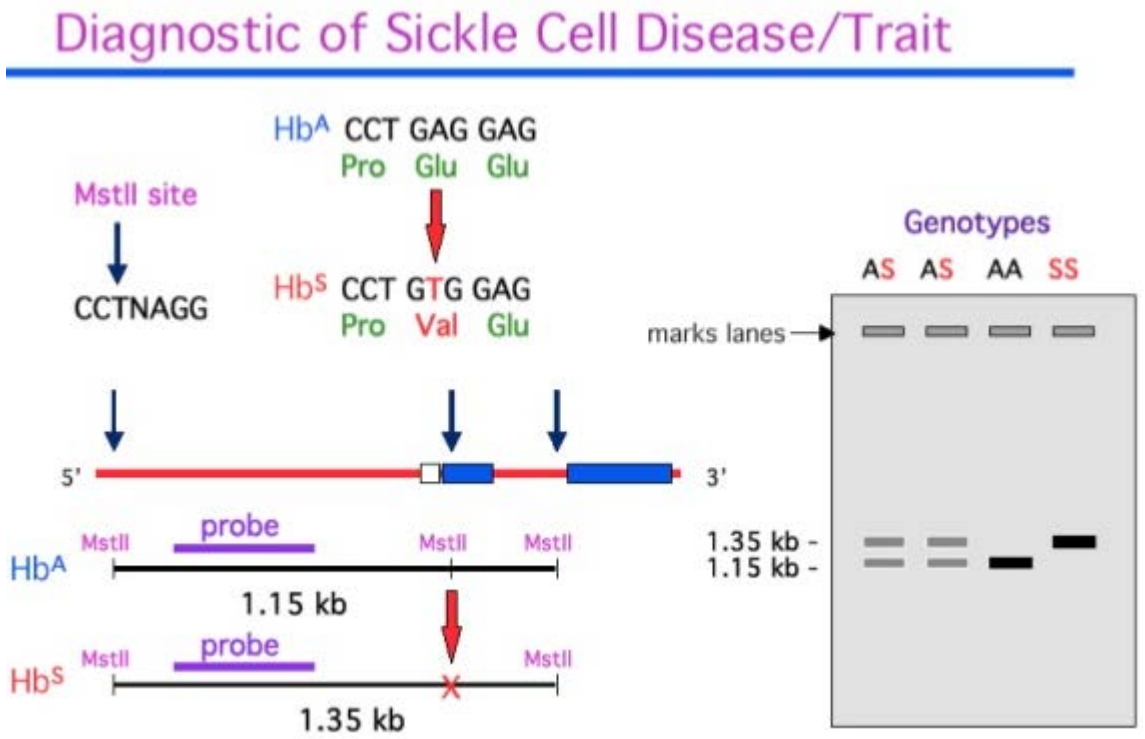
- Detects gene expression
- Detects proteins with labeled antibodies or probes
- Proteins separated with polyacrylamide gel electrophoresis
- Proteins transferred from gel to filter membrane
- Detect protein/antigen by incubation with labeled antibody
- Used for HIV confirmatory testing





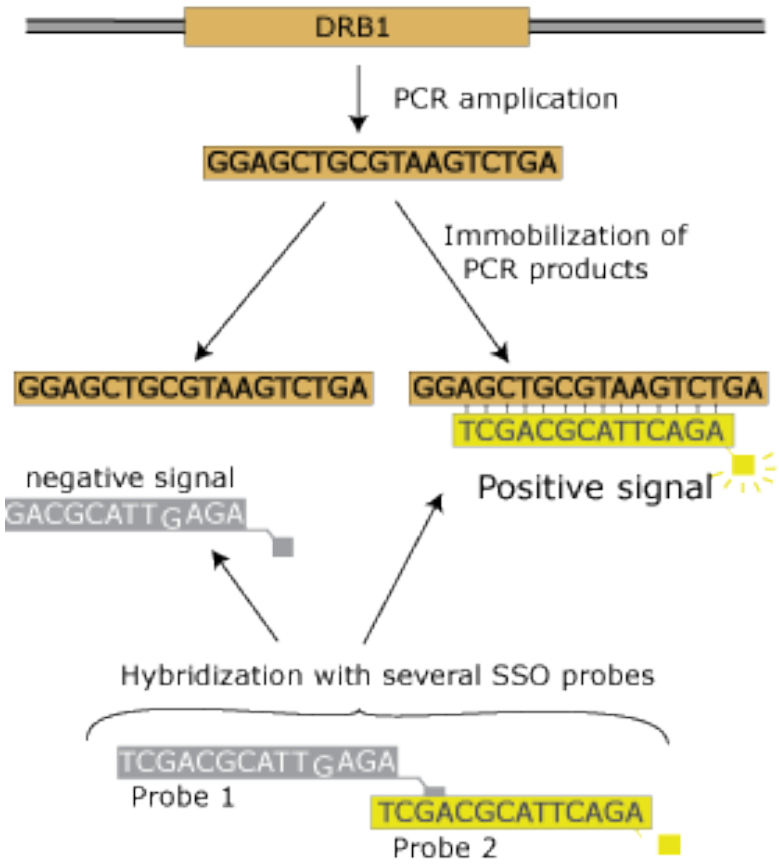
Techniques for Studying Gene Polymorphism

- Restriction Fragment Length Polymorphism (RFLP)
  - Used to detect glycosyl transferase gene in a person who is type AO or BO
  - Used to determine if individual carries the mutation for sickle cell anemia
  - Used for HLA typing, paternity testing, and in forensic science



Techniques for Studying Gene Polymorphism

- PCR and allele specific probes
  - PCR used to amplify polymorphic genes
  - PCR products are hybridized with specific probes - allows distinction of different known alleles
    - Called sequence-specific oligonucleotide probe
  - Commonly used for HLA typing
- DNA Sequencing
  - Used in HLA typing for allogeneic hematopoietic stem cell transplant



Red Blood Cell Genotyping

- Useful when serologic testing is impossible or inconclusive
- Detect single nucleotide polymorphisms
- Clinical Applications:
  - Fetal DNA Typing
  - Blood group typing of donors of alloimmunized patients
  - Screening of blood donors to find rare phenotypes
  - Blood group typing of patients with AIHA or other diseases

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Every life deserves world class care.