Chapter 16

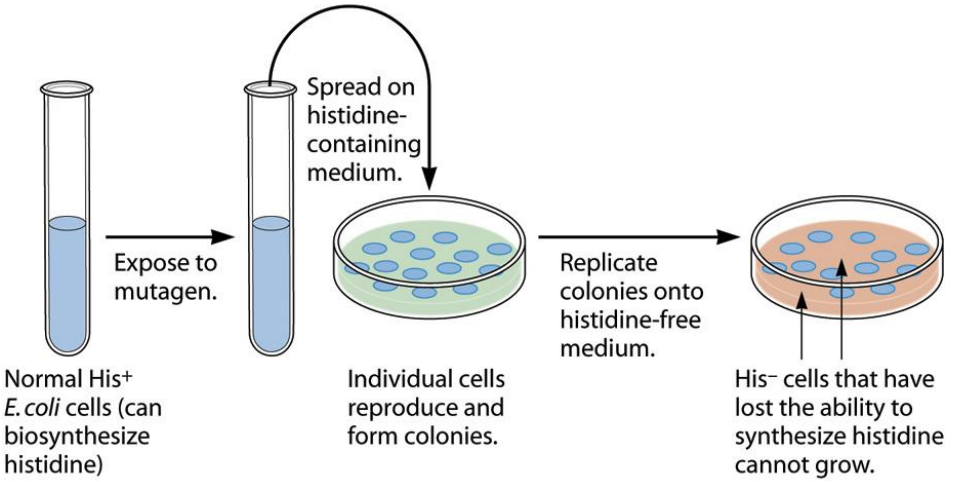
Biotechnology in the Research Laboratory

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

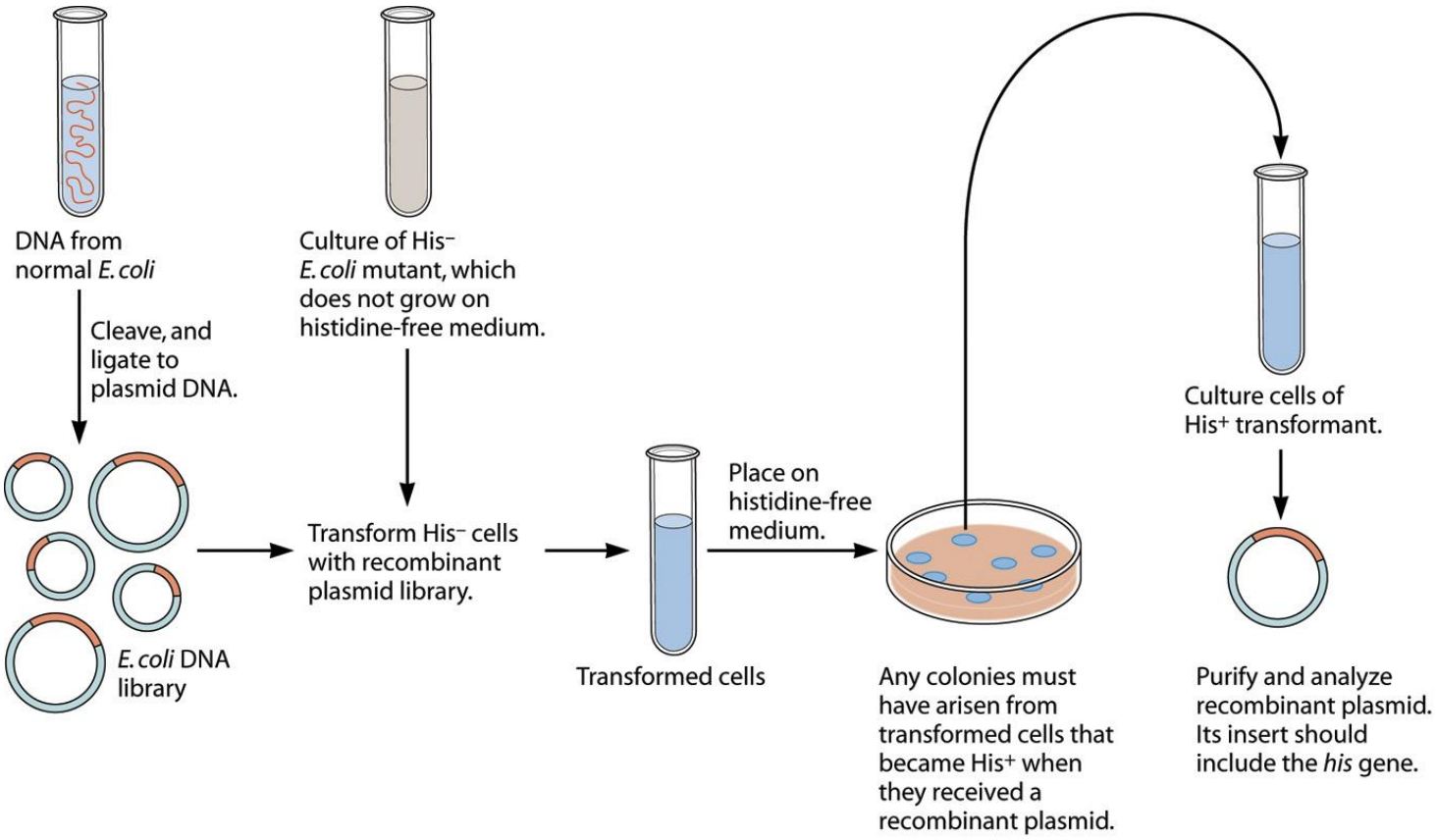
- Mutant
  - An organism with an alteration in its genotype, which leads to observable phenotype alteration
  - Provide information about the genes and involved in producing a trait
- Microorganism
  - *E. coli*



  - 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

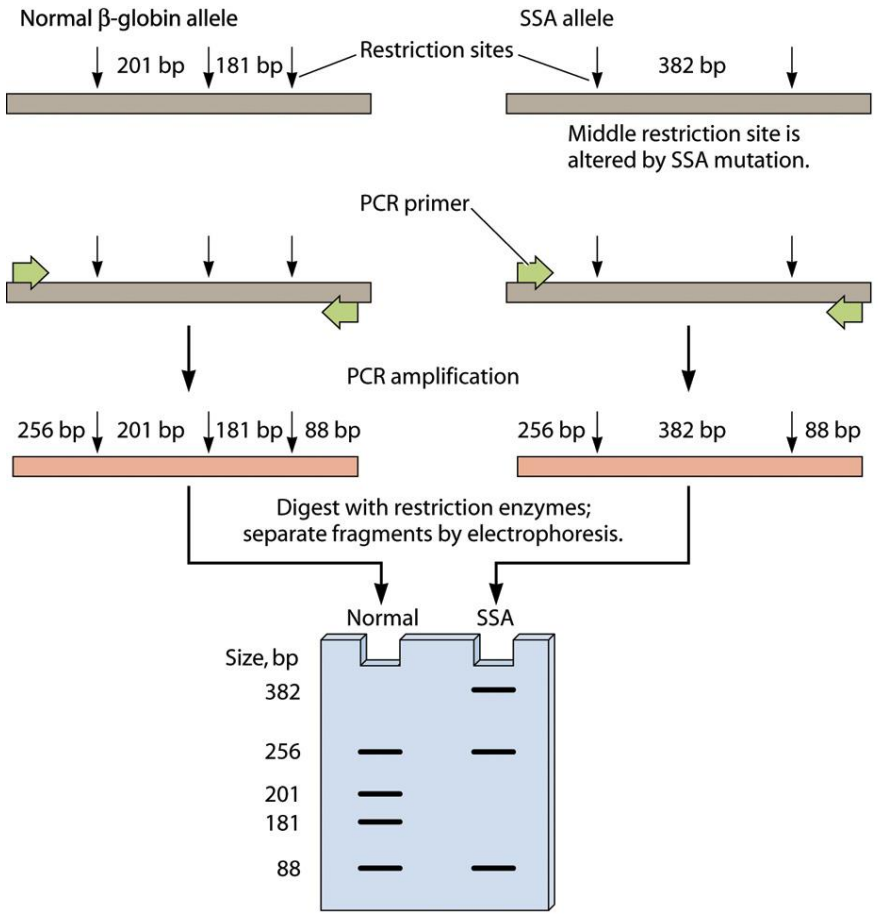
- Drosophila
  - Mutagenesis
    - Transposon : easy to find the integrated site
    - Chemical or UV mutagenesis : difficult to find the mutated site
- Animals
  - Finding genes from phenotype variant
    - Obese mouse, short legged dachshunds, disease etc.
    - Finding genetic markers inherited with 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

Testing for Sickle-Cell Anemia

- Mutation of hemoglobin (β-globin gene)
  - Alteration of restriction site
  - PCR of β-globin gene and cutting with restriction enzyme



Marker-Assisted Plant Breeding

- Using markers linked to desired traits for plant breeding
  - Tasty fruit
  - Drought resistance
- Save time compared to trial and error-based plant breeding

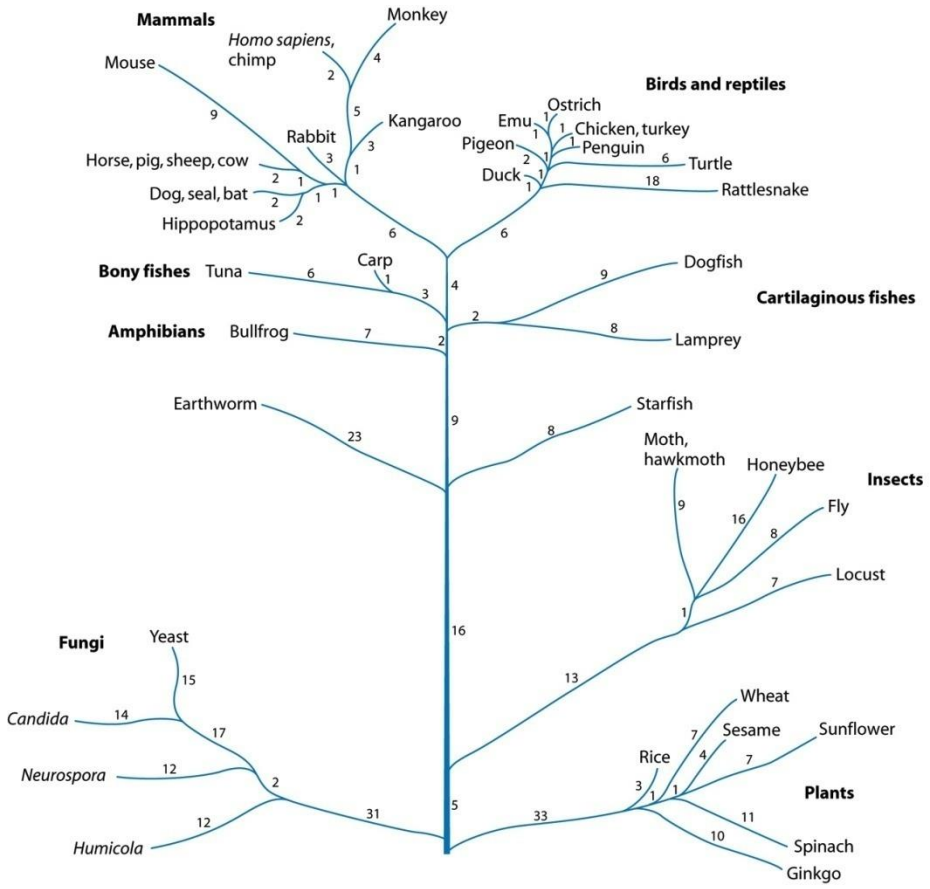
Drought-resistance rice developed in eastern India

Comparing Genotypes and Genomes

- Comparison of genotypes
  - 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
  - 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



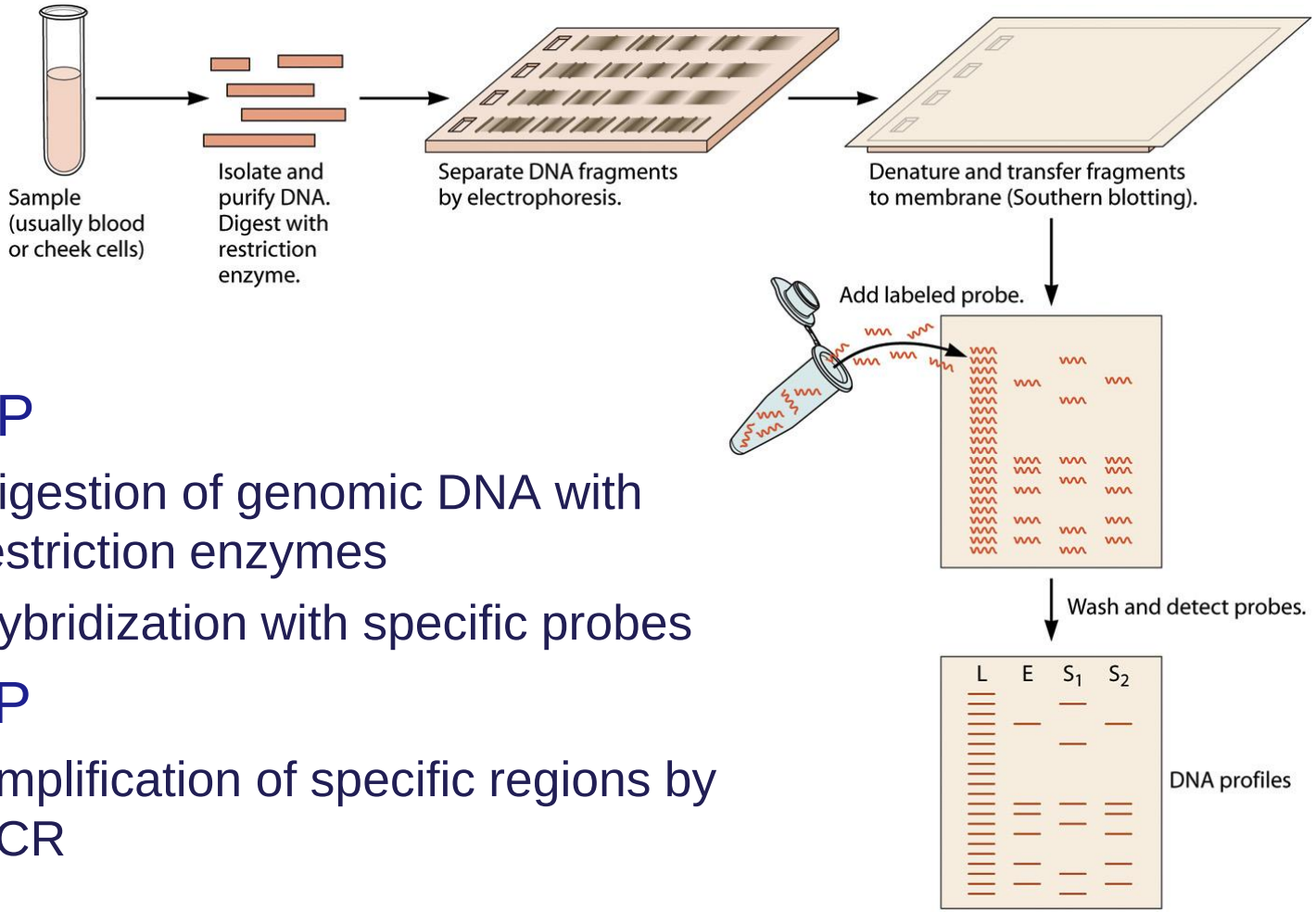
Ancient DNA

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

Timber beetle trapped in amber

Modern Neandertals

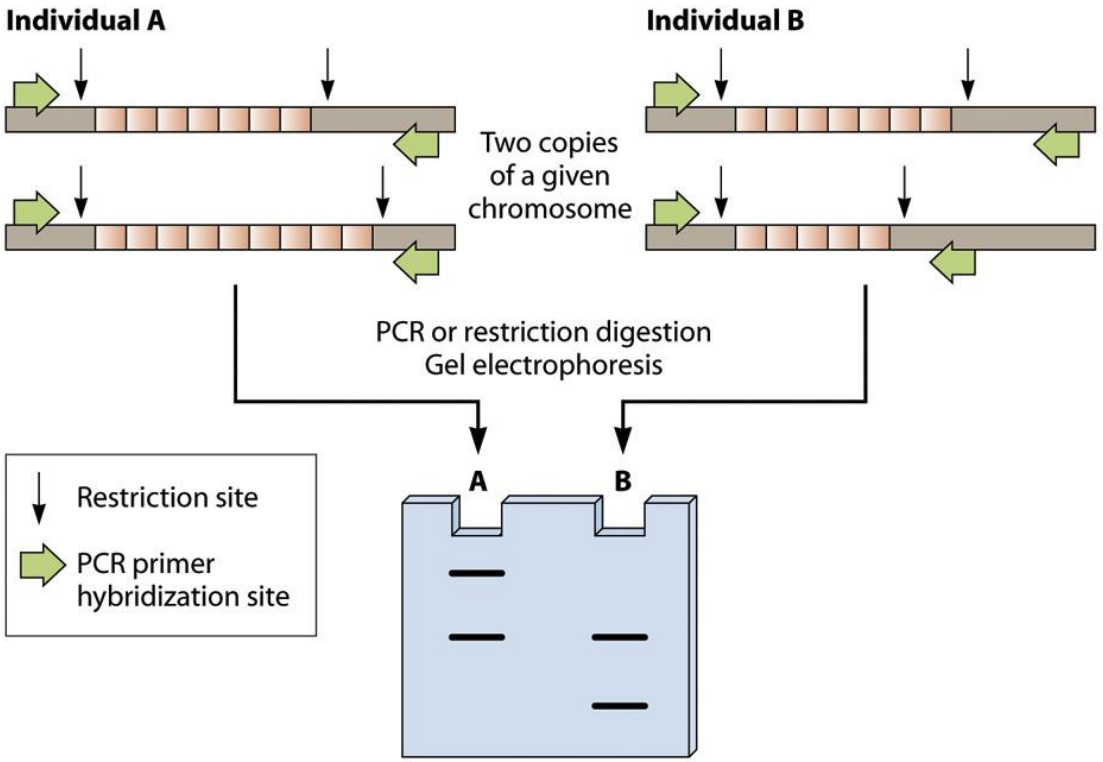
DNA Typing



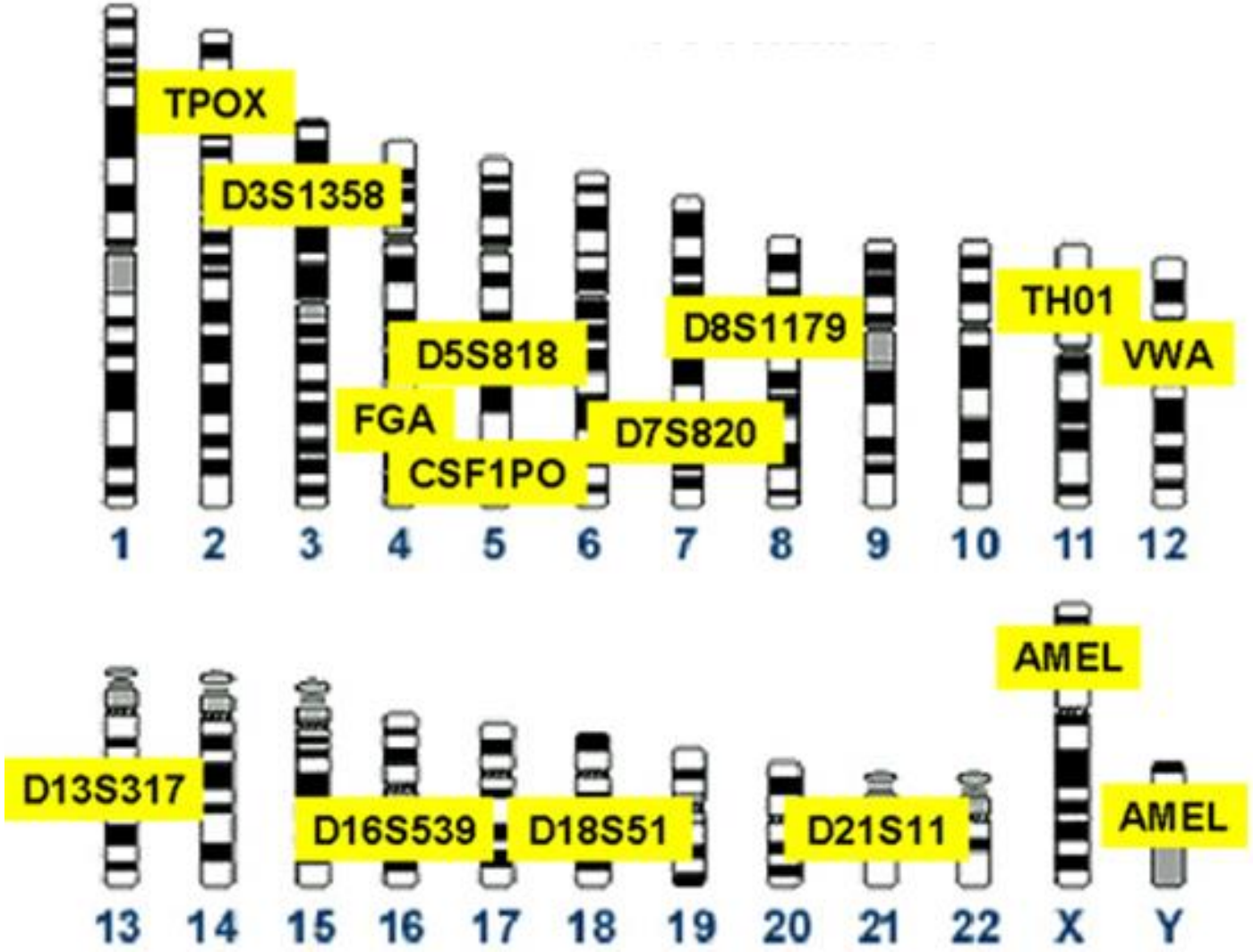
- RFLP
  - Digestion of genomic DNA with restriction enzymes
  - Hybridization with specific probes
- AFLP
  - Amplification of specific regions by PCR

DNA fingerprinting

- Detection of variable length of repetitive sequences

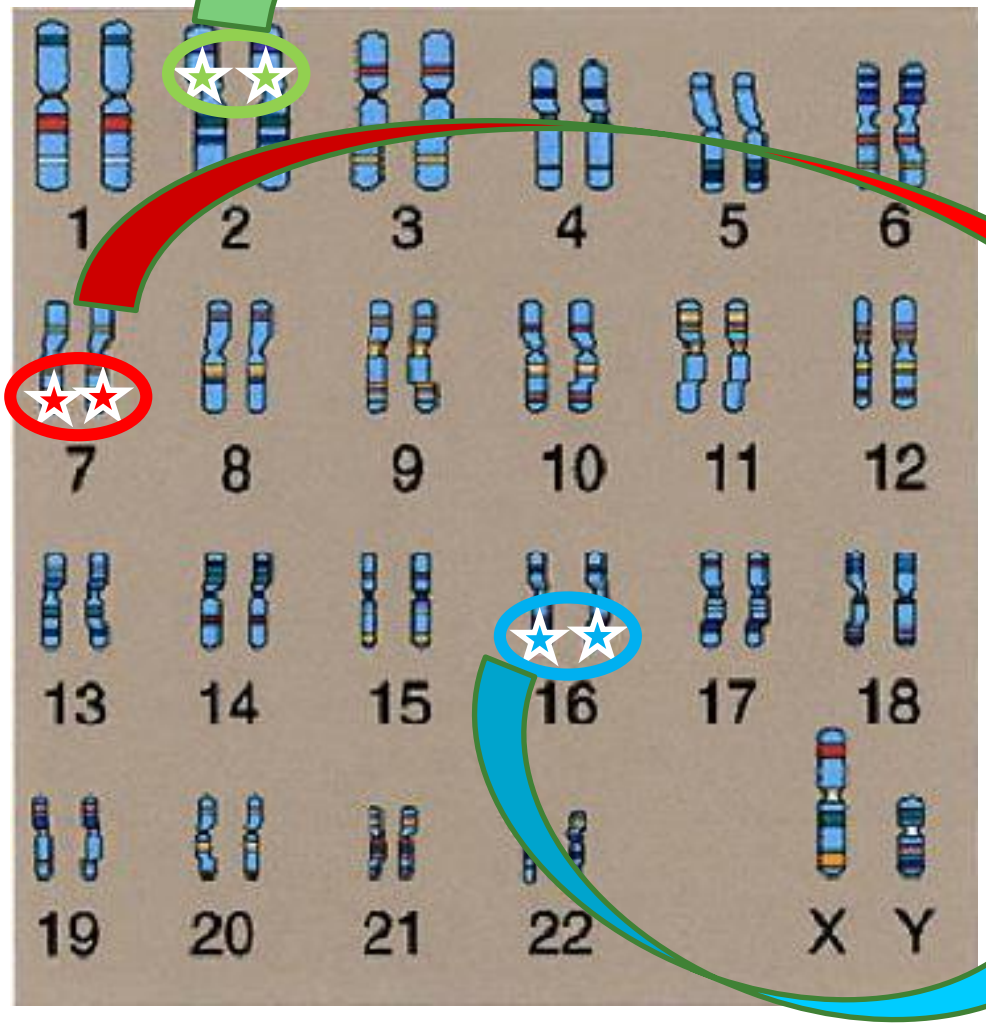


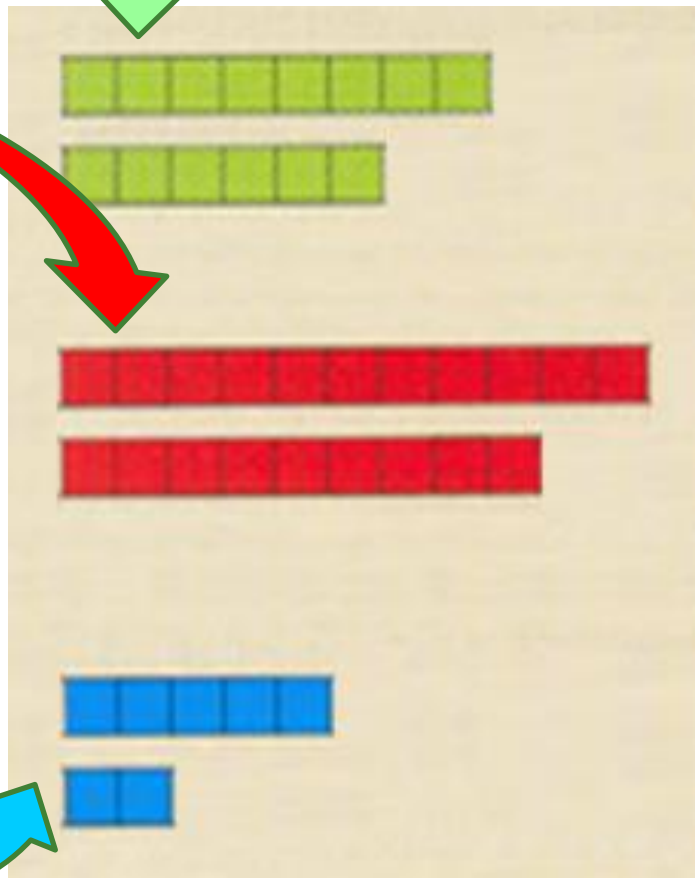
개인차가 많은 구간


TPOX
D3S1358
D5S818
FGA
CSF1PO
D7S820
D8S1179
TH01
VWA
1
2
3
4
5
6
7
8
9
10
11
12
D13S317
D16S539
D18S51
D21S11
AMEL
AMEL
13
14
15
16
17
18
19
20
21
22
X
Y

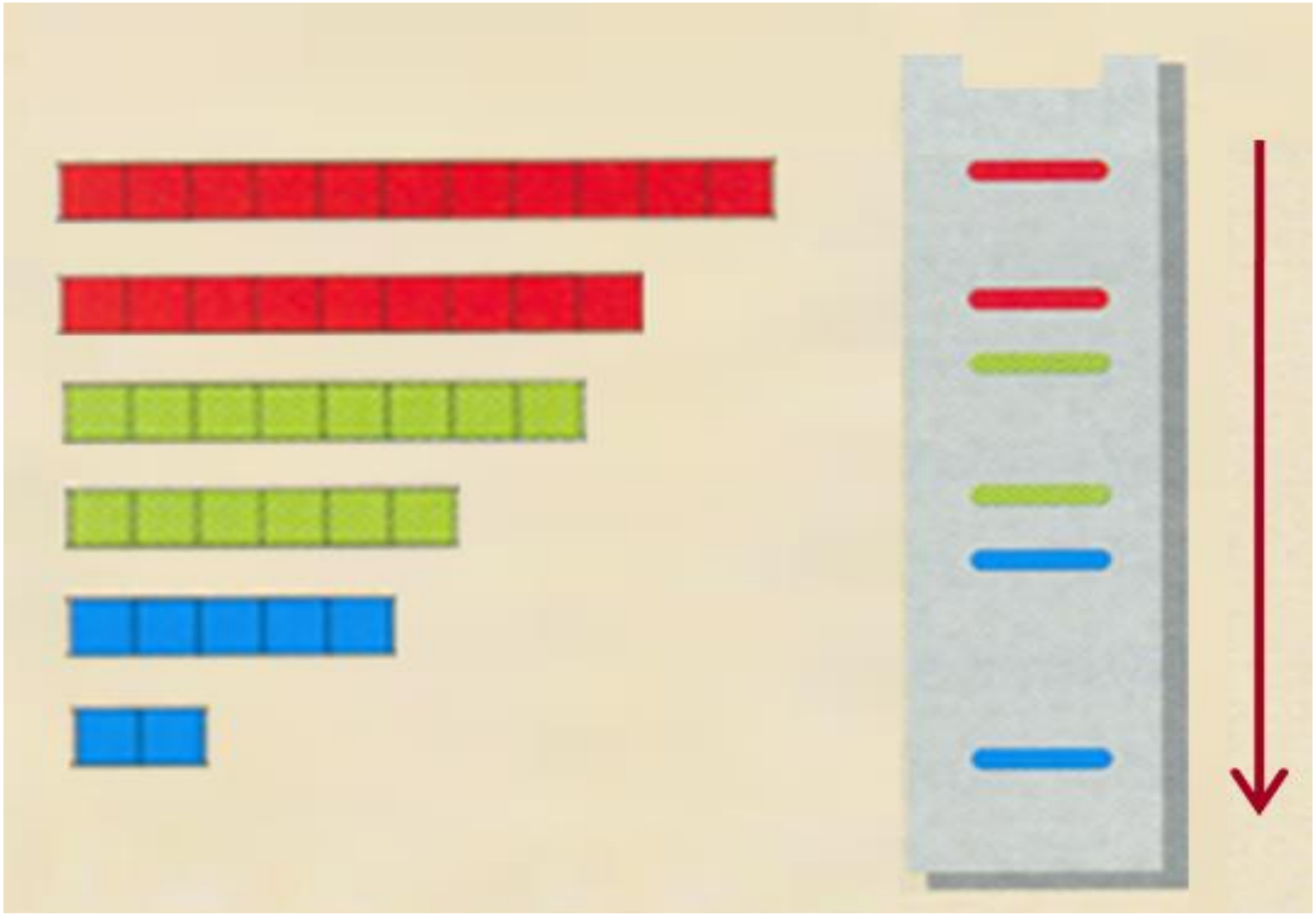
염 색 체 (23쌍)

STR
(Short Tandem Repeat)



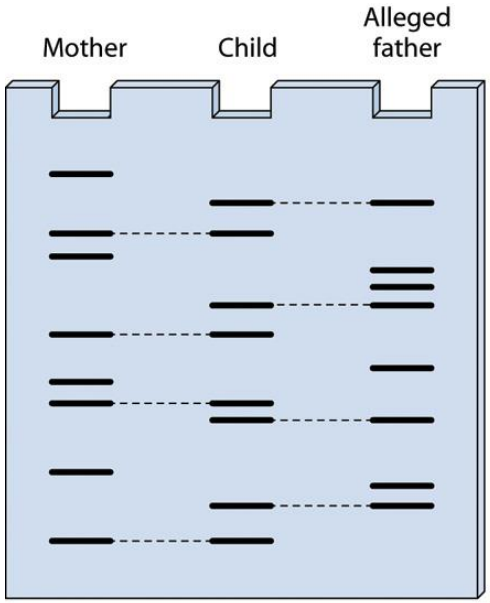


DNA Fingerprinting



Application of DNA Typing

- Forensic DNA typing
- Paternity/maternity testing
- Identification of human remains
- Conservation biology and ecology

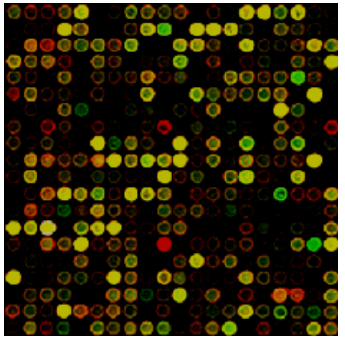


Genomics

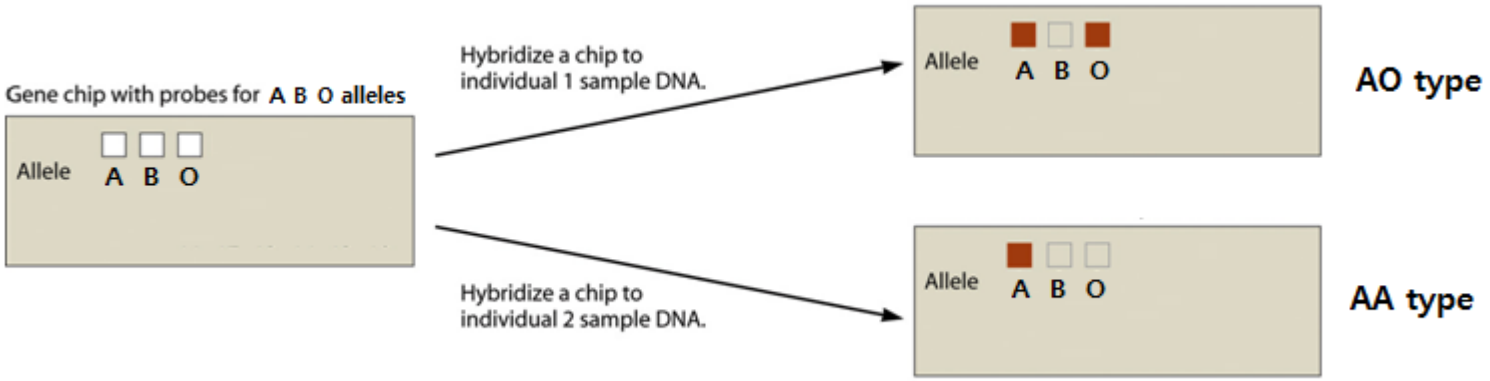
- Gene chip/ microarray
  - A grid of spots of DNA on a tiny glass or silicon
  - Fragment of DNA or synthetic oligonucleotide

- -omics : global analysis
  - Genomics: analysis of entire genome or global analysis of gene expression (mRNA)
  - Proteomics: global analysis of protein expression

Analyzing Genotypes and Gene Expression

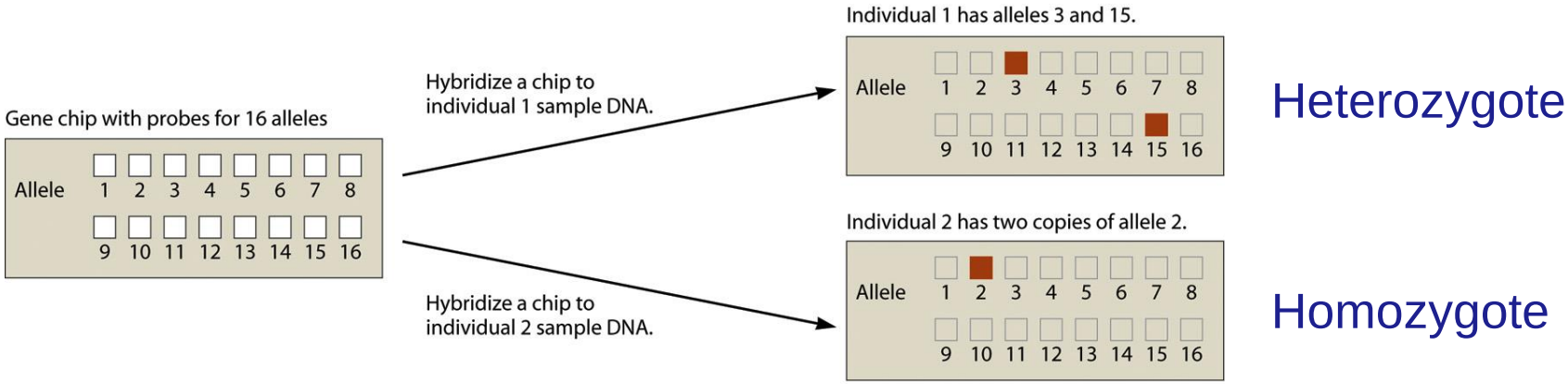


- Application of gene chip
  - Analysis of specific allele
  - Diagnosis of disease
  - Analysis of mRNA expression pattern
- Methods
  - The fluorescence-labeled sample DNA is allow to hybridize with the DNA on the gene chip.
  - Detection with computerized optical scanner



Analysis of Specific Allele

- It is now known that each of the A, B, and O alleles is actually a class of multiple alleles with different DNA sequences that produce proteins with identical properties: more than 70 alleles are known at the ABO locus. An individual with "Type A" blood may be an AO heterozygote, an AA homozygote, or an A'A heterozygote with two different 'A' alleles.

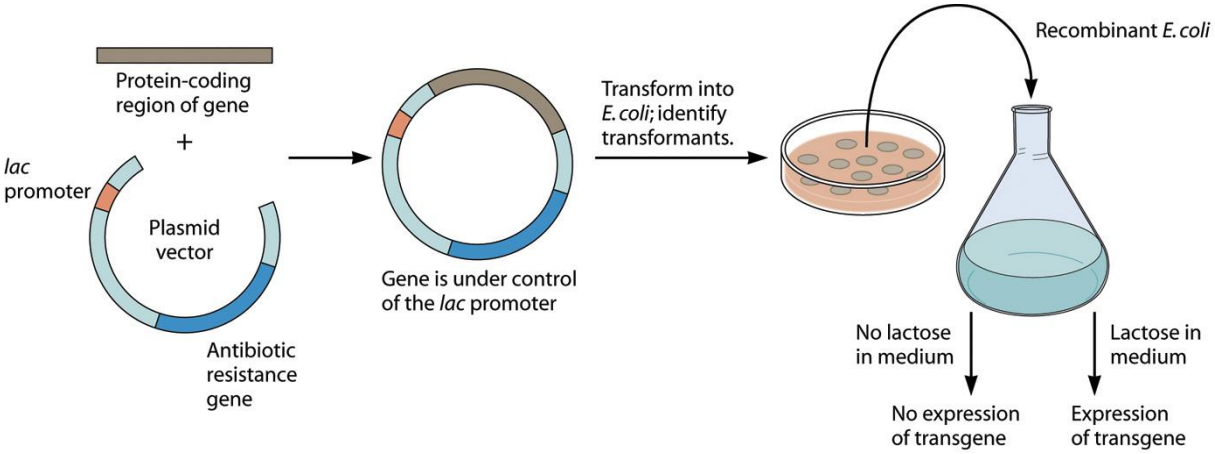


Genetic Engineering

- 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

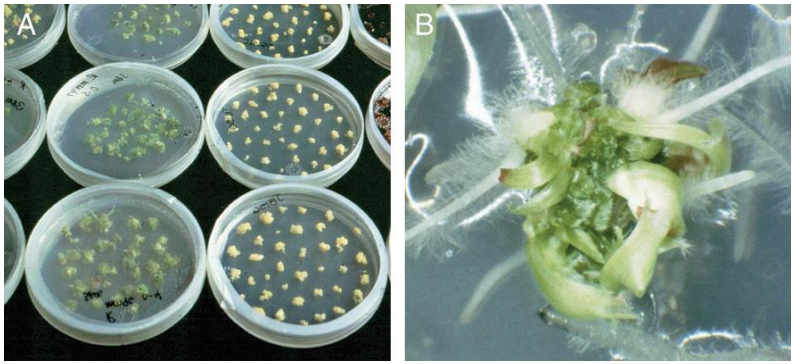
Genetic Engineering of Microorganisms

- 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 Plants

- Purpose of plant genetic engineering
  - Plant resistant to insect pests
  - Plant resistant to viral disease
  - Production of edible vaccine proteins in fruits
  - Production of medicinal proteins in plants
  - Plant resistant to frost
- Plant tissue culture
  - Regeneration of an entire plant from a single piece of tissue, or a single cell
  - Callus culture → regeneration of a plant by controlling hormone levels



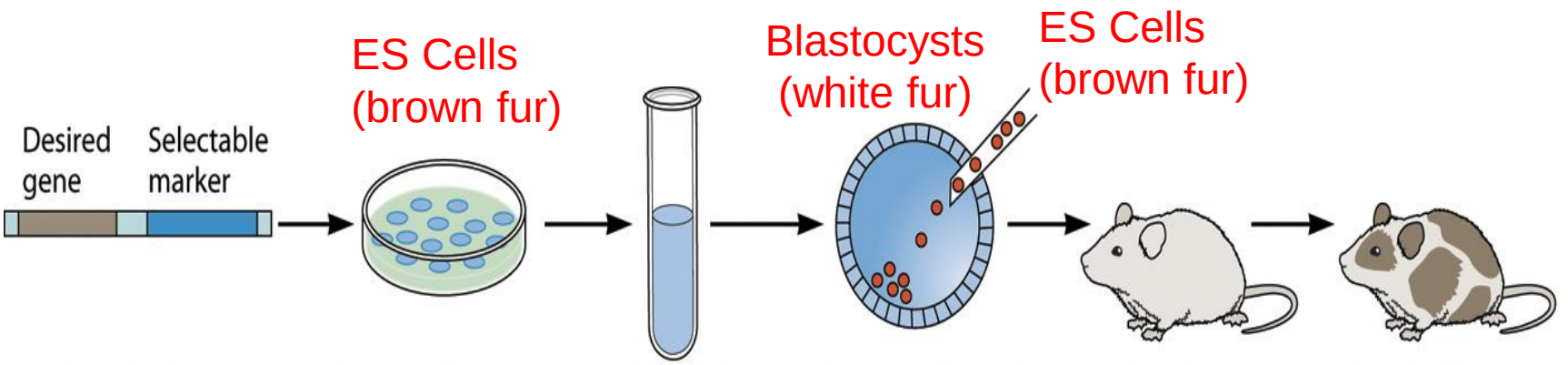
Genetic Engineering of Plants

- 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
- 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

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.

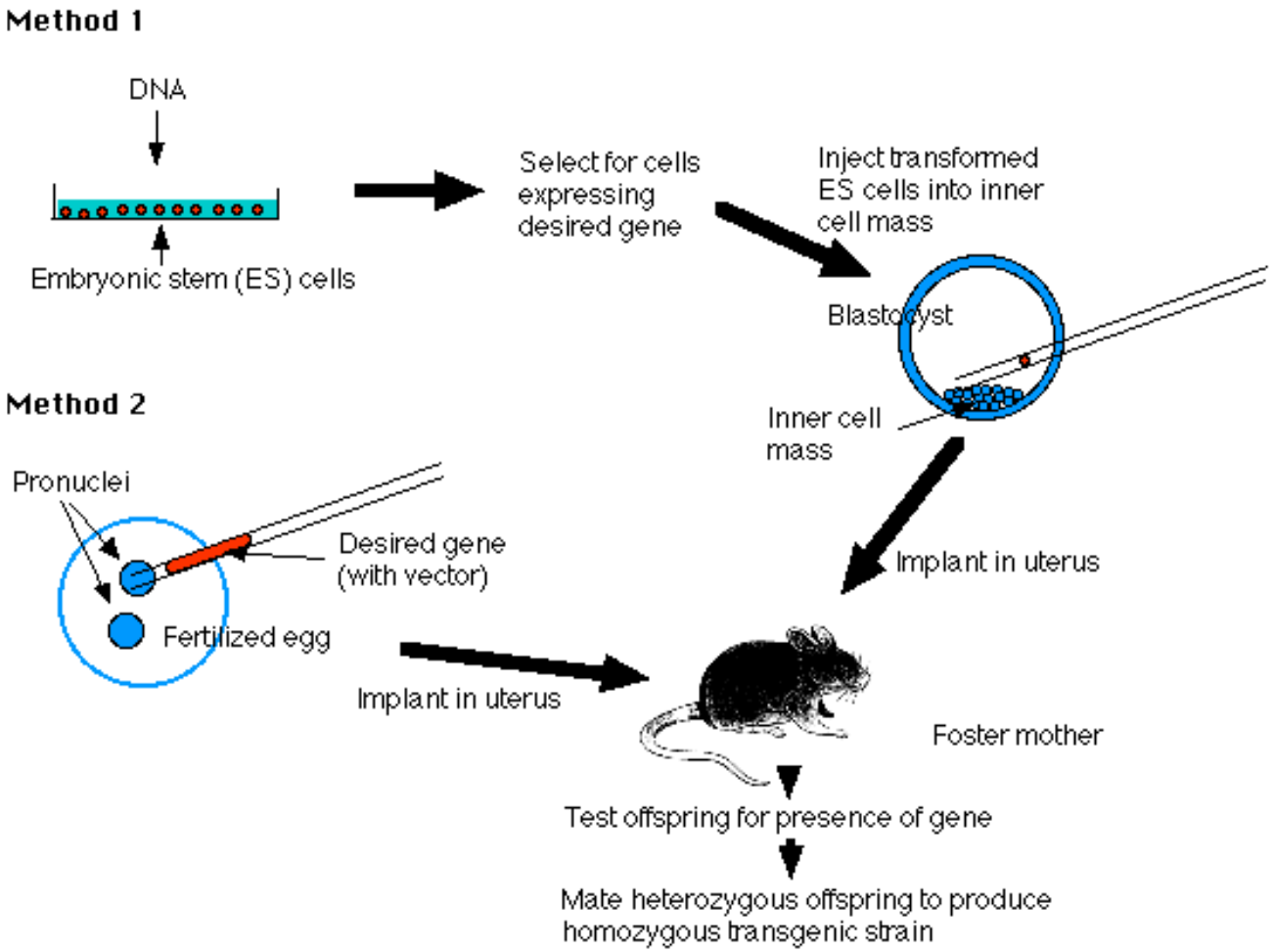
Implant injected blastocysts into female mouse.

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

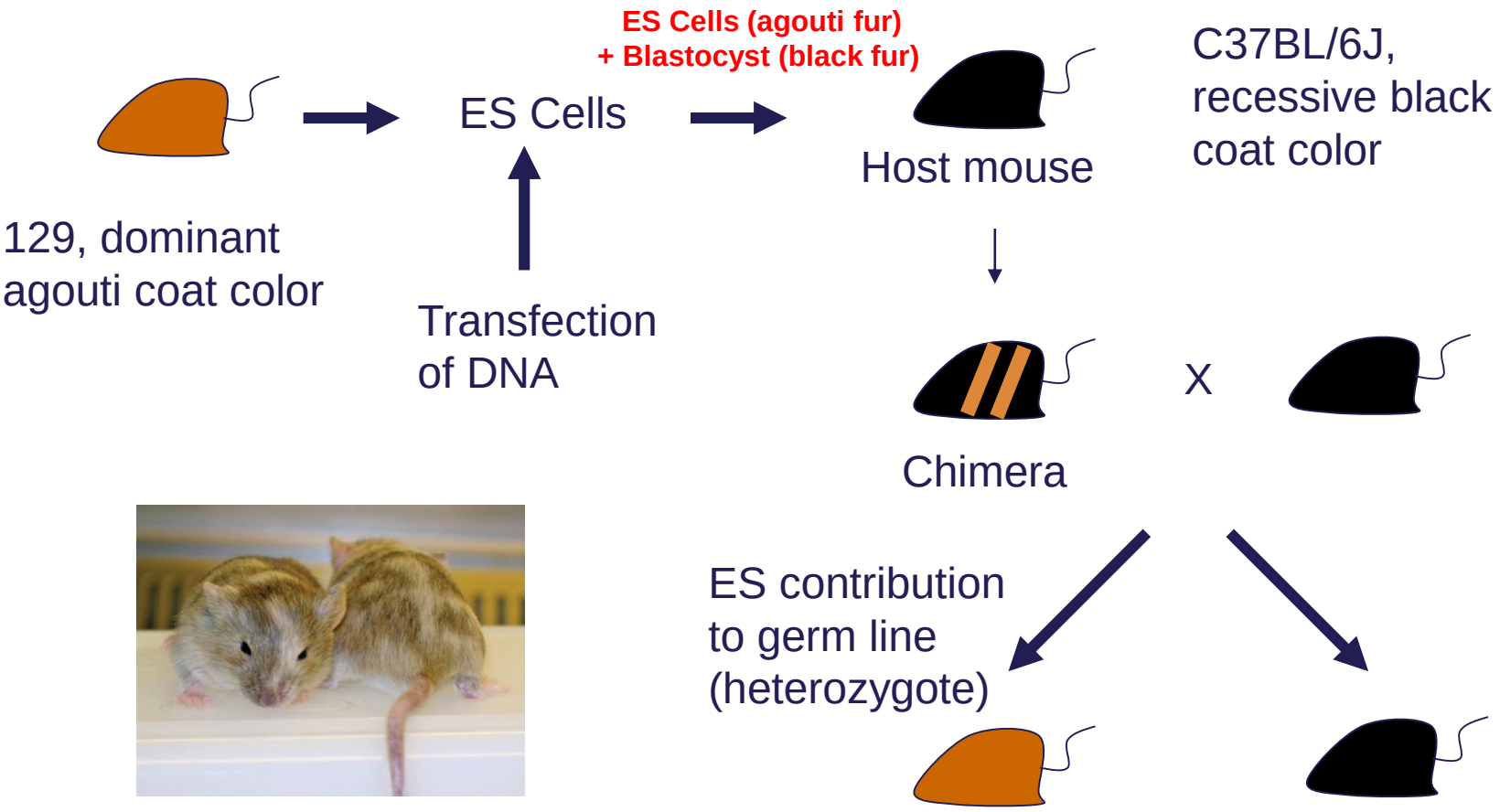
Genetic Engineering of Animals

Transfection of ES Cells

Pronuclear Microinjection



Selection for Germ Line Transmission



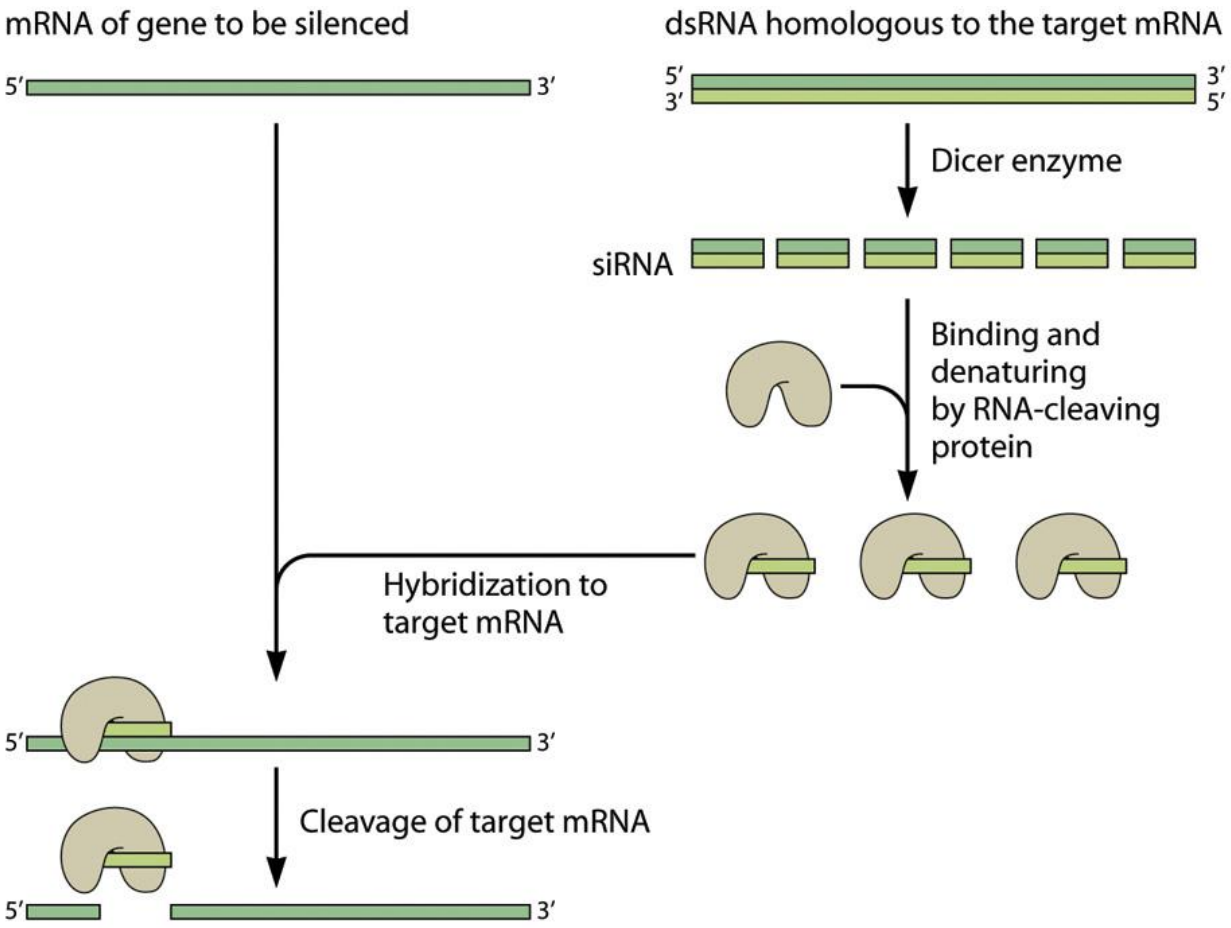
Knockout Mice

- 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

RNA Interference (RNAi)

- Antisense RNA
  - Inhibition of translation by hybridization to mRNA
  - Working in *C. elegans*
- RNA interference
  - dsRNA induces cleavage of homologous mRNA
  - Small interfering RNA (siRNA)
    - Produced from dsRNA by being digested with Dicer
    - Cut complementary mRNA
- Antisense RNA or siRNA as drugs

RNAi


mRNA of gene to be silenced
5′
3′
dsRNA homologous to the target mRNA
5′
3′
3′
5′
Dicer enzyme
siRNA
Binding and
denaturing
by RNA-cleaving
protein
Hybridization to
target mRNA
5′
3′
Cleavage of target mRNA
5′
3′