

Chapter 15

The Biotechnology Toolbox



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단백질 분석

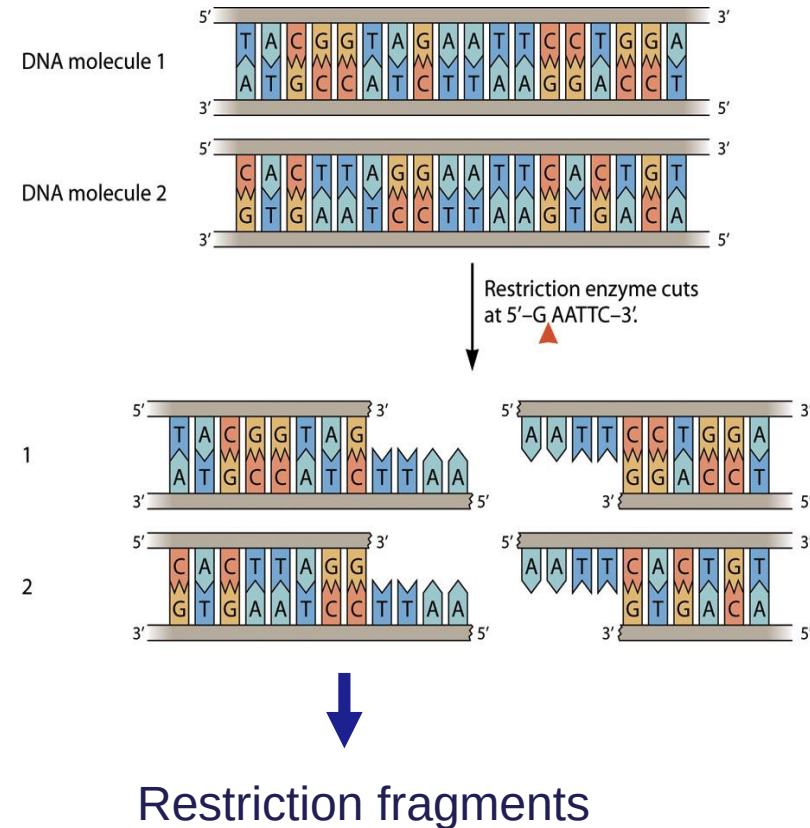
1. 재조합 DNA



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'



인식 부위 (Recognition site)

EcoRI G|A A T T C
 C T T A A|G
(Sticky end)

SmaI C C C C|G G G G
 G G G G|C C C C
(Blunt end)

■ Palindromes

eye

스위스

소주만병만주소

다시합창합시다

과학은좋은학과

Race Car

Madam, I'm Adam

I love Tevoli

Palindrome

- AD 79, Pompei
 - “Sator Arepo Tenet Opera Rotas”

S A T O R
A R E P O
T E N E T
O P E R A
R O T A S

Separating mixtures of DNA fragments

■ Electrophoresis

■ Gels

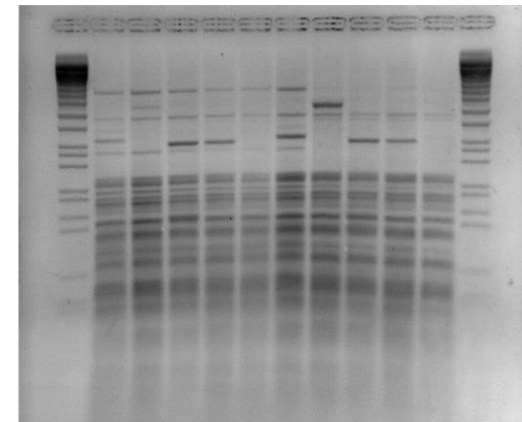
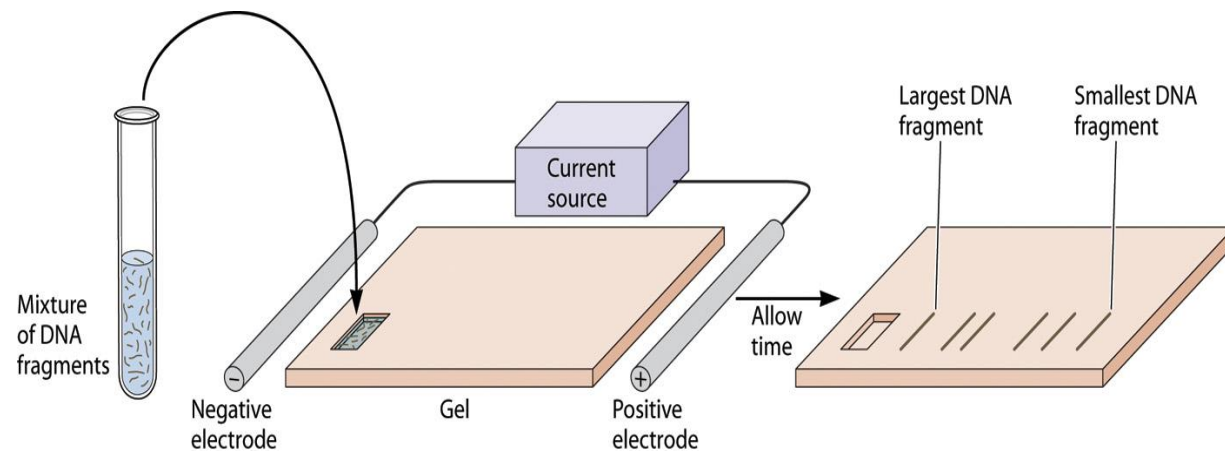
- Agarose : broad range of resolution
- Polyacrylamide : high resolution for smaller DNA

■ Migration of DNA to the positive electrode under the electric current

■ Separation of DNA molecules by molecular weight and shape

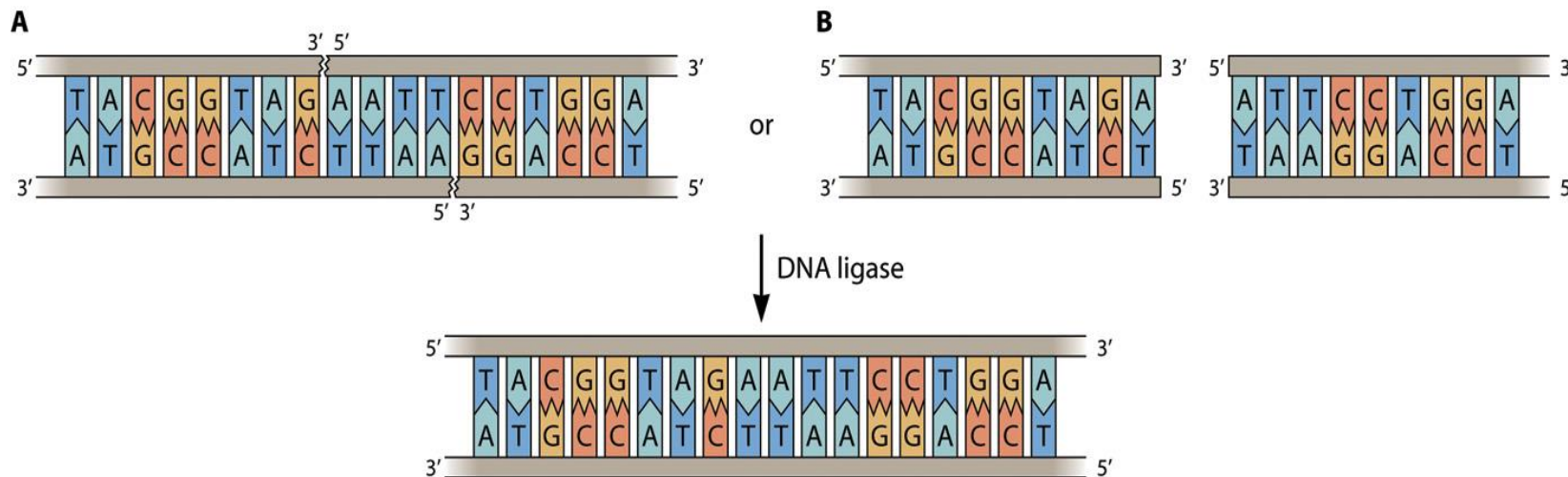
- $L = k \frac{1}{\log_{10} MW}$ for linear DNA

■ Staining of DNA for visualization (Ethidium bromide, EtBr)



Pasting DNA

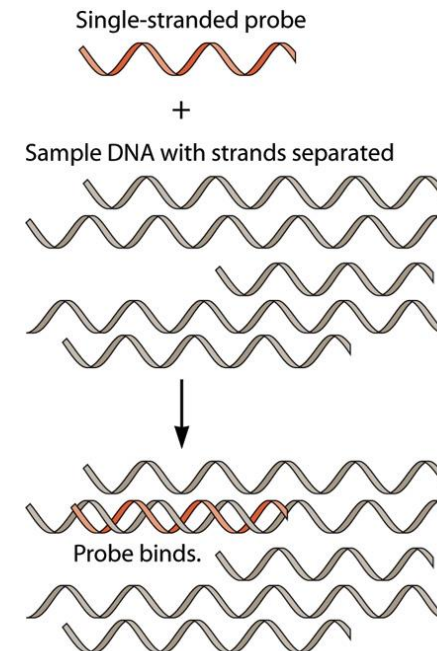
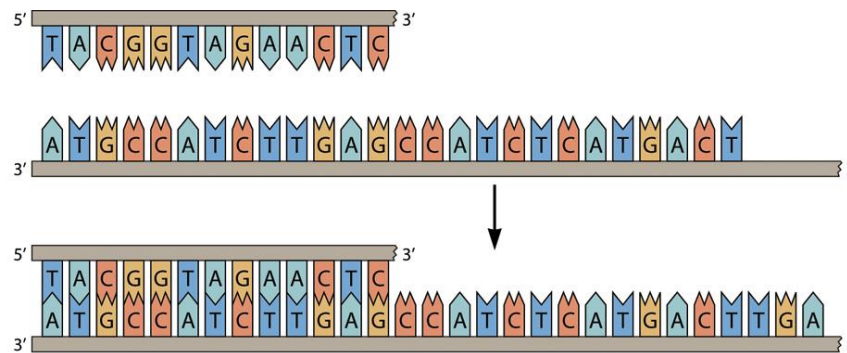
- DNA ligase
 - Joins DNA by forming new phosphodiester bond
- Recombinant DNA
 - DNA generated by joining DNA pieces from different sources



Hybridization Analysis

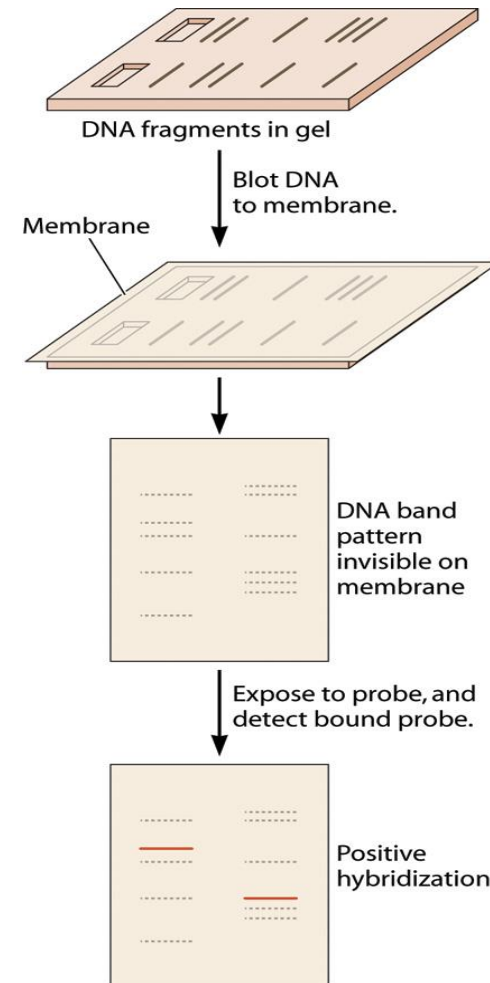
■ Hybridization

- 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

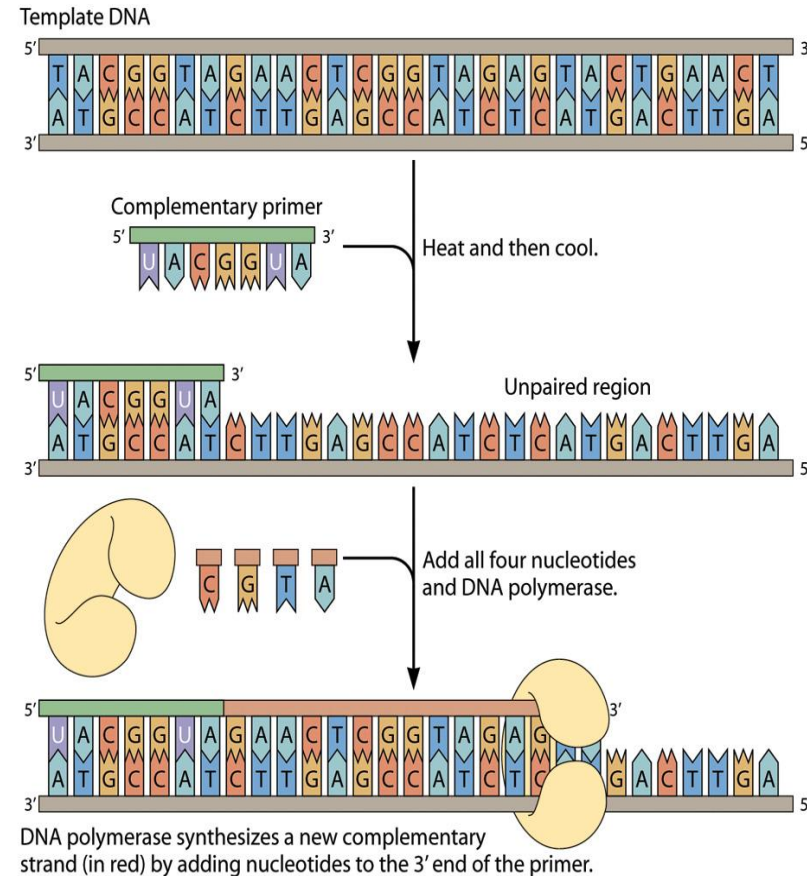


2. DNA 대량 복제



Making DNA in vitro

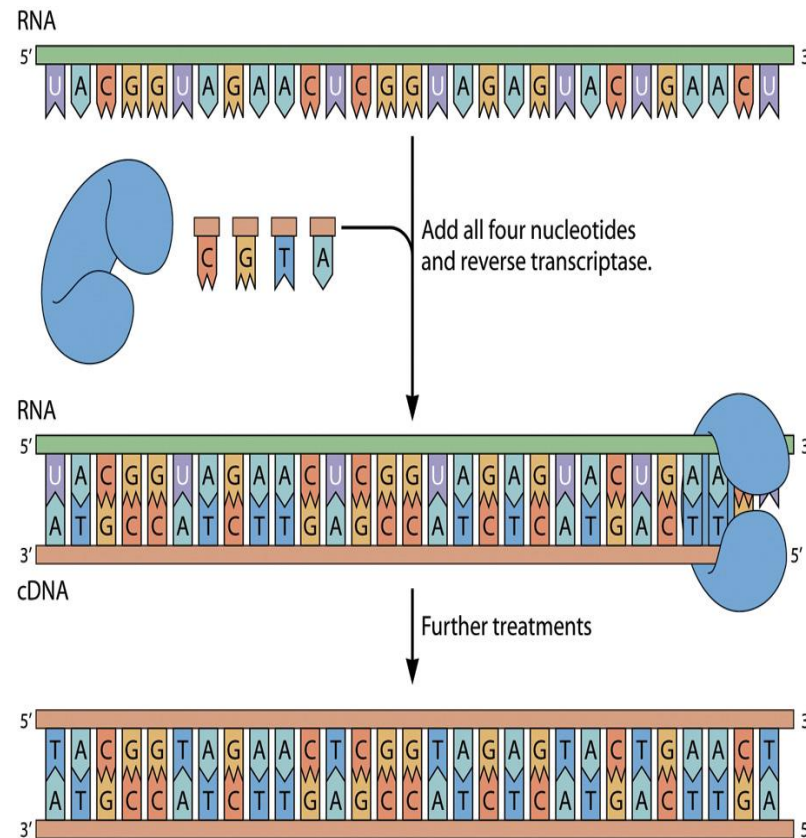
- DNA polymerase
 - Denaturation of DNA
 - Primer binding
 - RNA primer (within the cell)
 - DNA primer
 - DNA synthesis by DNA polymerase



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



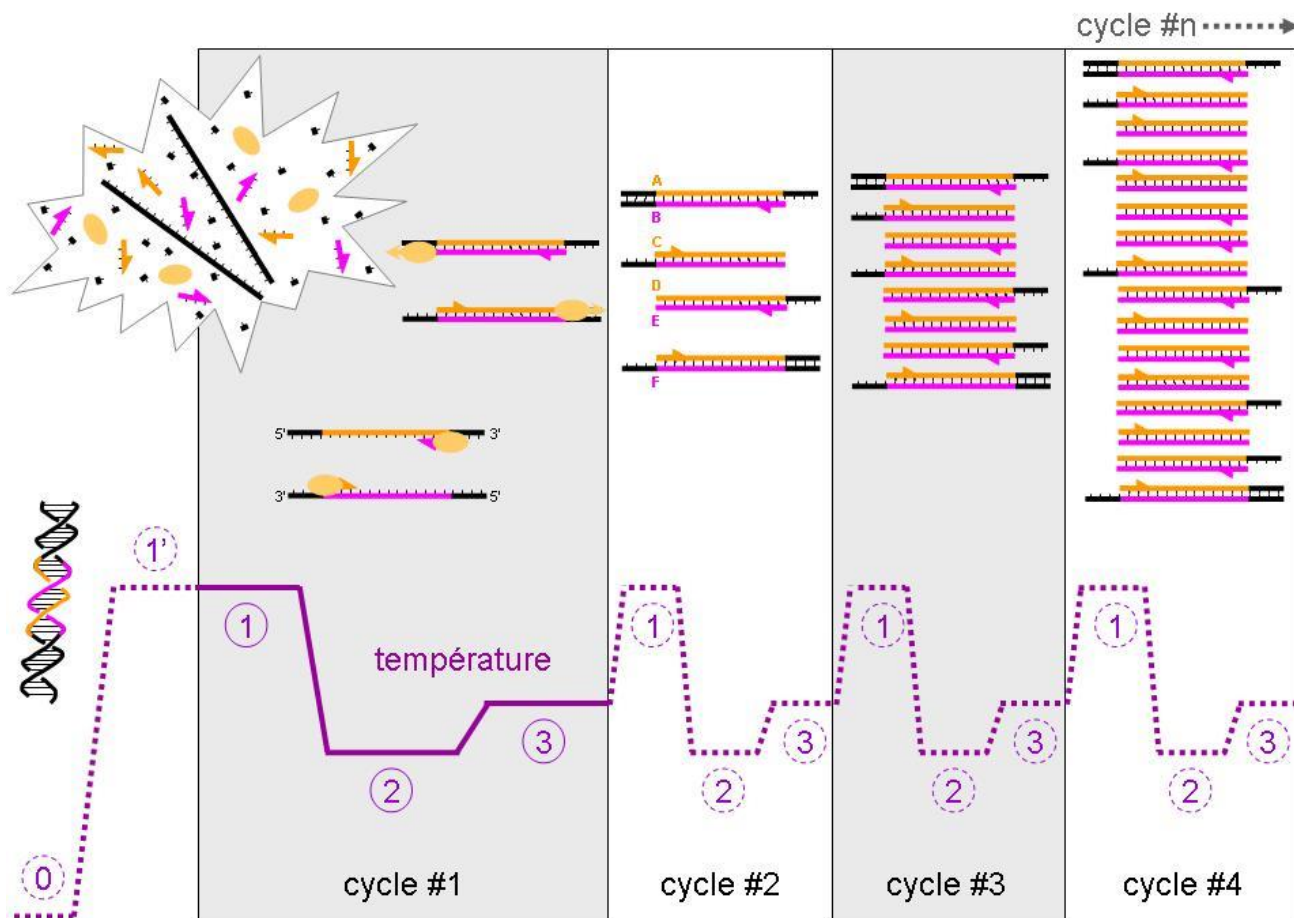
Polymerase Chain Reaction (PCR)

■ 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



PCR



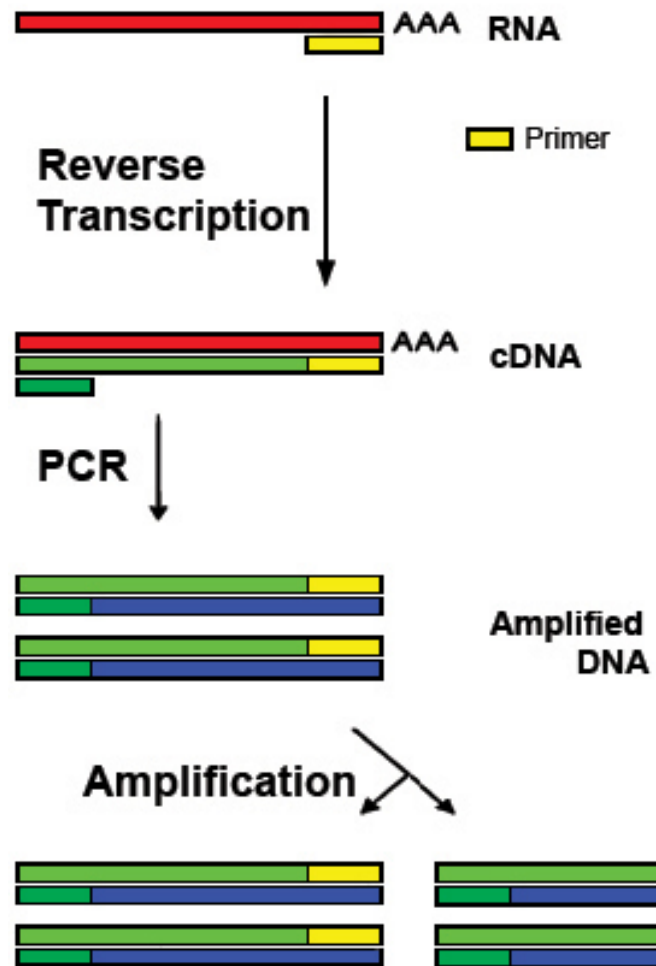
■ 반응 혼합물

- DNA 주형
- 프라이머(2종류)
- DNA 중합효소
- dNTPs

■ 반응 조건

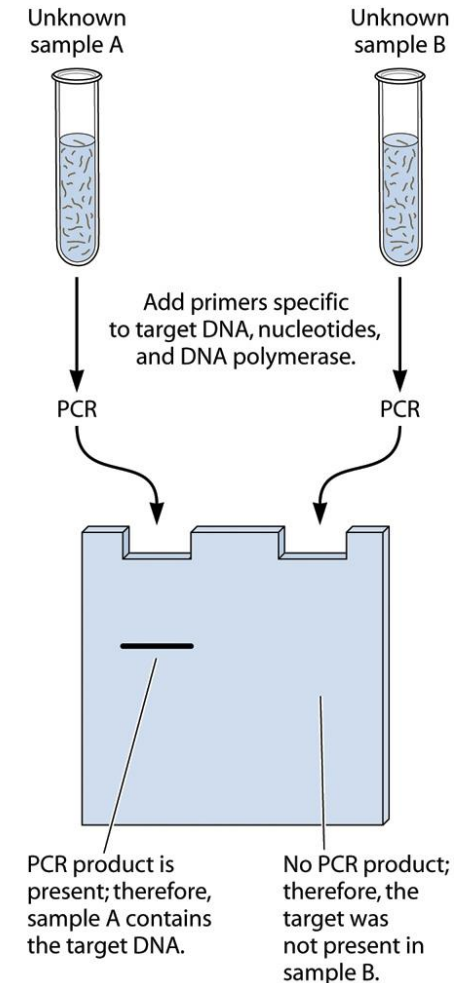
- ① DNA 변성
(95°C)
- ② 프라이머 부착
(54°C)
- ③ DNA 중합
(72°C)

Reverse transcription and PCR



PCR as a Detection Method

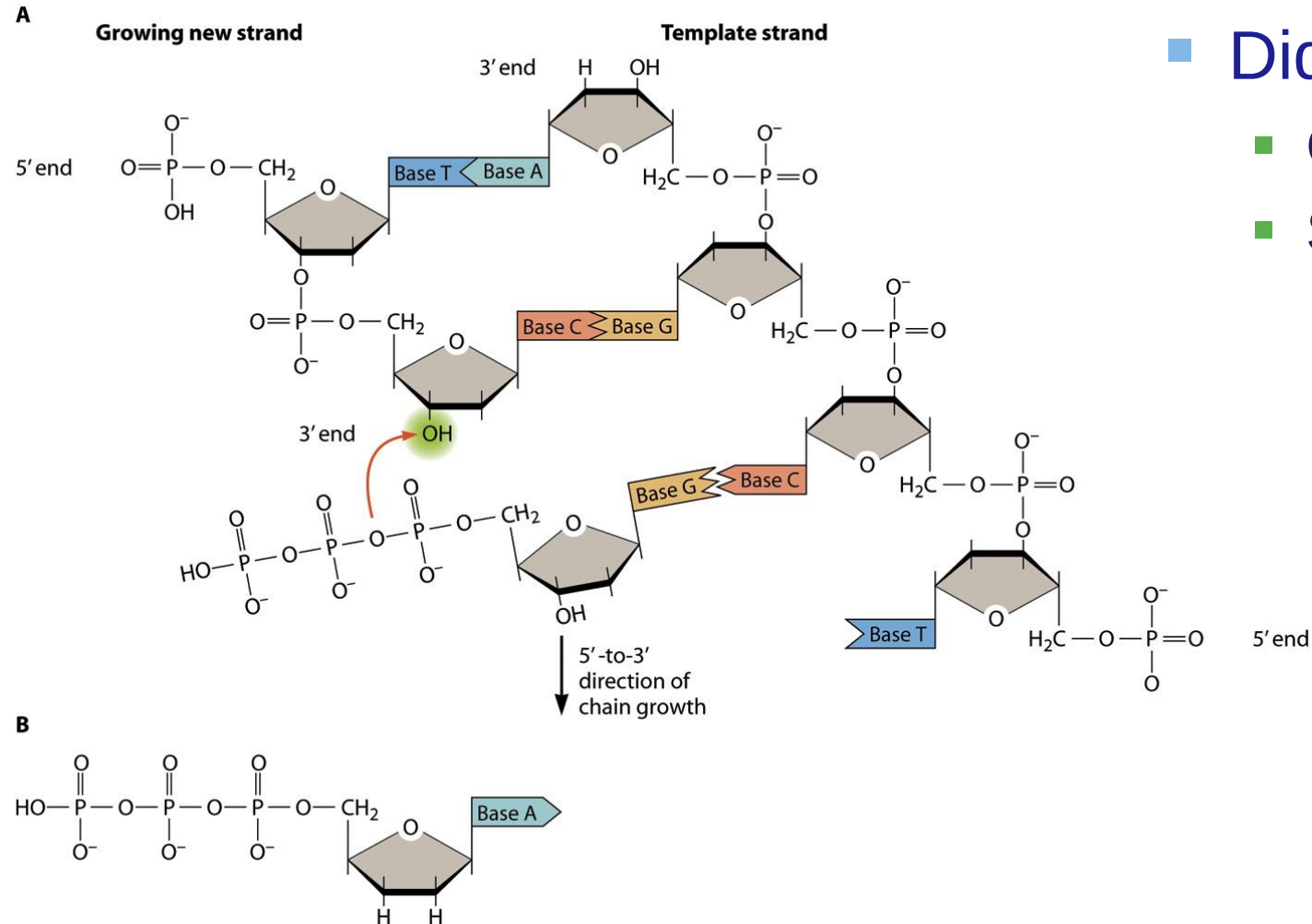
- More sensitive than hybridization in detecting DNA
- Diagnosing disease
 - Traditional method for diagnosis of infectious disease
 - Culturing the pathogenic bacteria for identification
 - Time consuming
 - PCR-base detection
 - Fast and sensitive



3. DNA 염기서열 결정

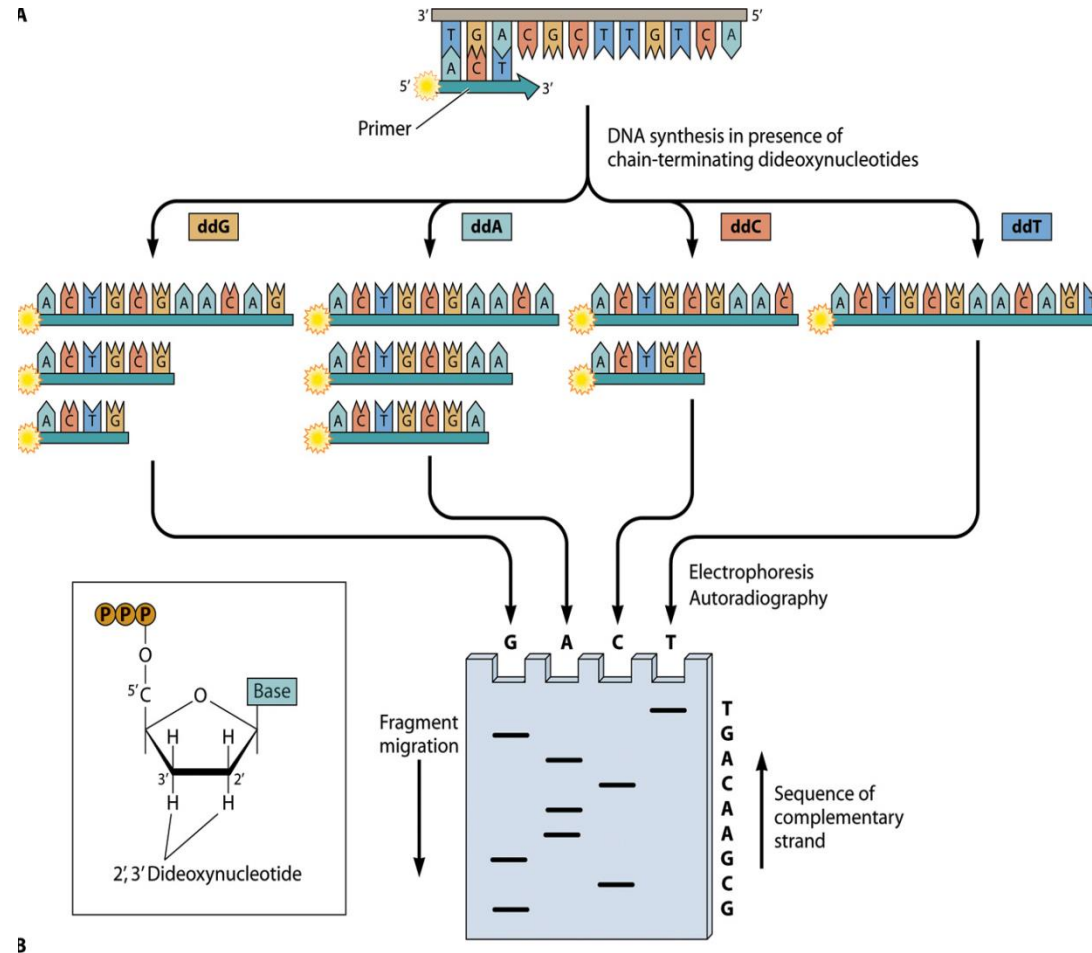


Chain termination by ddNTP



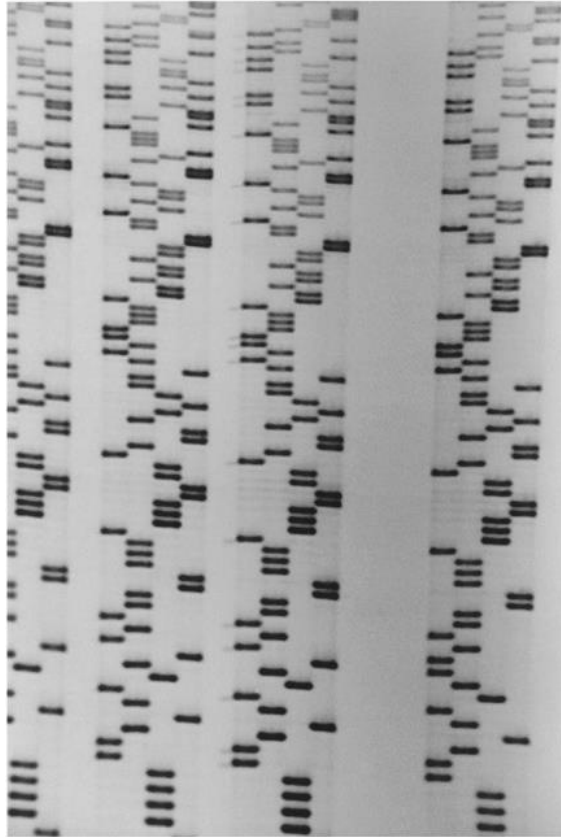
- DideoxynTP
- Chain termination
- Sanger (1977)

Chain Termination Sequencing



Chain Termination Sequencing

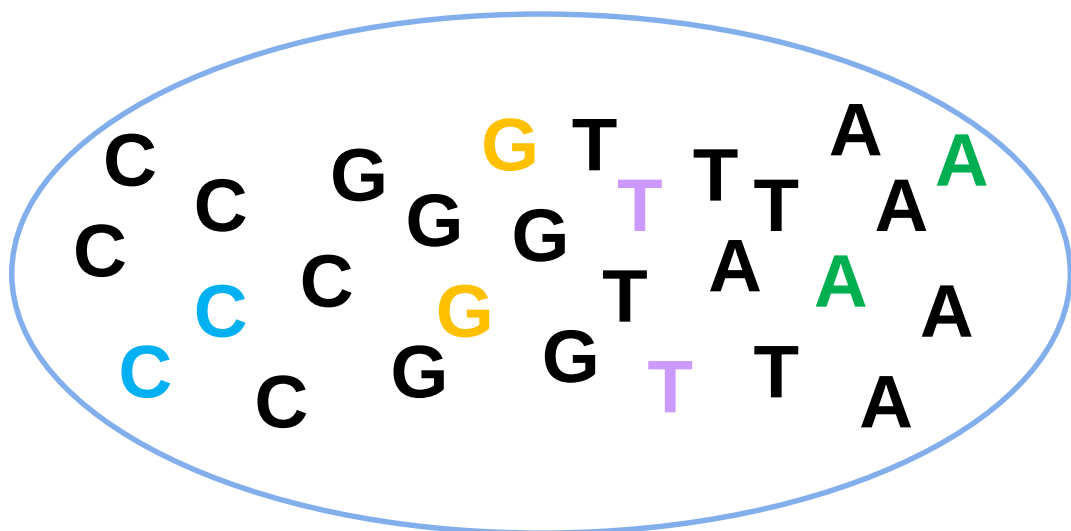
B



Automated DNA Sequencing

3' A C G G C A T T C G G T T 5'
5' T G C C G T A A G C C A A 3'

3' A C G G C A T T C G G T T 5'
5' T G C C G T A A G C C A A 3'



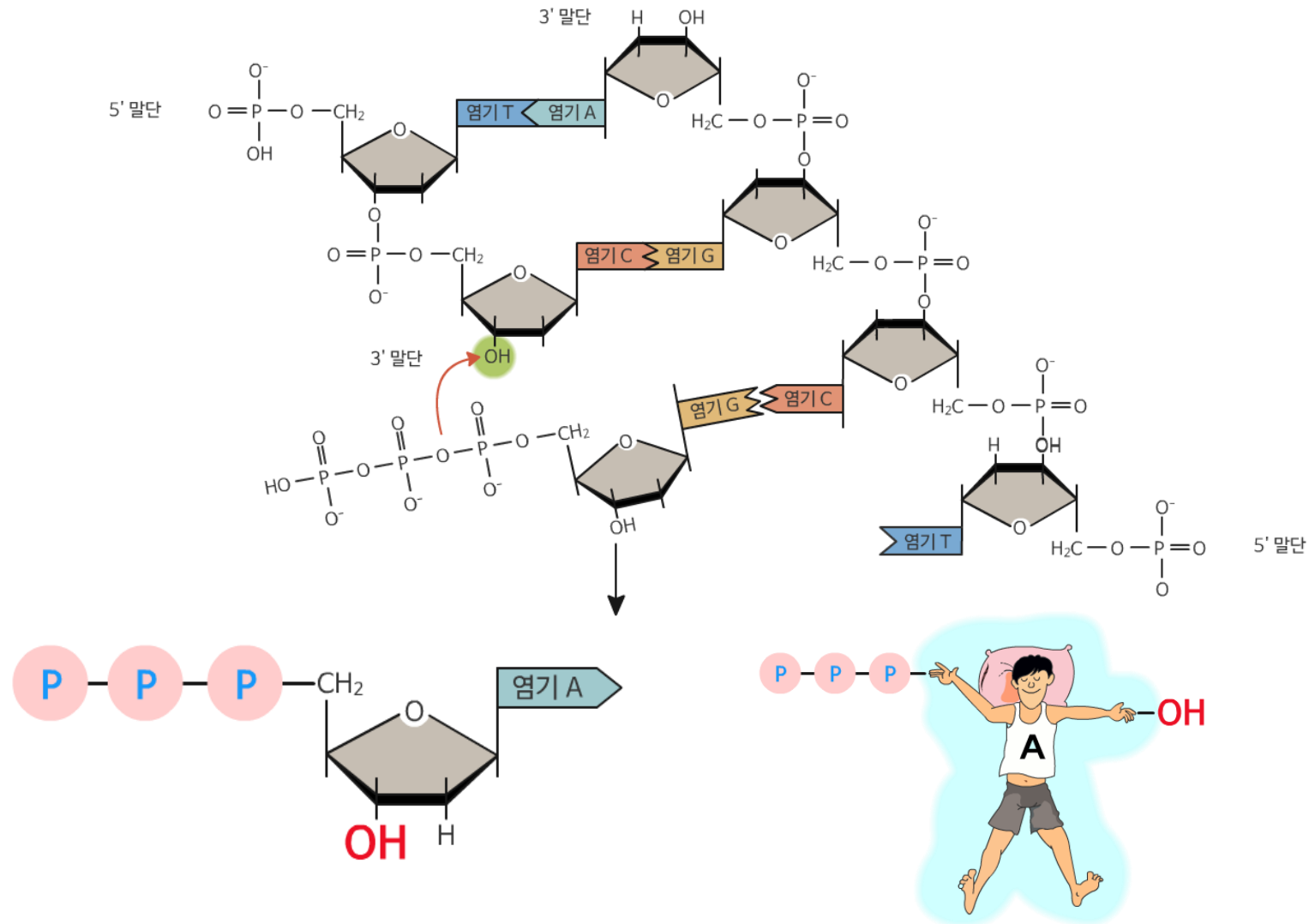
dNTP

--- A, T, G, C

ddNTP (4-color fluorescence labelled)

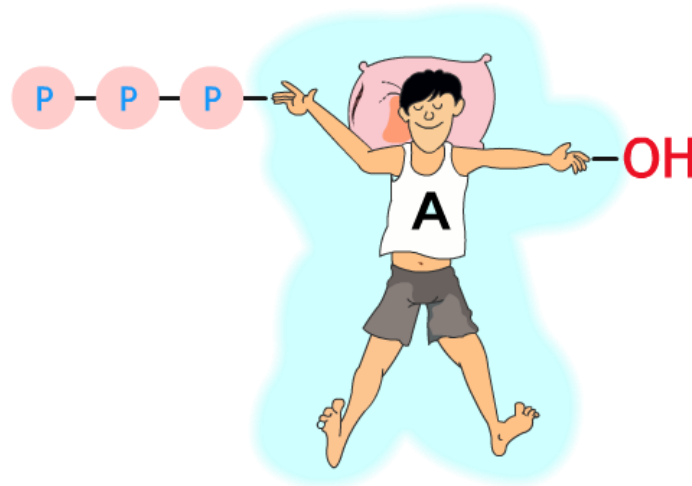
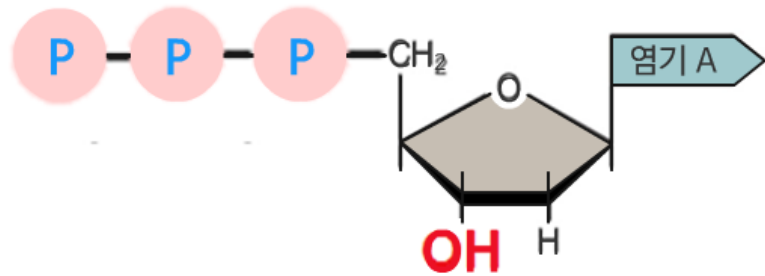
--- A, T, G, C

Automated DNA Sequencing

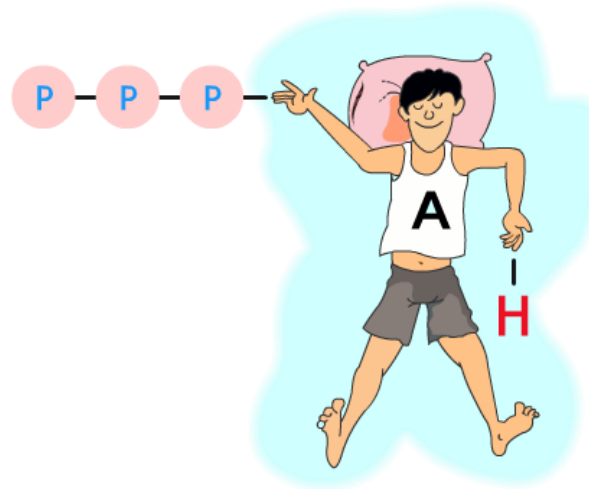
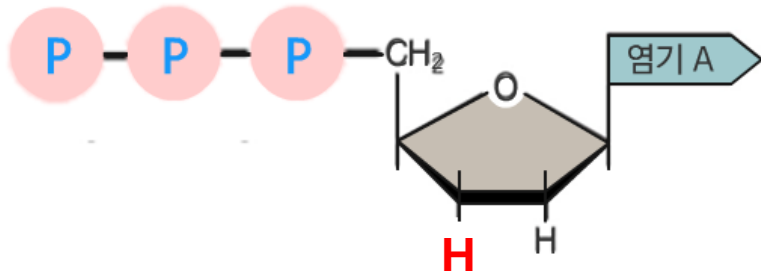


Automated DNA Sequencing

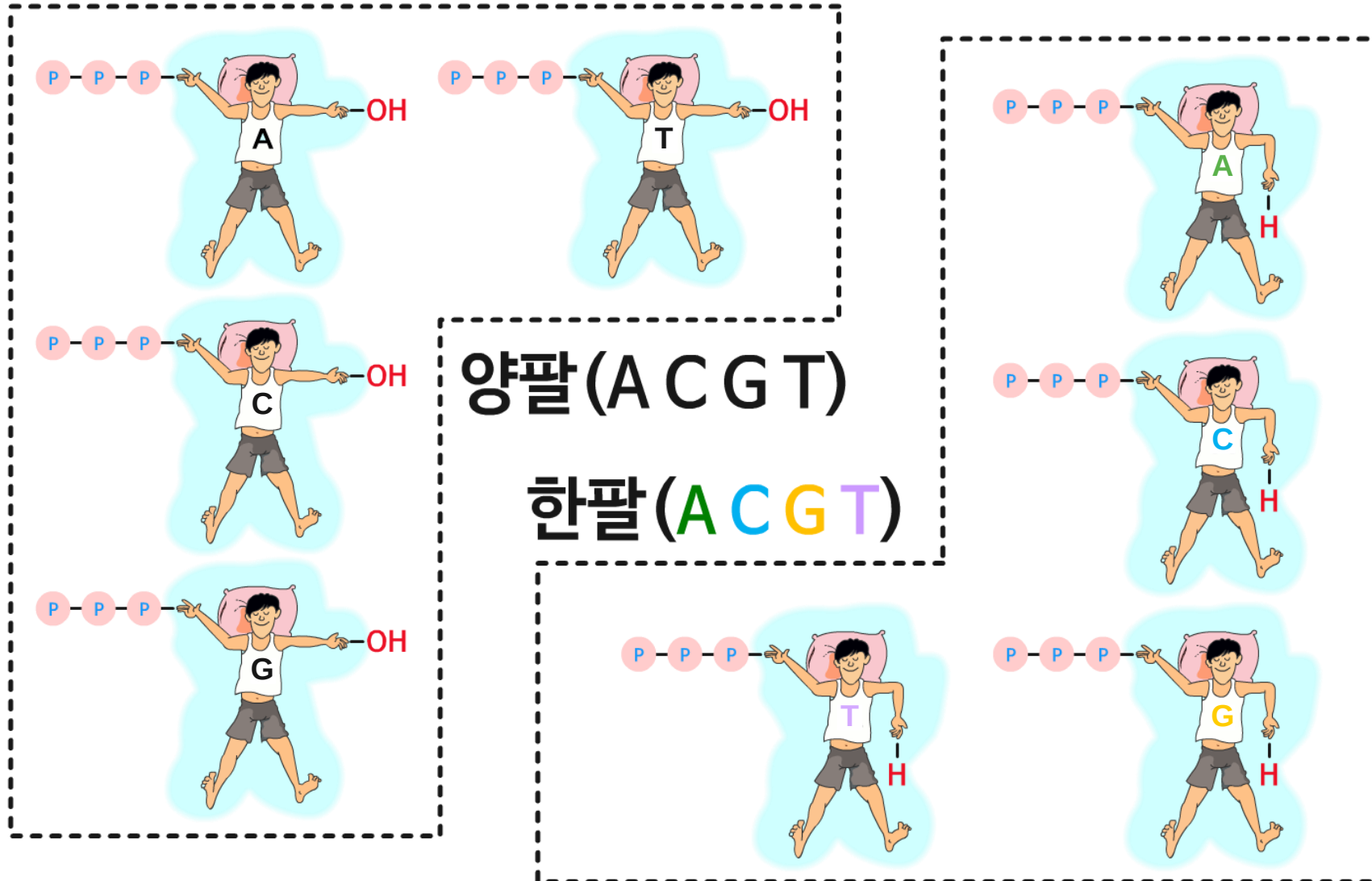
<dATP>



<ddATP>



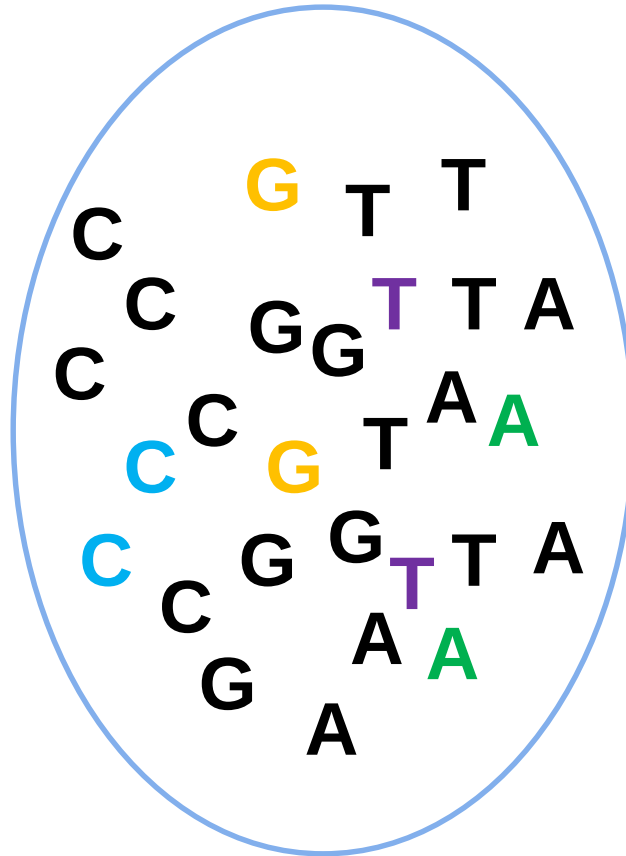
Automated DNA Sequencing



Automated DNA Sequencing

A C G G C A T T C G G T T
T G C *C G T A A G C C A A*

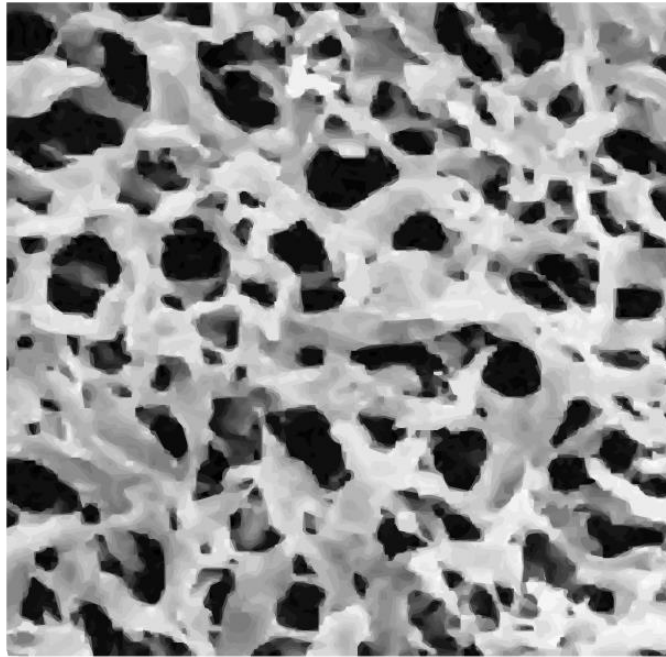
T G C C
T G C C G
T G C C G T
T G C C G T A
T G C C G T A A
T G C C G T A A G
T G C C G T A A G C
T G C C G T A A G C C
T G C C G T A A G C C A



Gel Electrophoresis

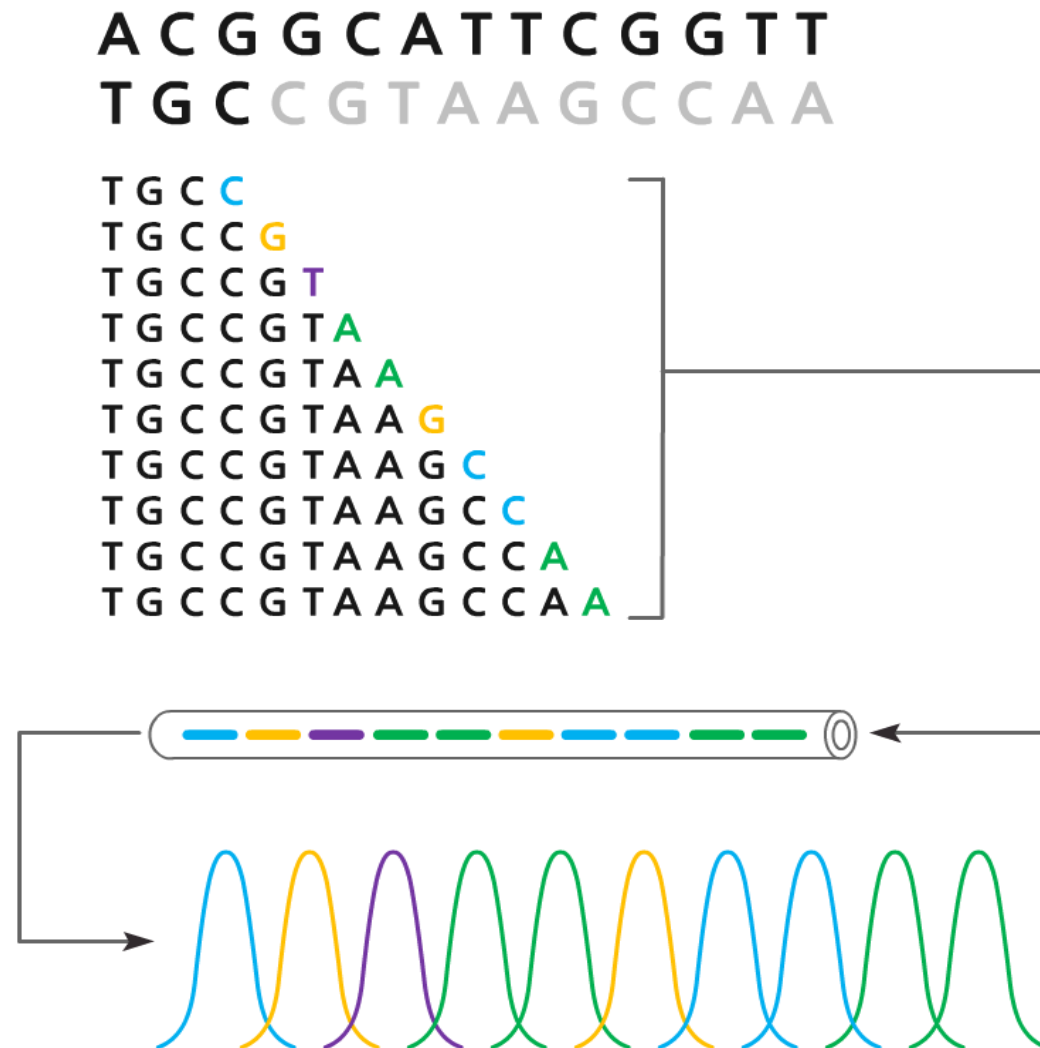
DNA 가닥의 장애물 경기

T G C C
T G C C G
T G C C G T
T G C C G T A
T G C C G T A A G
T G C C G T A A G C
T G C C G T A A G C C A
T G C C G T A A G C C A A



다공성 젤

Automated DNA Sequencing



4. 클로닝 (Cloning)



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

클로닝 운반체 (Cloning Vector)

- 플라스미드 (Plasmid)

- 자기 자신의 복제 기점(replication origin)을 가지고 있는 작은 원형 DNA



- 바이러스 운반체 (Viral vector)

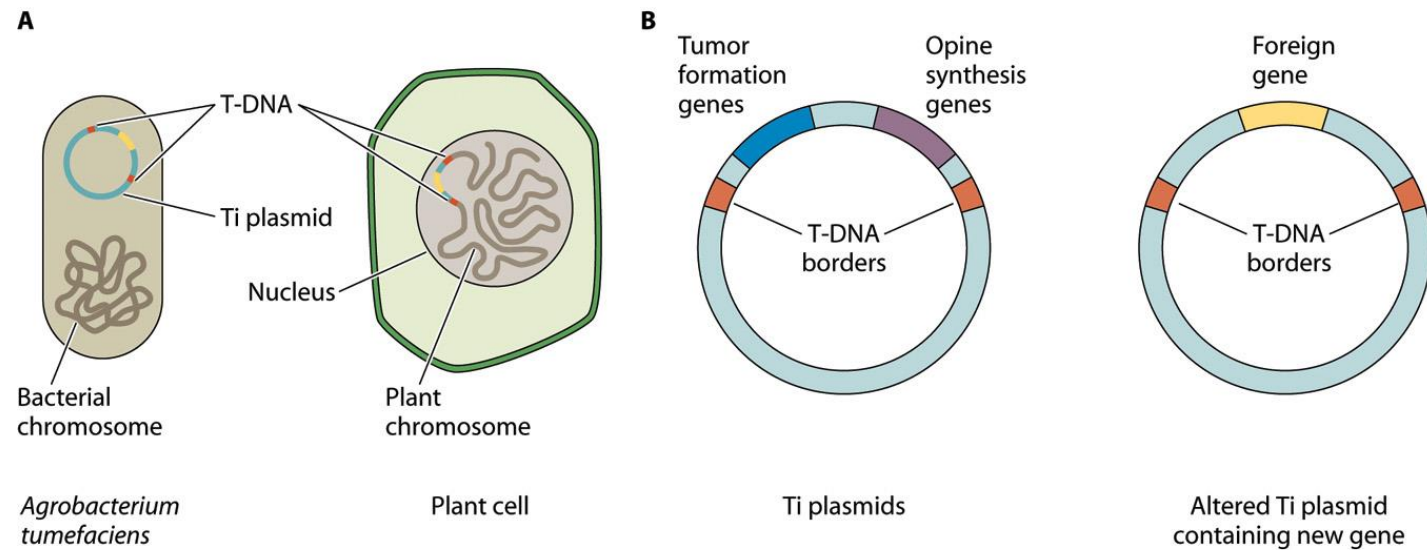
- 바이러스 자체
- 바이러스 증식에 꼭 필요하지는 않은 바이러스 DNA 부분을 우리가 복제하고자 하는 DNA로 대체하여 사용

- 효모 인공 염색체 (Yeast artificial chromosome)

- 복제기점(replication origin), 중심체(centromere), 말단소체(telomere)를 포함시켜 제조한 인공 염색체

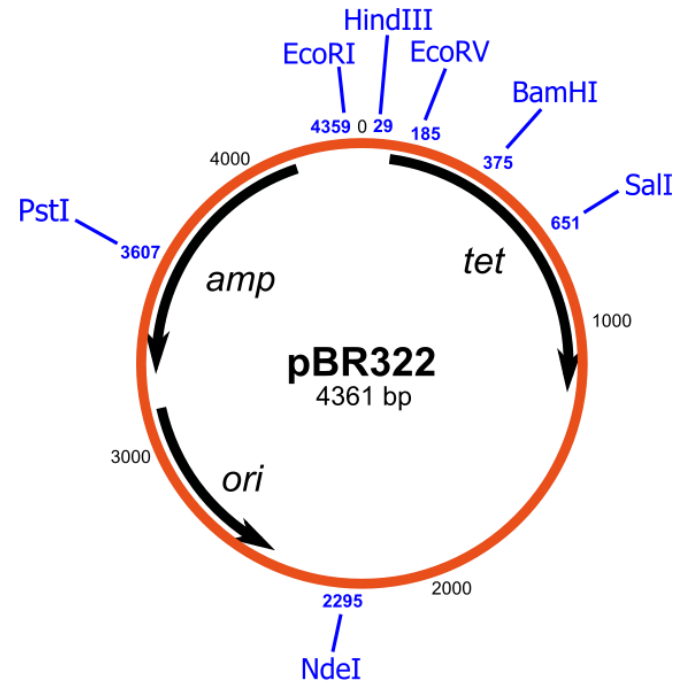
Ti Plasmid

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



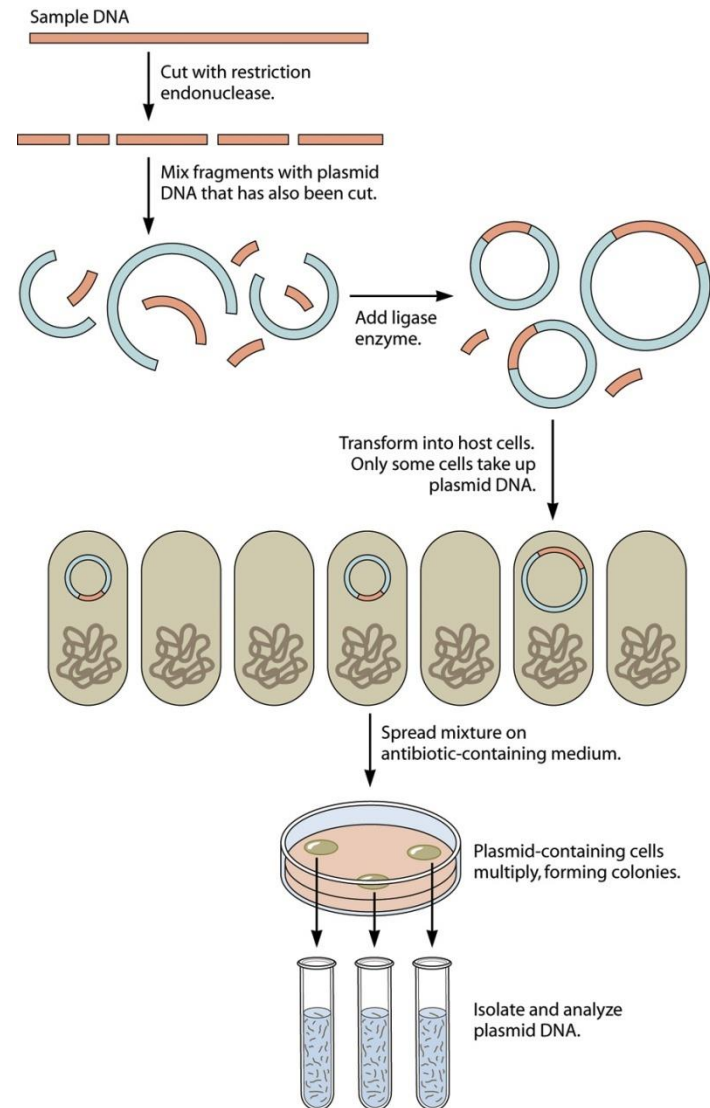
플라스미드 운반체 (Plasmid Vector)

- 복제 기점
 - *ori* 사이트
 - 많은 카피 수
- 마커 유전자 (Marker gene)
 - 예) 항생제 내성 유전자
 amp^R , tet^R 등
- 다수의 클로닝 사이트
 - 각 제한효소 인지 부위가 한 군데 씩만 존재



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



클로닝된 DNA 확인

- PCR
 - 양끝에 맞는 프라이머 사용
 - 복제된 DNA 크기 확인 (젤 전기영동)
- 염기서열 분석 (Sequencing)
- 제한효소 절단 (Restriction digestion)
 - 삽입 DNA가 올바른 개수와 크기로 절단되었는지 확인 (전기영동에 의해)

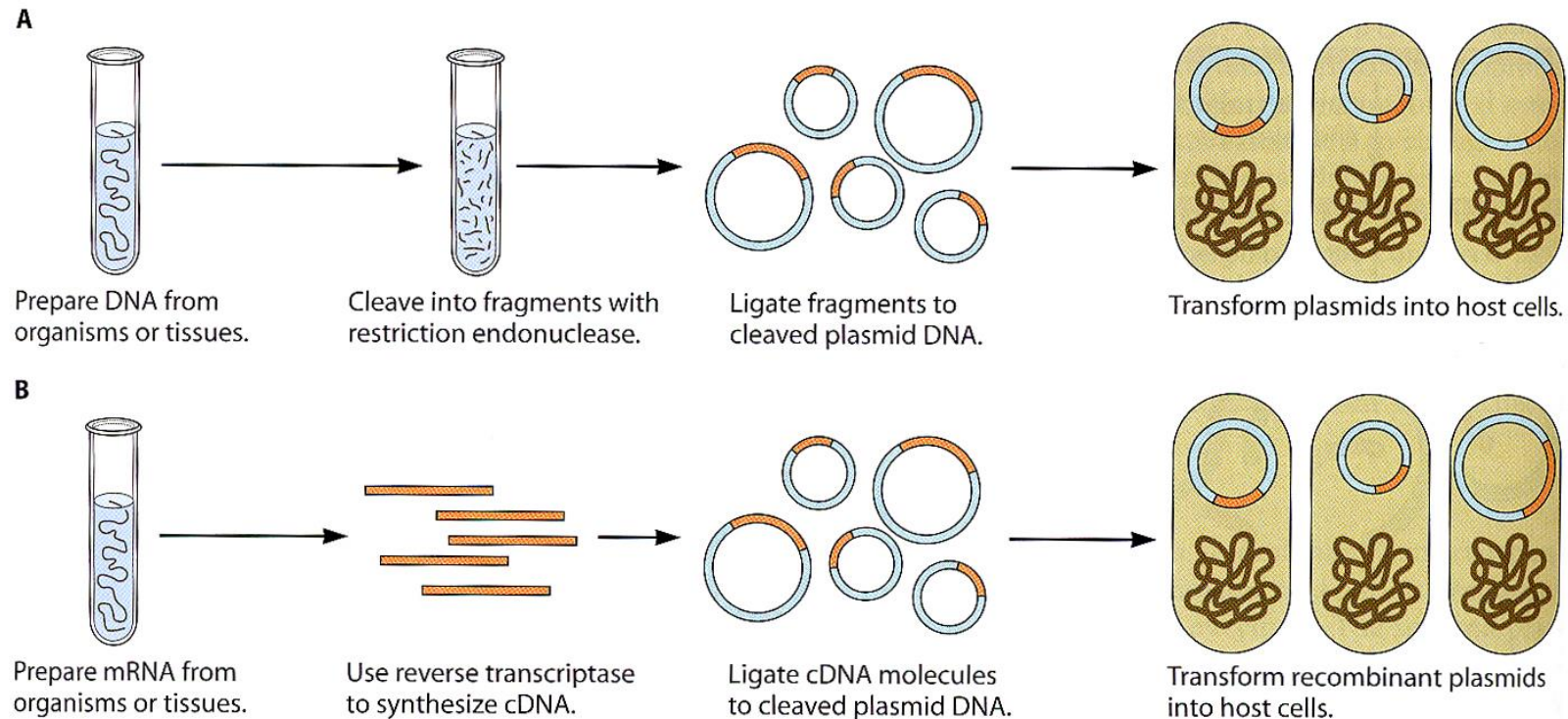
DNA Library

- 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

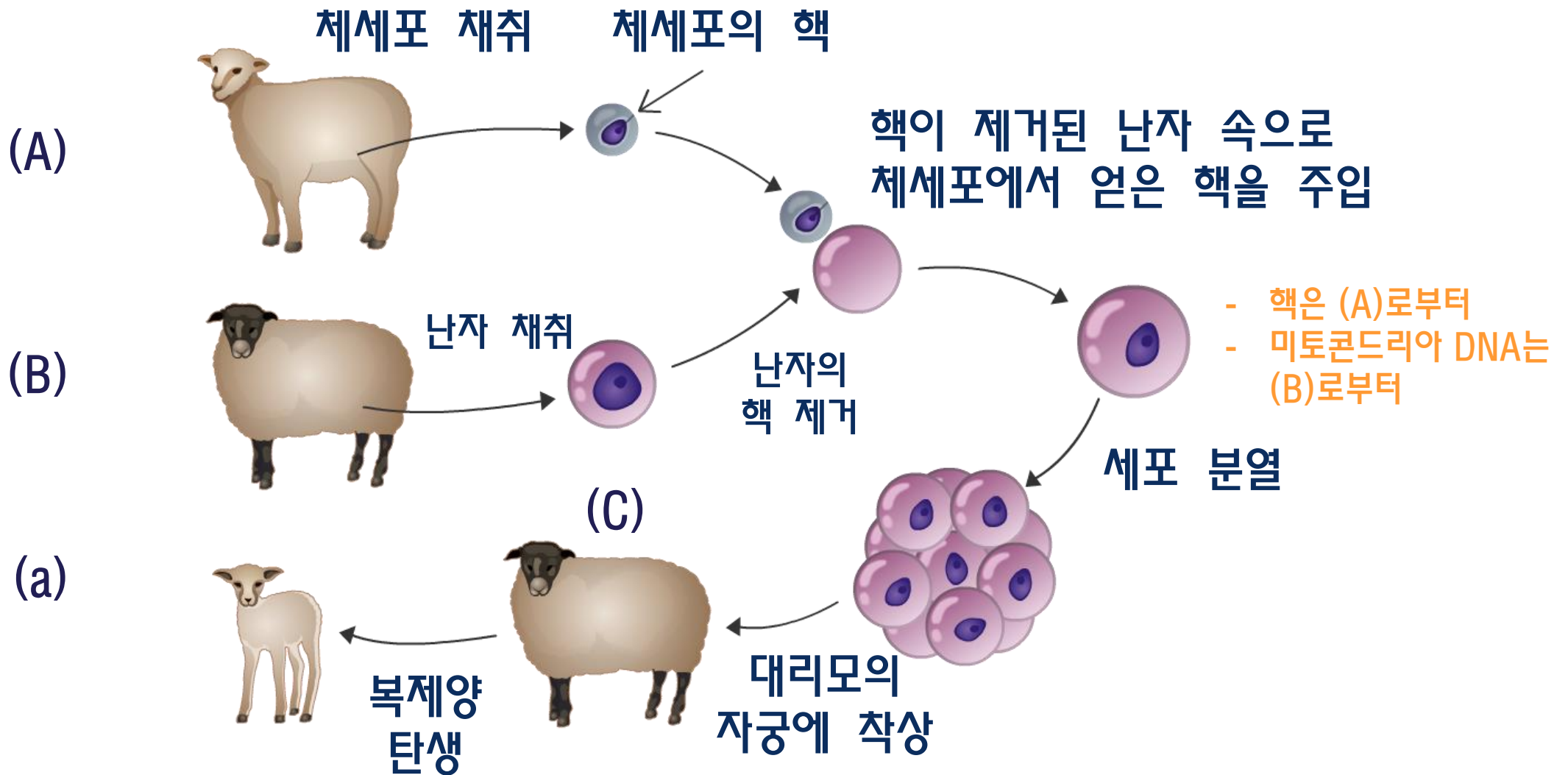
(B) cDNA library



Cloning Complex Organisms

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

핵 이식 (Nuclear Transfer)



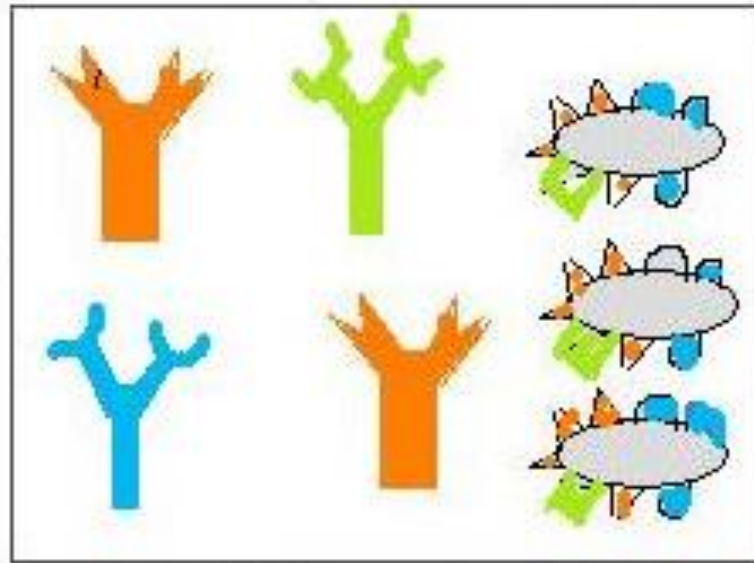
5. 단백질 분석



Analyzing Proteins by Antibody

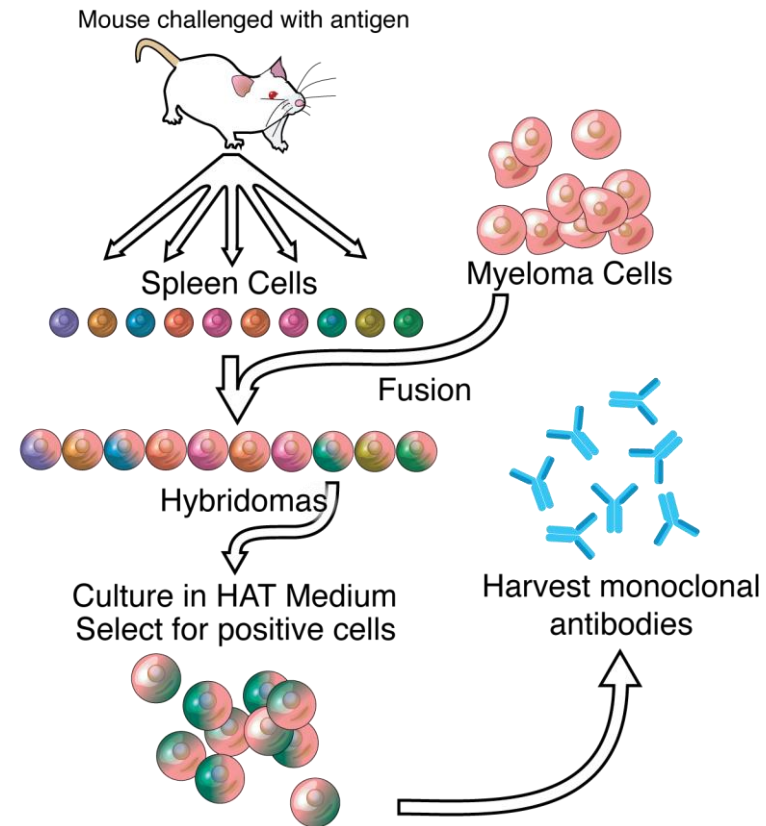
- 다클론 항체 vs. 단일클론 항체

(Polyclonal antibody vs. Monoclonal antibody)



Analyzing Proteins by Antibody

- 단일클론 항체 (Monoclonal antibody)
 - 항체: B 세포에서 생산됨
 - B 세포:
 - 지라(spleen)에서 채취
 - 세포분열 안함 (배양 불가)
 - B 세포를 골수종 세포 (myeloma cell)와 융합
 - 무한한 세포 분열 (배양 가능)
 - 항체 생산
 - 원하는 항체를 생산하는 세포 선별

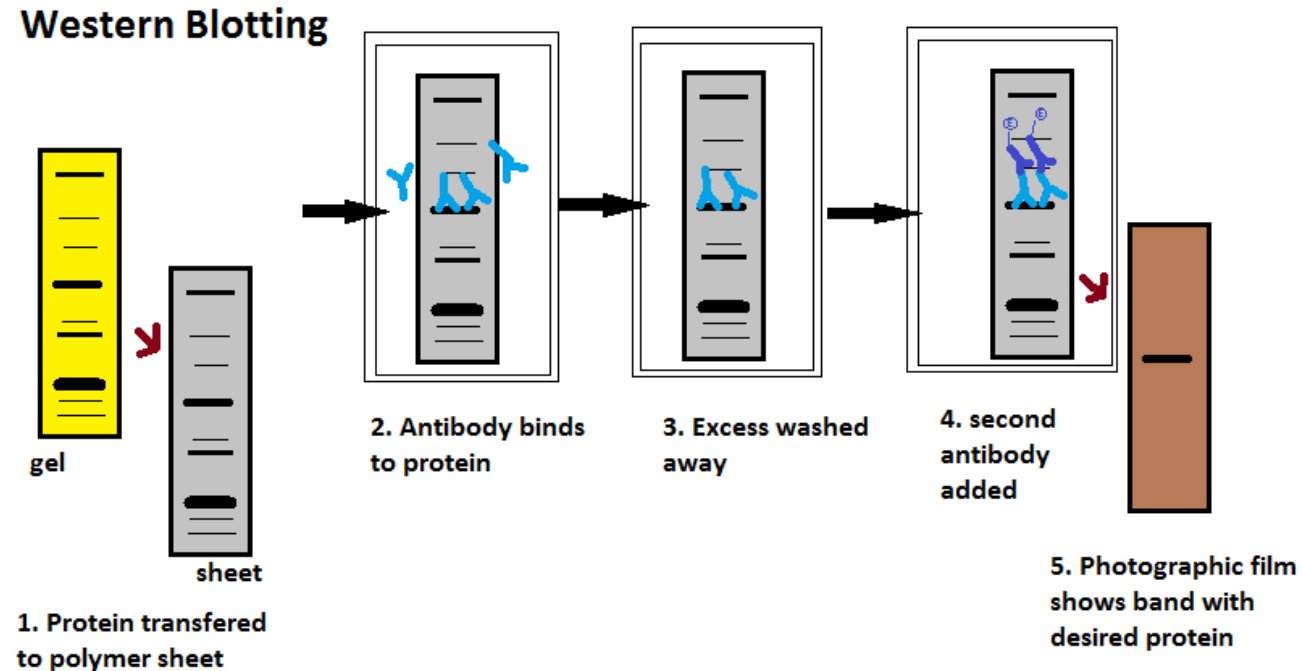


Analyzing Proteins by Antibody

- 웨스턴 블랏 (Western blot)
 - 특정 단백질의 유무 혹은 양 분석
 - 단백질/항체 결합
- 서던 블랏 (Southern blot)
 - 특정 DNA 서열의 유무 혹은 양 분석
 - DNA/DNA 결합
 - Edwin Southern에 의해 개발
- 노던 블랏 (Northern blot)
 - 특정 RNA 서열의 유무 혹은 양 분석
 - RNA/DNA(혹은 RNA) 결합
- 이스턴 블랏 (Eastern blot)
 - 번역후 수정(post translational modification) 분석
 - 예) 탄수화물/렉틴 결합

Analyzing Proteins by Antibody

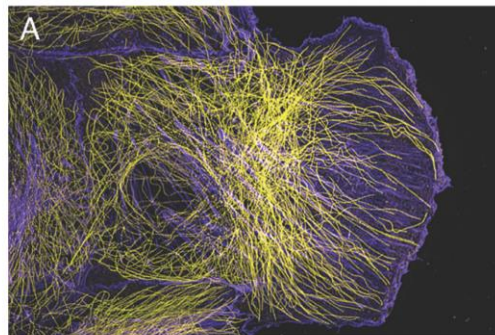
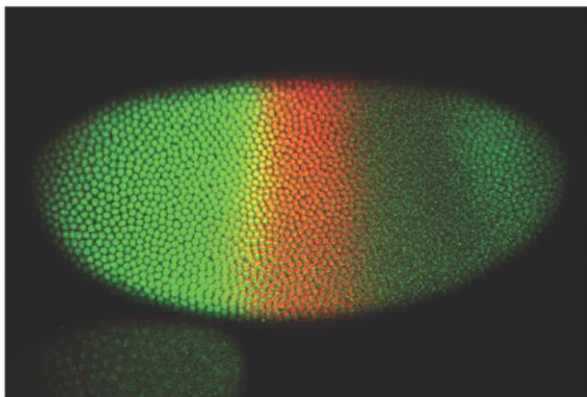
- 웨스턴 블랏 (Western blot)
 - 항체를 이용하여 특정 단백질 확인



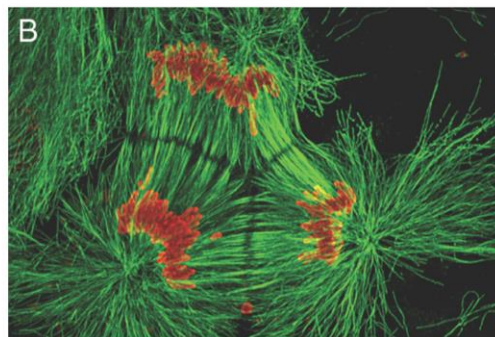
Analyzing Proteins by Antibody

- 특정 단백질의 생체 내 혹은 세포 내 위치 분석
 - 형광 표지된 항체 사용

초파리 분절 특이적 단백질



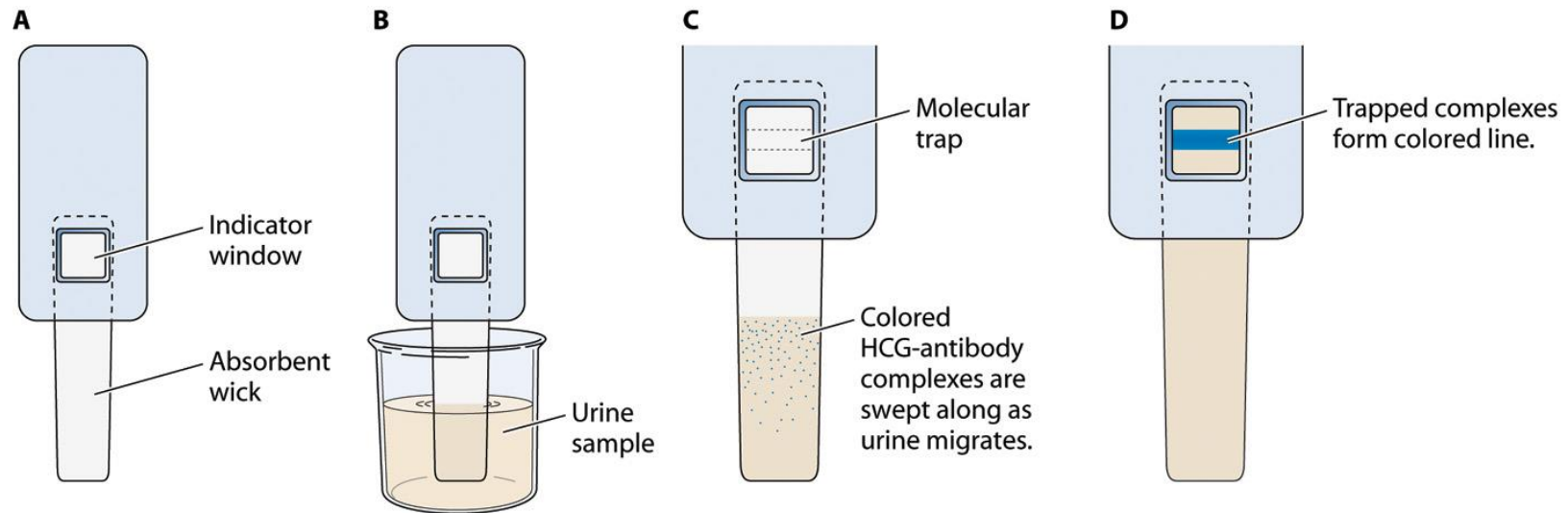
세포골격
(cytoskeleton)
두 종류의
필라멘트



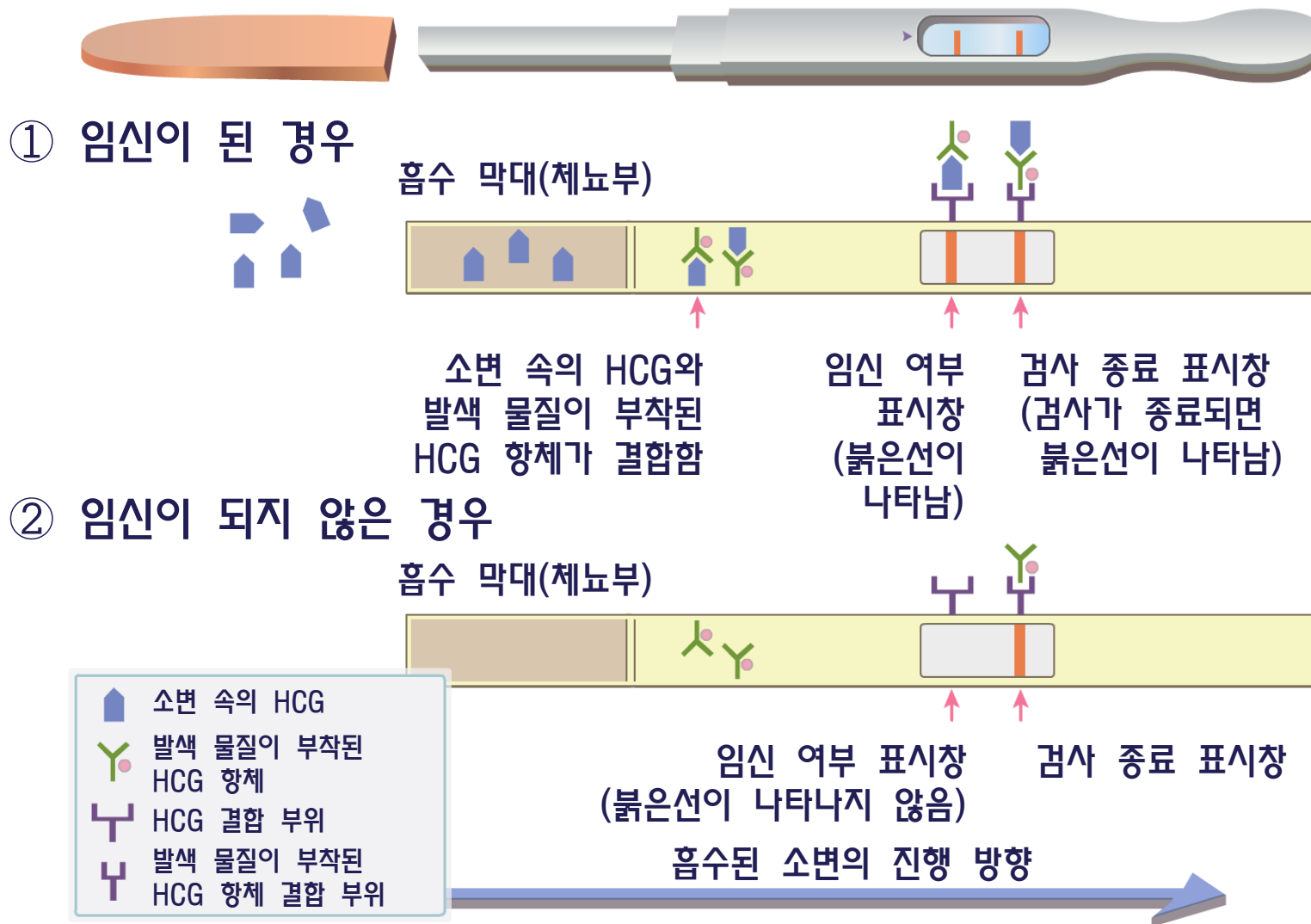
유사분열 중인 세포
(초록색: 방추사,
붉은색: DNA)

Antibodies for Diagnosis

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



Antibodies for Diagnosis



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