

Chapter 15

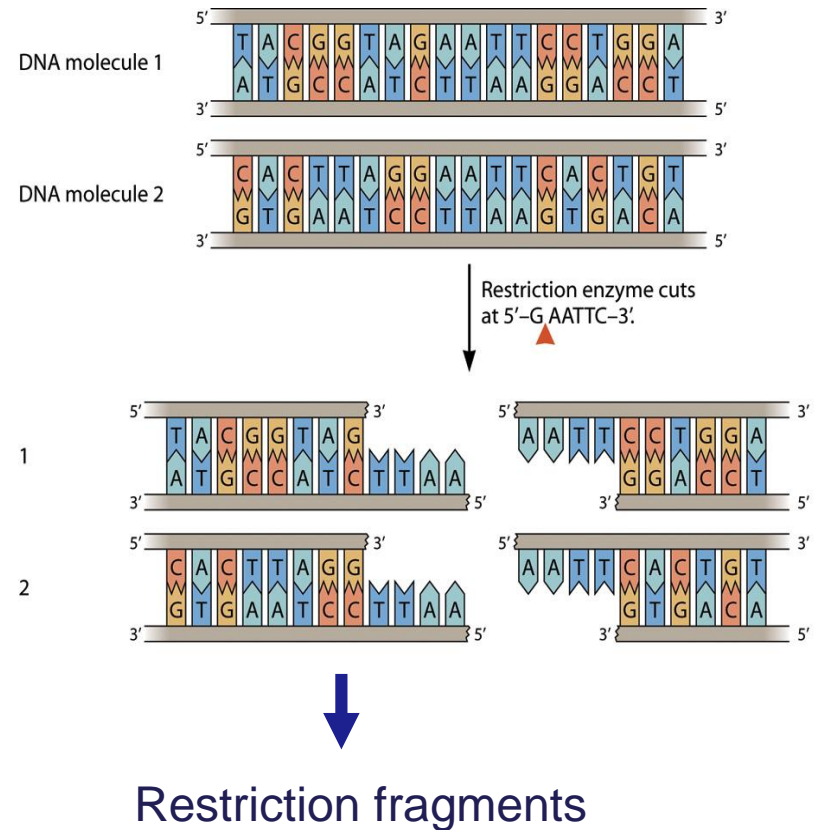
# The Biotechnology Toolbox



# Cutting and Pasting DNA

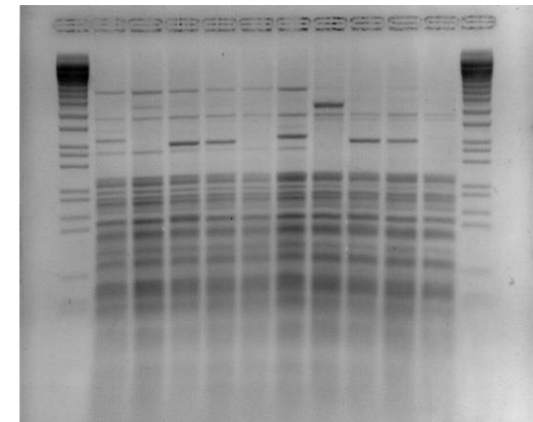
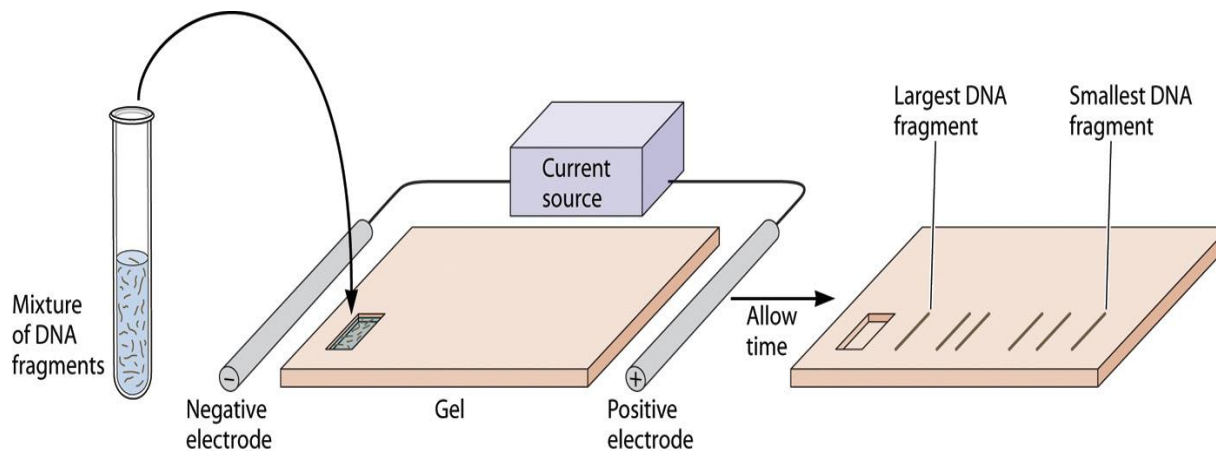
## ■ Cutting DNA

- Restriction endonuclease or restriction enzymes
- Cellular protection mechanism for infected foreign DNA
- Recognition and cutting specific sites of DNA
  - Recognition sites are usually palindromic
    - e.g. 5'-GAATTC-3'



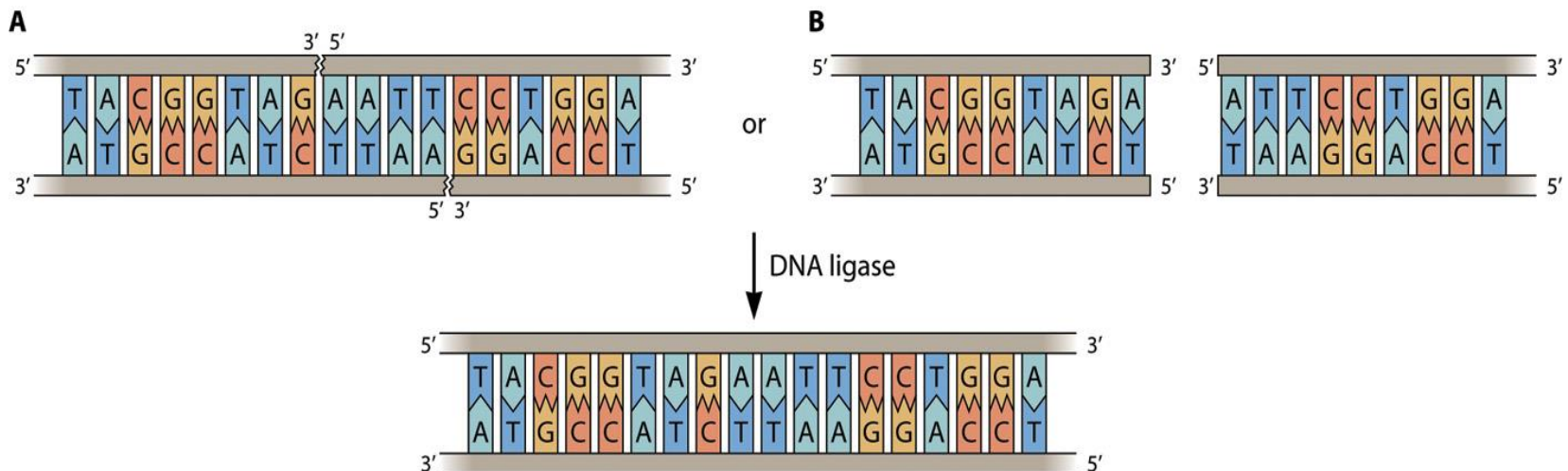
# Separating mixtures of DNA fragments

- Electrophoresis
  - Gels
    - Agarose : broad range of resolution
    - Polyacrylamide : high resolution for smaller DNA
  - Migration of DNA to the positive electrode under the electric current
  - Separation of DNA molecules by molecular weight and shape
    - $L = k 1/\log_{10}MW$  for linear DNA
  - Staining of DNA for visualization (Ethidium bromide, EtBr)



# Pasting DNA

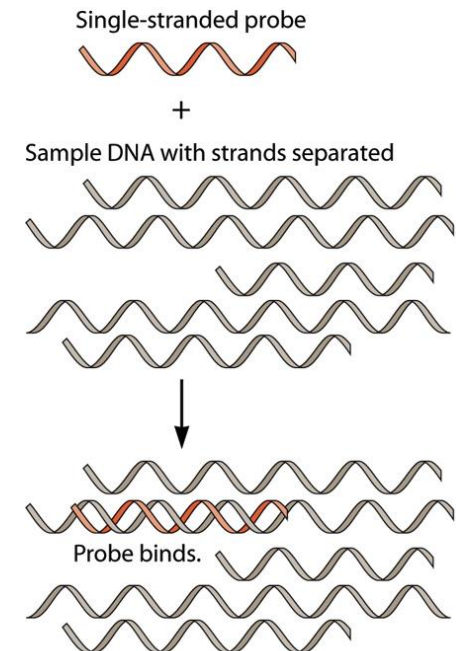
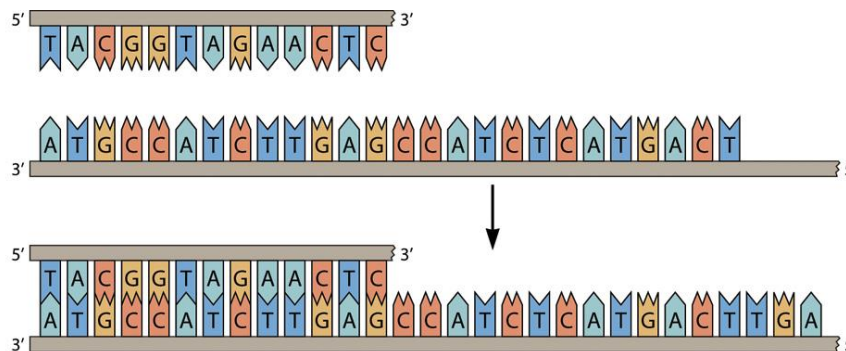
- DNA ligase
  - Joins DNA by forming new phosphodiester bond
- Recombinant DNA
  - DNA generated by joining DNA pieces from different sources



# Hybridization Analysis

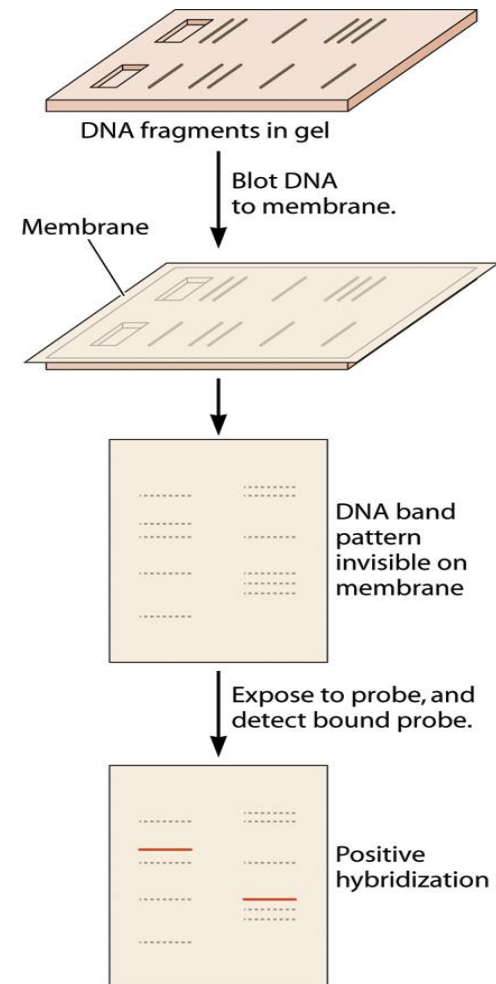
## ■ Hybridization

- Forming double strand DNA by complementary base pairing
- Procedure
  - Denaturation: making ssDNA by heating
  - Hybridization with labeled ssDNA or ssRNA probe
    - Radioisotope labeling
    - Fluorescence labeling
  - Detection of hybridized products



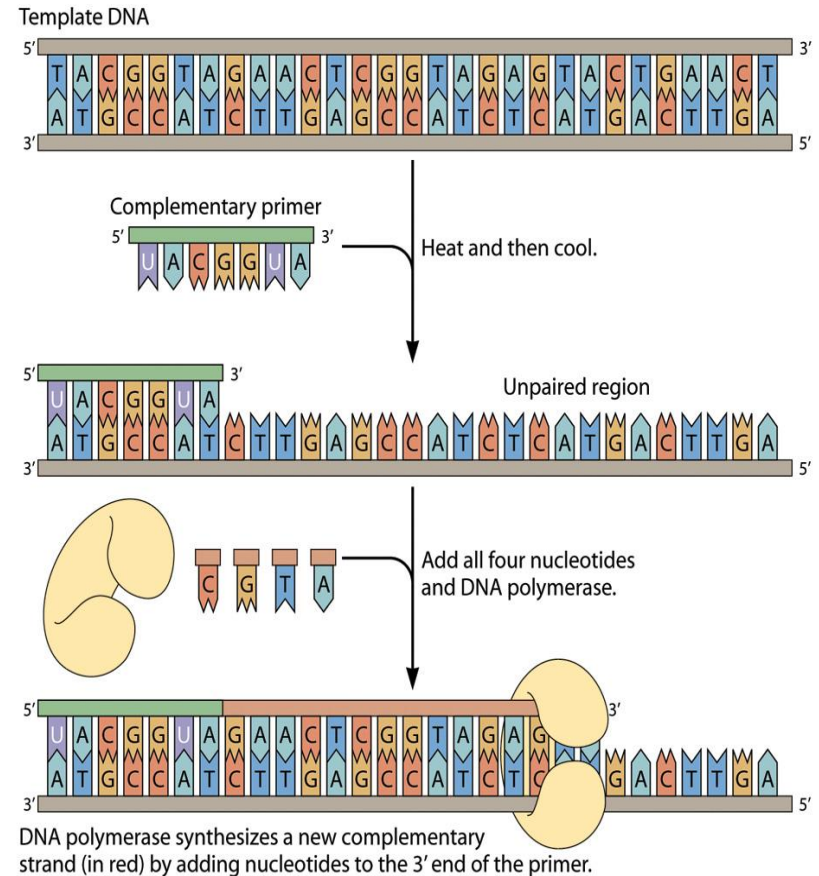
# Hybridization Analysis

- Locating a specific DNA sequence
  - Gel electrophoresis of restriction fragments
  - Blotting on a membrane
  - Hybridization with labeled probe
    - Synthetic oligonucleotides: chemically produced ssDNA
    - Denatured natural DNA fragment
  - Detection of the hybridized bands



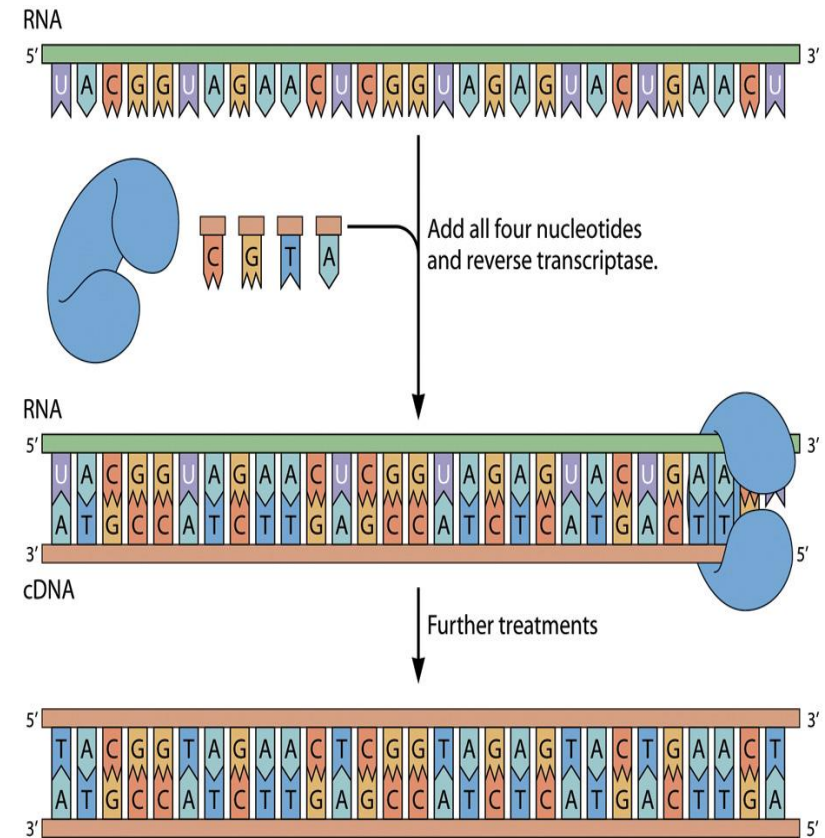
# Making DNA in vitro

- DNA polymerase
  - Denaturation of DNA
  - Primer binding
    - RNA primer (within the cell)
    - DNA primer
  - DNA synthesis by DNA polymerase



# Making DNA from an RNA template

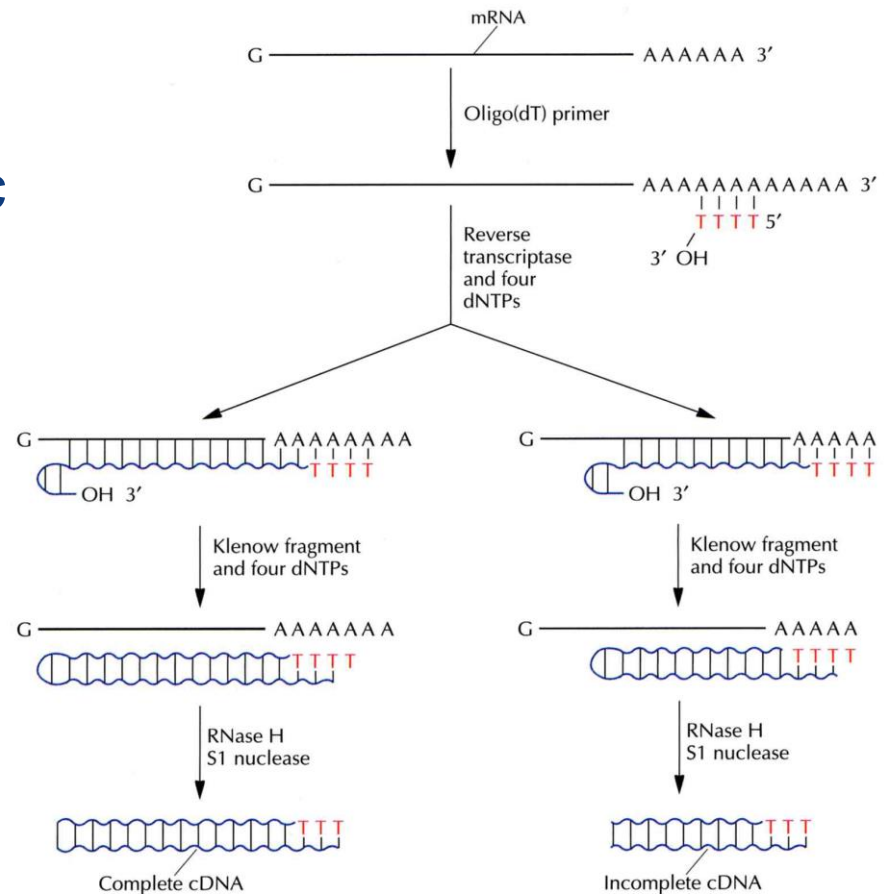
- Reverse transcriptase
  - Making complementary DNA (cDNA)
  - Made by RNA viruses
  - Important for expressing eukaryotic gene in bacteria
    - No intron after reverse transcription





# Reverse Transcription

- Klenow fragment
  - a product of proteolytic digest of the DNA polymerase I
- RNase H
  - hydrolyzes mRNA
- S1 nuclease
  - removes hairpin loop

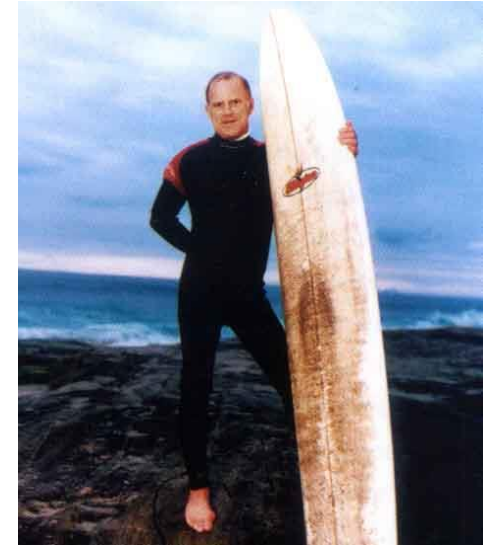


# Polymerase Chain Reaction (PCR)

## ■ PCR

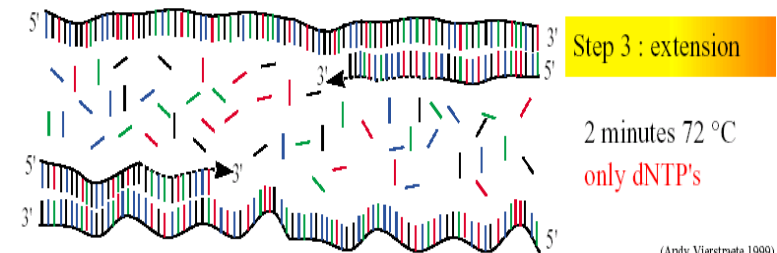
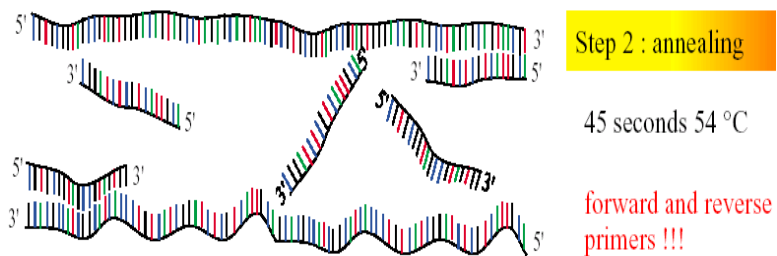
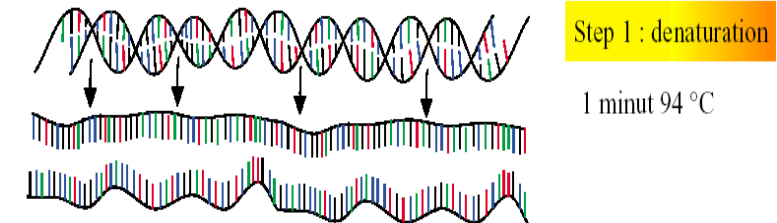
- Invented by Kary Mullis (1983)
- Amplification of specific DNA sequence
- Reaction mixture
  - DNA template, 2 primers, DNA polymerase (heat-resistant), dNTPs
- Reaction conditions
  - Denaturation of DNA at 95°C
  - Primer annealing at 54°C
  - DNA synthesis at 72°C

Repeat

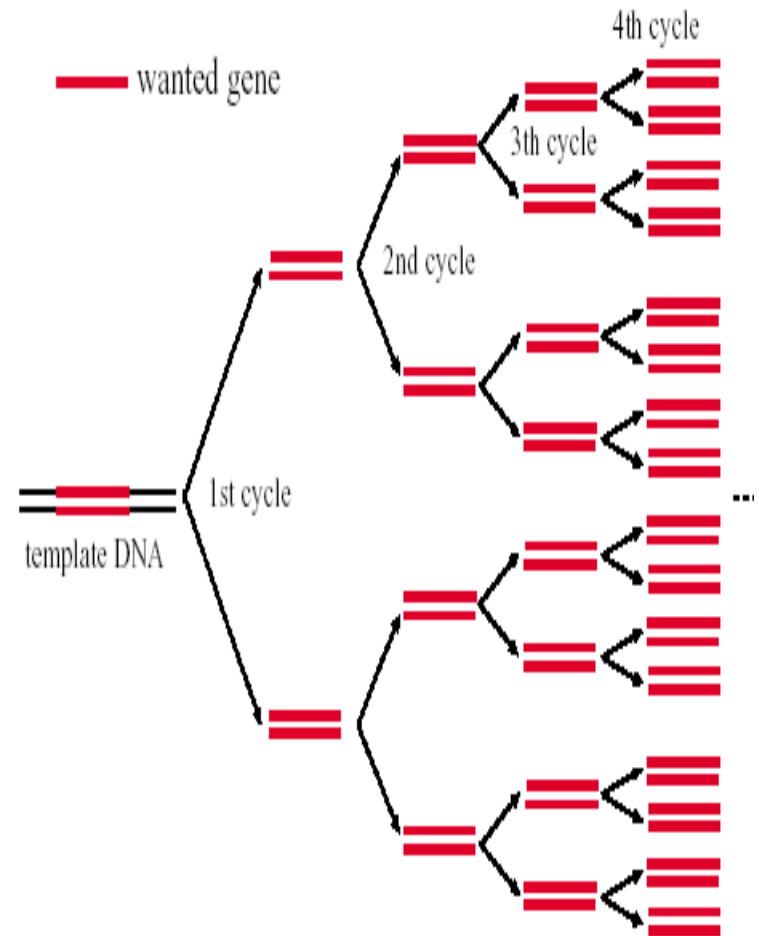


# PCR

30 - 40 cycles of 3 steps :

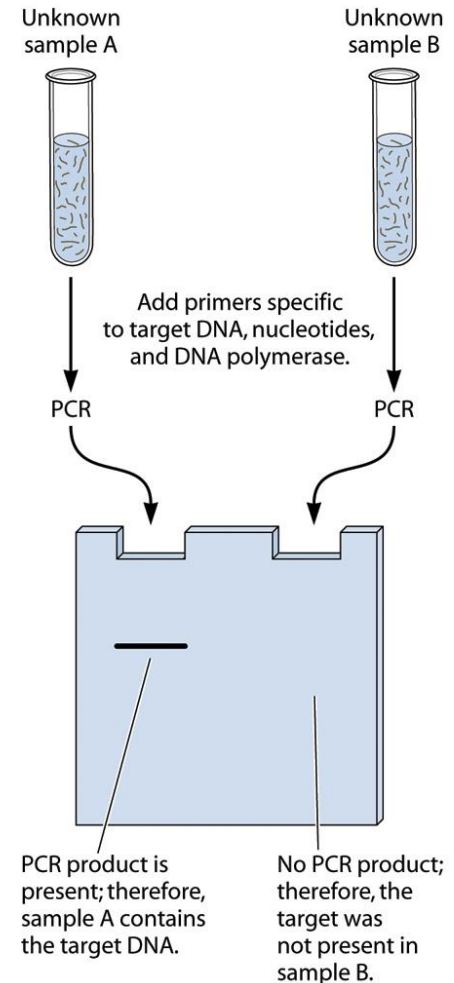


(Andy Vierstraete 1999)



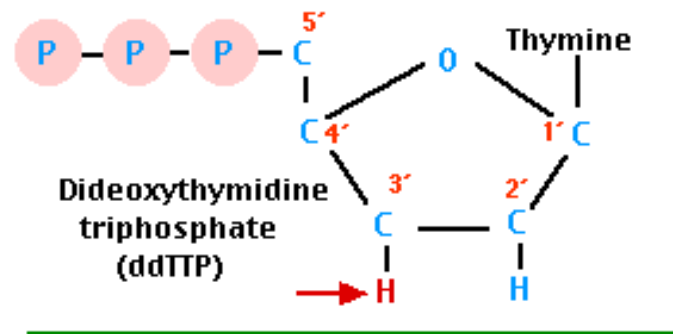
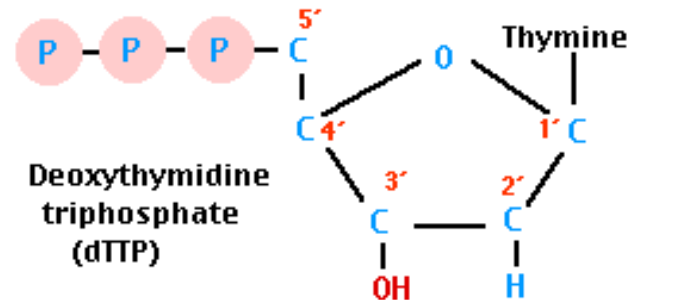
# PCR as a Detection Method

- More sensitive than hybridization in detecting DNA
- Diagnosing disease
  - Traditional method for diagnosis of infectious disease
    - Culturing the pathogenic bacteria for identification
    - Time consuming
  - PCR-base detection
    - Fast and sensitive

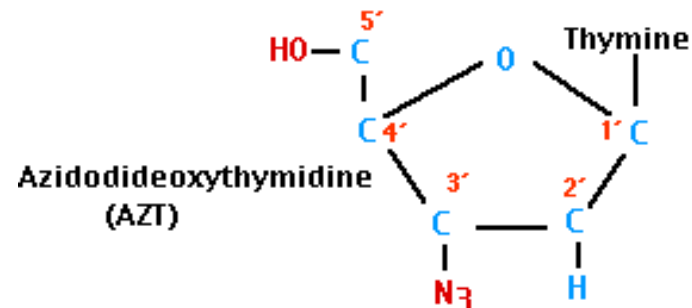


# DNA Sequencing with terminators

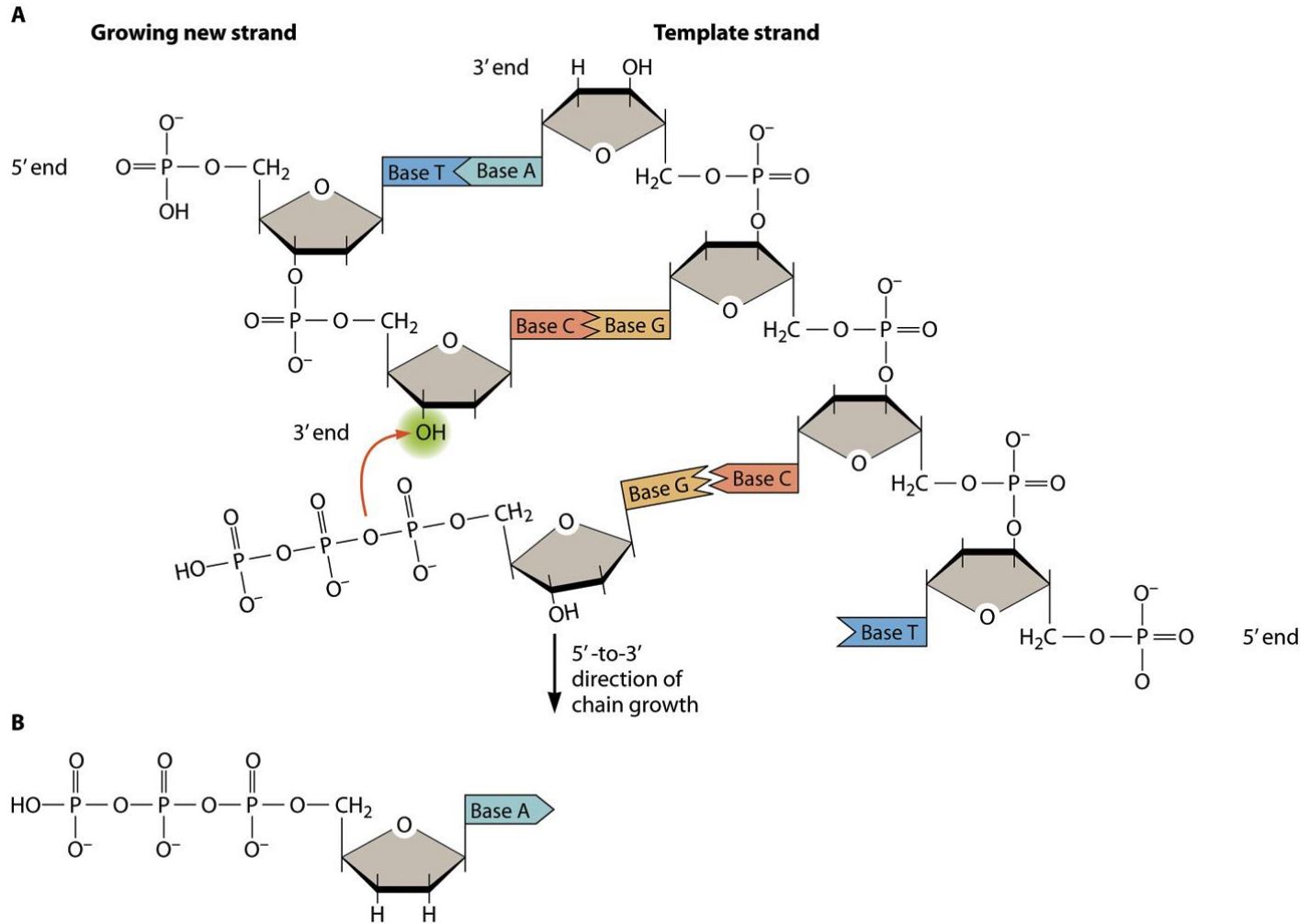
- DideoxynTP
  - Chain termination
  - Sanger (1977)



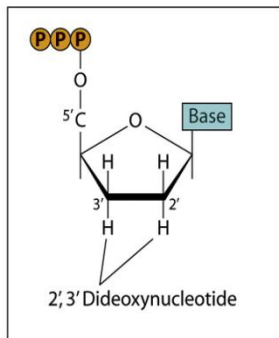
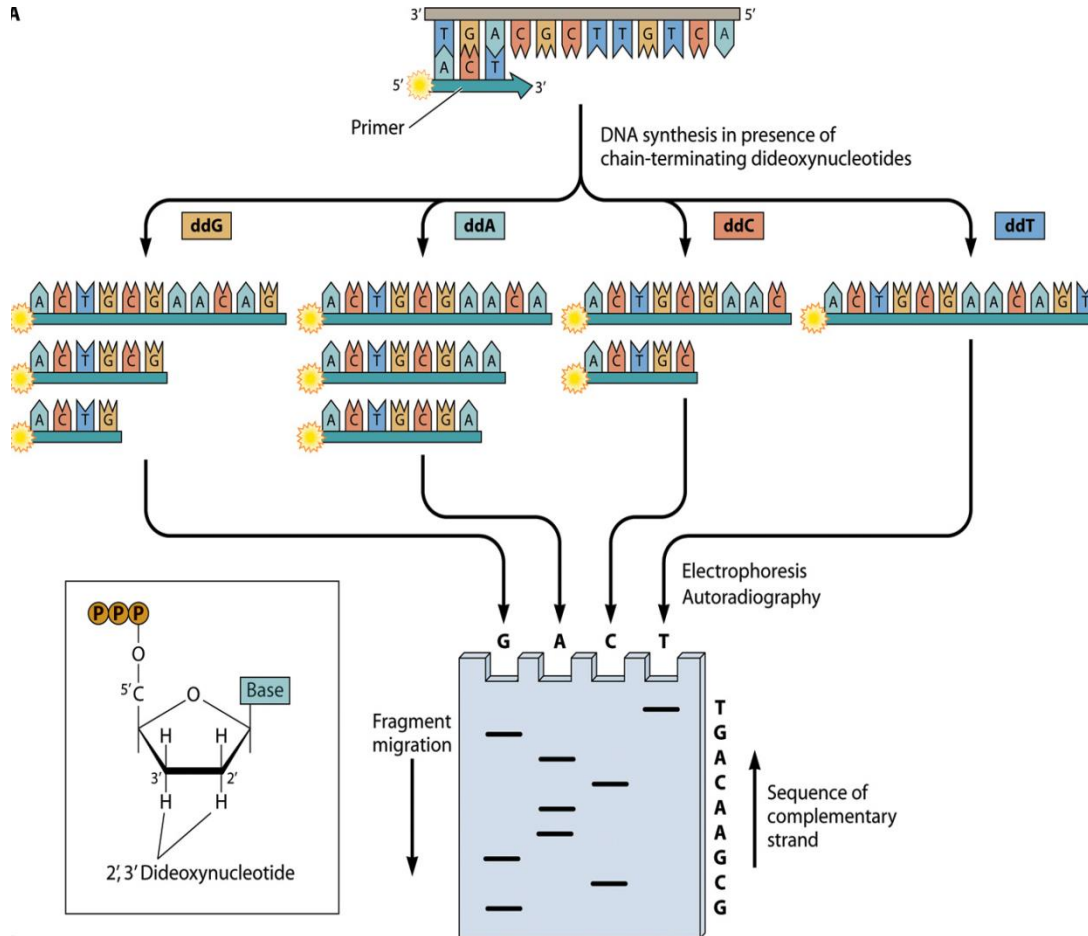
- Anti-AIDS drug (AZT)
  - HIV is an RNA virus.
  - Reverse transcriptase is an sloppy enzyme.



# Chain termination by ddNTP



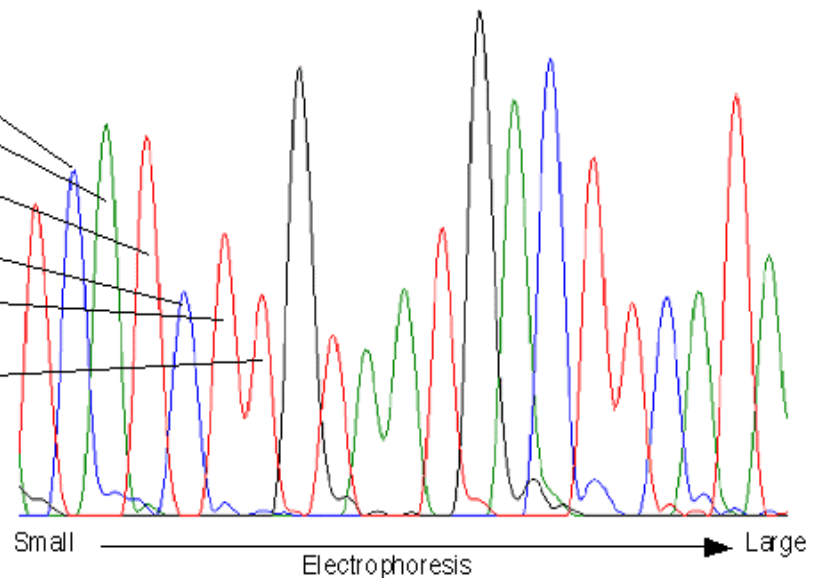
# Chain Termination Sequencing



# Automated DNA Sequencing

Label four ddNTP with different fluorescent dyes

Run in one gel lane or capillary tube



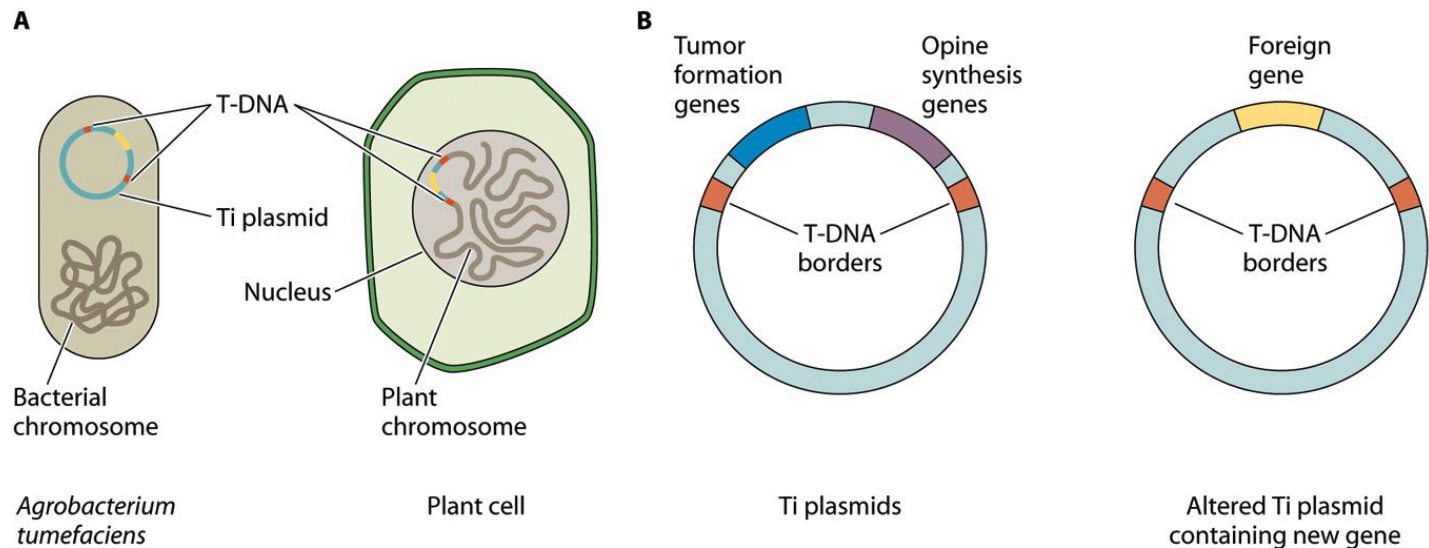


# Cloning

- Cloning
  - Production of identical copies of something
    - e.g. asexual reproduction
- DNA cloning
  - Producing identical copies of DNA (replication) inside of a cell
  - Cloning vectors
    - Plasmid : small circular DNA with own replication origin
    - Viral vector: Replacement of non-essential viral DNA to gene of interest
    - Yeast artificial chromosome
      - Replication origins, centromere, and telomeres

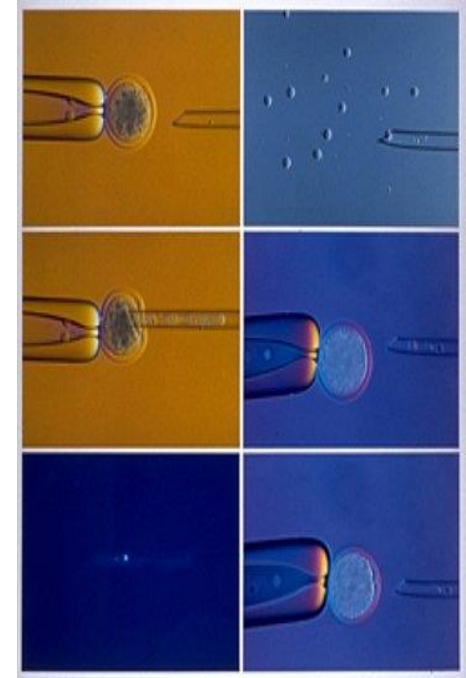
# Ti Plasmid

- Ti plasmid in *Agrobacterium tumefaciens*
  - Transfer T-DNA into plant DNA and induce tumor
  - Replace T-DNA with the gene of interest

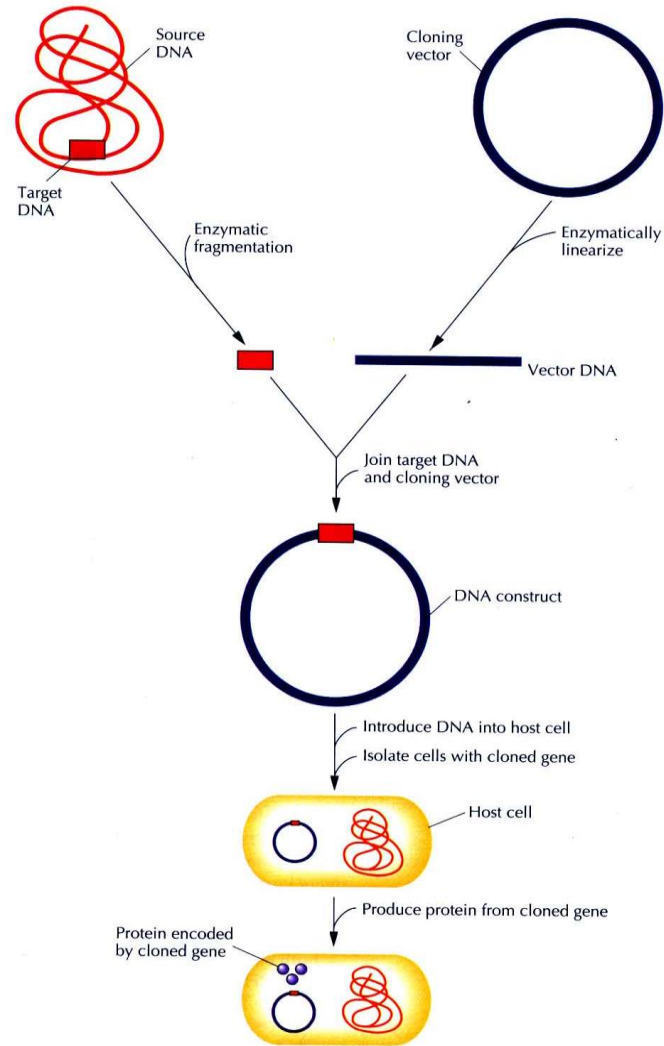


# Introduction of DNA

- Methods for introduction of DNA
  - Microinjection
  - Chemical
  - Physical : gene gun, electroporation
- Selection of cells with plasmids
  - Marker genes
    - Antibiotics
    - Auxotrophic markers
  - Confirmation of the presence of the gene of interest
    - PCR
    - Sequencing
    - Restriction digestion

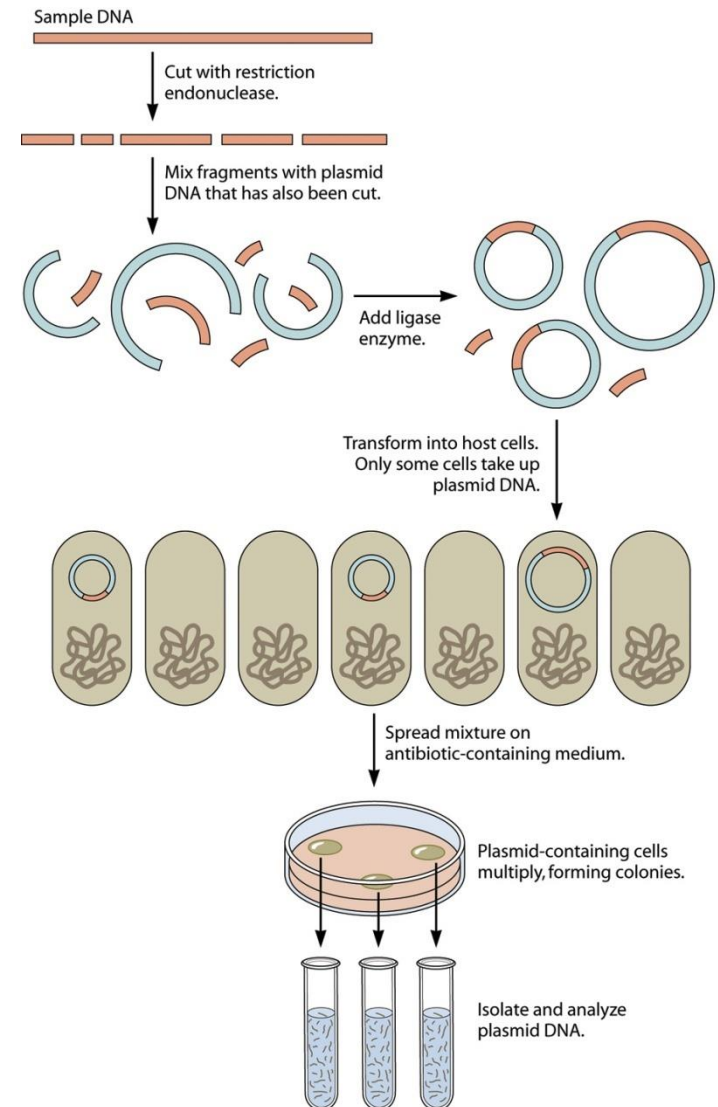


# Cloning Procedure



# Cloning Procedure

- Ligation of vector and insert
  - Insert DNA : restriction fragment or PCR product
- Introduction into host
- Selection of plasmid-containing cells using marker
- Isolation and analysis of plasmids



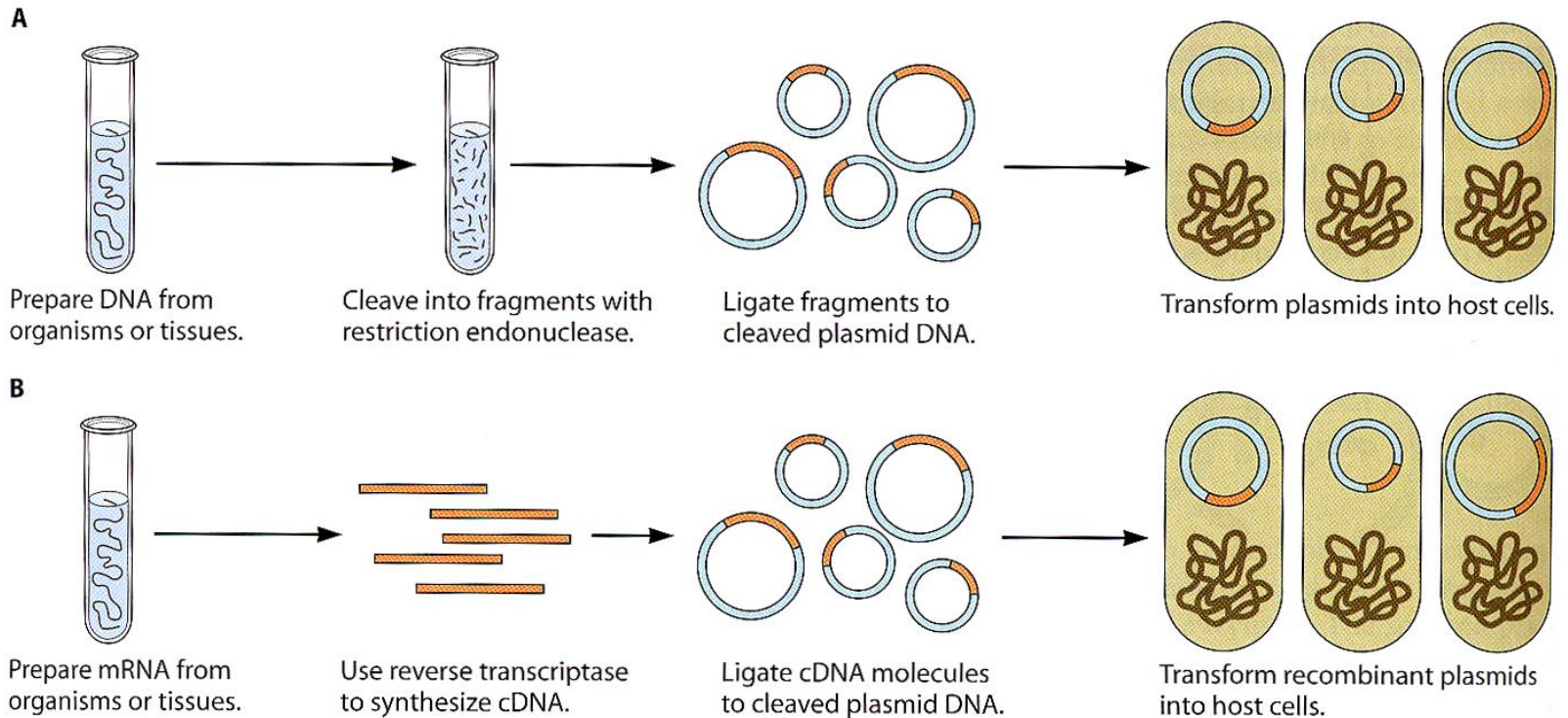
# DNA Library

- DNA library
  - Collection of clones from one organism
- Genomic DNA library
  - DNA fragments covering the whole genome
- cDNA library
  - Library generated from mRNA
  - Representing only expressed genes
  - Reverse transcription with reverse transcriptase

# DNA Library

## (A) Genomic DNA library

## (B) cDNA library

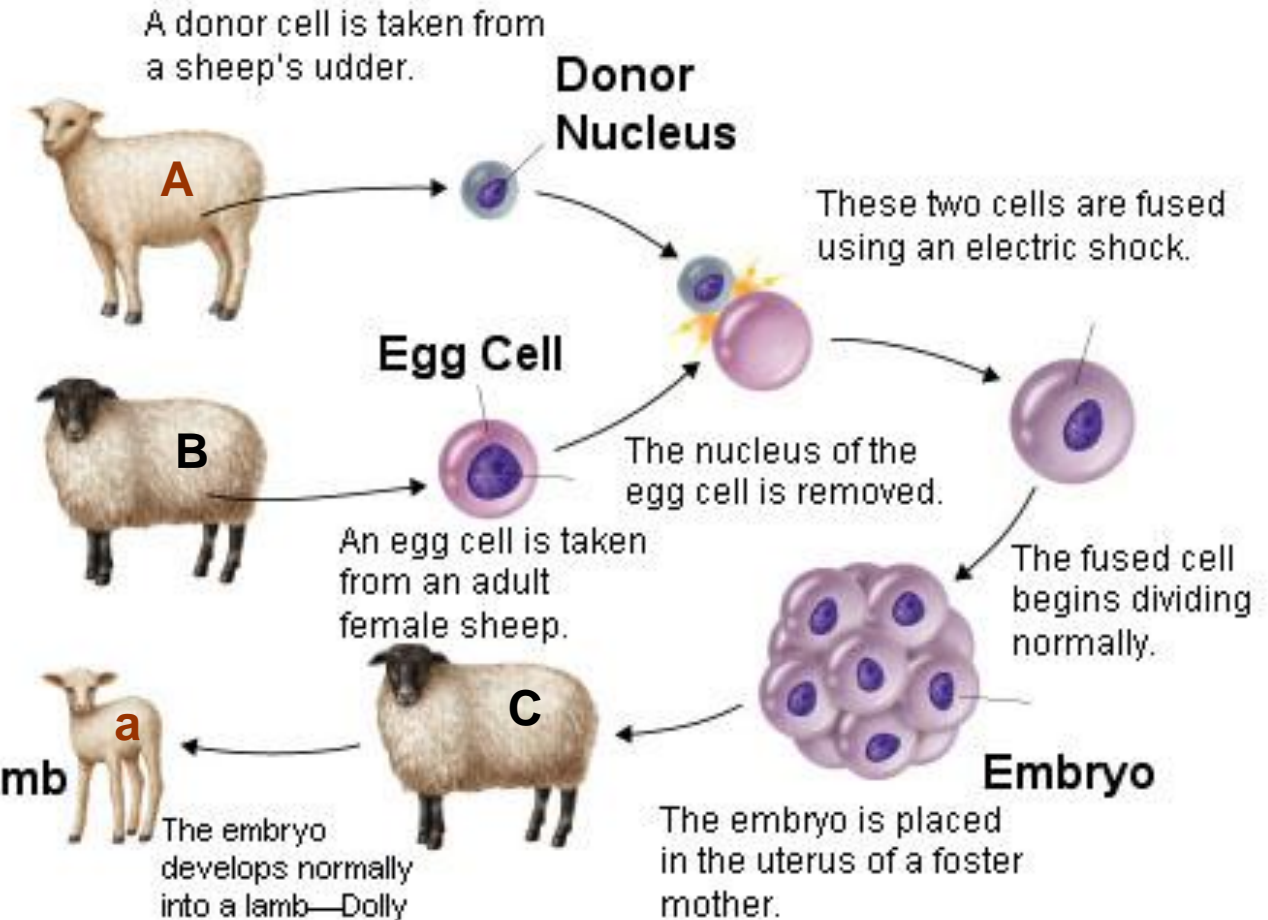


# Cloning Complex Organisms

- Identical twins
  - Development of embryos from splits of early embryo
  - Twining: artificial splitting of animal embryos
- Nuclear transfer
  - Donor DNA + egg without nucleus
  - Still contains mitochondrial DNA of the egg donor



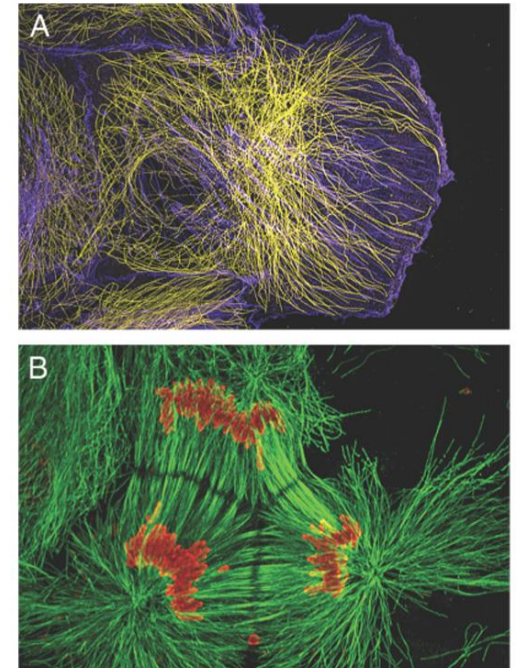
# Nuclear Transfer



**Dolly, 1997**

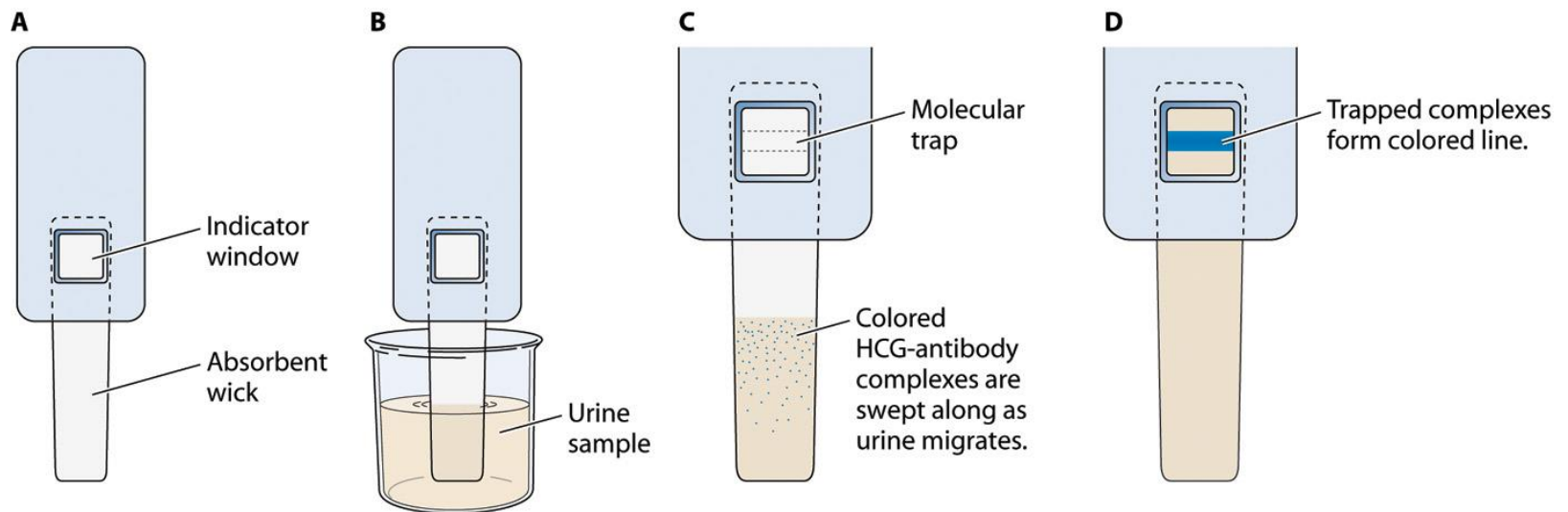
# Analyzing Proteins by Antibody

- Monoclonal antibodies
  - Pure antibody: generated by B cells → no cell division in culture
  - Fusion of B cells with cancerous cells (myeloma cells)
    - indefinite division in cell culture
    - Production of monoclonal antibody
    - Screening cells producing desired antibody
- Protein detection using antibody
  - Detection of specific protein: Western blotting
  - Localization of protein : fluorescence-labeled antibody



# Antibodies for Diagnosis

- Home pregnancy test
  - Detection of a pregnancy hormone human chorionic gonadotropin (HCG)



# Three-Dimensional Protein Structure Analysis

- Protein Structure
  - Protein structure is related to its function
  - Information to study the function of proteins or design new proteins
- X-ray crystallography
  - X-ray diffraction
    - Determination of DNA structure (by Watson and Crick)
  - X-ray crystallography
    - Pure protein crystals : regular packed arrays of molecules
    - Deduction of arrangement of atoms using X-ray diffraction data
- NMR (Nuclear Magnetic Resonance)
  - Magnetic properties of certain atomic nuclei (H, C)
  - Use highly concentrated pure solutions of protein
  - Application to medical imaging