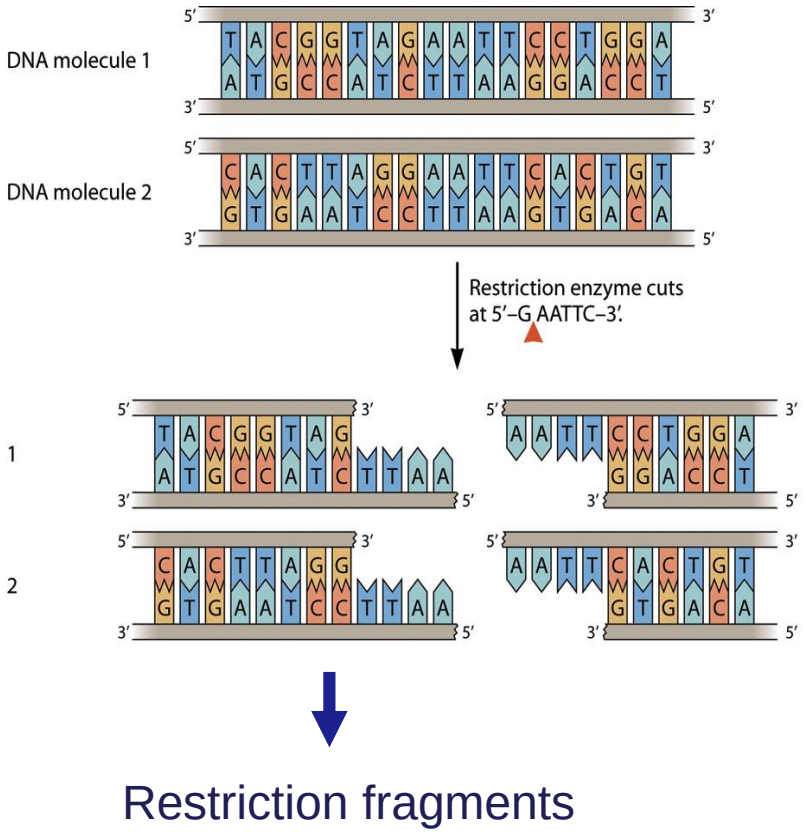
Chapter 15

The Biotechnology Toolbox

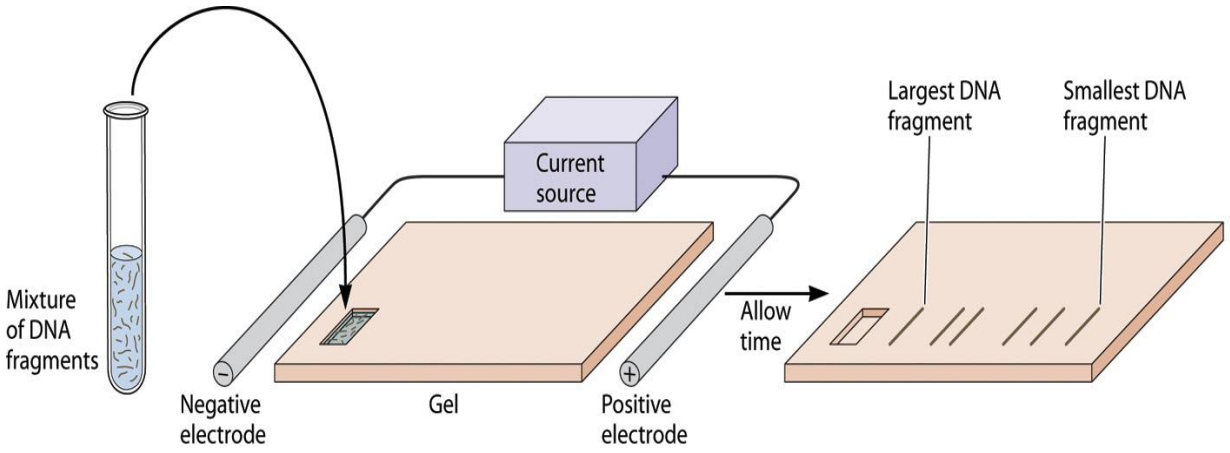
Cutting and Pasting DNA

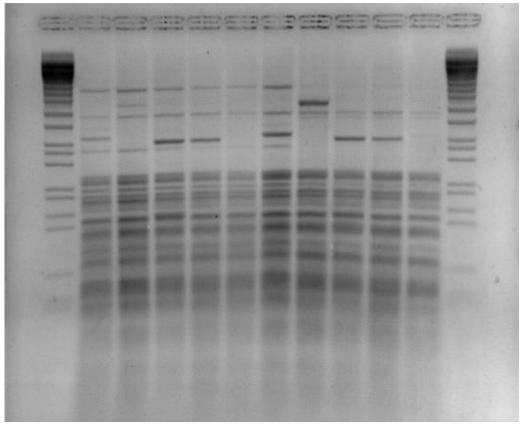
- Cutting DNA
  - Restriction endonuclease or restriction enzymes
  - Cellular protection mechanism for infected foreign DNA
  - Recognition and cutting specific sites of DNA
    - Recognition sites are usually palindromic
      - e.g. 5’-GAATTC-3’



Separating mixtures of DNA fragments

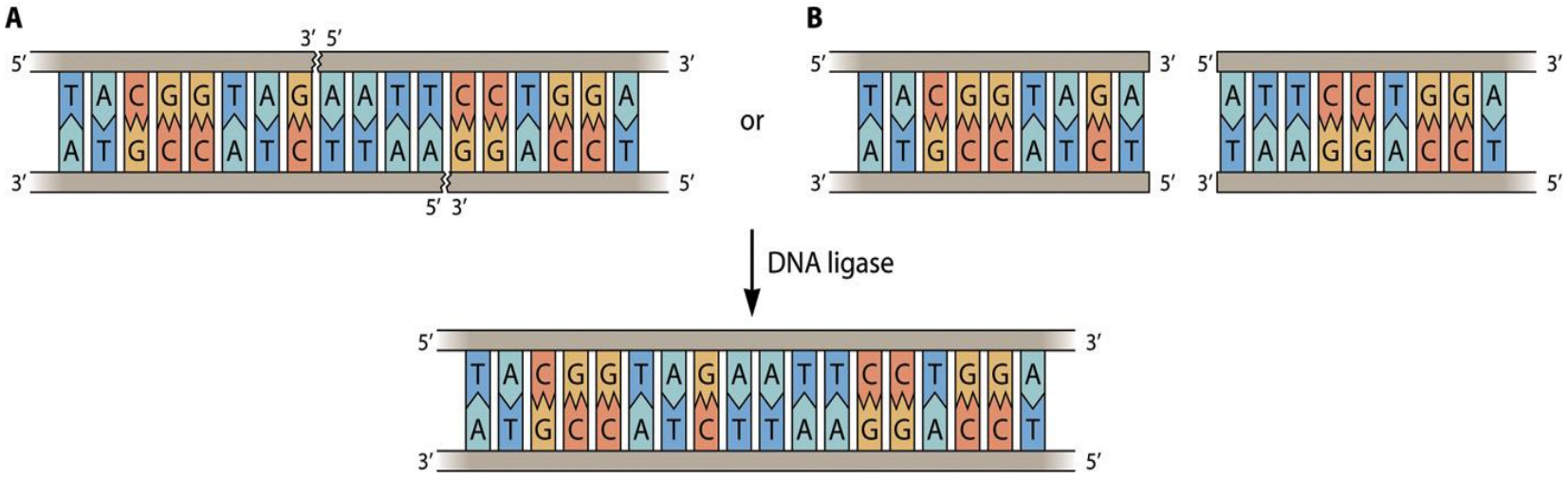
- Electrophoresis
  - Gels
    - Agarose : broad range of resolution
    - Polyacrylamide : high resolution for smaller DNA
  - Migration of DNA to the positive electrode under the electric current
  - Separation of DNA molecules by molecular weight and shape
    - $L = k\ 1/\log_{10}MW$ for linear DNA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





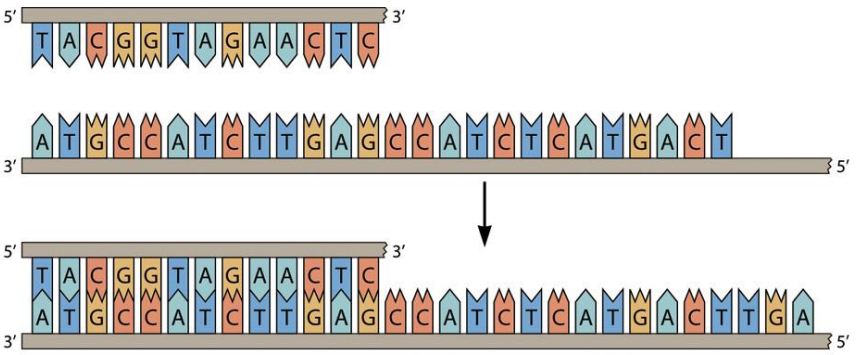
Pasting DNA

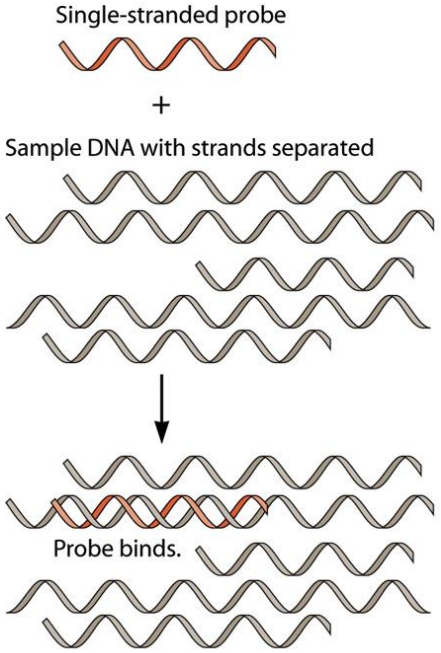
- DNA ligase
  - Joins DNA by forming new phosphodiester bond
- Recombinant DNA
  - DNA generated by joining DNA pieces from different sources



Hybridization Analysis

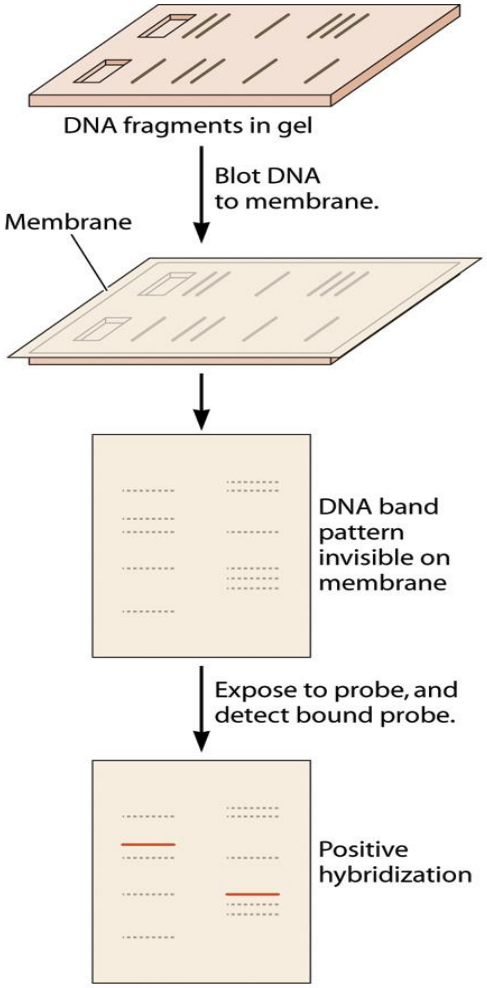
- Hybridization
  - Forming double strand DNA by complementary base paring
  - Procedure
    - Denaturation: making ssDNA by heating
    - Hybridization with labeled ssDNA or ssRNA probe
      - Radioisotope labeling
      - Fluorescence labeling
    - Detection of hybridized products





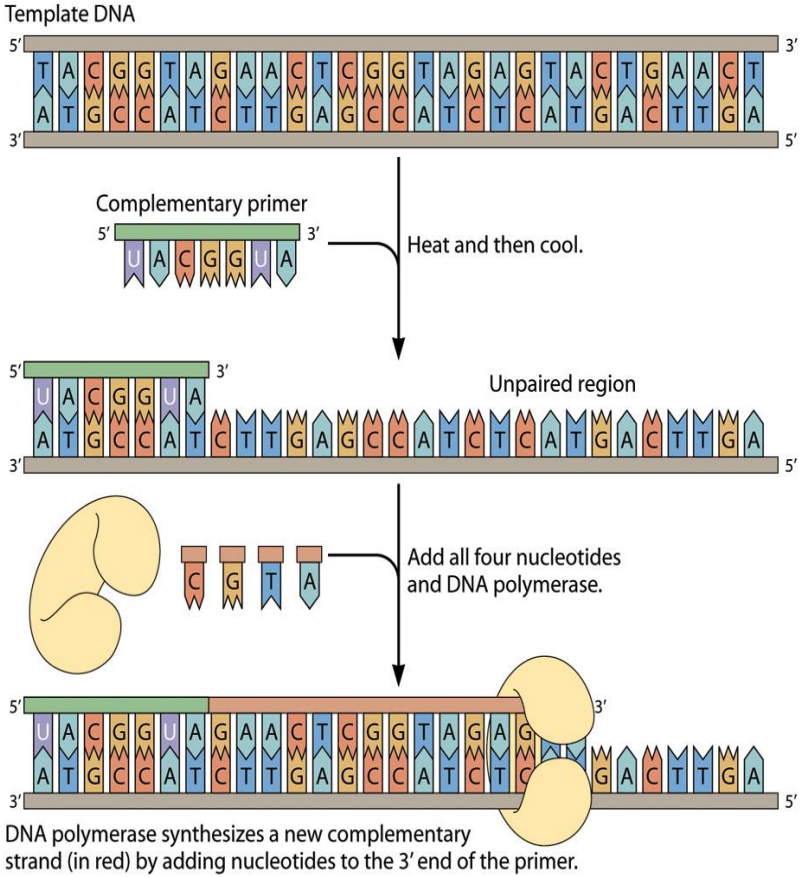
Hybridization Analysis

- 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



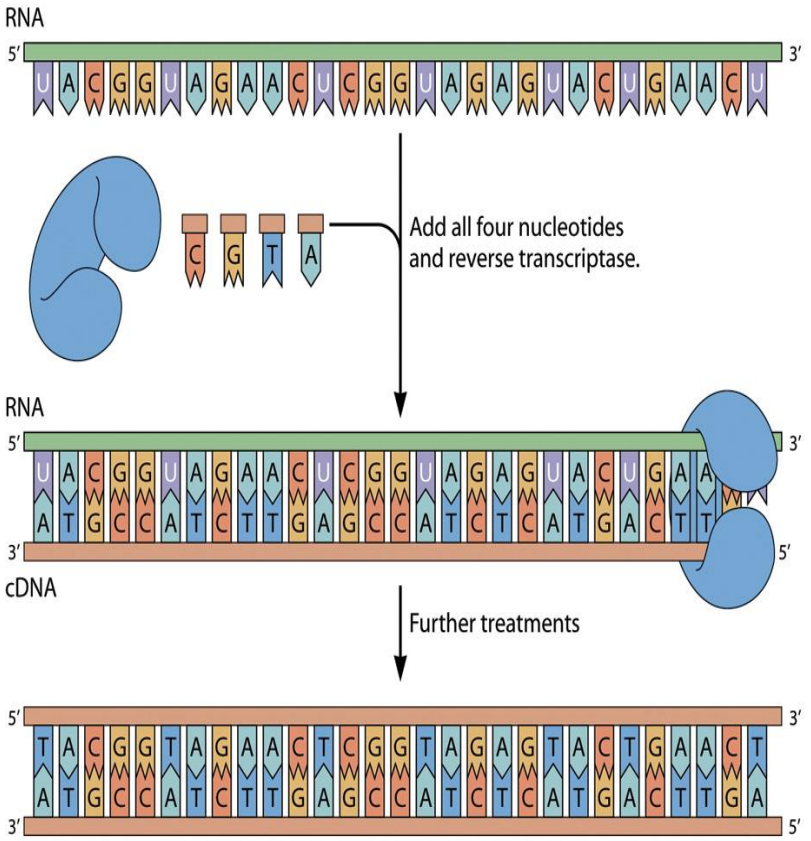
Making DNA in vitro

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



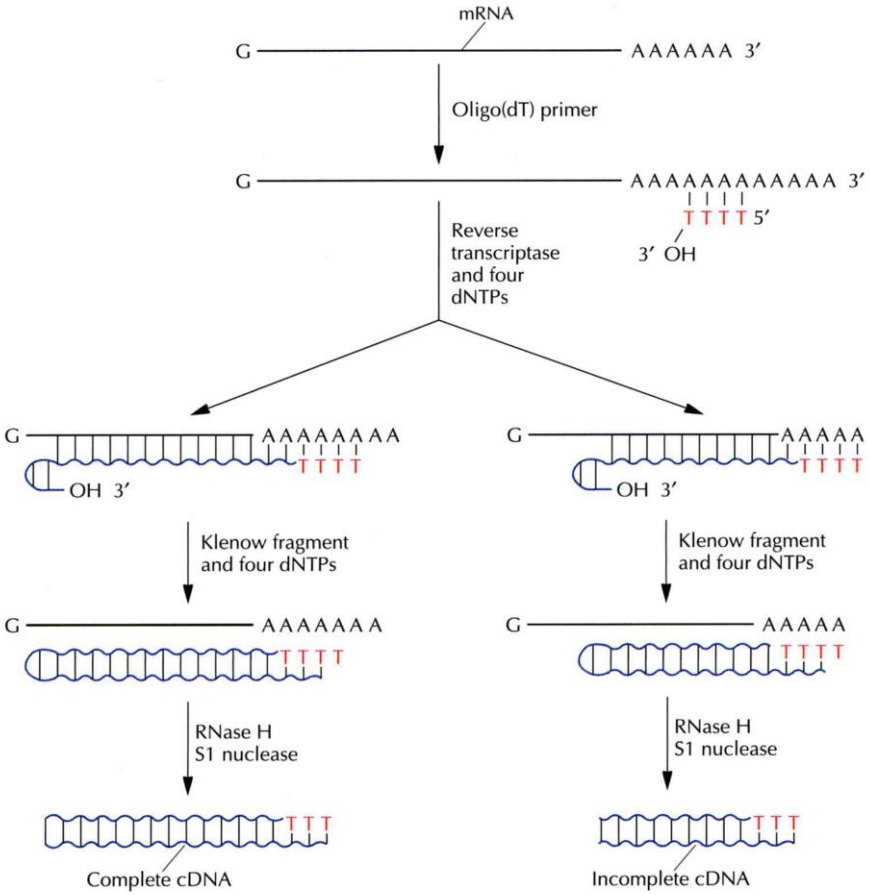
Making DNA from an RNA template

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



Reverse Transcription

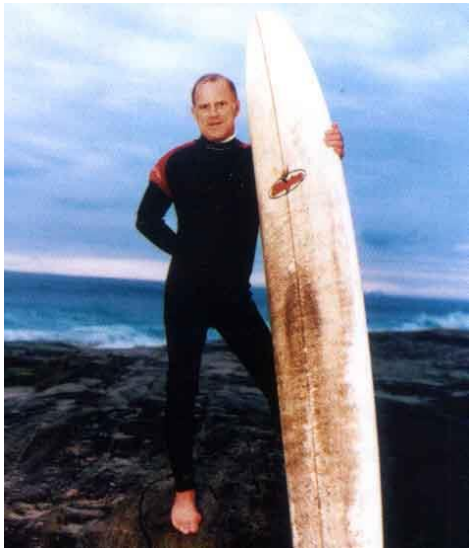
- Klenow fragment
  - a product of proteolytic digest of the DNA polymerase I
- RNase H
  - hydrolyzes mRNA
- S1 nuclease
  - removes hairpin loop



Polymerase Chain Reaction (PCR)

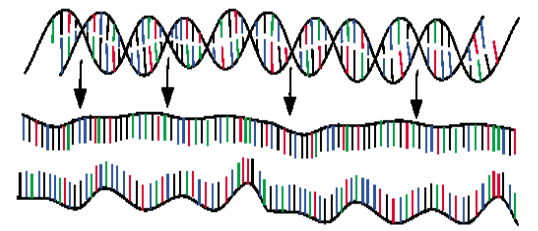
- PCR
  - Invented by Kary Mullis (1983)
  - Amplification of specific DNA sequence
  - Reaction mixture
    - DNA template, 2 primers, DNA polymerase (heat-resistant), dNTPs
  - Reaction conditions
    - Denaturation of DNA at 95^{o}C
    - Primer annealing at 54^{o}C
    - DNA synthesis at 72^{o}C

Repeat



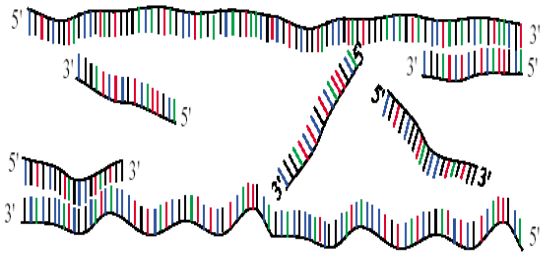
PCR

30 - 40 cycles of 3 steps :



Step 1 : denaturation

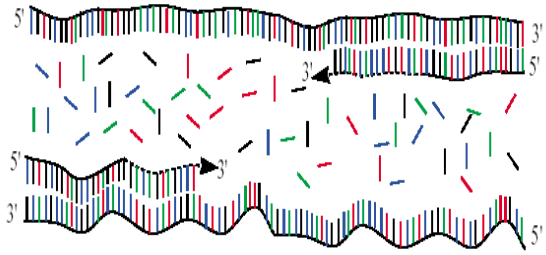
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

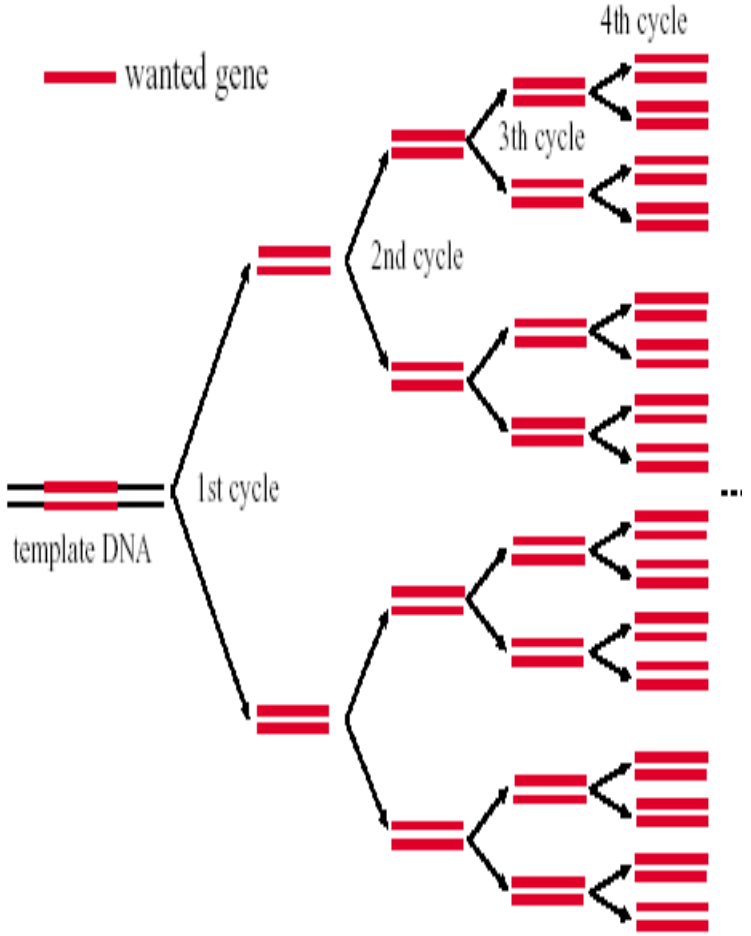


Step 3 : extension

2 minutes 72 °C

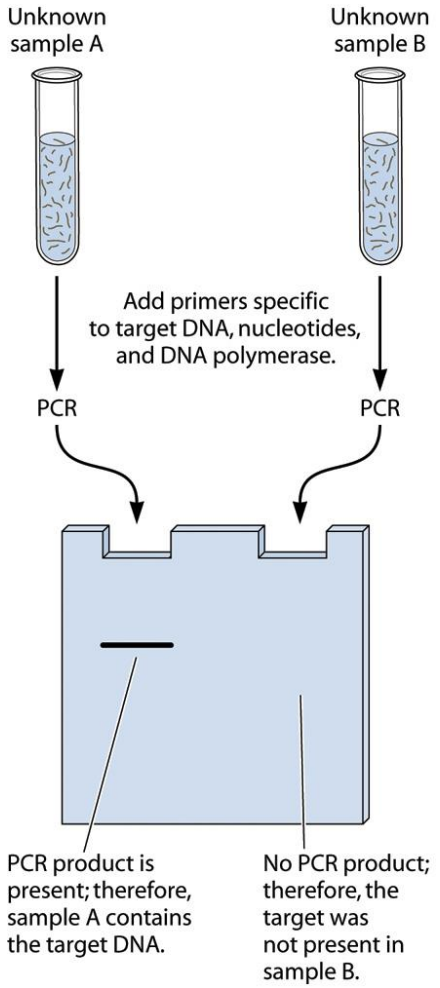
only dNTP's

(Andy Vierstraete 1999)



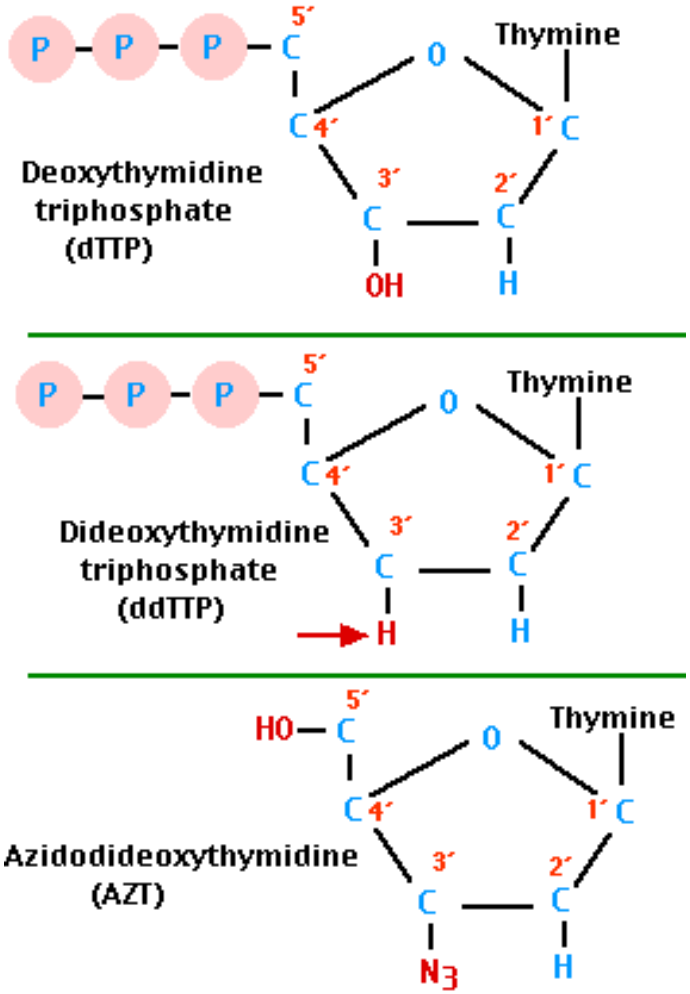
PCR as a Detection Method

- 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



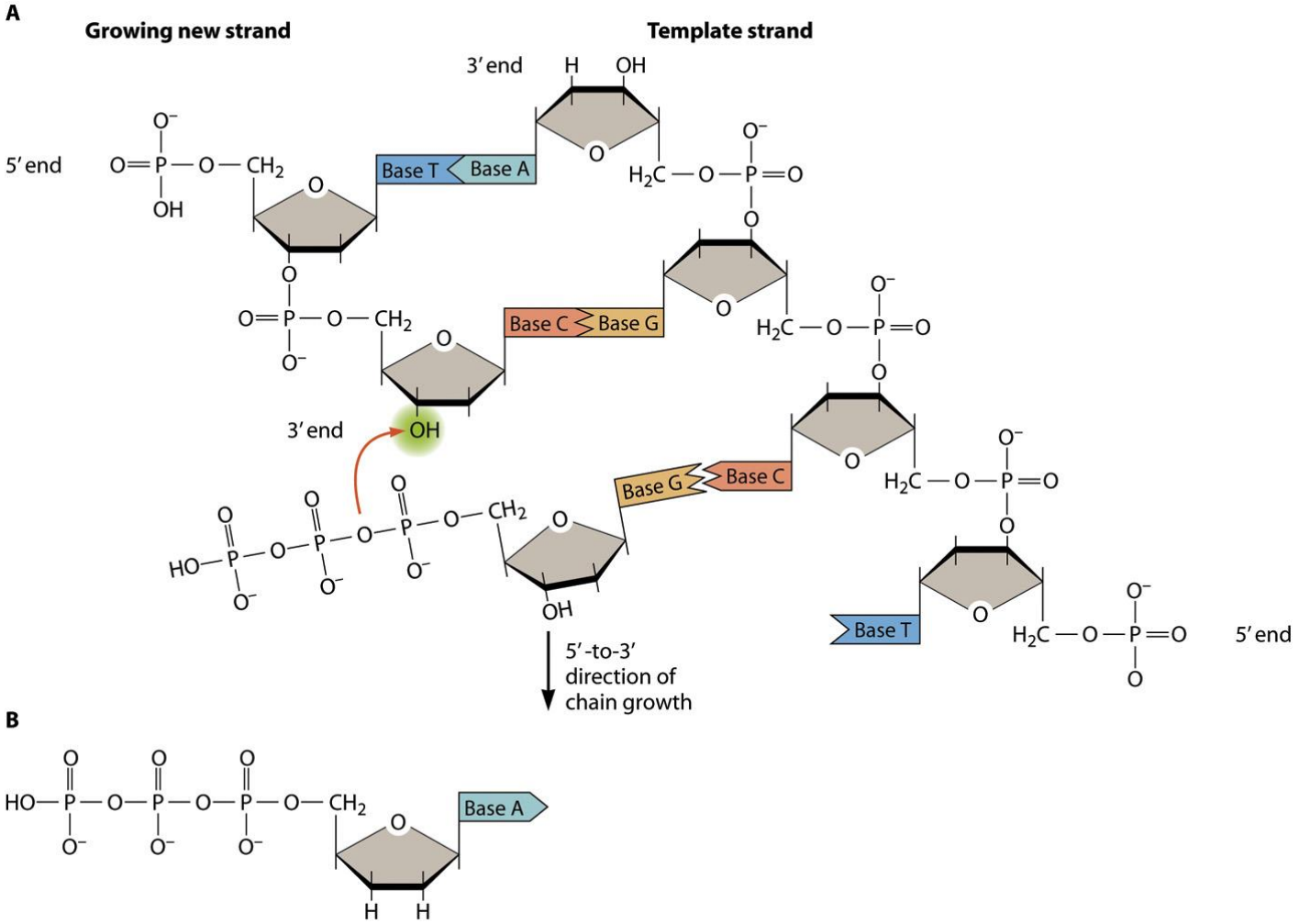
DNA Sequencing with terminators

- DideoxyNTP
  - Chain termination
  - Sanger (1977)

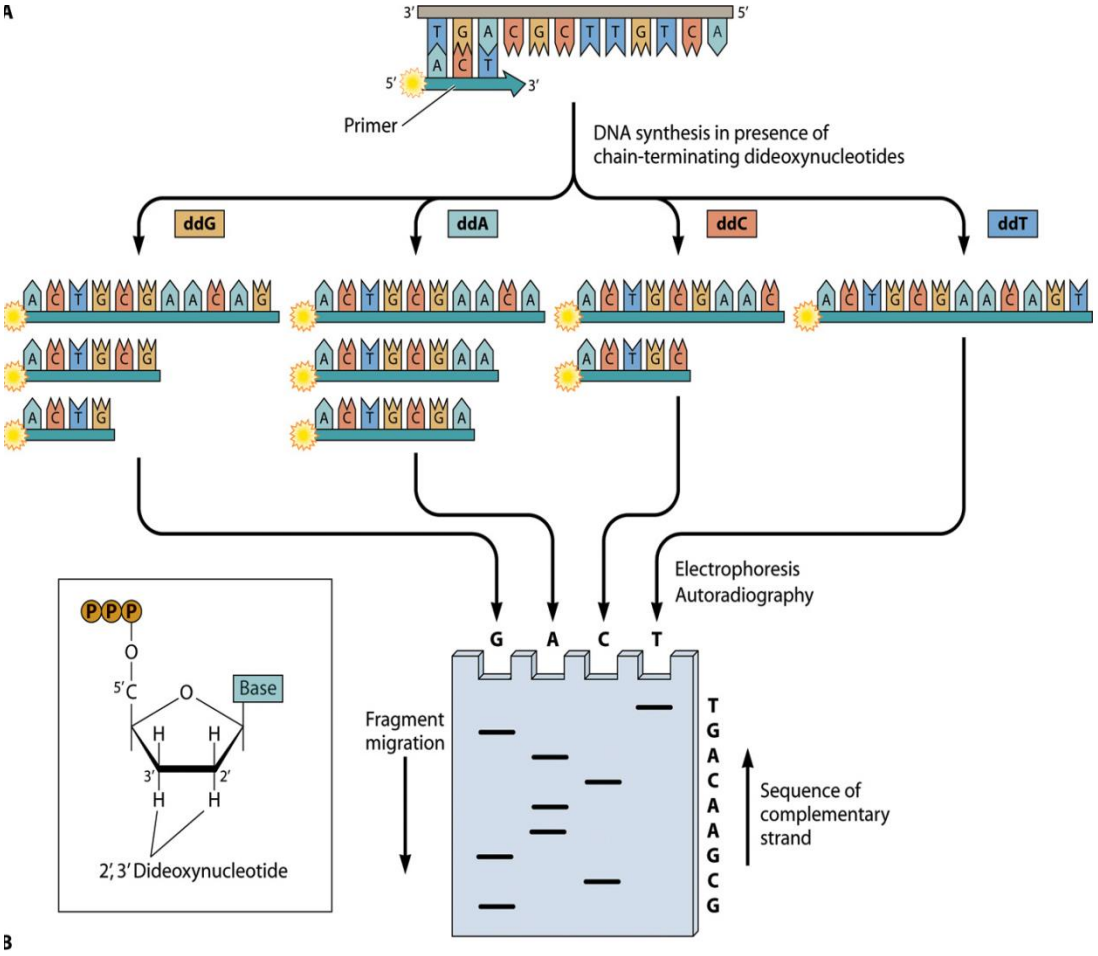


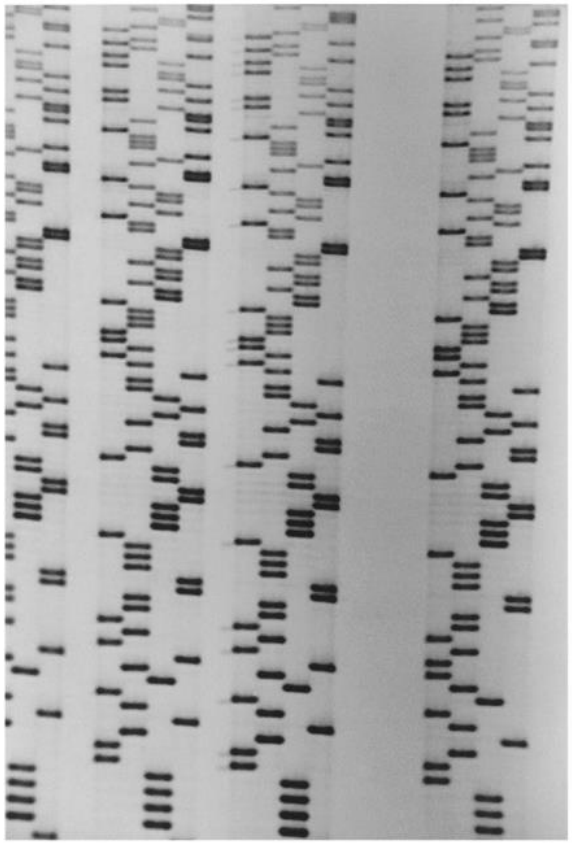
- Anti-AIDS drug (AZT)
  -- HIV is an RNA virus.
  -- Reverse transcriptase is an sloppy enzyme.

Chain termination by ddNTP



Chain Termination Sequencing

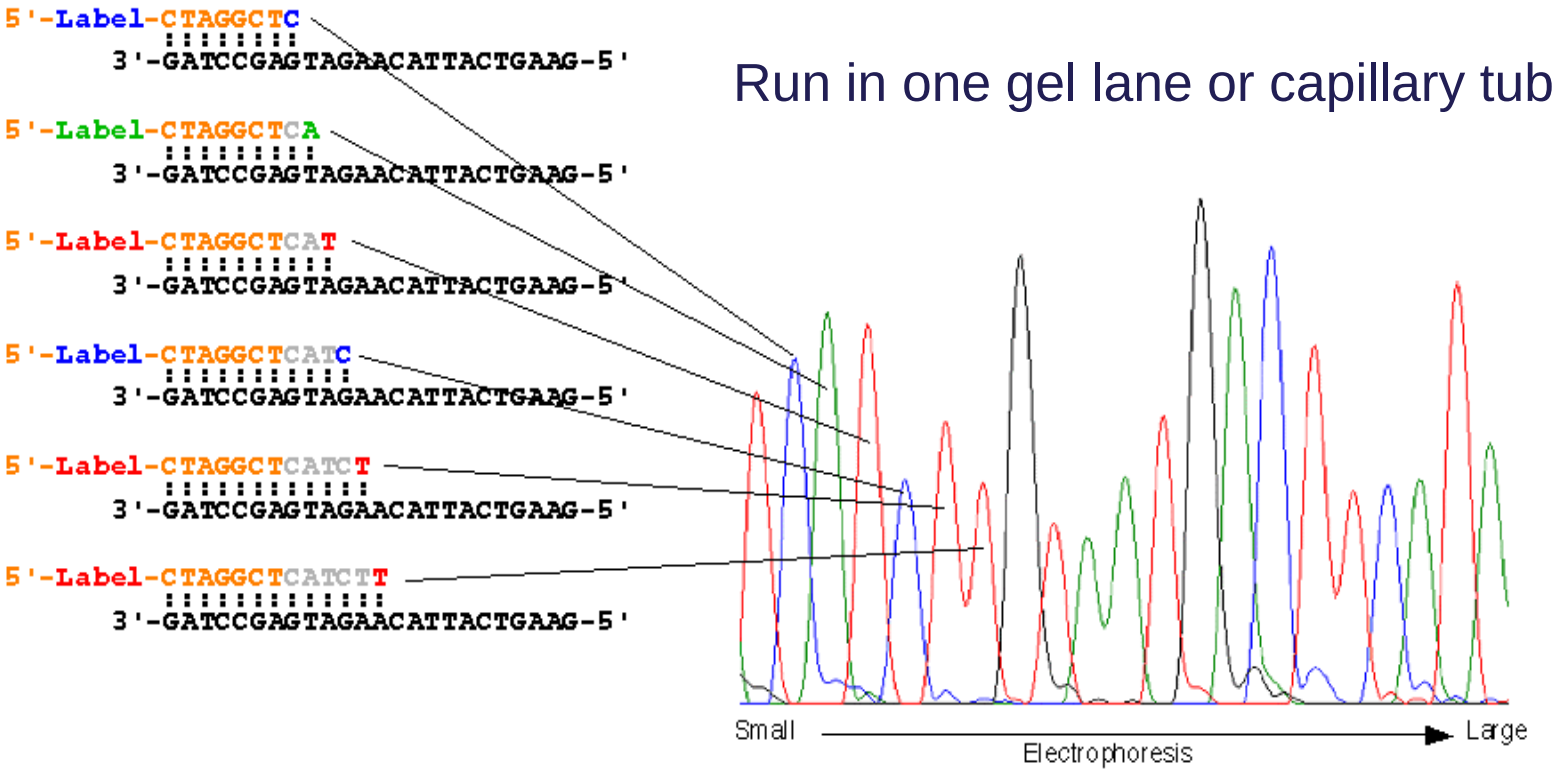




Automated DNA Sequencing

Label four ddNTP with different fluorescent dyes

Run in one gel lane or capillary tube

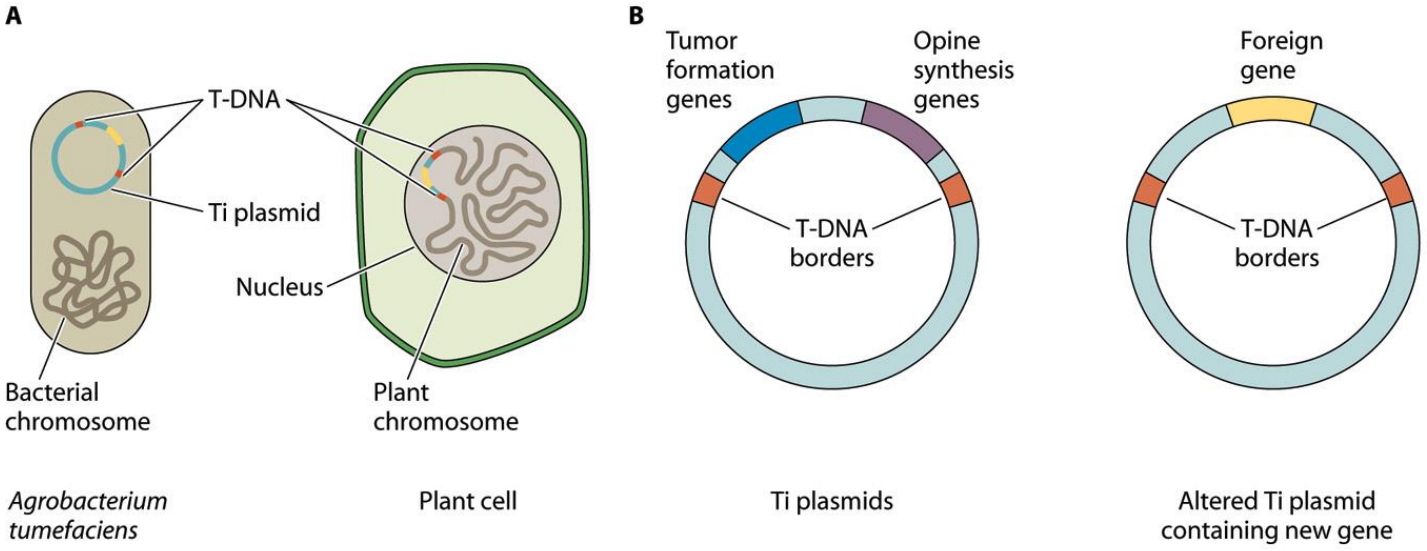


Cloning

- 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

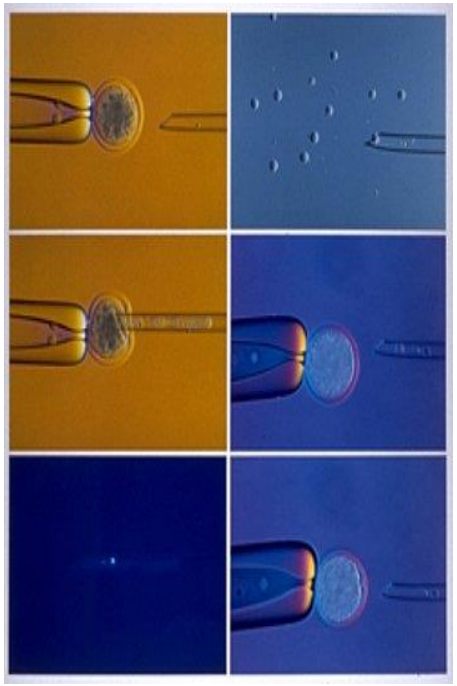
Ti Plasmid

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

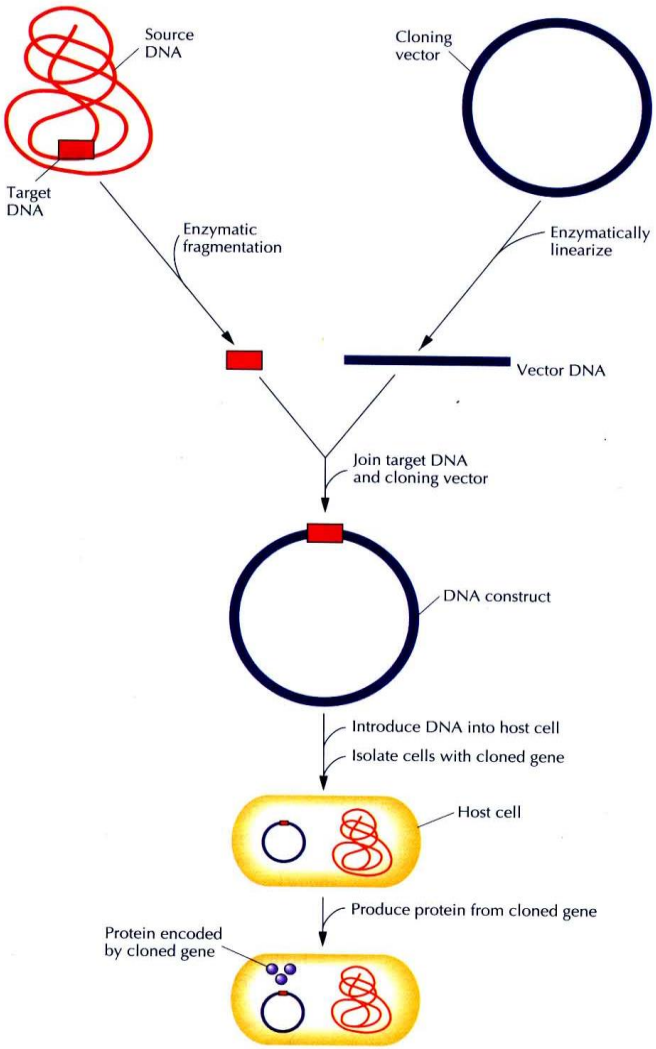


Introduction of DNA

- 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

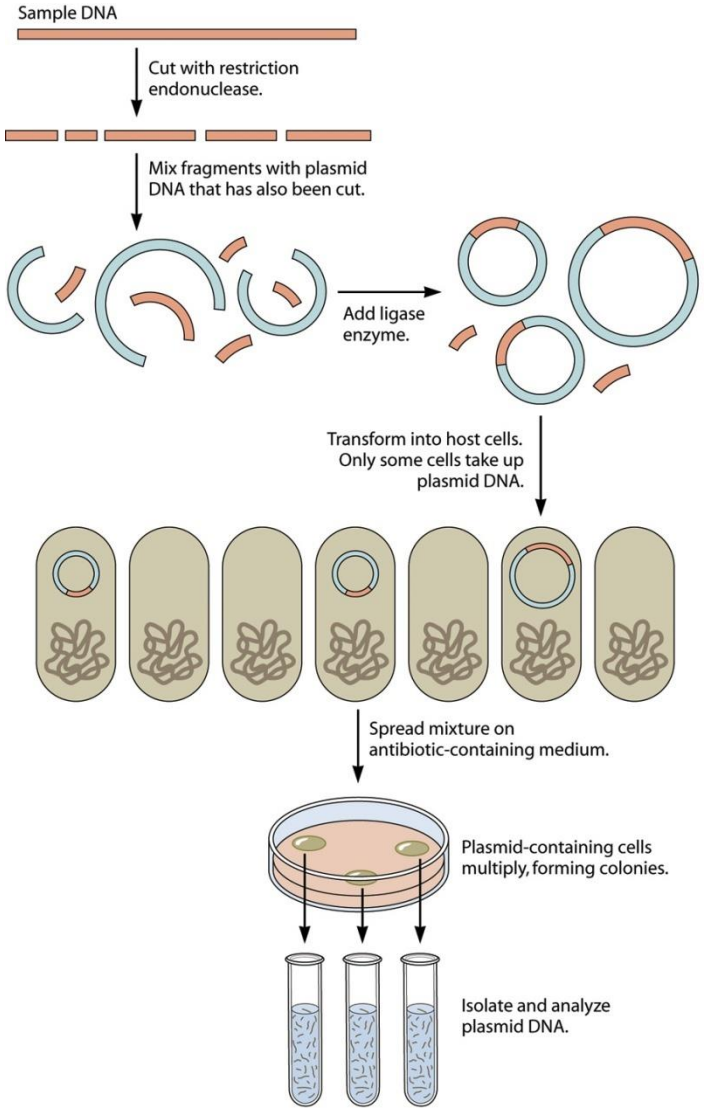


Cloning Procedure



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

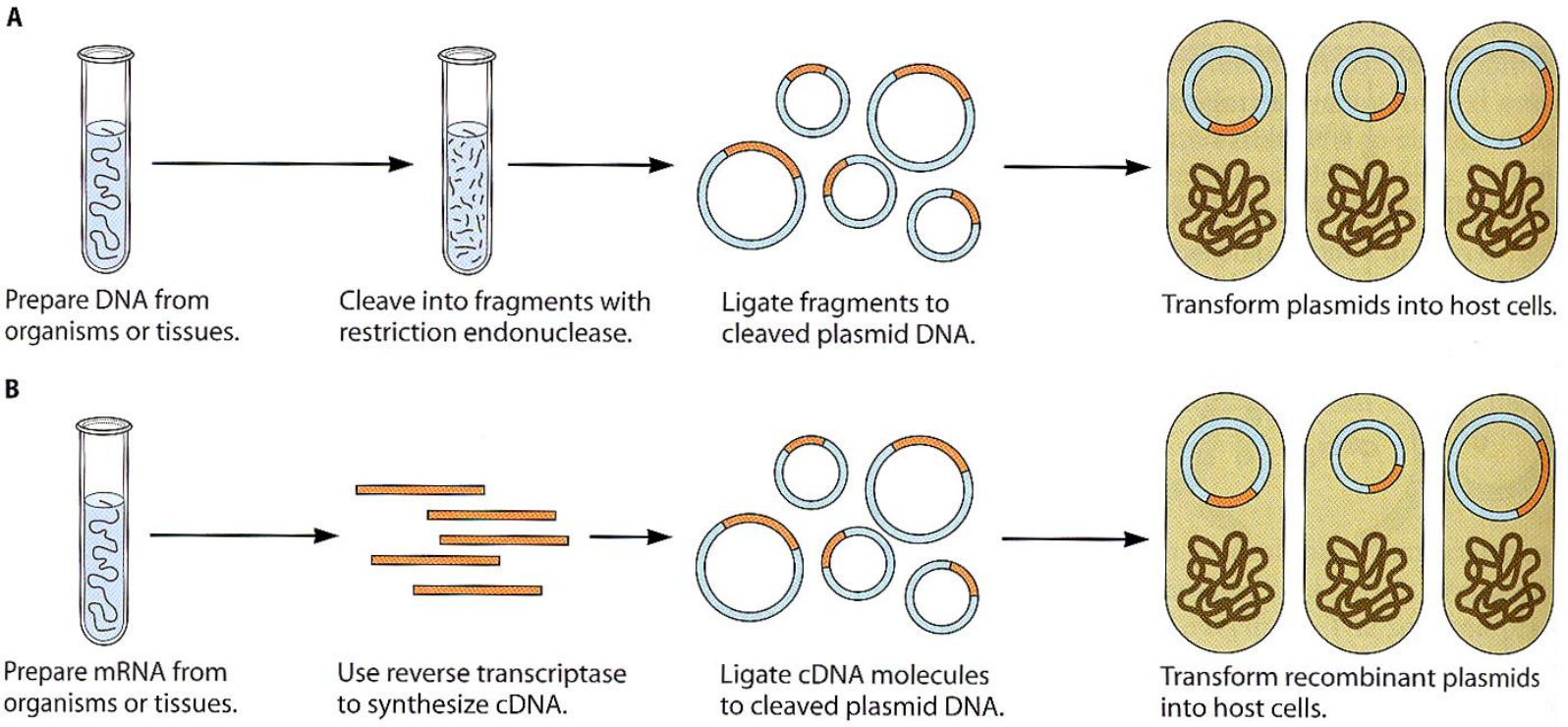


DNA Library

- 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

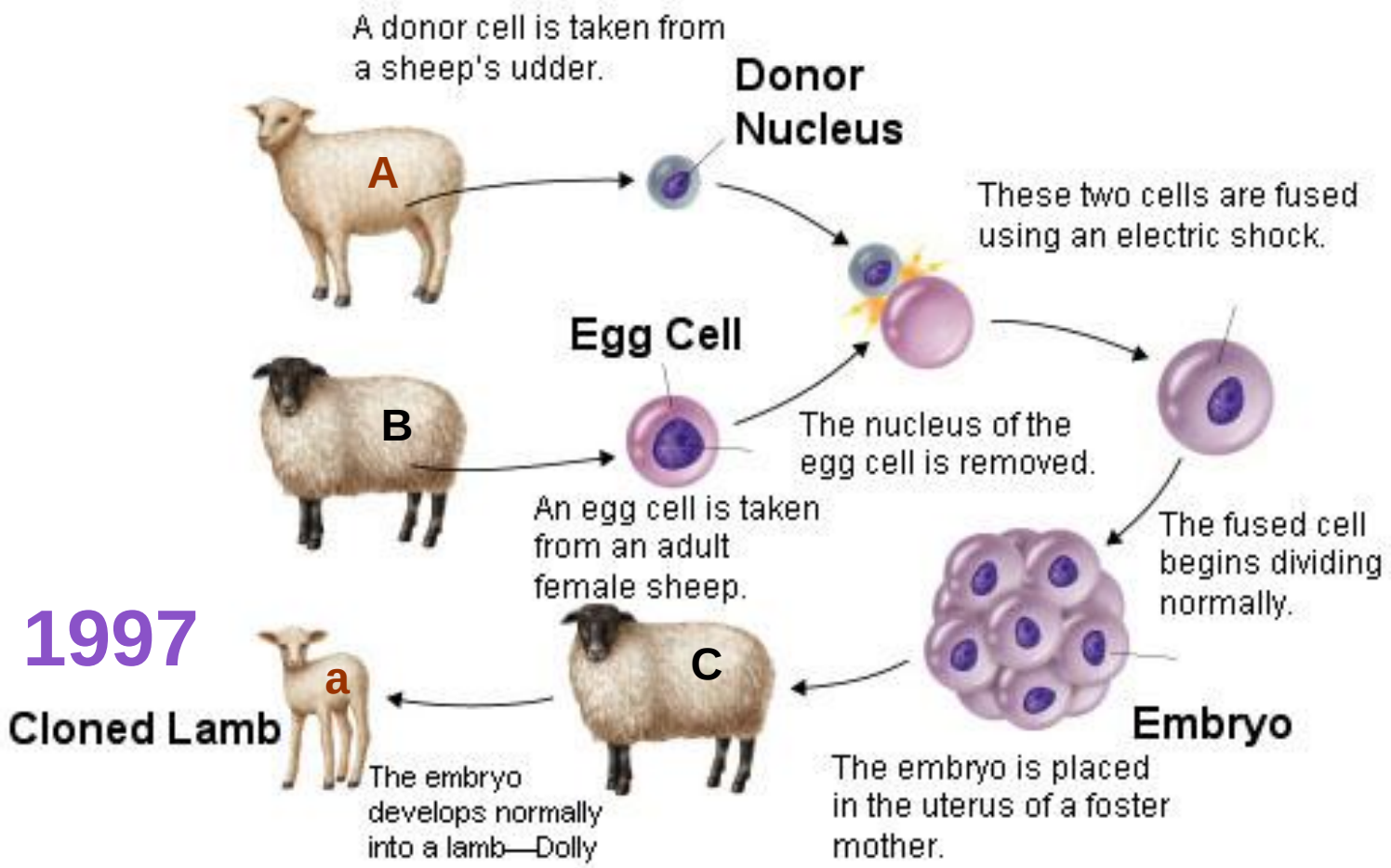
(A) Genomic DNA library (B) cDNA library



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

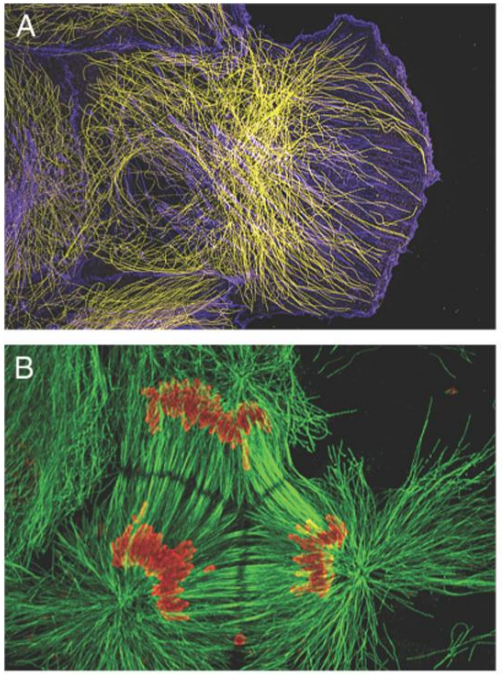
Nuclear Transfer



Dolly, 1997

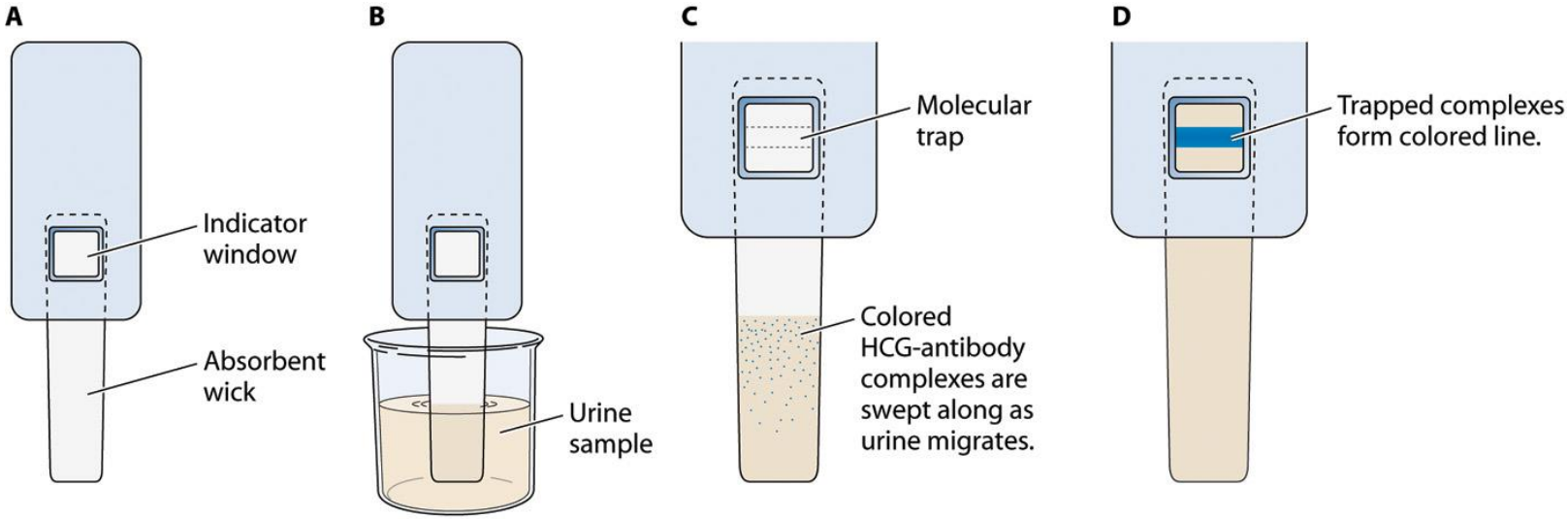
Analyzing Proteins by Antibody

- 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