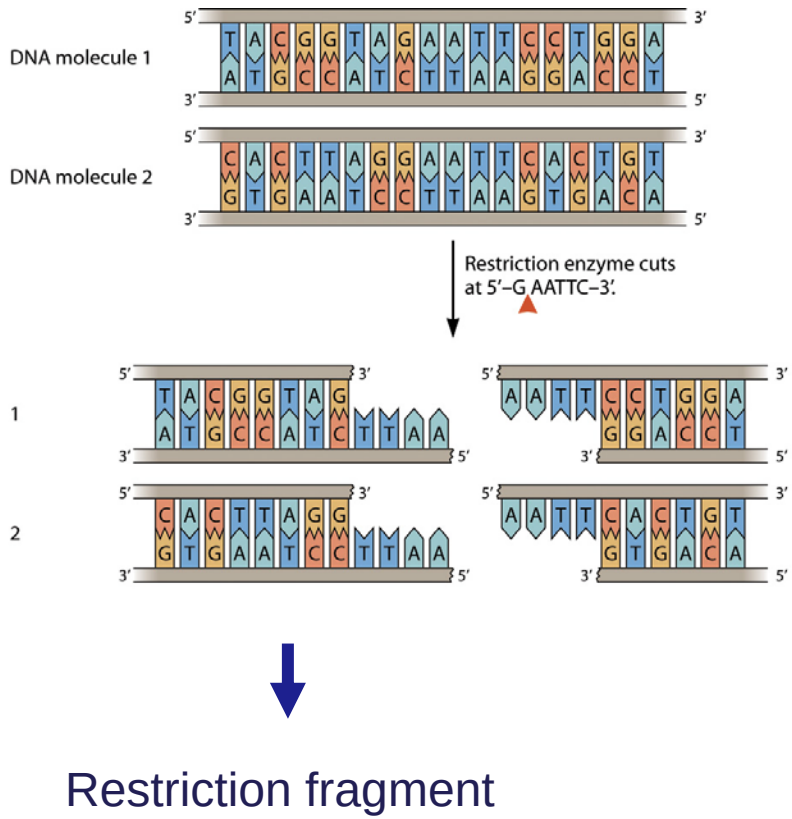
Chapter 15

The Biotechnology Toolbox

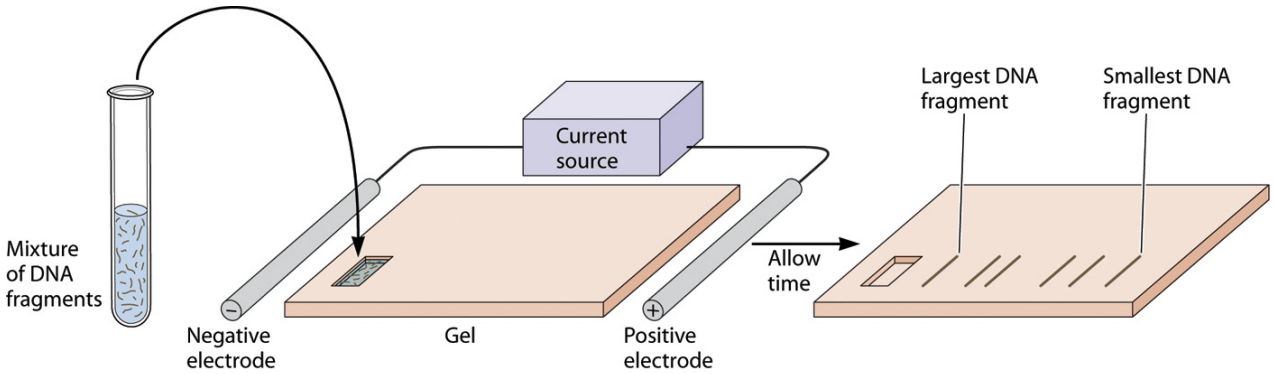
Cutting and Pasting DNA

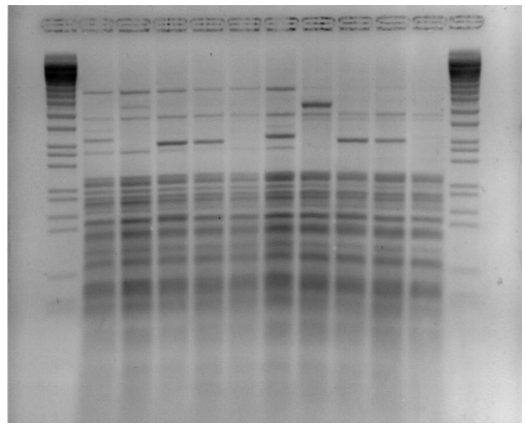
- Cutting DNA
  - Restriction endonuclease or restriction enzymes
  - Cellular protection mechanism for infected foreign DNA
  - Recognition and cutting specific sites of DNA
    - Many recognition sites are palindromic
      - e.g. 5’-GAATTC-3’



Separating mixtures of DNA fragments

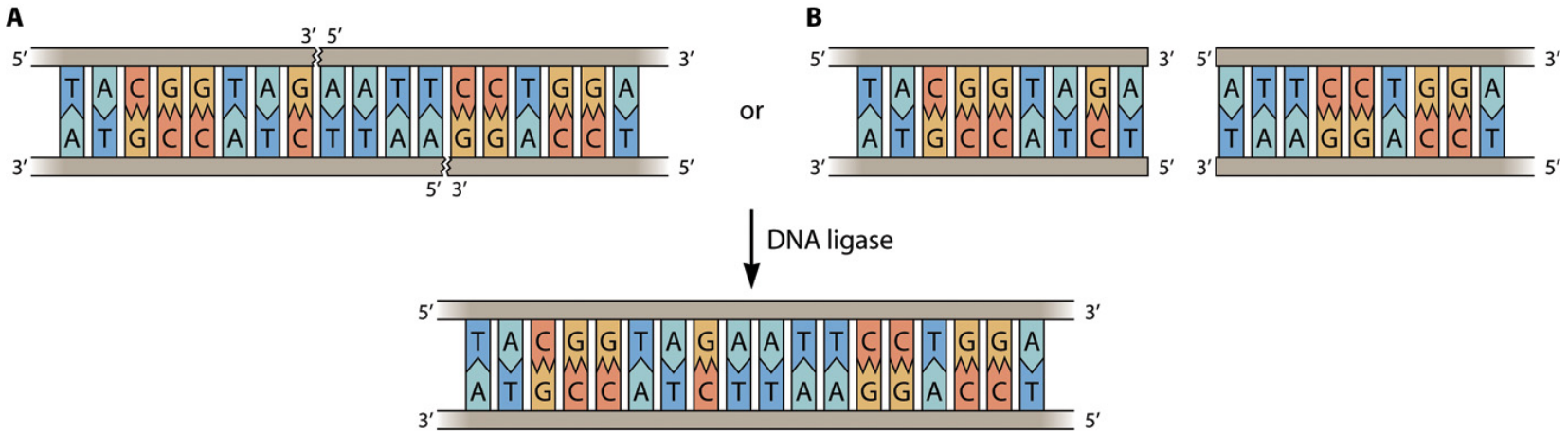
- Electrophoresis
  - Gels
    - Agarose : broad range of resolution
    - Polyacrylamide : high resolution for smaller DNA
  - Migration of DNA to the positive electrode under the electric current
  - Separation of DNA molecules by molecular weight ($L=k1/\log_{10}MW$) and shape
  - Staining of DNA for visualization (Ethidium bromide)





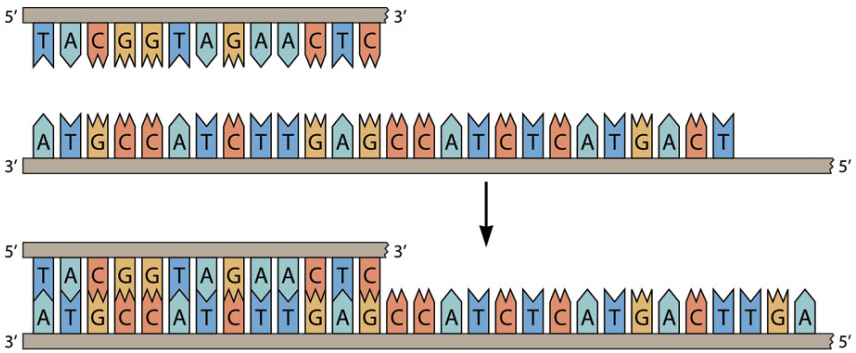
Pasting DNA

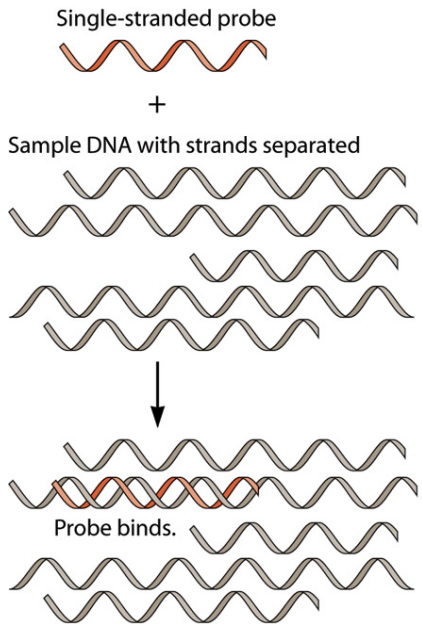
- DNA ligase
  - Joins DNA by forming new phosphodiester bond
- Recombinant DNA
  - DNA generated by joining DNA pieces from different sources



Hybridization Analysis

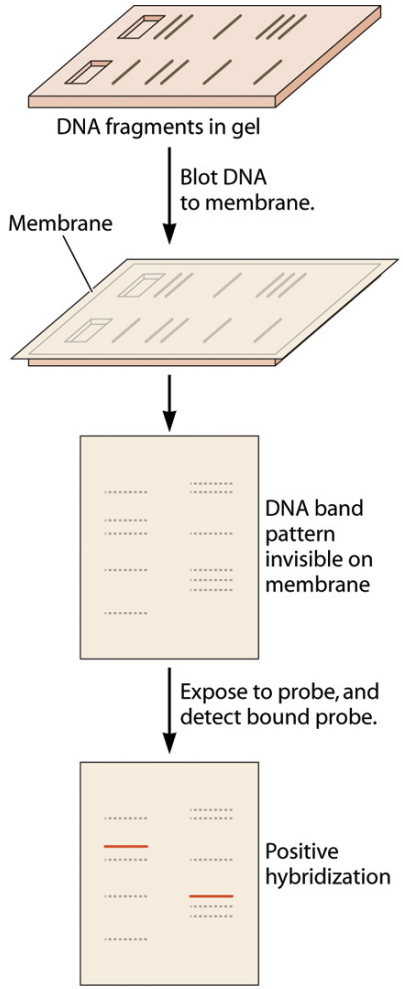
- Hybridization
  - Forming double strain DNA by complementary base paring
  - Procedure
    - Denaturation: making ssDNA by heating
    - Hybridization with labeled ssDNA or ssRNA probe
      - Radioisotope labeling
      - Fluorescence labeling
    - Detection of hybridized products





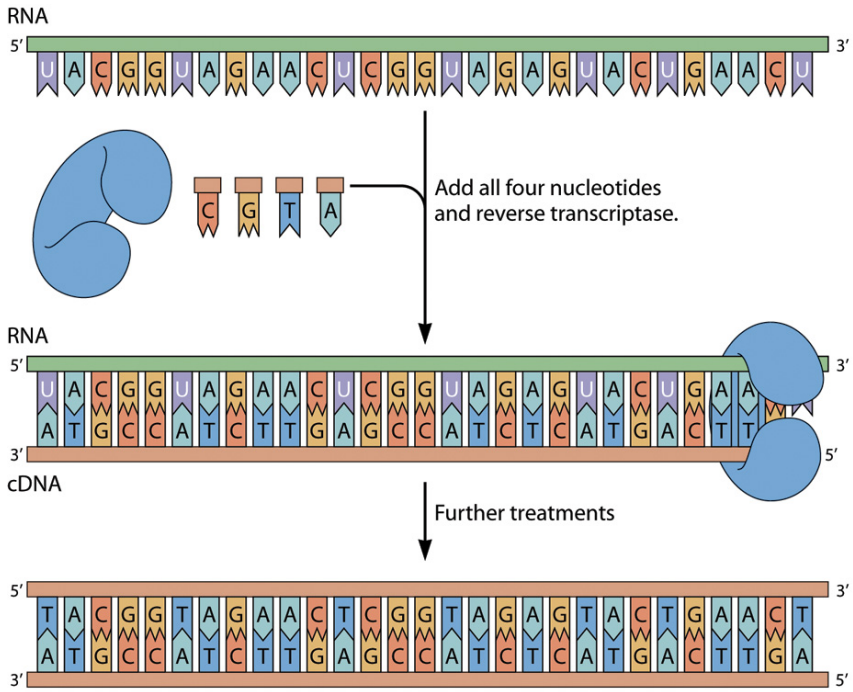
Hybridization Analysis

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



Polymerase Chain Reaction (PCR)

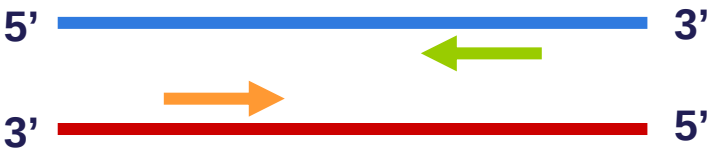
- PCR
  - Invented by Kary Mullis (1983)
    - Nobel prize in chemistry in 1993
  - Amplification of specific DNA sequence
  - Reaction mixture
    - DNA template, 2 primers, DNA polymerase (heat-resistant), dNTPs
  - Reaction conditions
    - Denaturation of DNA (95^{o}C)
    - Primer annealing (30~60^{o}C)
    - DNA synthesis (72^{o}C)

Repeat

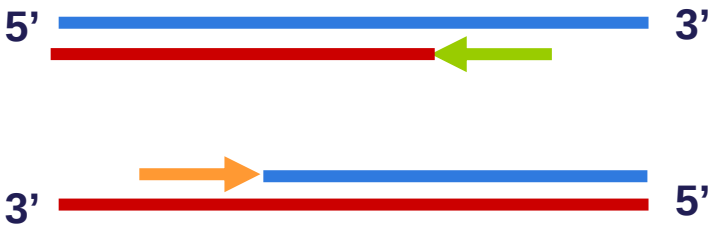
Polymerase Chain Reaction (PCR)

First cycle

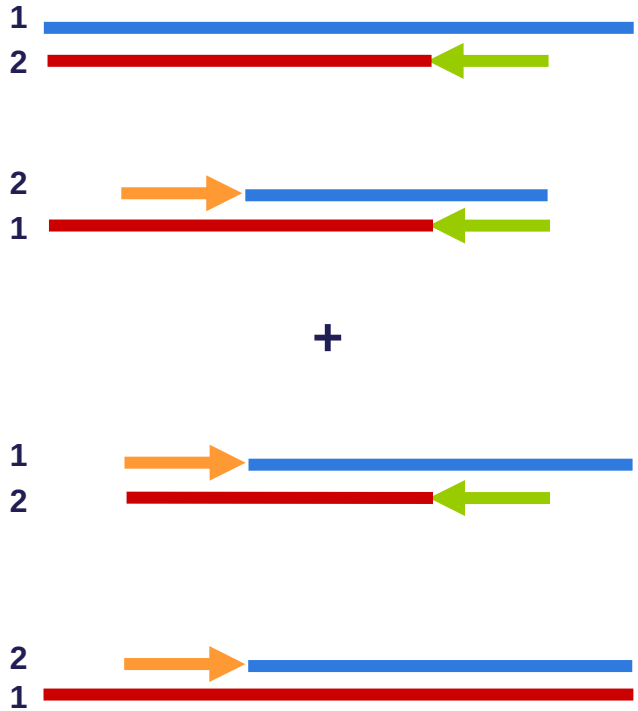
Denaturation
Primer annealing



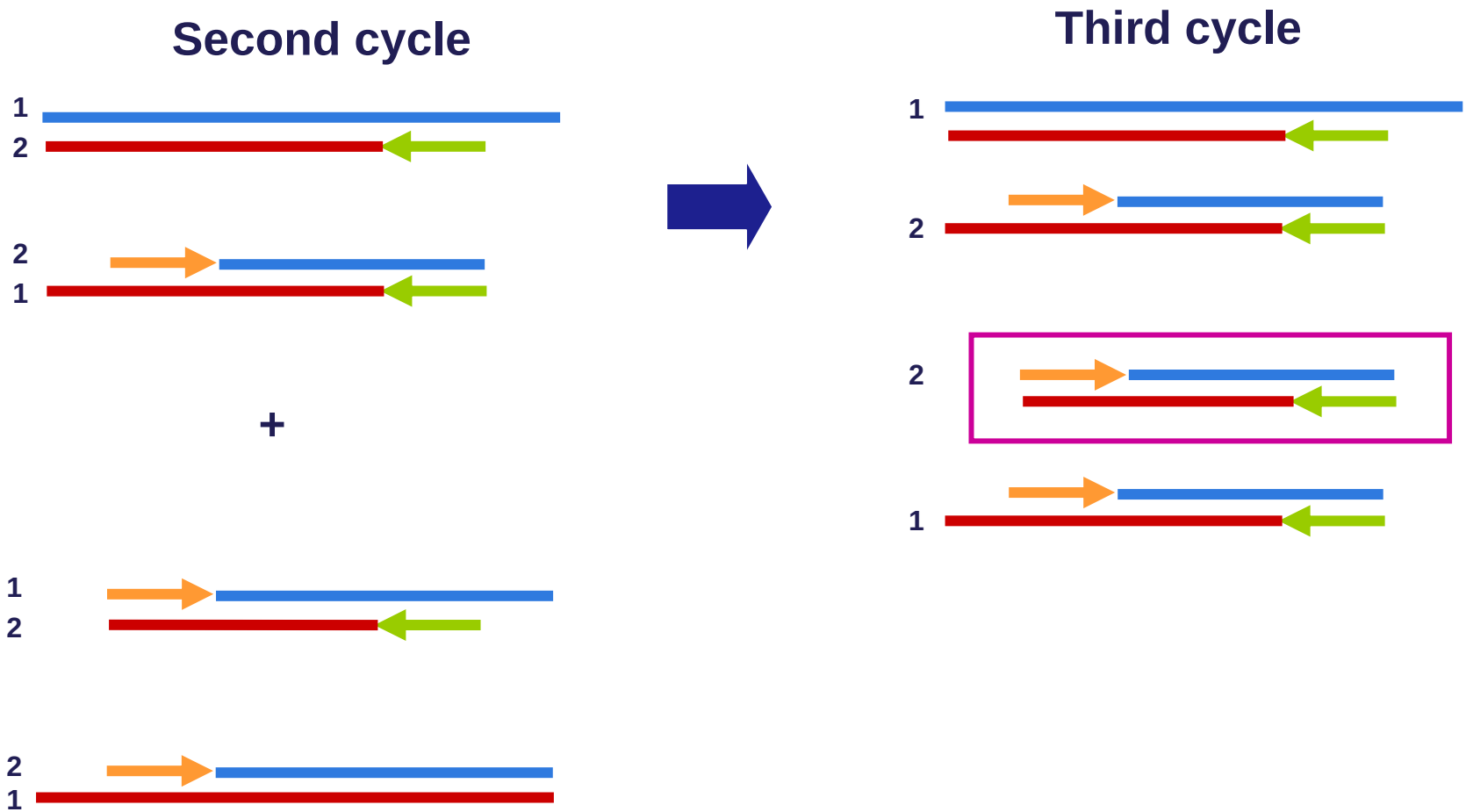
DNA synthesis



Second cycle

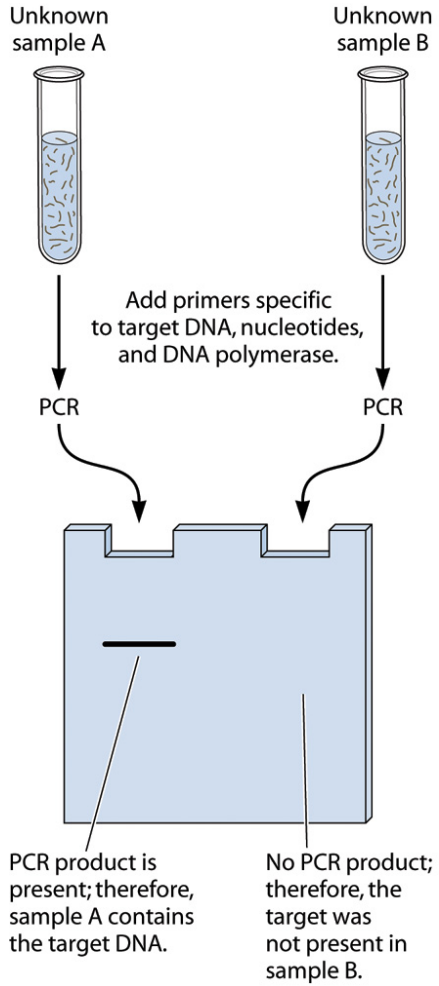


Polymerase Chain Reaction (PCR)



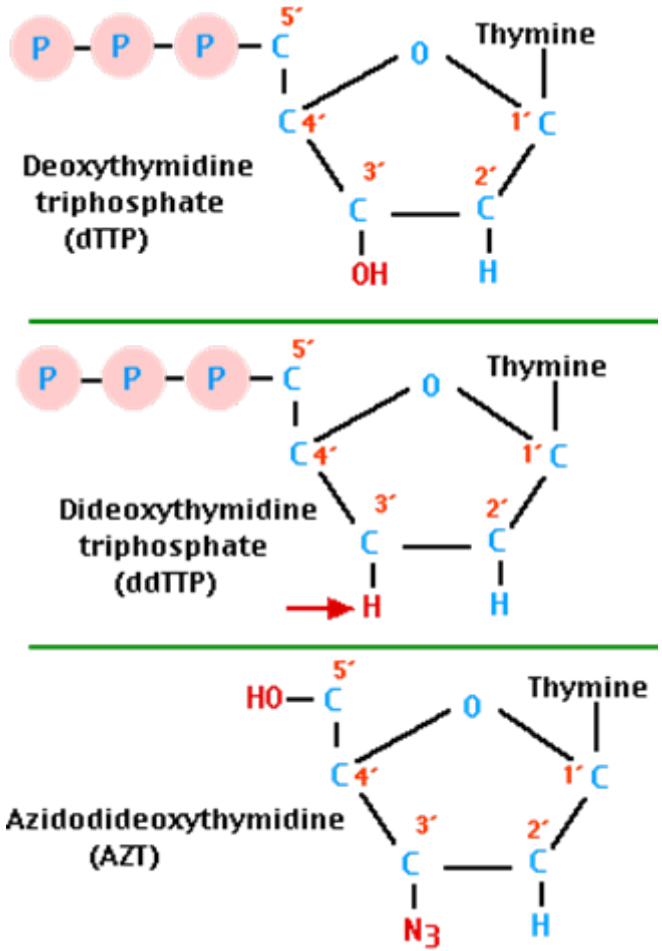
PCR As a Detection Method

- 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



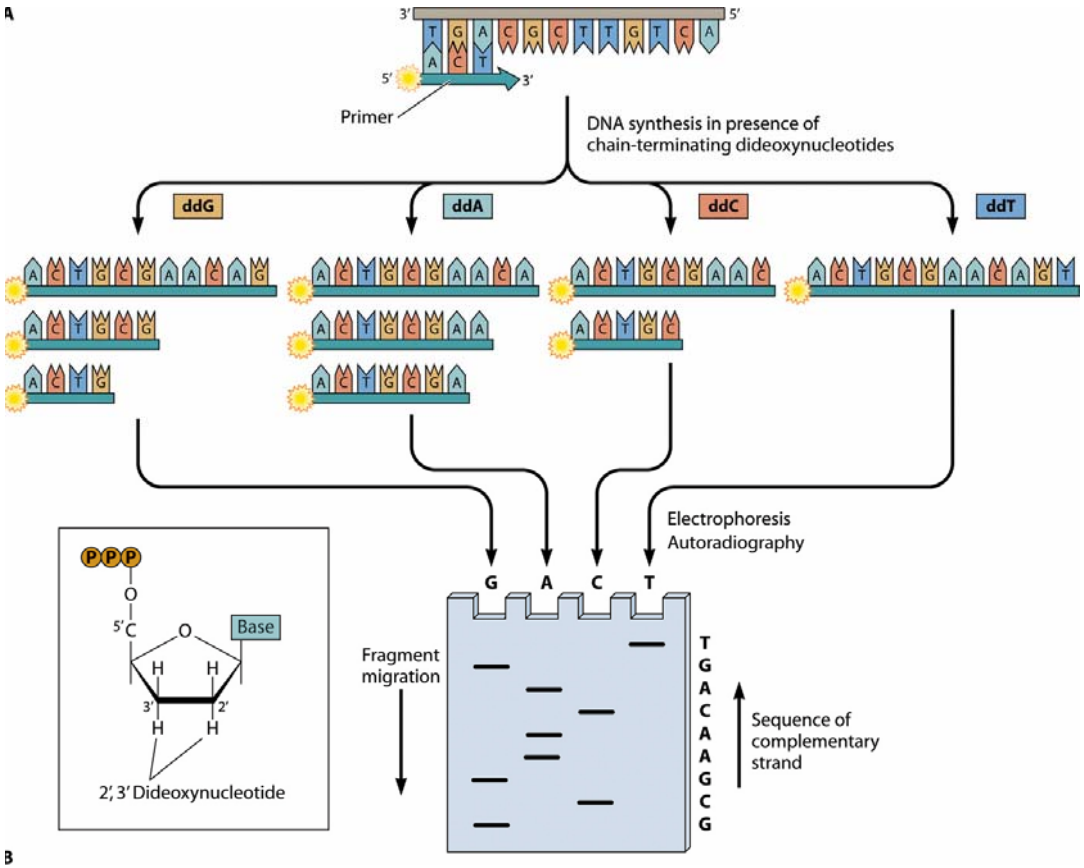
DNA Sequencing

- DideoxyNTP
  - Chain termination
  - Sanger (1977)



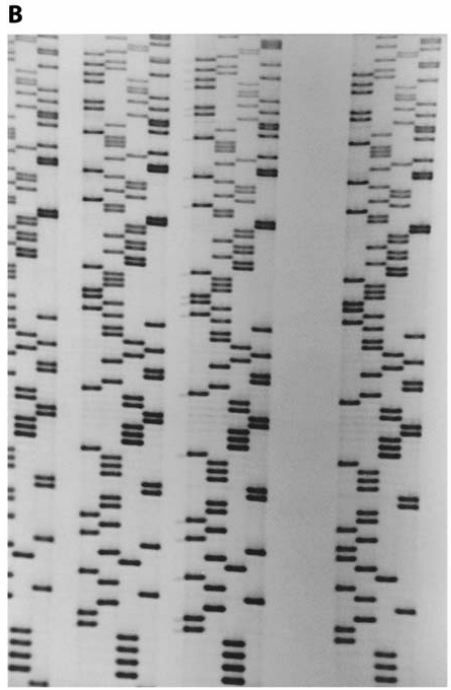
Anti-AIDS drug

Chain Termination Sequencing



T7 DNA polymerase (Sequenase)

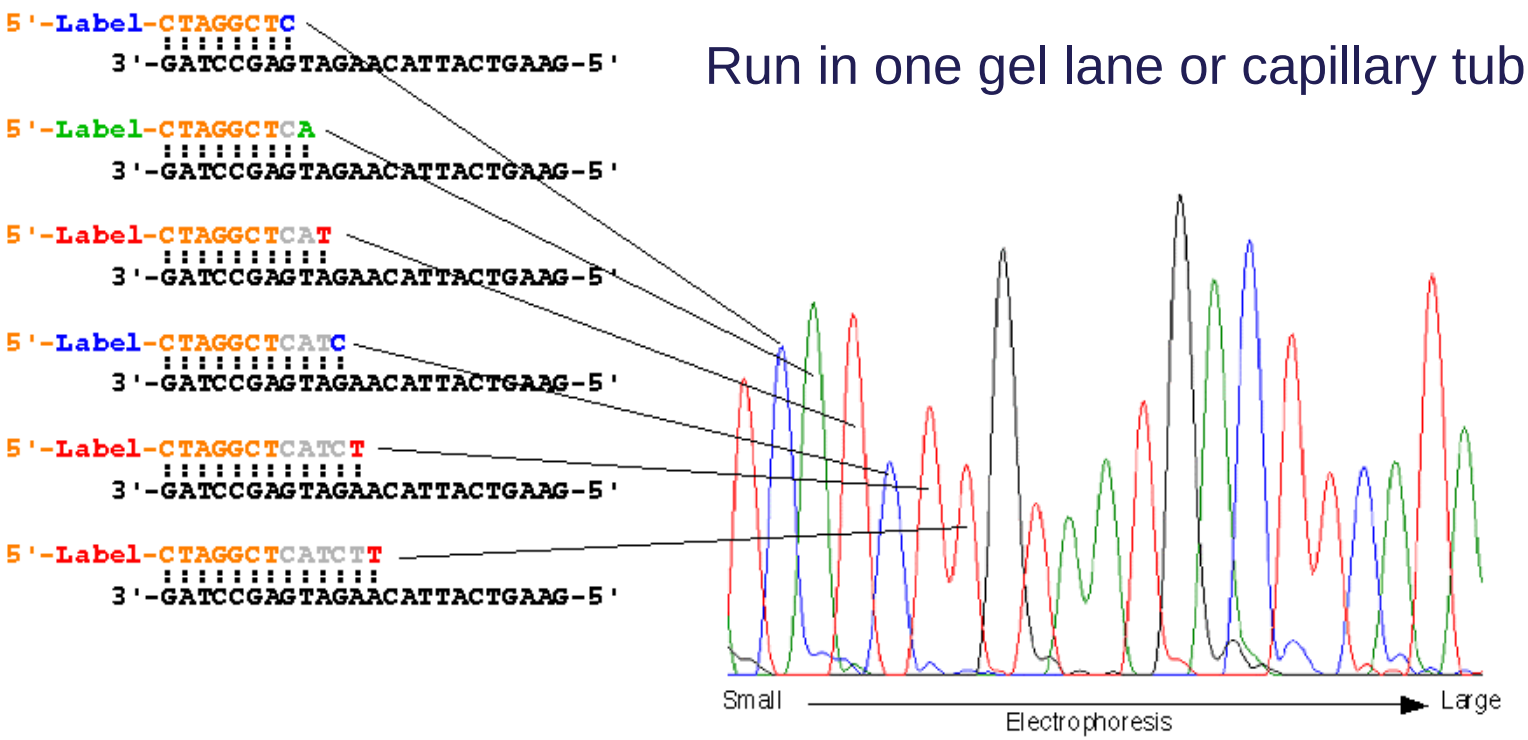
Taq polymerase



Automated DNA Sequencing

Label four ddNTPs with different fluorescent dyes

Run in one gel lane or capillary tube

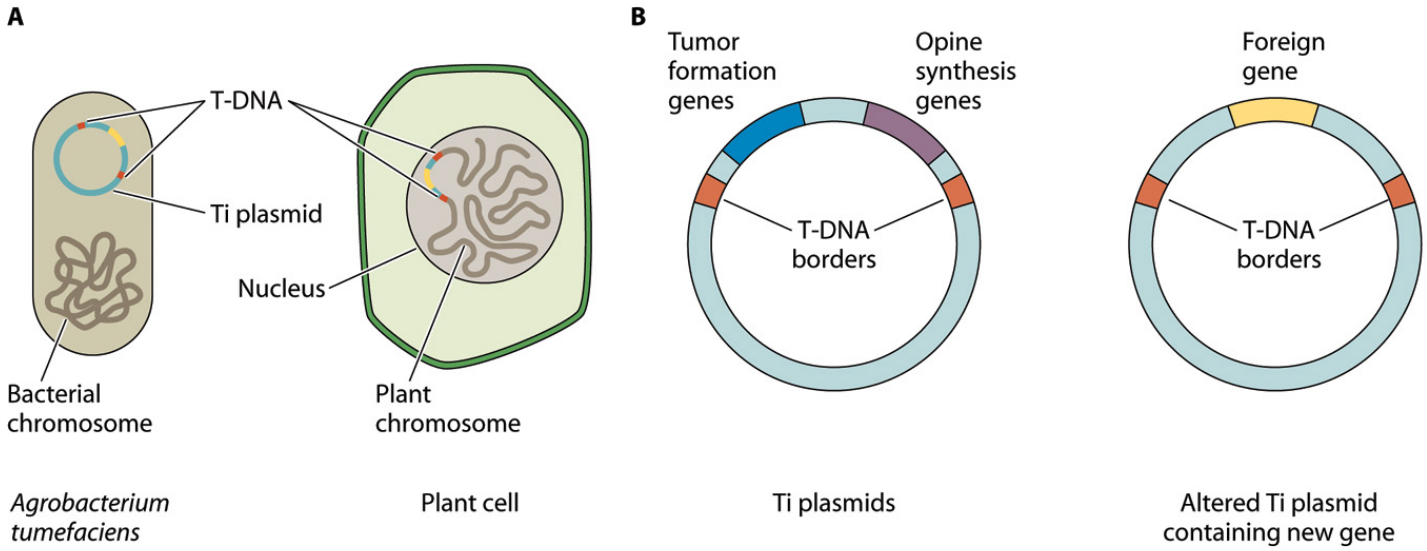


Cloning

- 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 plsmid in Agrobacterium tumefaciens
  - Transfer T-DNA into plant DNA and induce tumor
  - Replace T-DNA with gene of interest

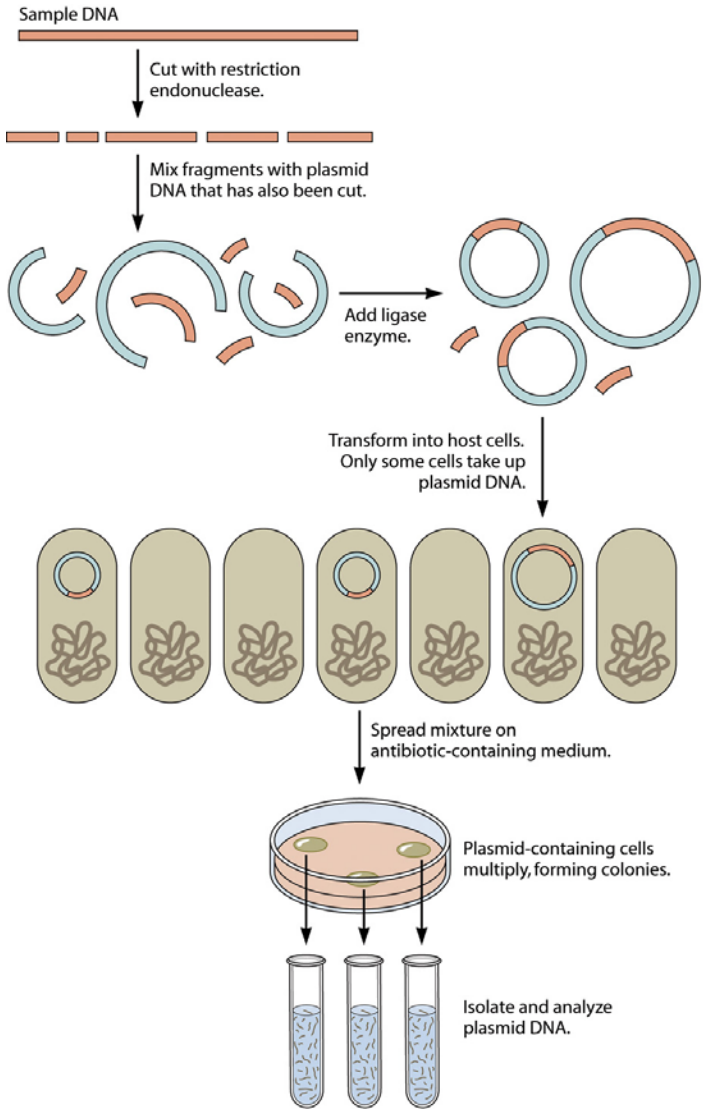


Introduction of DNA

- 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 gene of interest
    - PCR
    - Sequencing
    - Restriction digestion

Cloning Procedure

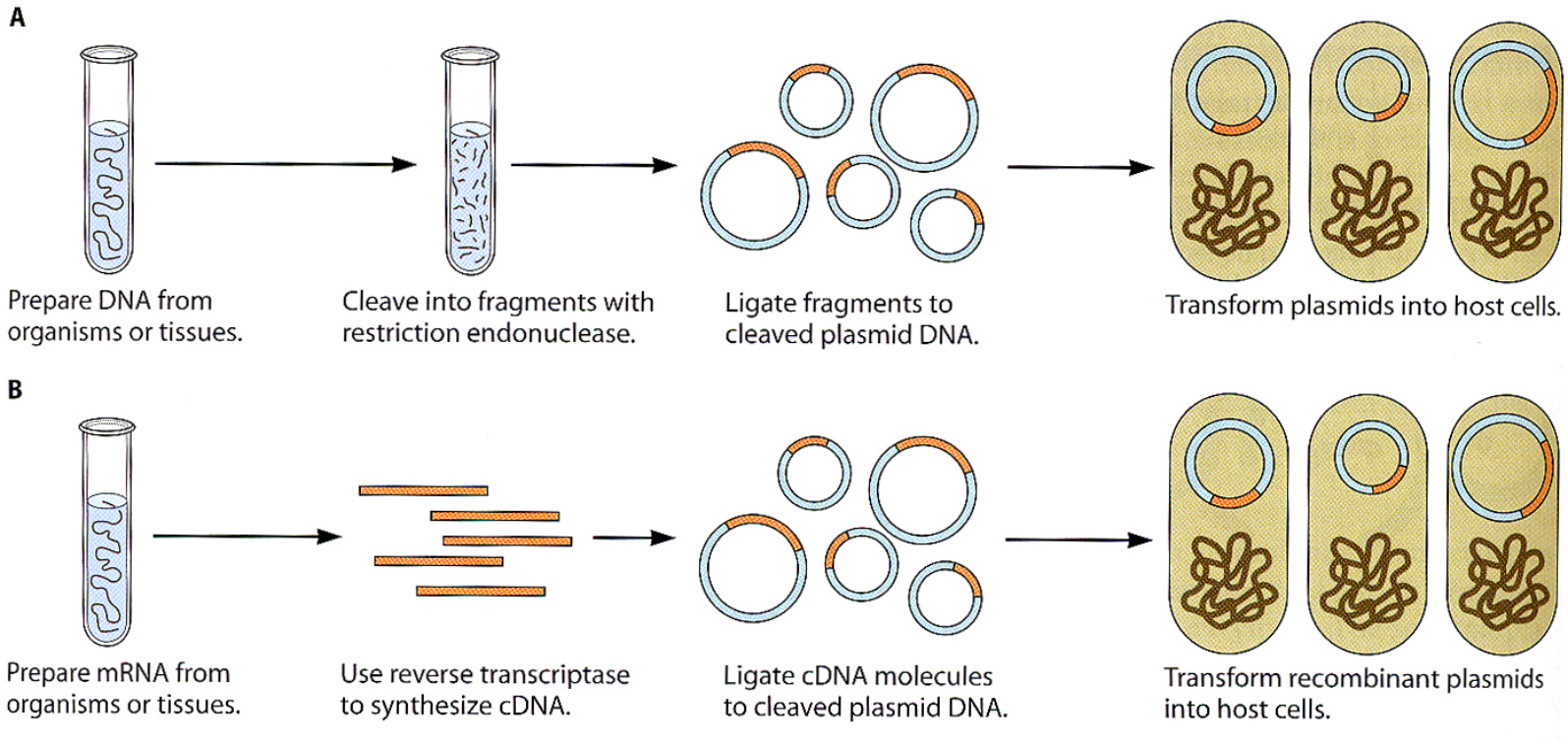
- Ligation of vector and insert
  - Insert DNA : restriction fragment or PCR product
- Introduction into host
- Selection of plasmid-containing cells using marker
- Isolation and analysis of plasmids



DNA Library

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

Genomic vs. cDNA Library



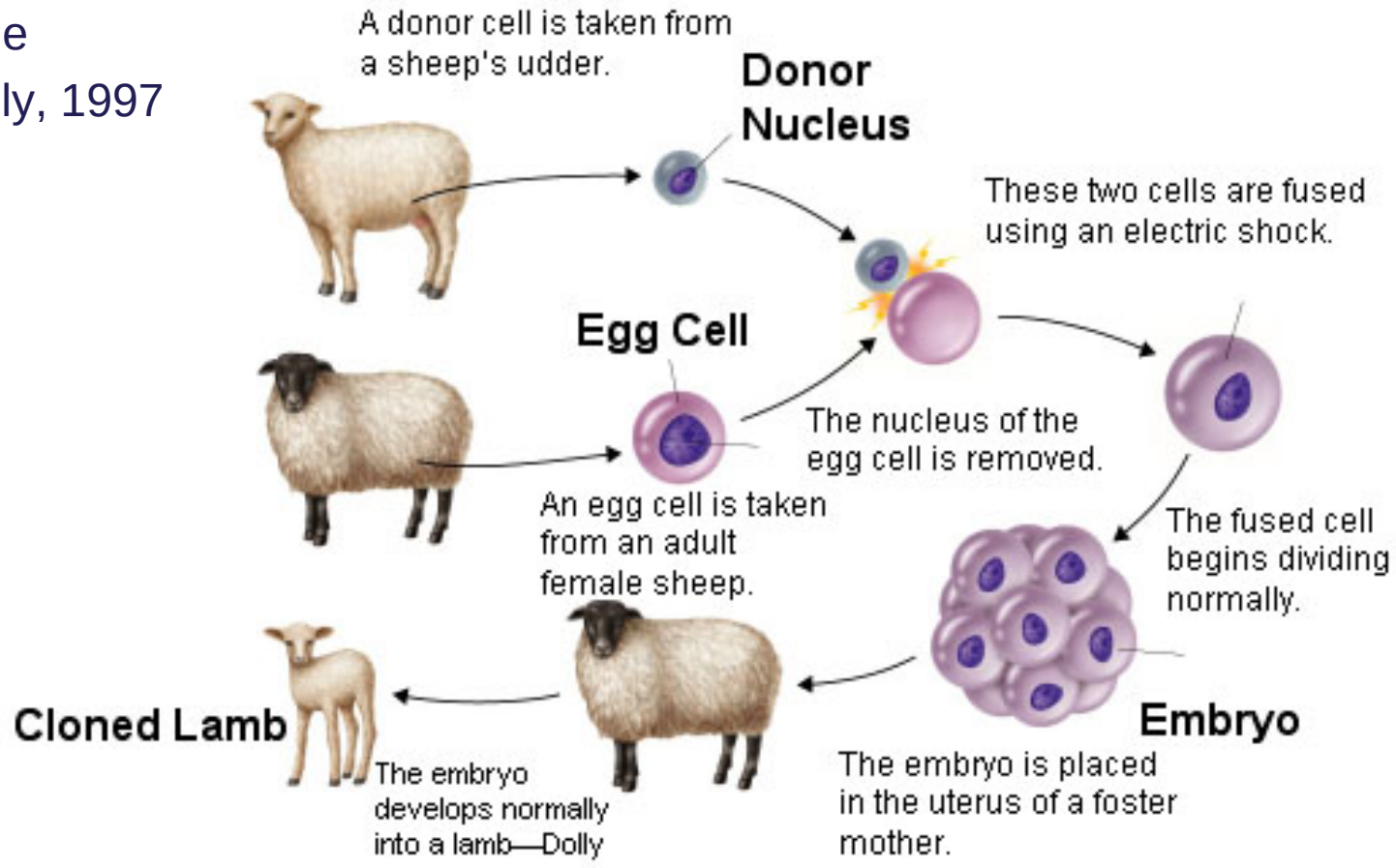
Cloning Complex Organisms

- 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

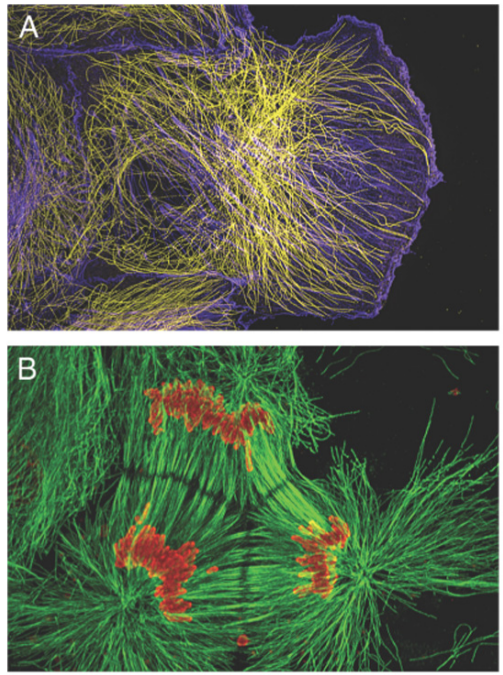
Adult mammary epithelial cell line

→ Dolly, 1997



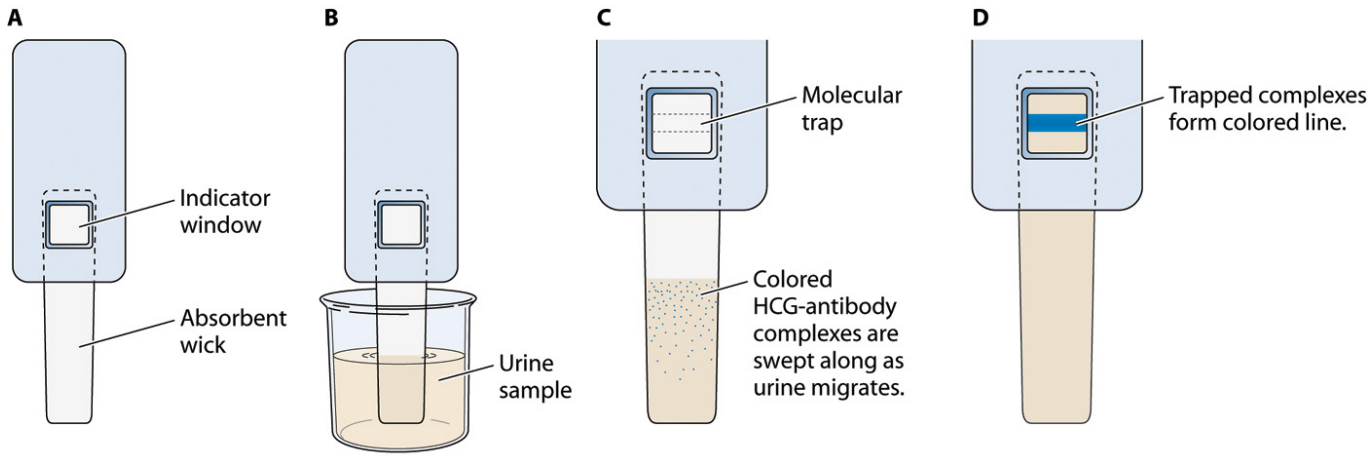
Analyzing Proteins by Antibody

- Monoclonal antibodies
  - Pure antibody: generated by B cells→ no cell division in culture
  - Fusion of B cells with cancerous cells (myeloma cells)
    - infinite 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)



- Test for strep throat
  - Detection of Streptococcus pyogenes using antibody

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
  - X-ray crystallography
    - Pure protein crystals : regular packed arrays of molecules
    - Deduction of arrangement of atoms using X-ray diffraction data
- NMR
  - Magnetic properties of certain atomic nuclei (H, C)
  - Use highly concentrated pure solutions of protein
  - Application to medical imaging