Chapter 16

Biotechnology in the Research Laboratory

목 차

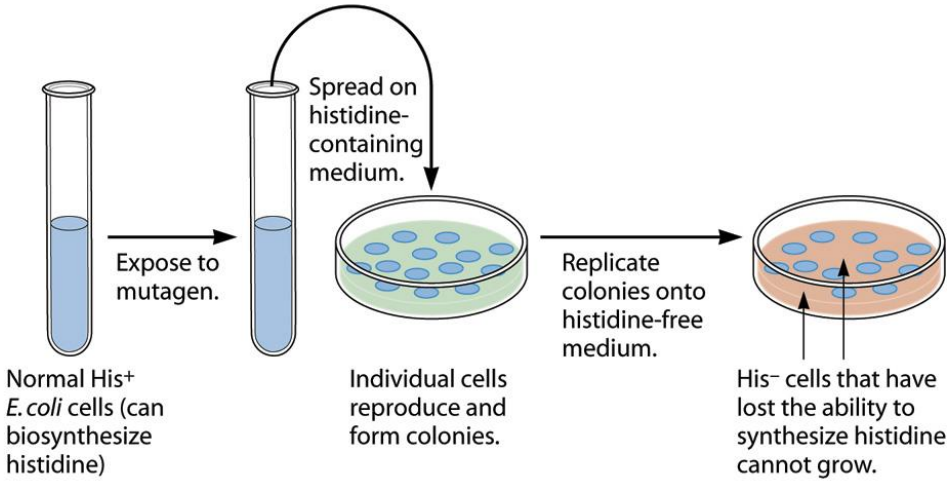
1. 유전자 탐색 (Finding Genes)

Biotechnology in Research Laboratory

- Finding genes
  - Isolation of genes with specific functions
- Genetic testing
  - Detection of the presence of a specific sequence in the sample
    - Diagnosis of infectious disease
  - Detection of the similarity of sequences from different individuals
    - Diagnosis of genetic disease and forensic DNA typing
    - Evolutionary studies
- Genetic engineering
  - Genetic engineering of microorganisms
  - Transgenic plants
  - Transgenic animals

Finding Genes

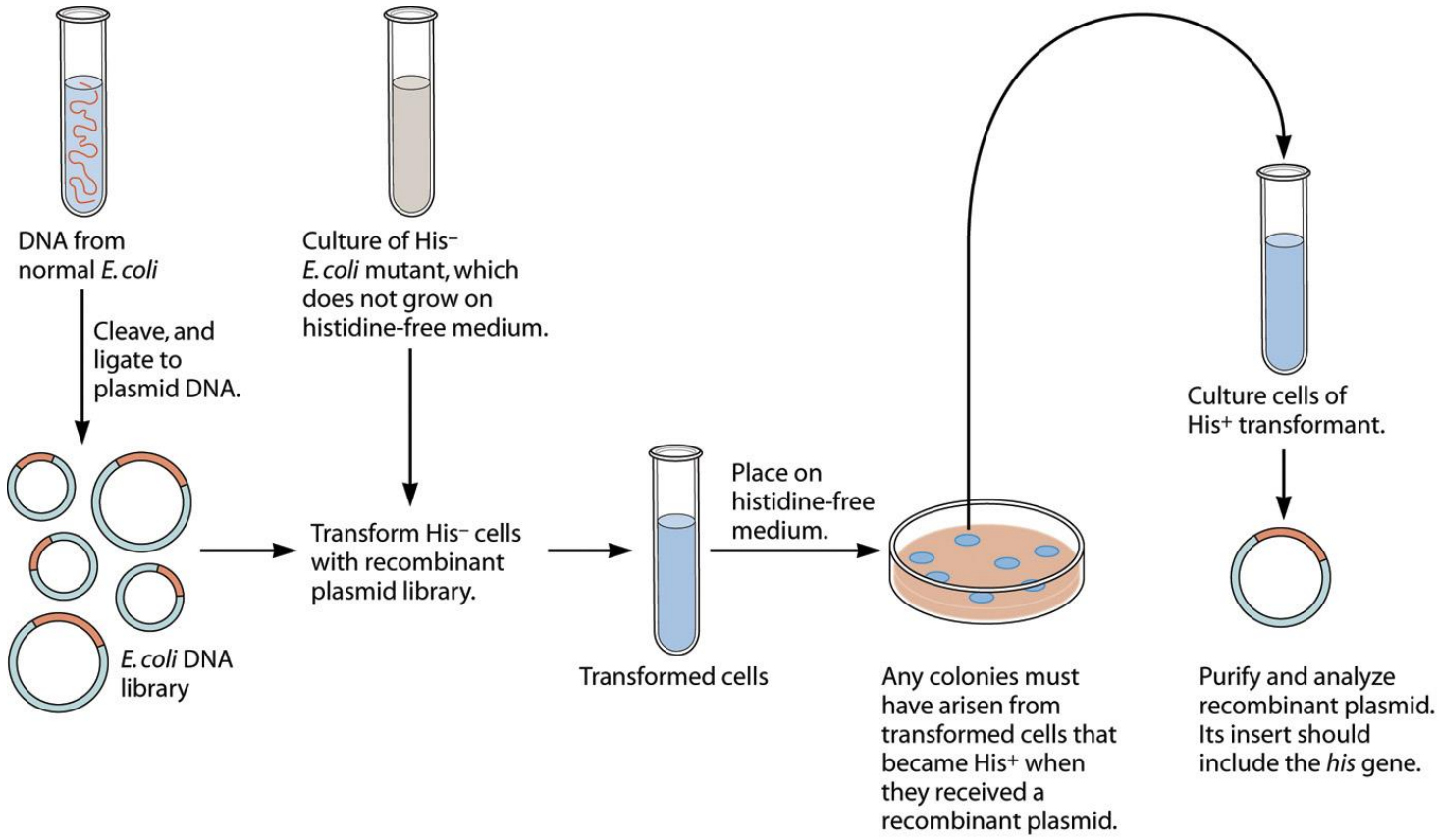
- Mutant
  - An organism with an alteration in its genotype, which leads to observable phenotype alteration
  - Provide information about the genes and involved in producing a trait
- Microorganism
  - *E. coli*



  - Yeast
    - Ease-to-use system to find genes of higher eukaryotes

Finding of His Synthesis Gene

- Screening for His synthesis gene by complementation



Finding Genes

- Drosophila
  - Mutagenesis
    - Transposon : easy to find the integrated site
    - Chemical or UV mutagenesis : difficult to find the mutated site
- Animals
  - Finding genes from phenotype variant
    - Obese mouse, short legged dachshunds, disease etc.
    - Finding genetic markers inherited with the trait
      - Unique restriction fragments
        - » Restriction Fragment Length Polymorphism (RFLP)
      - A single nucleotide difference
        - » Single-Nucleotide Polymorphism (SNP)
    - Searching for candidate gene around the marker

Related Organisms Usually Have Similar Genes

- Gene finding using model organisms
  - Yeast, Drosophila
- Finding homologous genes in higher organisms
  - Homology search of DNA sequence database
  - Confirmation of the predicted function
    - Knock-out mouse

2. 유전형 비교 (Comparing Genotypes)

Comparing Genotypes and Genomes

- Hybridization of DNA from two different species
  - DNA sequence similarity can be measured by melting temperature of the hybridized DNA

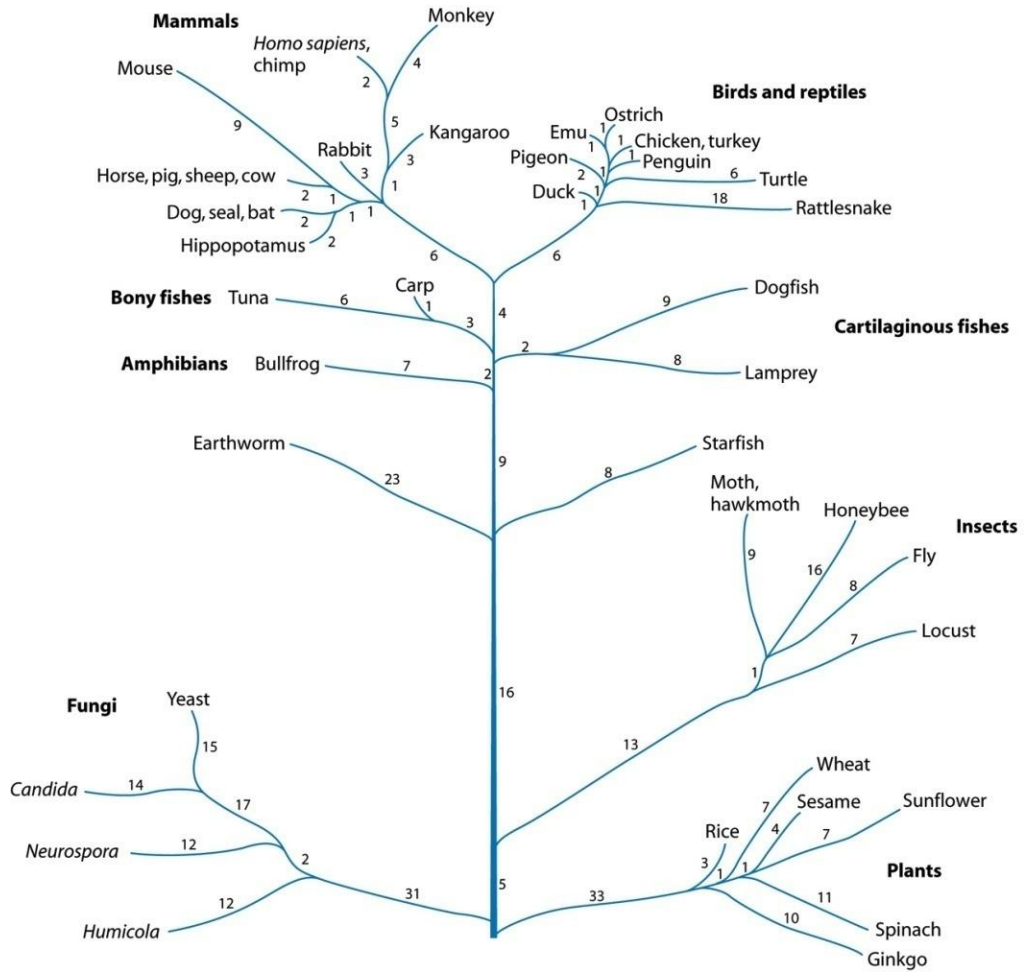
- DNA sequencing
  - To compare distantly related species
    - Use common DNA with slow evolution
  - To compare genotypes within a single species
    - Use rapidly evolving DNA, e.g. mitochondrial DNA

Comparing Genotypes and Genomes

- RFLP
  - Estimation of similarity of DNA by comparison of the similarity of restriction fragment length polymorphism (RFLP)
- AFLP
  - Compare amplified fragment length polymorphism using various PCR primer pairs
- SNPs
  - Single-nucleotide polymorphisms

Genotyping for Evolutionary Studies

- Comparison of DNA and protein sequences
  - Measure the degree of difference
  - Generation of evolutionary tree



Ancient DNA

- Isolation of ancient DNA
  - Samples preserved in bogs or amber
  - Bones and teeth
  - Can be used for archaeology

Timber beetle trapped in amber

Ancient DNA

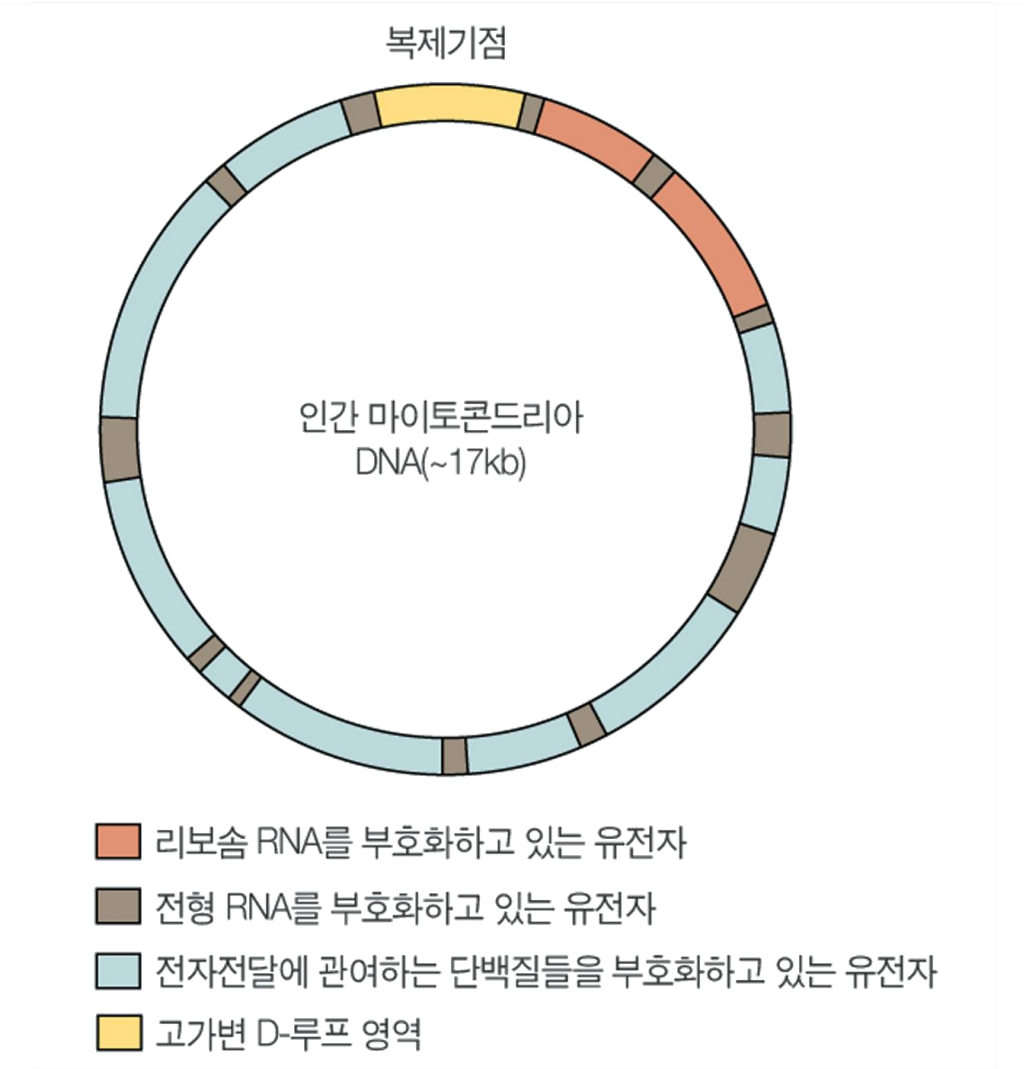
- Mitochondrial DNA from Neanderthal human fossil
  - Lived in the Near East and Europe (125,000 to 30,000 years ago)
  - Mitochondrial DNA showed no relationship to modern human

Modern Neandertals

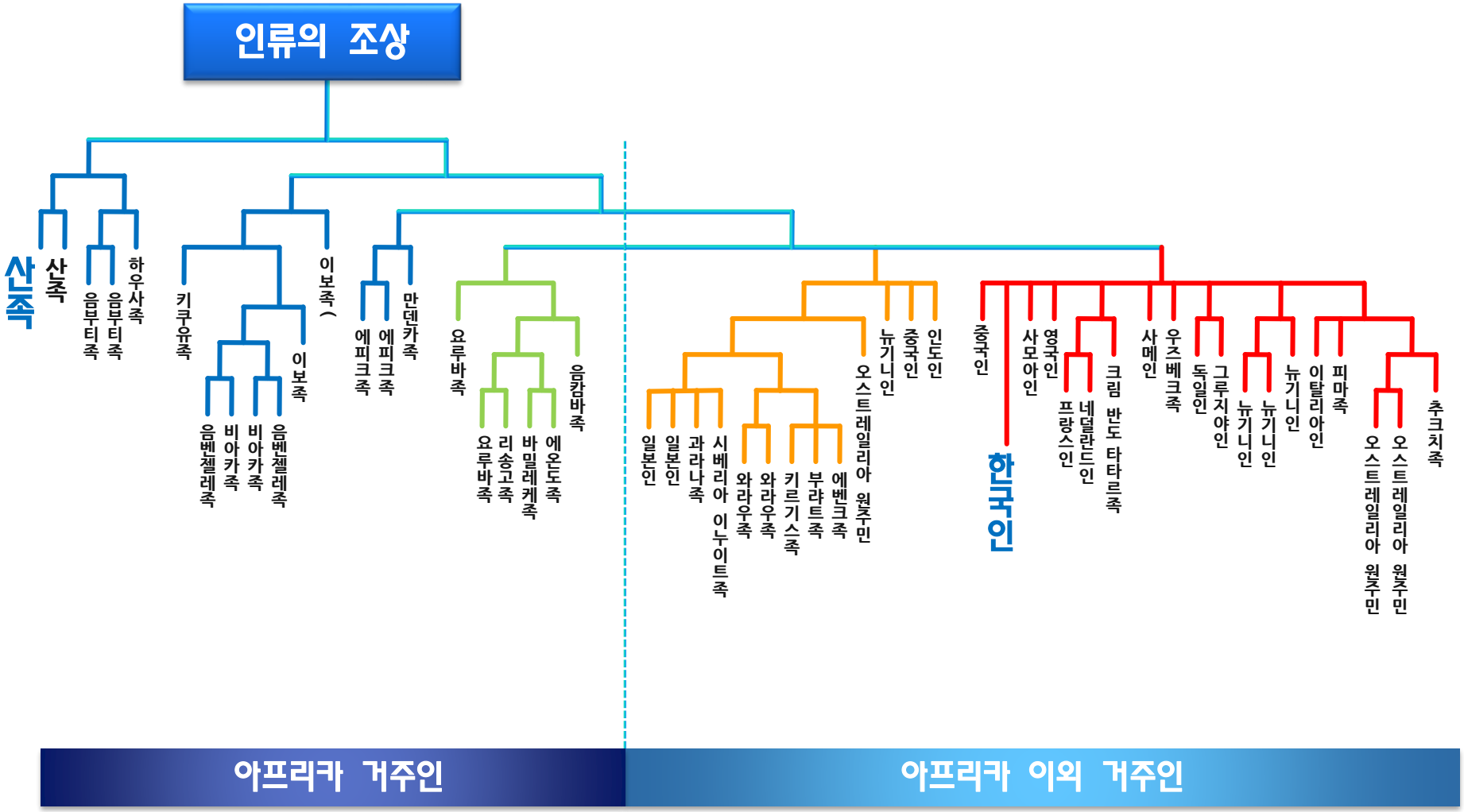
미토콘드리아 DNA 유형

- 미토콘드리아 DNA는 모계에서 유래
- 복제 기점(replication origin) 주위: 돌연변이 발생률 높음
- 잃어버린 가족 찾기, 유해 확인에 활용
  - 아르헨티나 군부의 잔혹한 집권 시에 잃어버린 가족들 찾아 주기
  - 러시아 공주 아나스타샤 확인 작업
  - 전쟁으로 인해 발생한 미확인 유해 확인 작업

미토콘드리아 DNA



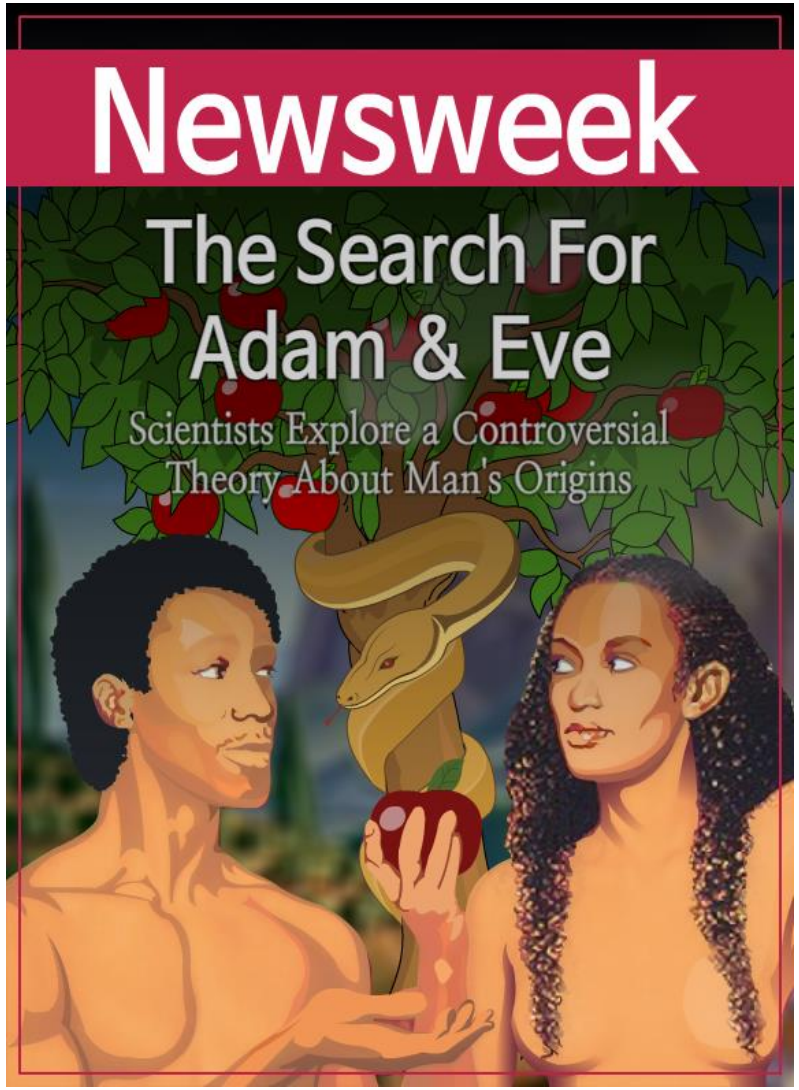
mt DNA를 이용한 인간 가계도



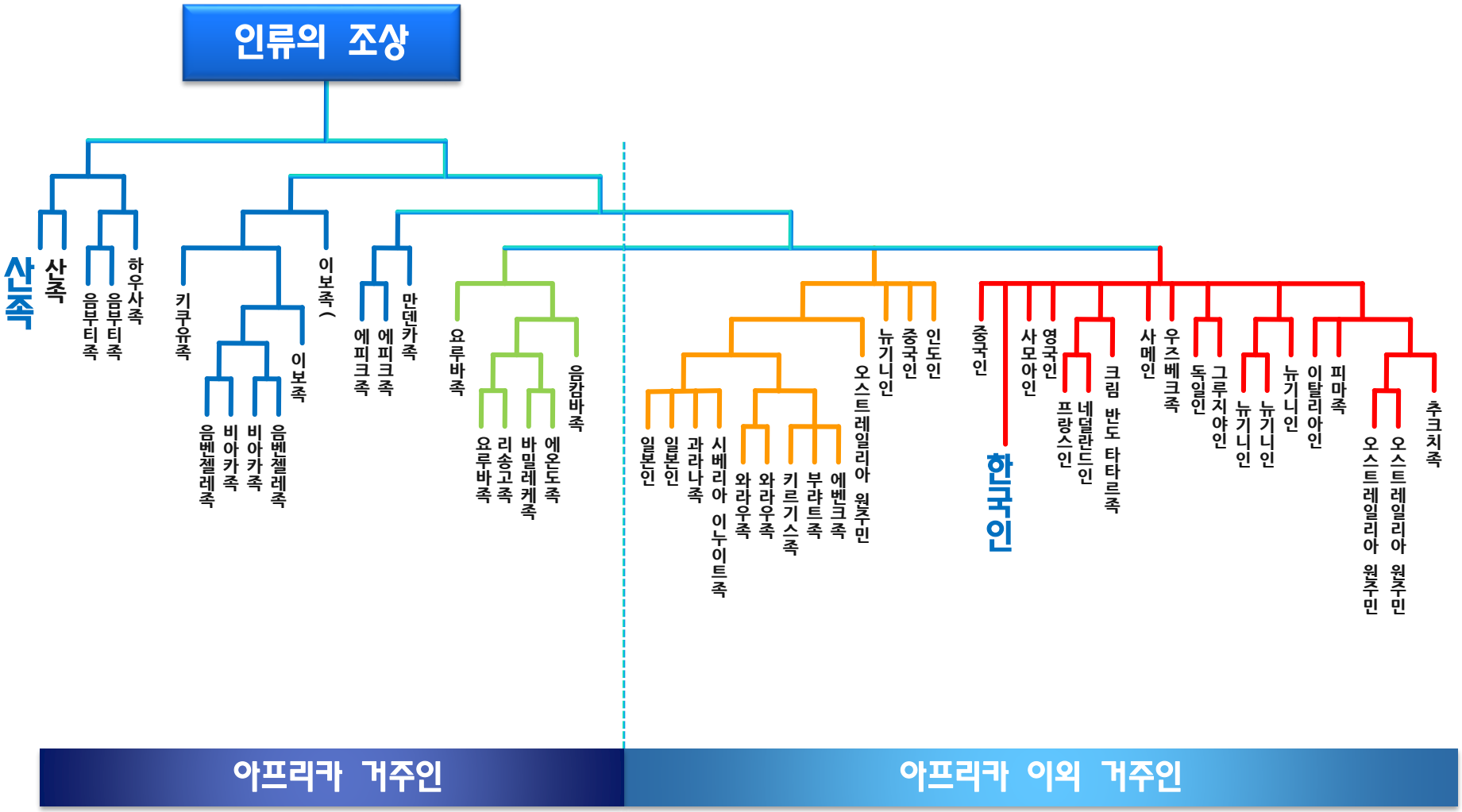
mt DNA를 이용한 인간 가계도

- 두 개의 굵은 가지
  - 아프리카 내의 집단들로만 이루어진 가지
  - 아프리카를 포함한 다른 모든 지역의 집단들로 이루어진 가지
- 우리 모두의 공통 조상은 아프리카로부터 유래
- mt DNA “Eve”
  - 인류 공통의 시조 할머니
  - 약 15만년 전의 아프리카 여성

아담과 이브


Newsweek
The Search For
Adam & Eve
Scientists Explore a Controversial
Theory About Man's Origins

mt DNA를 이용한 인간 가계도



부 시 먼

※ 출처 : pixabay.com

칼라하리 사막의 산족 공동체

※ 출처 : svgsilh.com

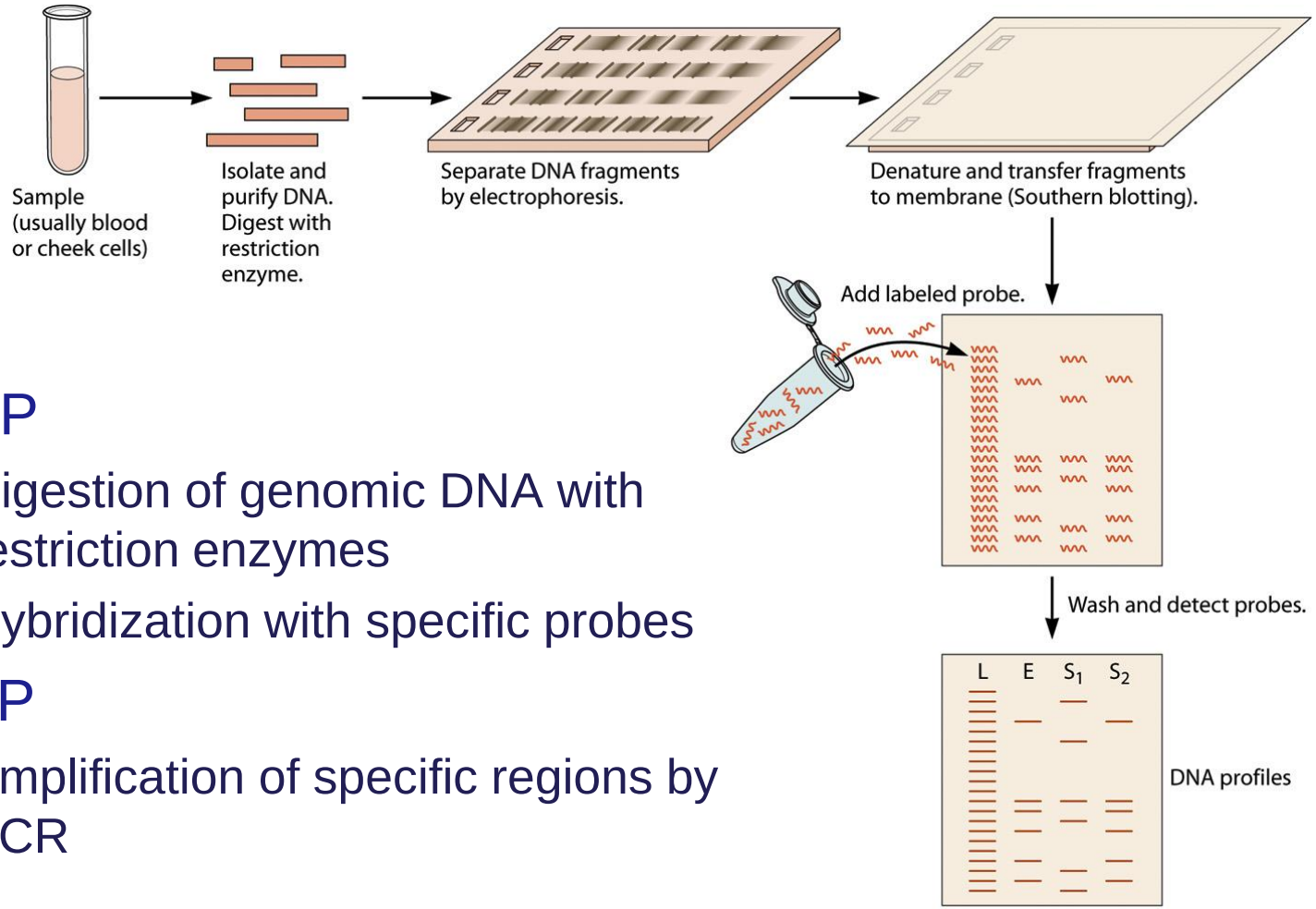
산(San) 족

- 부시먼
  - 17세기말 네덜란드 식민지 정착민들이 산(San) 족을 경멸적으로 부른 이름

- 독특한 언어
  - 혀를 차는 소리
  - 지구상의 대부분의 언어: 20~40가지의 소리
  - 산족 언어: 141개의 소리

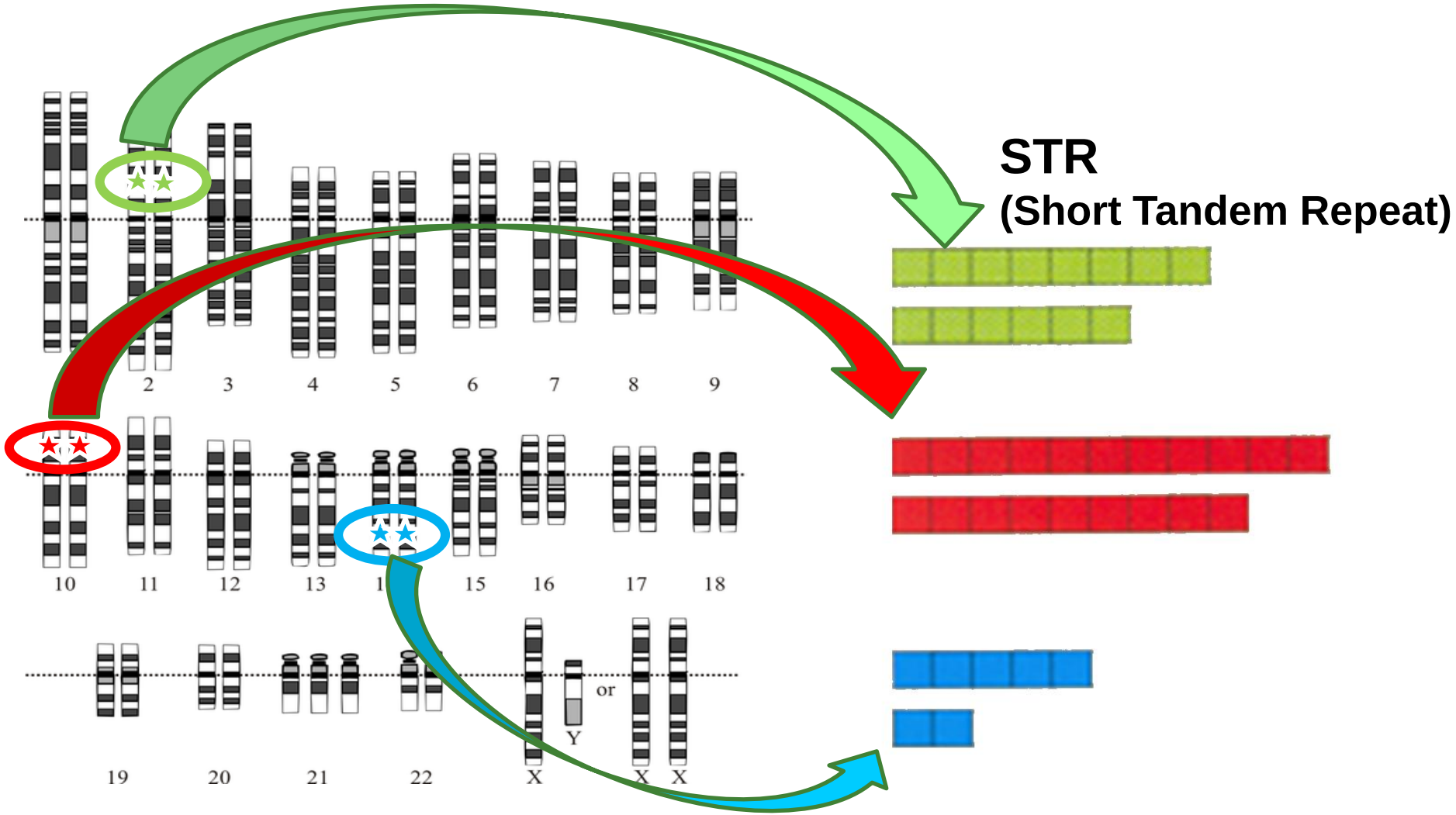
3. DNA 지문 (DNA Fingerprint)

DNA Typing



- RFLP
  - Digestion of genomic DNA with restriction enzymes
  - Hybridization with specific probes
- AFLP
  - Amplification of specific regions by PCR

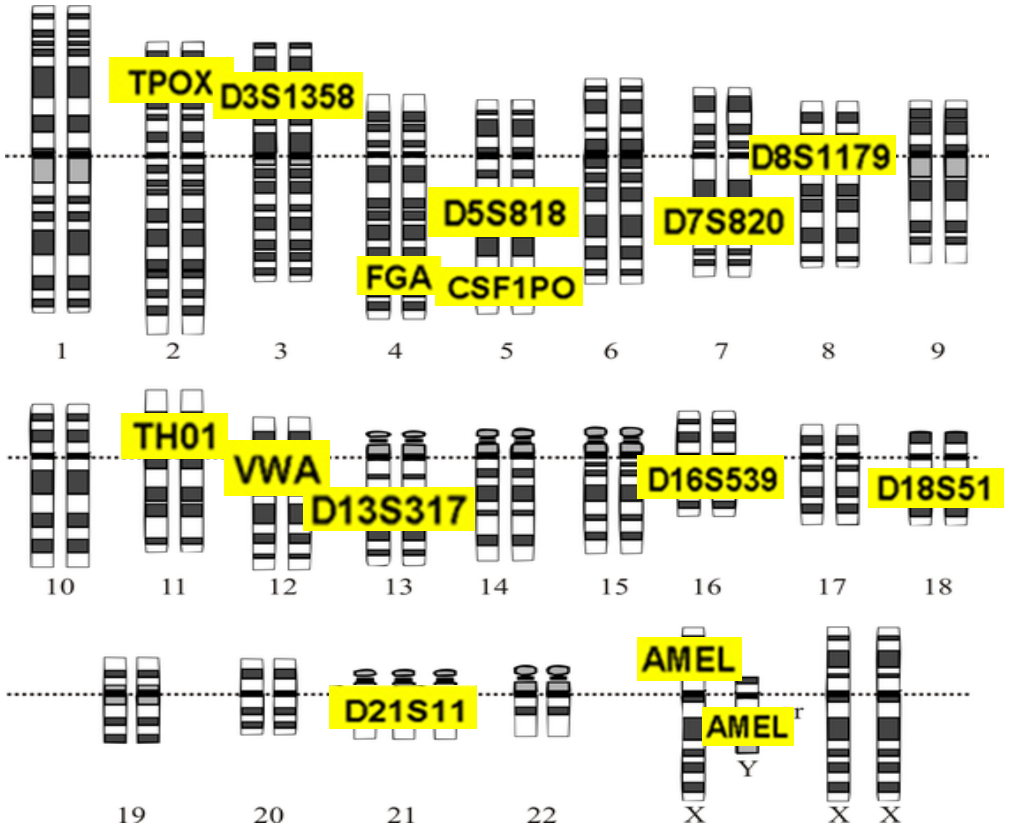
DNA Fingerprinting



※ 출처 : ko.wikipedia.org

DNA Fingerprinting

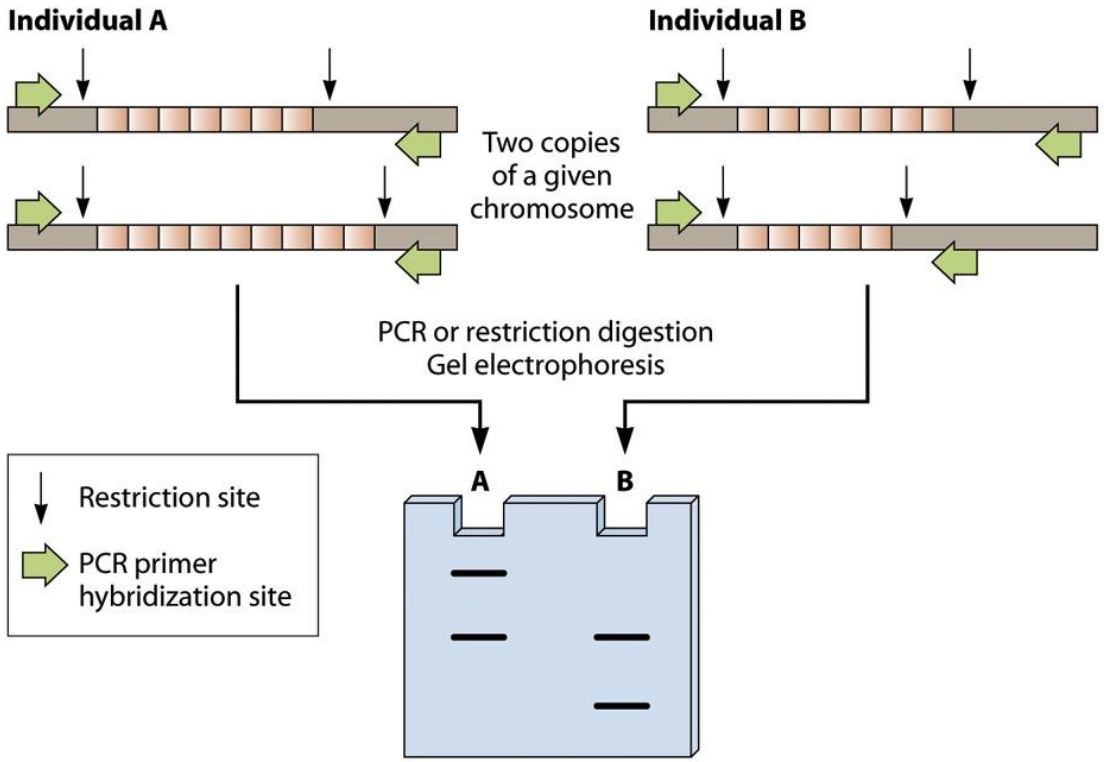
- 짧은 반복 서열(STR)의 길이 차이 분석
- 개인 간 반복 서열의 길이 차이가 많은 부분



※ 출처 : ko.wikipedia.org

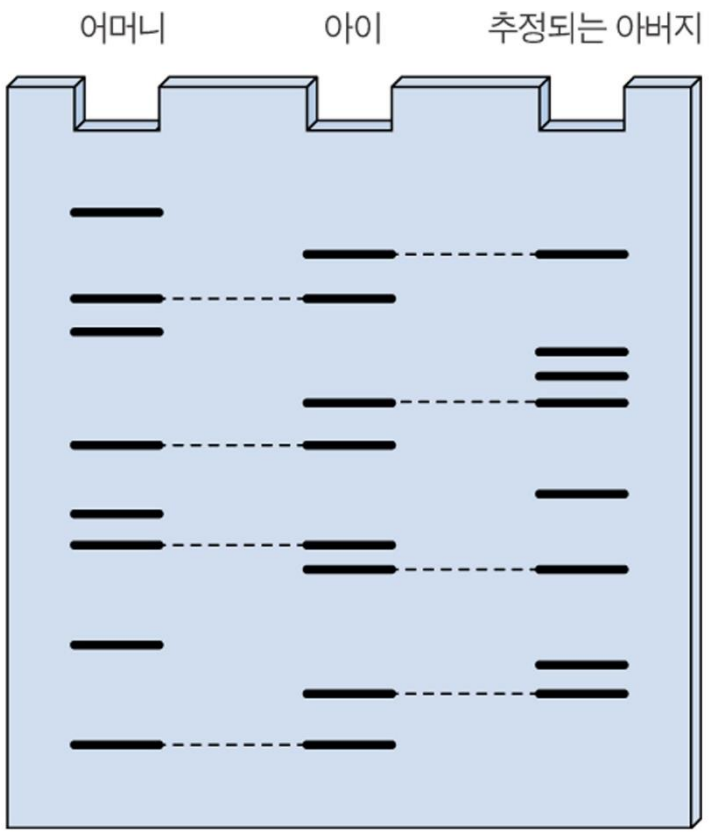
DNA Fingerprinting

- Detection of variable length of repetitive sequences



DNA 유형 분석의 응용

- 범죄 수사
- 친자 확인
- 유해 신원 확인
  - (전사자, 사망자 등)
- 보존 생물학, 보존 생태학 등



Genomics

- Gene chip/ microarray
  - A grid of spots of DNA on a tiny glass or silicon
  - Fragment of DNA or synthetic oligonucleotide
- -omics : global analysis
  - Genomics: analysis of entire genome or global analysis of gene expression (mRNA)
  - Proteomics: global analysis of protein expression

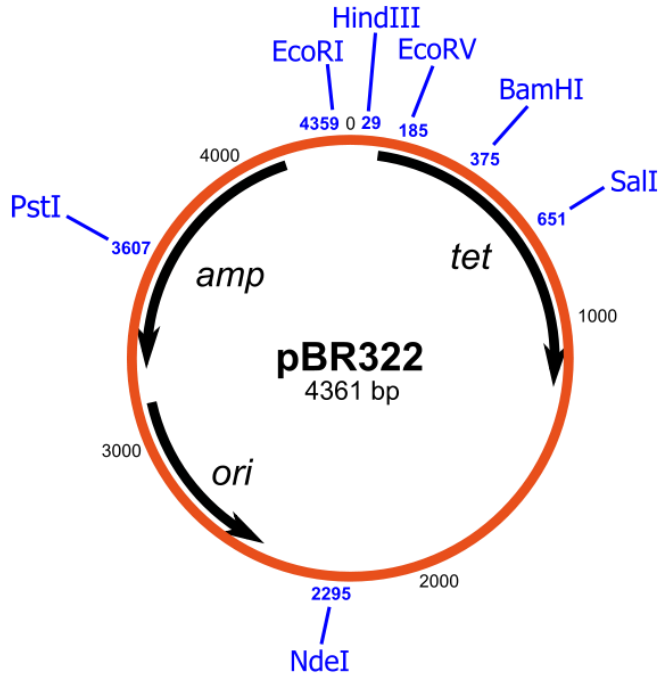
4. 유전공학 (Genetic Engineering)

Genetic Engineering

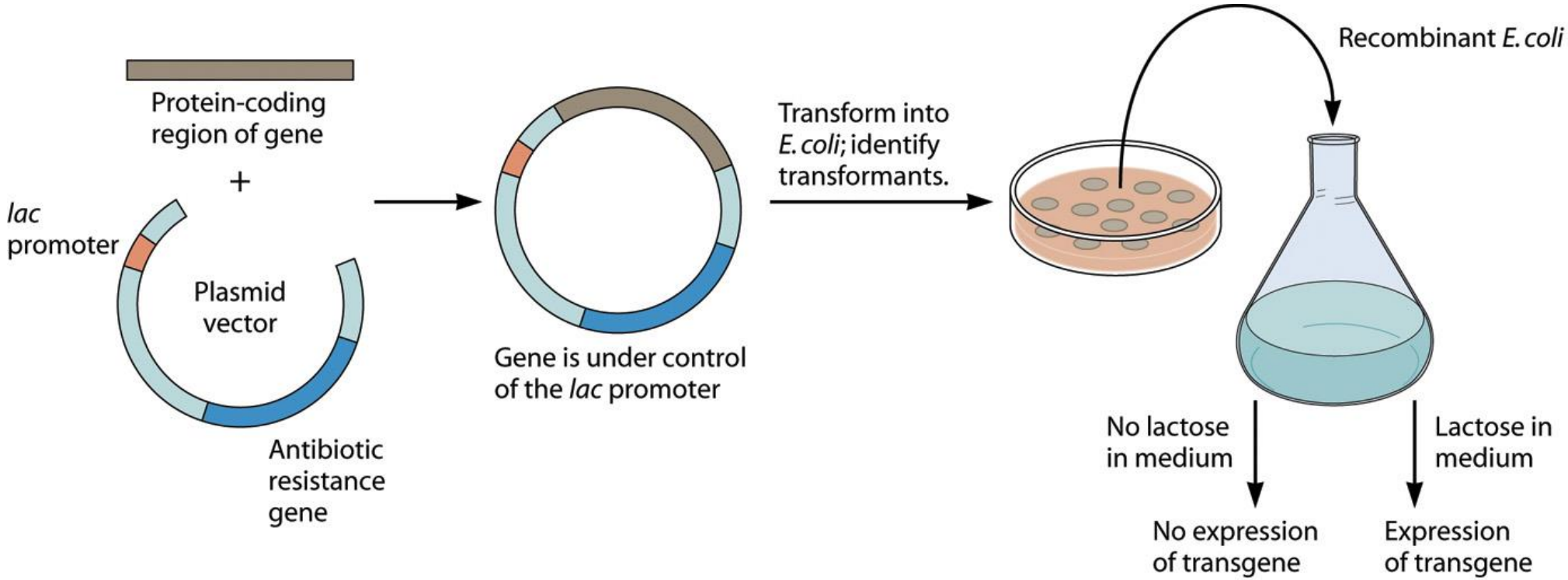
- Genetic engineering
  - The process of directed manipulation of the genome of an organism
- Transgenic organism
  - An organism containing a gene (transgene) from another source
- Purpose of genetic engineering
  - To analyze gene function
  - To obtain large quantities of a protein
    - Therapeutic proteins, enzymes etc

Genetic Engineering of Microorganisms

- Cloning of eukaryotic gene to express in *E. coli*
  - Making cDNA
  - Cloning vector
    - Prokaryotic promoter
      - Lac promoter: inducible by lactose or its imitates
    - Multicloning sites for inserting DNA
    - *E. coli* replication origin
    - Selection markers



Genetic Engineering of Microorganisms



Genetic Engineering of Plants

- Purpose of plant genetic engineering
  - Plant resistant to insect pests
  - Plant resistant to viral disease
  - Plant resistant to drought
  - Plant resistant to frost
  - Production of edible vaccine proteins in fruits
  - Production of medicinal proteins in plants

Genetic Engineering of Plants

- Resistance to viral diseases
  - Expression of coat protein gene of the tobacco mosaic virus
    - Resistance to TMV
- Fighting aluminum toxicity
  - Aluminum toxicity in the humid tropical climates or acidified soil
  - Transgenic plant expressing citrate synthase
    - Citric acid binds to soil aluminum and prevents entering the plant roots

Genetic Engineering of Animals

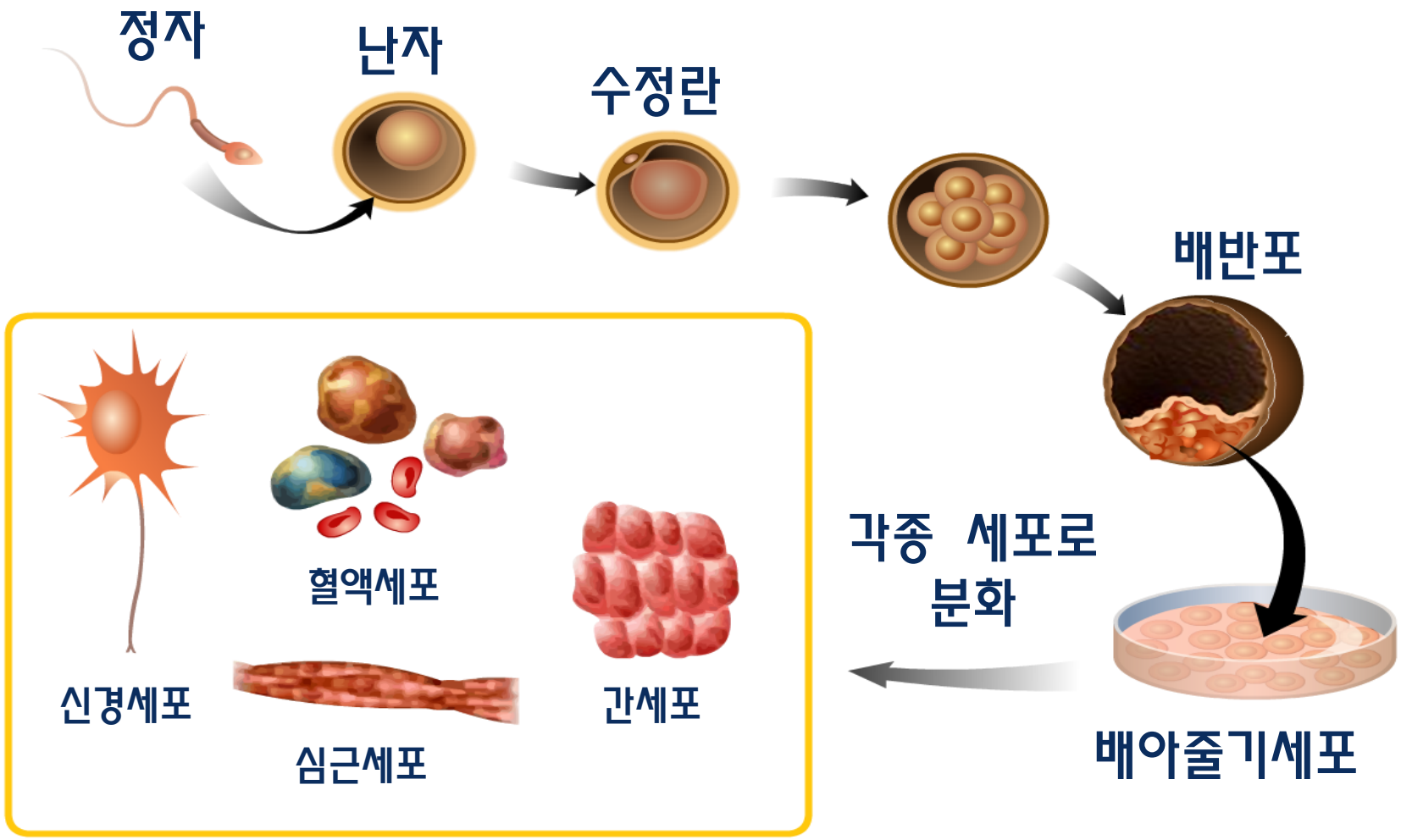
- Microinjection of DNA into fertilized egg
  - Very low chance of proper integration of DNA into genome
  - Random integration

Transgenic pigs that secrete human blood-clotting proteins in their milk

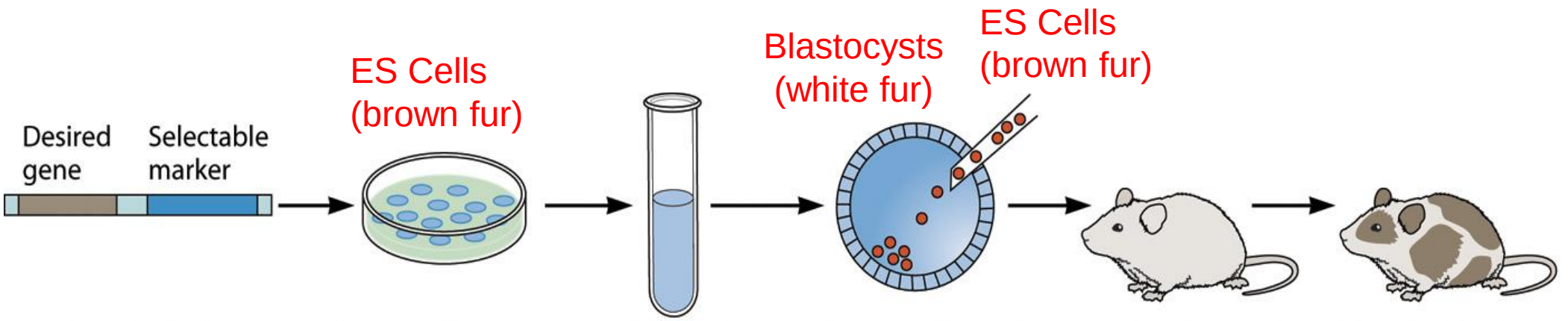
Genetic Engineering of Animals

- Gene replacement in ES cells
  - Introduction of linear DNA containing a manipulated gene into ES cells
  - Selection for the homologous recombination using markers
  - Injection of the selected ES cells to blastocysts
  - Implantation of the blastocyst into surrogate mother
  - Isolation of chimera mice containing manipulated ES cells
  - Selection for heterozygote mice with germ line transmission
  - Selection for a homozygote mouse by mating heterozygotes

배아줄기세포 (ES Cell)



Making Transgenic Mouse with ES



Introduce desired gene into linear vector with selectable marker gene such as antibiotic resistance.

Select transformants on antibiotic medium. Transformants have integrated the DNA into their genomes since the linear vector cannot be maintained independently.

Isolate and test DNA from individual transformants to verify correct insertion event.

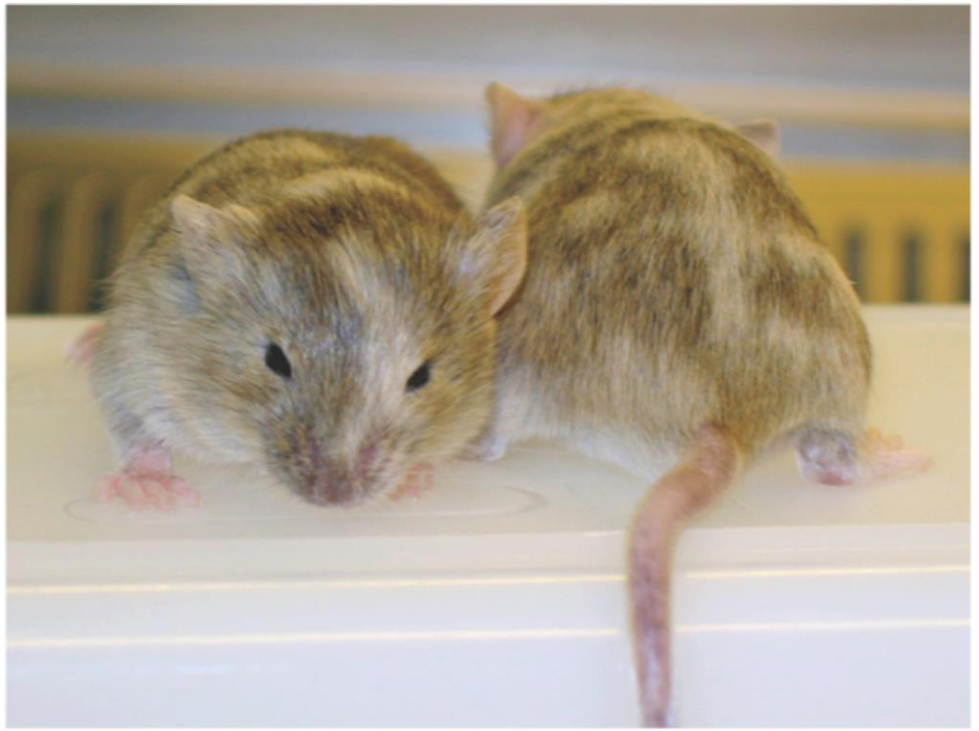
Inject transformed ES cells into mouse blastocyst.

Implant injected blastocysts into female mouse.

Chimeric offspring have cells descended from the ES cells (brown fur) and the blastocyst (white fur).

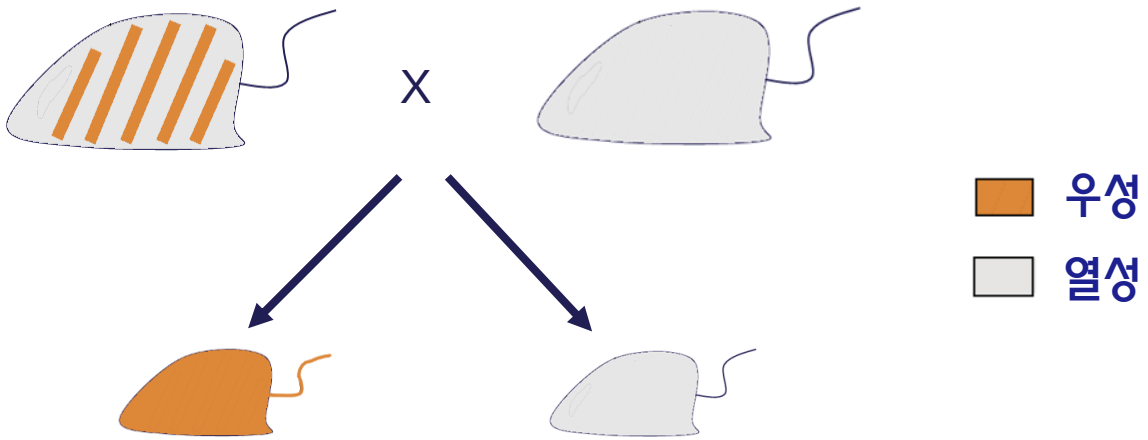
Chimeric Mice

- The white fur is derived from blastocyst cells, while the brown fur is derived from the ES cells.



Chimeric Mice

- 전체 세포가 형질 전환된 마우스 제조
  - 생식세포 계열 형질 전환



갈색 이형접합체
(heterozygote)

  - 갈색 이형접합체(heterozygote) 간에 새끼를 낳아서 동형접합체(homozygote) 생산

Knockout Mice

- Confirmation of the gene function using knockout mice
  - Mice with a gene deletion
- Model system for human disease
  - Genomes of human and mouse are 80% similar
  - Useful for developing and testing new therapies and drugs