

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
 - Evolutionary studies
- Genetic engineering
 - Genetic engineering of microorganisms
 - Transgenic plants
 - Transgenic animals

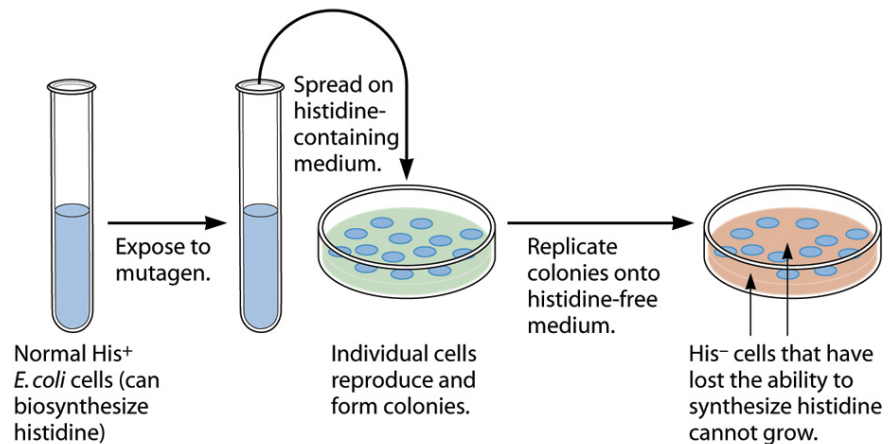
Finding Genes

■ 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*

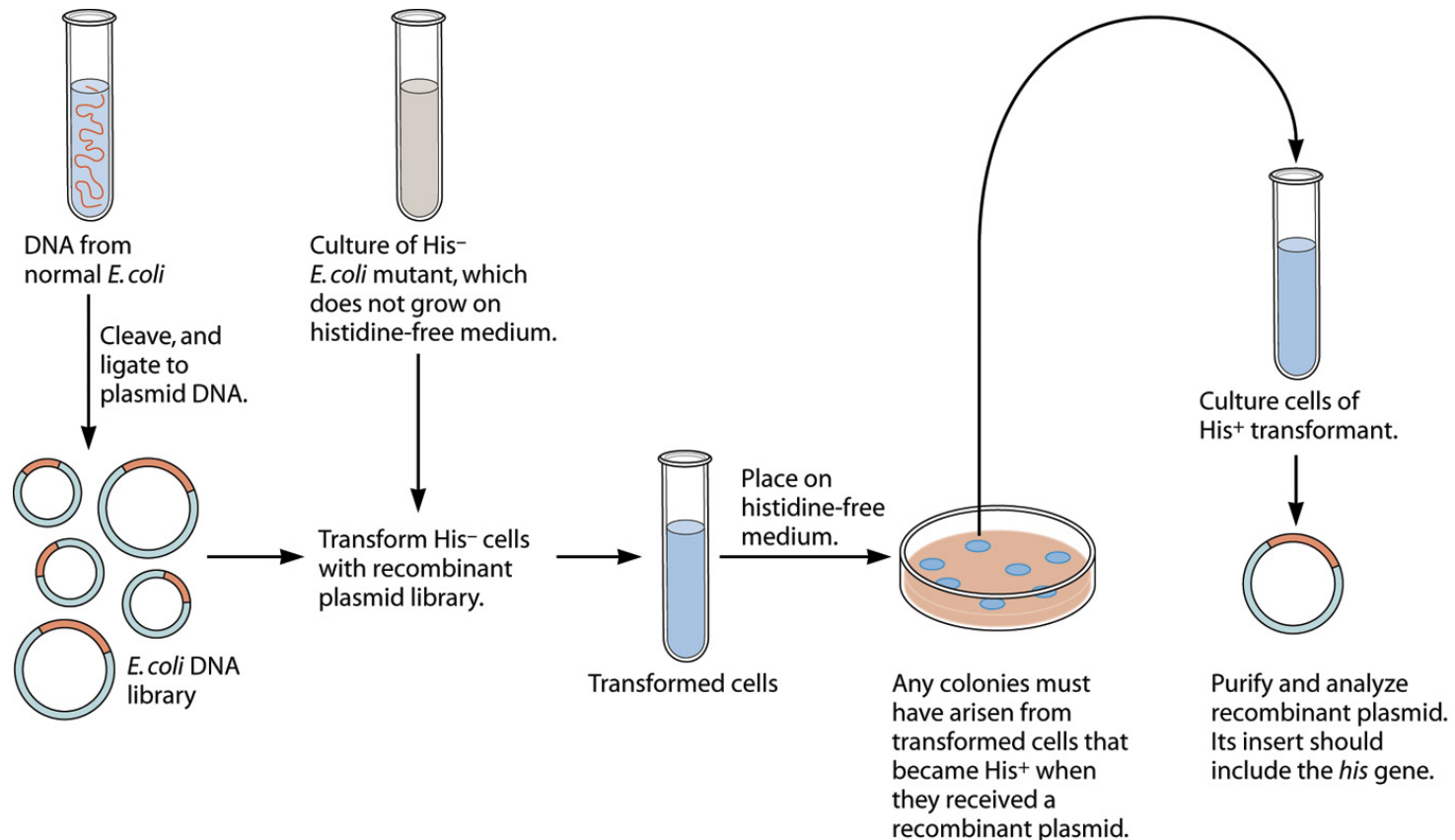


■ Yeast

- 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 (RFLP)
 - A single nucleotide difference (SNP)
- Searching for candidate gene around the marker

Related Organisms Usually Have Similar Genes

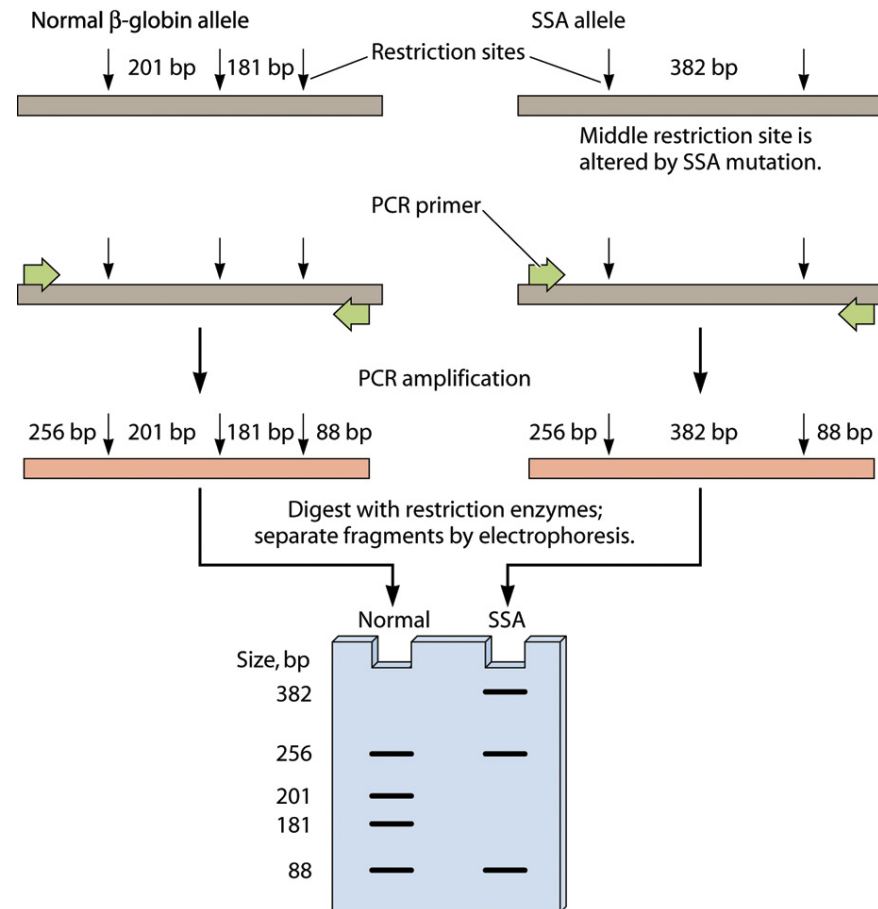
- Gene finding using model organisms
 - Yeast, Drosophila
- Finding homologous genes in higher organisms
 - Homology search of DNA sequence database
 - Confirmation of the predicted function
 - Knock-out mouse

Genetic Testing with Gene Sequences

- Diagnosis of inherited disease
 - BRCA1, BRCA2 for breast cancer
 - Testing for recessive allele of genetic disease
 - Parents who can possibly possess a recessive allele
 - In vitro fertilized egg
 - Markers instead of the real gene
 - Presence of the marker is not a proof of the presence of a disease gene
 - Marker can be separated from the disease gene by recombination
 - Absence of the marker does not guarantee the absence of the disease gene

Testing for Sickle-Cell Anemia

- Mutation of homoglobin (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
 - Better tasting fruit
 - Drought resistance
- Save time compared to trial and error-based plant breeding



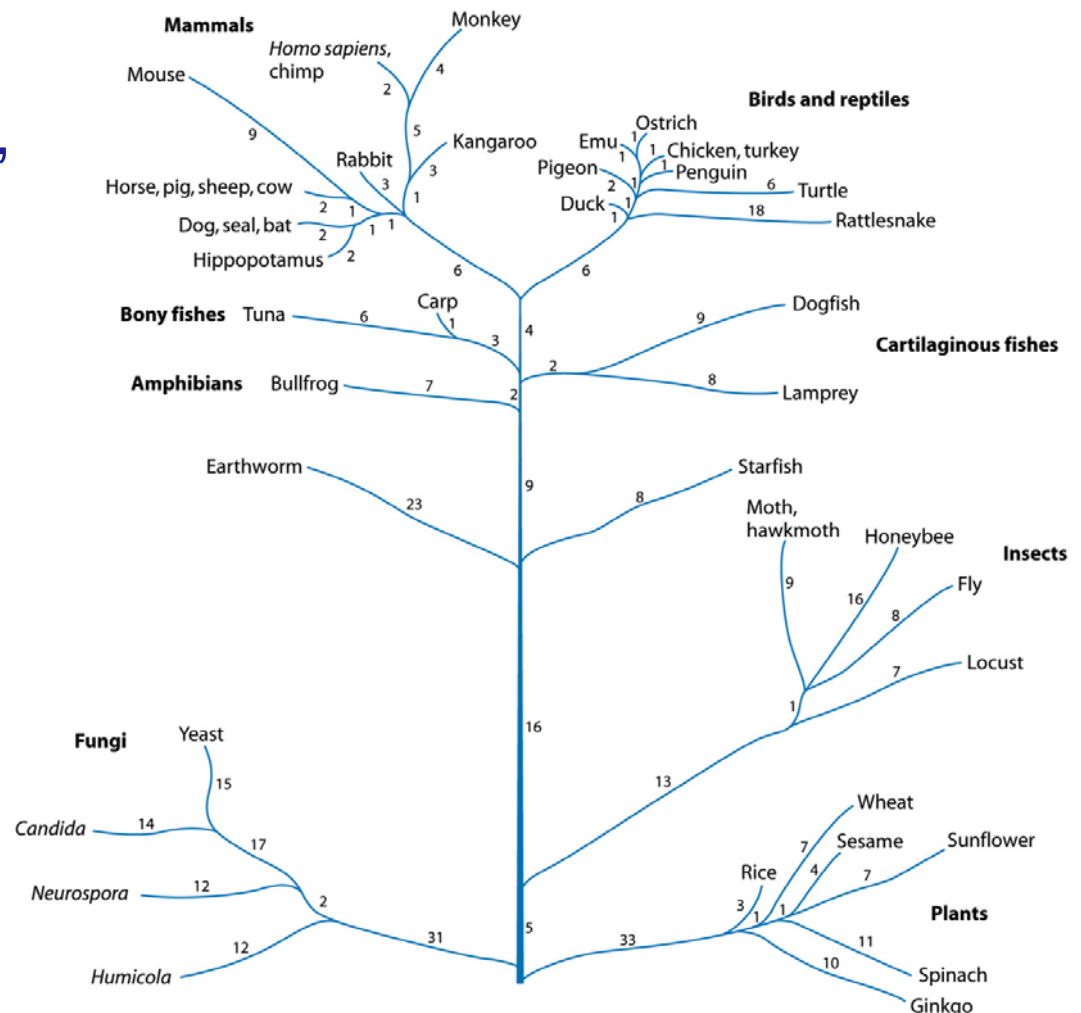
Drought-resistance rice developed in eastern India

Comparing Genotypes and Genomes

- Comparison of genotypes
 - Hybridization of DNA from two different species
 - Determination of the melting temperature
 - 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 amplification fragment length polymorphism using various PCR primer pairs
 - SNPs
 - Single-nucleotide polymorphism

Genotyping for Evolutionary Studies

- Evolution studies using morphological, ecological, and behavioral comparisons between species
- 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 near East and Europe (125,000 to 30,000 years ago)
 - Mitochondrial DNA showed no relationship to modern human

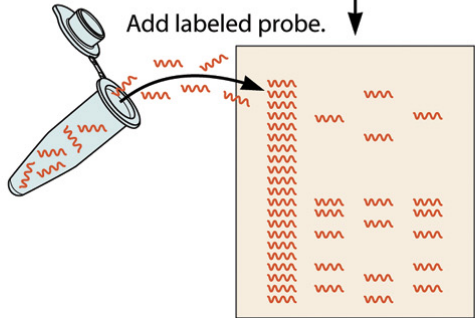


Modern



Neandertals

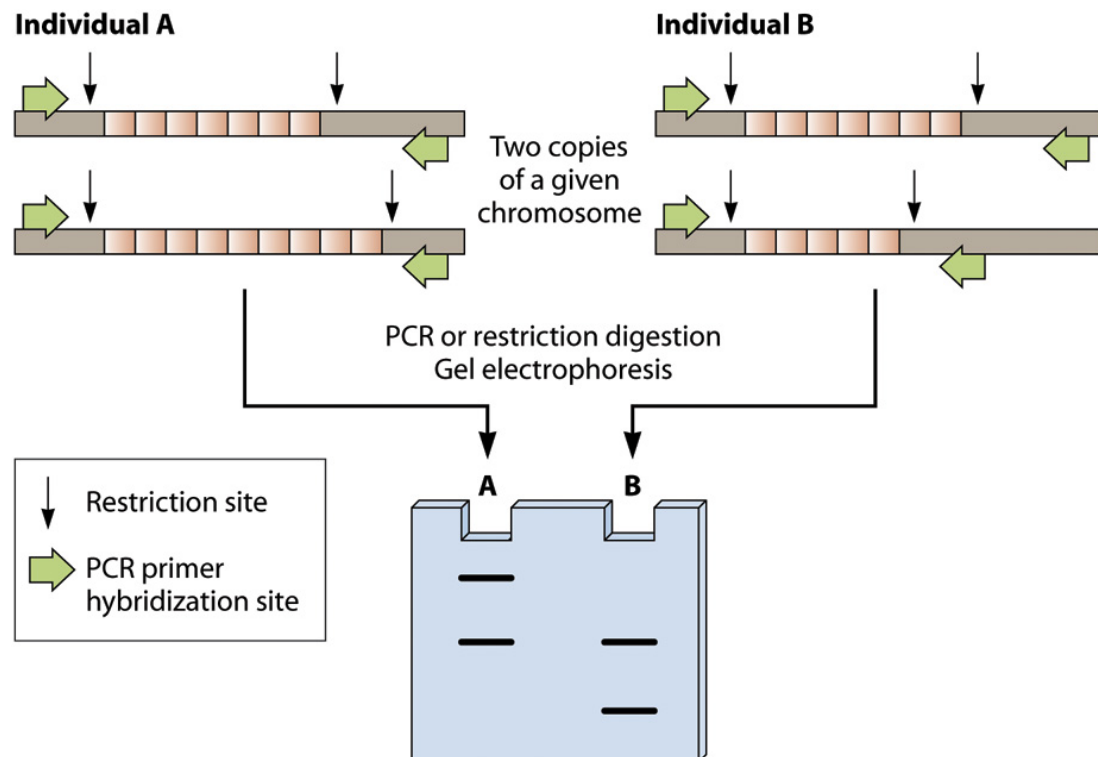
DNA Typing



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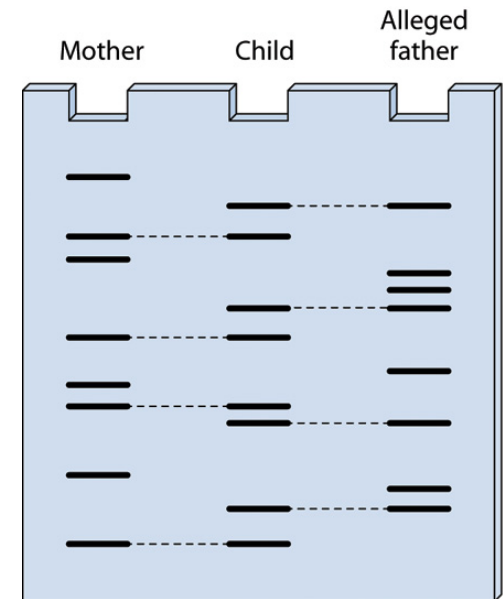
DNA fingerprinting

- Detection of variable length of repetitive sequences



Application of DNA Typing

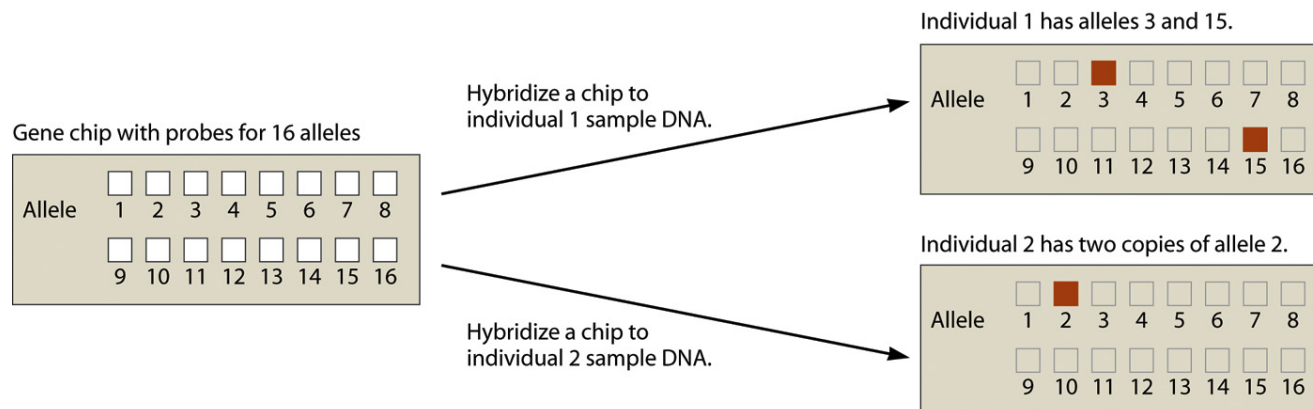
- Forensic DNA typing
- Paternity/maternity testing
- Identification of human remains
- Conservation biology and ecology



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: global analysis of gene expression (mRNA)
 - Proteomics: global analysis of protein expression



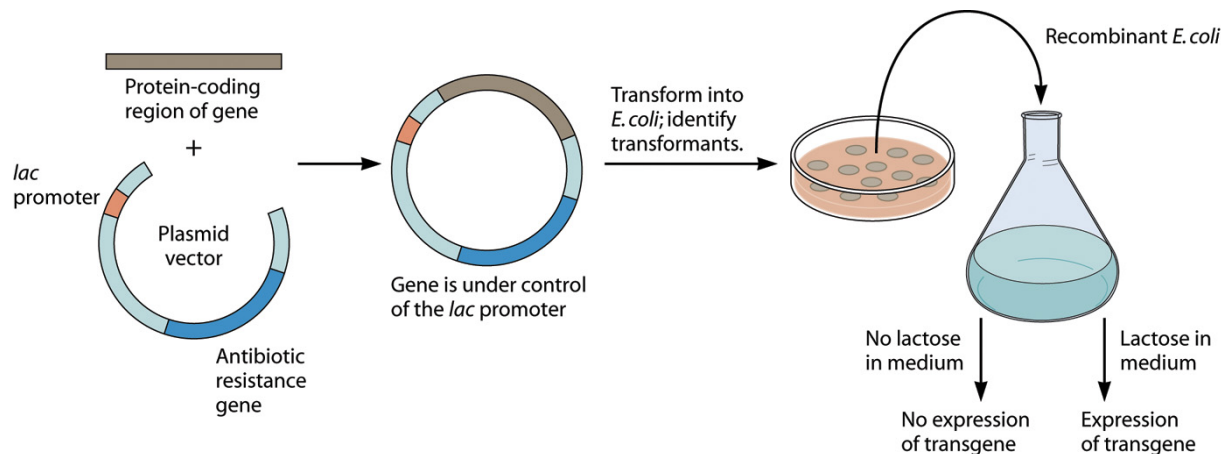


Genetic Engineering

- 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

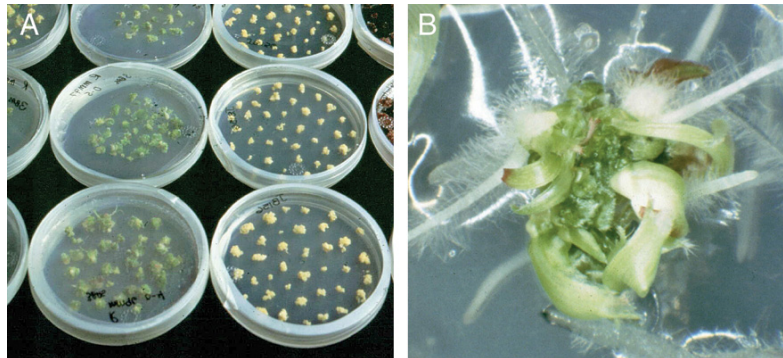
Genetic Engineering of Microorganisms

- 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



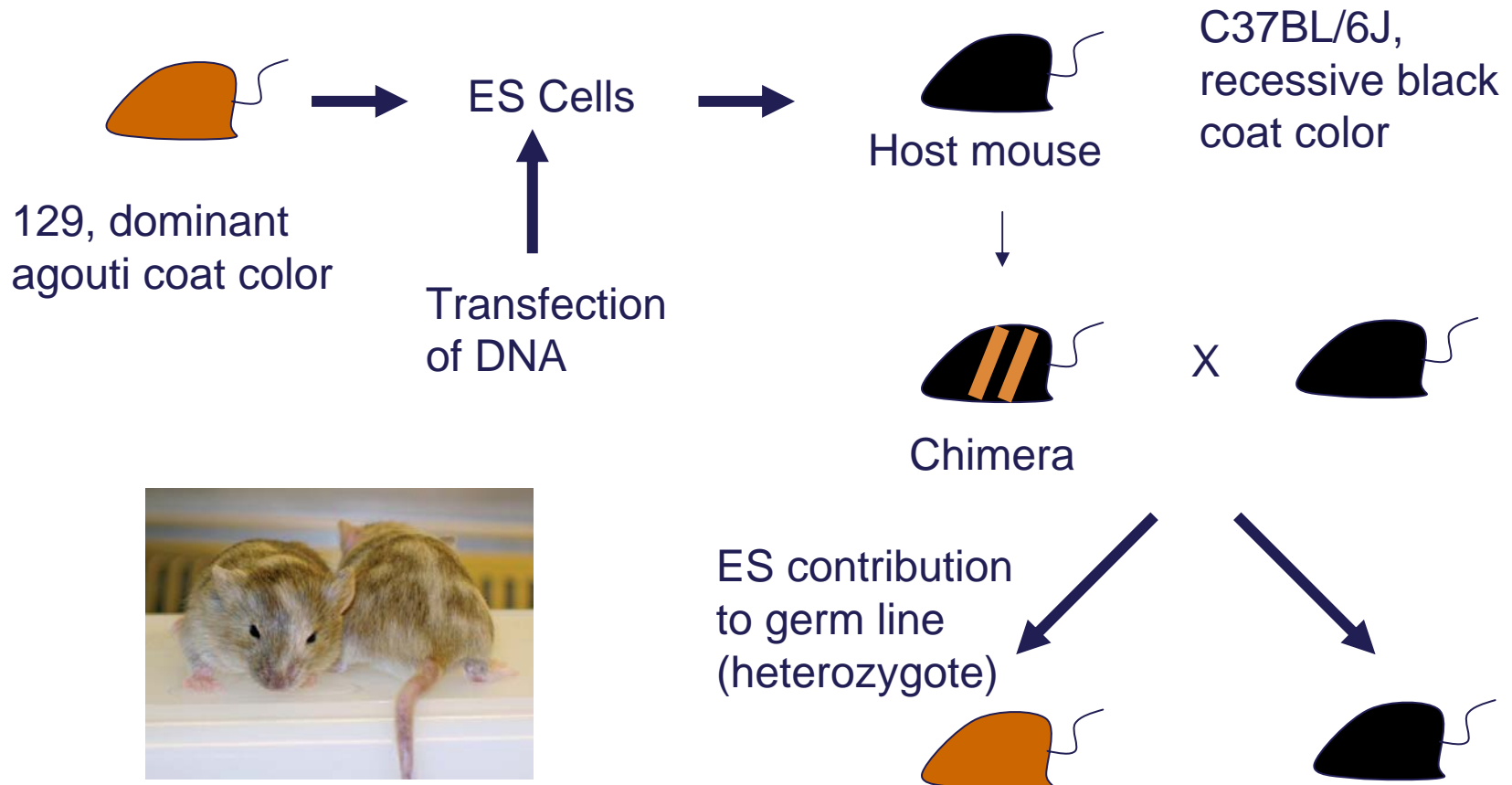
Genetic Engineering of Plants

- 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

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

