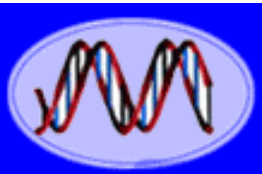
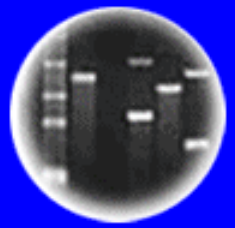


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

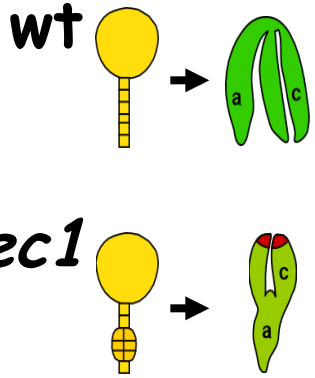
Cloning: Ethical Issues
and Future Consequences

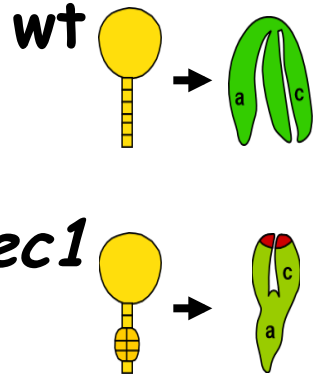
Plants of Tomorrow

HC70AL Spring 2011
Gene Discovery Laboratory

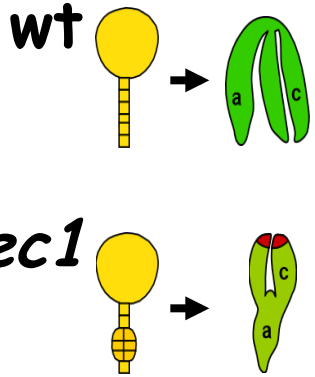
Knocking-Out Seed Transcription Factor Genes

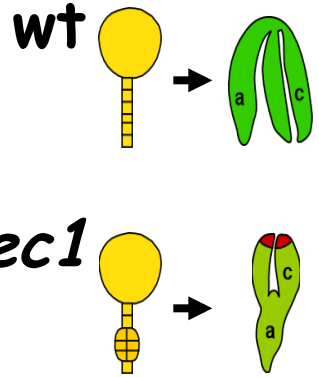
4/5/11





Using Reverse Genetics To Find Genes That Control Seed Development





Recall…..Arabidopsis Features That Make It a Model Plant & Crop

1. Fast Generation Time
2. Small Size
3. Small Genome (~120 Mb & 25,000 Genes)
4. Sequenced Genome
5. Large Scientific Community-Many Resources For Genomics & Functional Studies
6. Knock-Out in Every Gene

Arabidopsis thaliana

Figure 1-13c
Introduction to Genetic Analysis, Ninth Edition

Table 1–1 Some Genomes That Have Been Completely Sequenced

SPECIES	SPECIAL FEATURES	HABITAT	GENOME SIZE (1000s OF NUCLEOTIDE PAIRS PER HAPLOID GENOME)	ESTIMATED NUMBER OF GENES CODING FOR PROTEINS
ARCHAEA				
Methanococcus jannaschii	lithotrophic, anaerobic, methane-producing	hydrothermal vents	1664	1750
Archaeoglobus fulgidus	lithotrophic or organotrophic, anaerobic, sulfate-reducing	hydrothermal vents	2178	2493
Nanoarchaeum equitans	smallest known archaean; anaerobic; parasitic on another, larger archaean	hydrothermal and volcanic hot vents	491	552
EUCARYOTES				
Saccharomyces cerevisiae (budding yeast)	minimal model eucaryote	grape skins, beer	12,069	~6300
Arabidopsis thaliana (Thale cress)	model organism for flowering plants	soil and air	~142,000	~26,000
Caenorhabditis elegans (nematode worm)	simple animal with perfectly predictable development	soil	~97,000	~20,000
Drosophila melanogaster (fruit fly)	key to the genetics of animal development	rotting fruit	~137,000	~14,000
Homo sapiens (human)	most intensively studied mammal	houses	~3,200,000	~24,000

Genome size and gene number vary between strains of a single species, especially for bacteria and archaea. The table shows data for particular strains that have been sequenced. For eucaryotes, many genes can give rise to several alternative variant proteins, so that the total number of proteins specified by the genome is substantially greater than the number of genes.

Table 1-1 (part 2 of 2) *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Arabidopsis Has Same Number of Genes as Humans

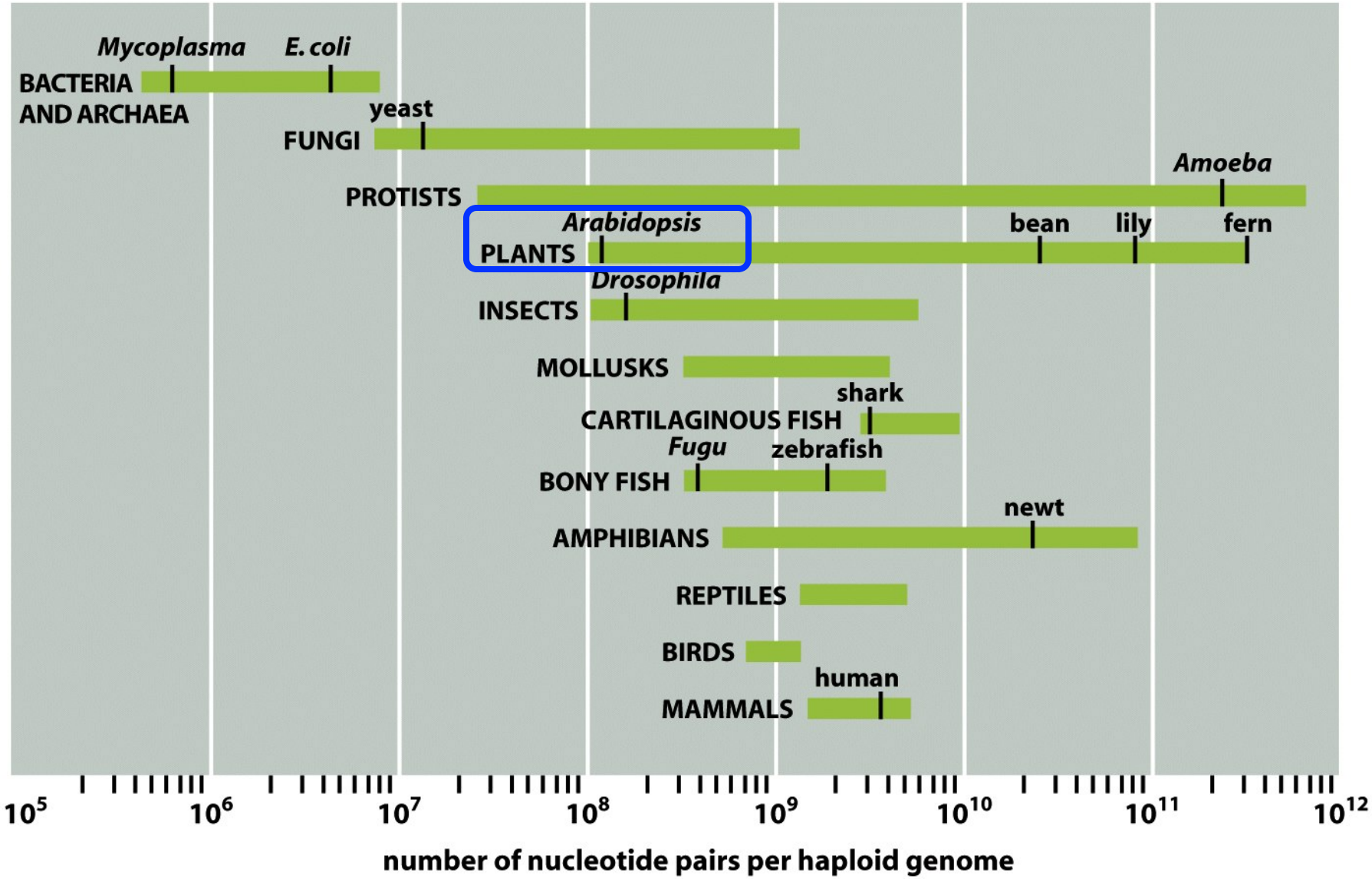


Figure 1-37 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Recall….Plant Life Cycle

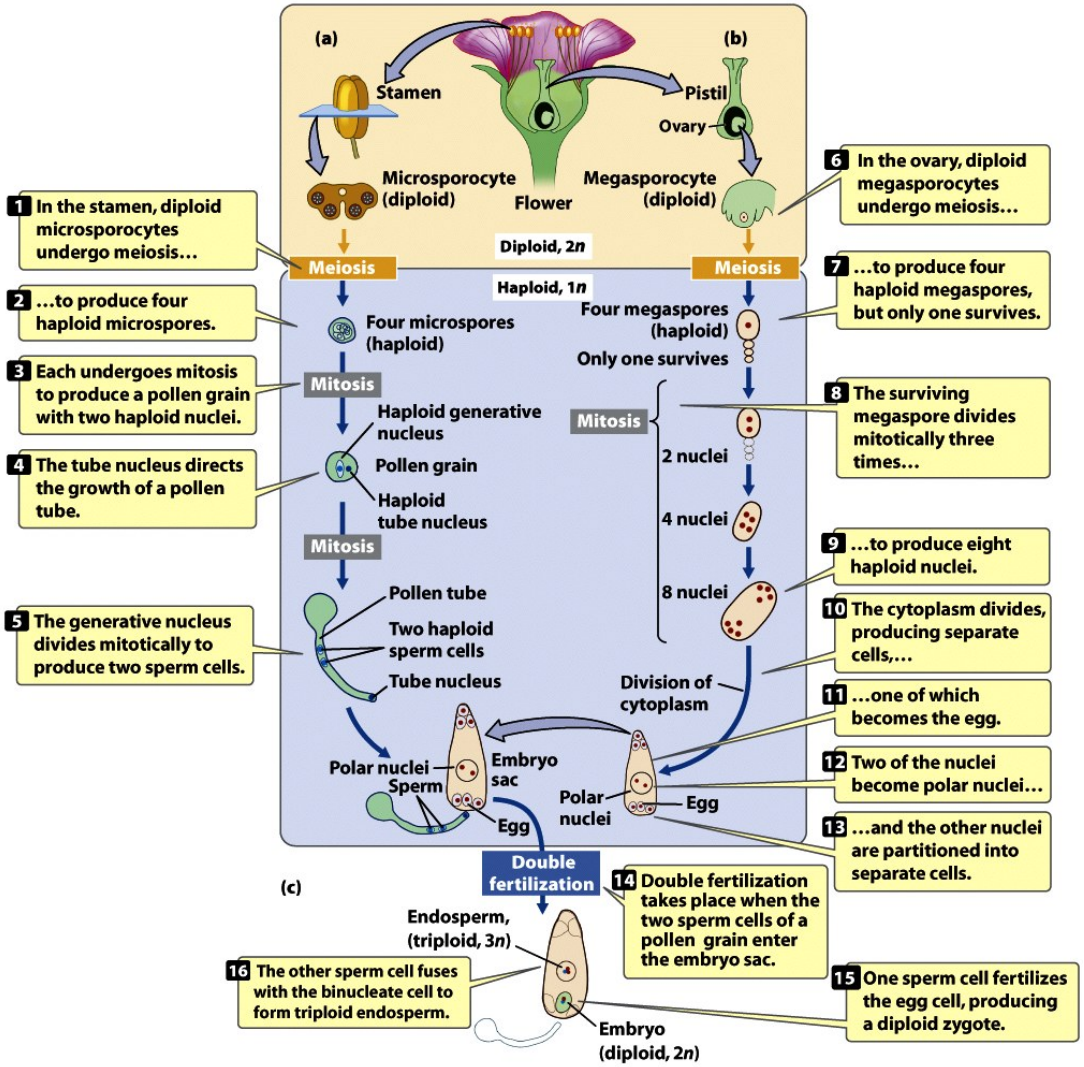
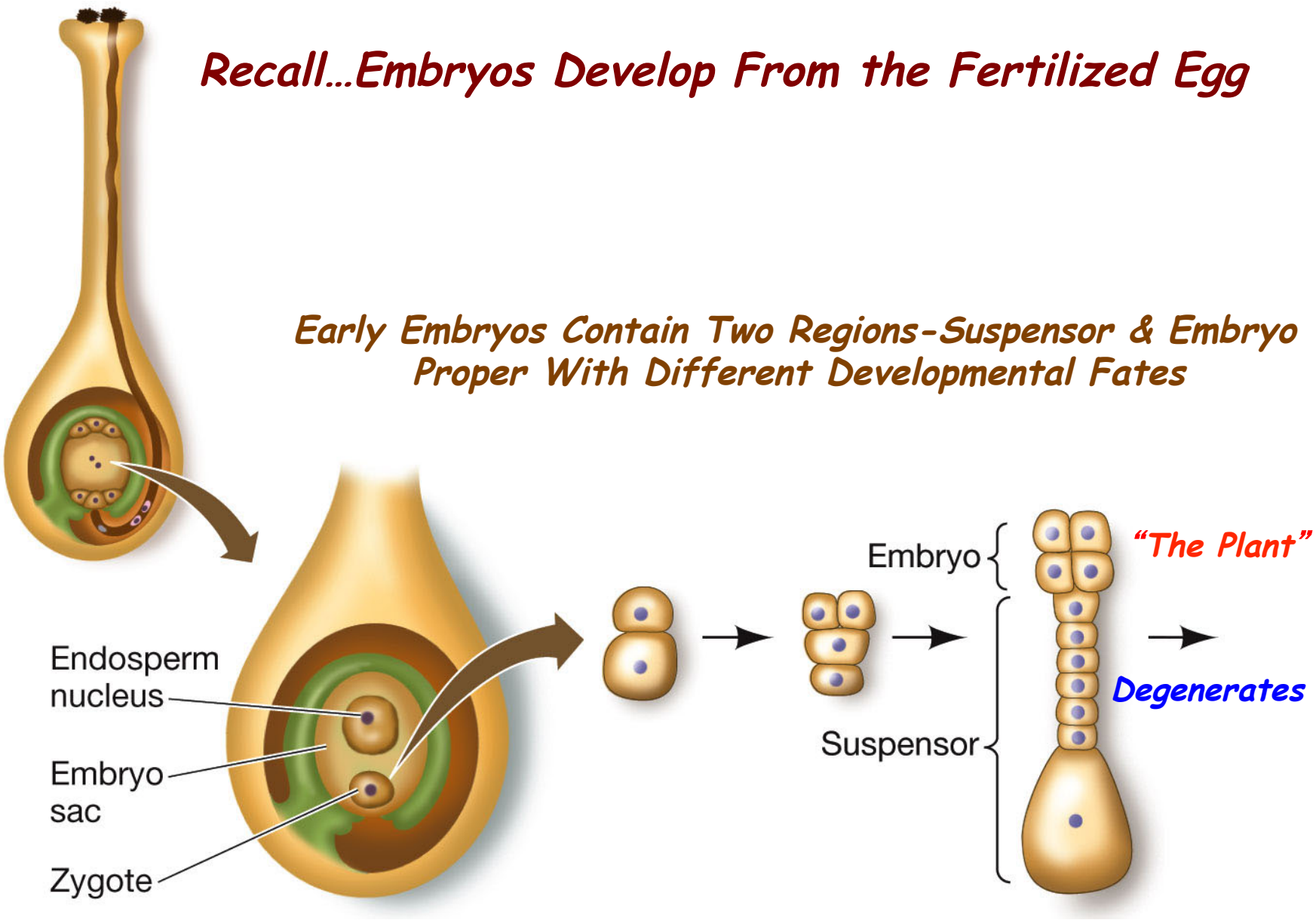


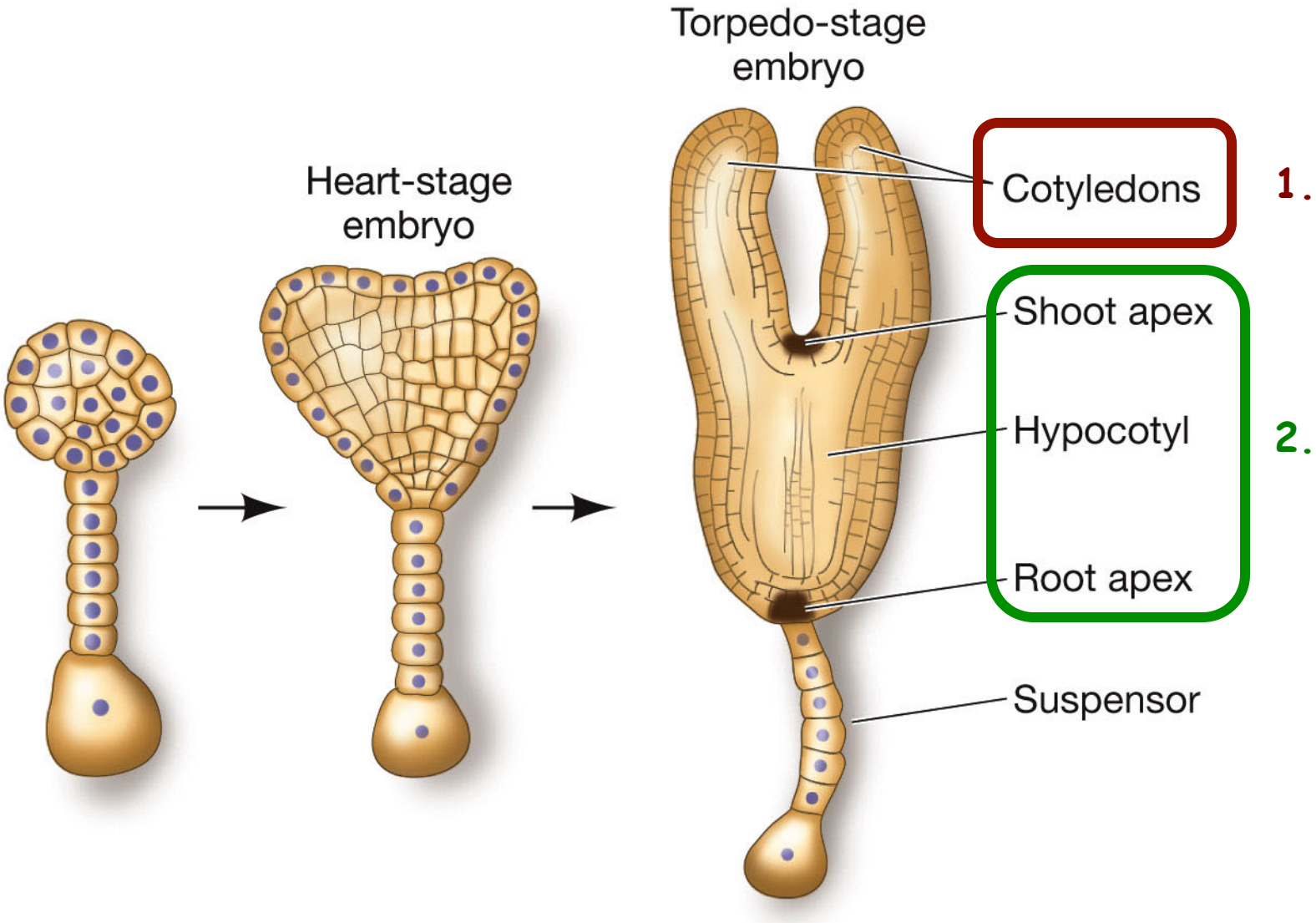
Figure 2-22
Genetics: A Conceptual Approach, Third Edition

Recall…Embryos Develop From the Fertilized Egg

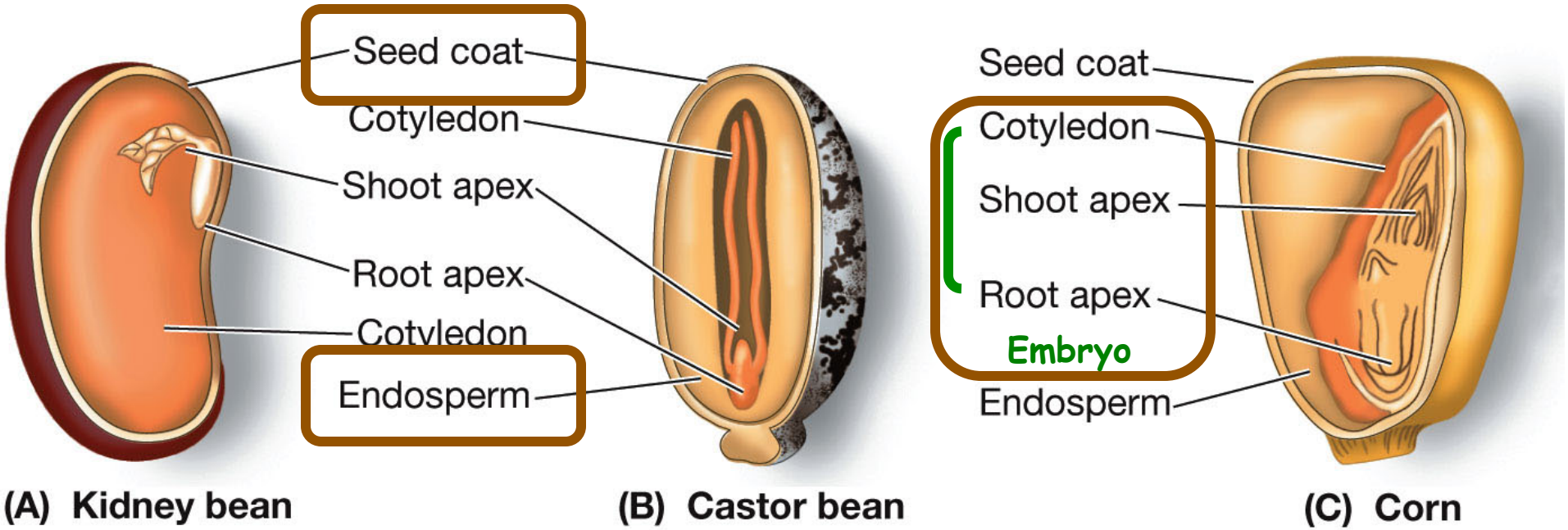
Early Embryos Contain Two Regions-Suspensor & Embryo Proper With Different Developmental Fates



Recall…Embryos Undergo a Series of Events Leading to a Dormant Embryo in a Mature Seed



Recall…The "End Product" is Dormant Seed With a Mature Embryo!

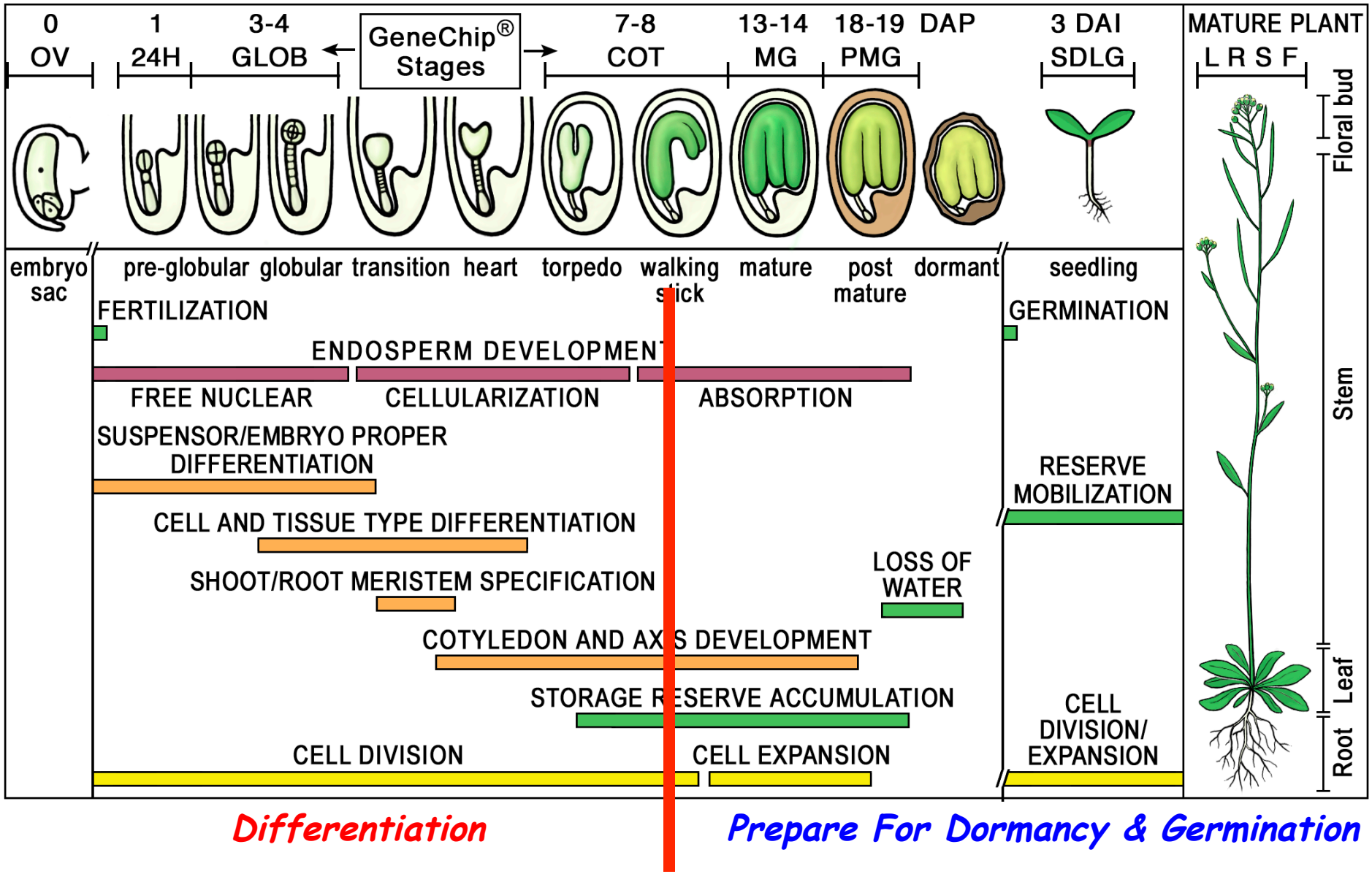


A Seed Consists of Three Parts With Distinct Genetic Origins

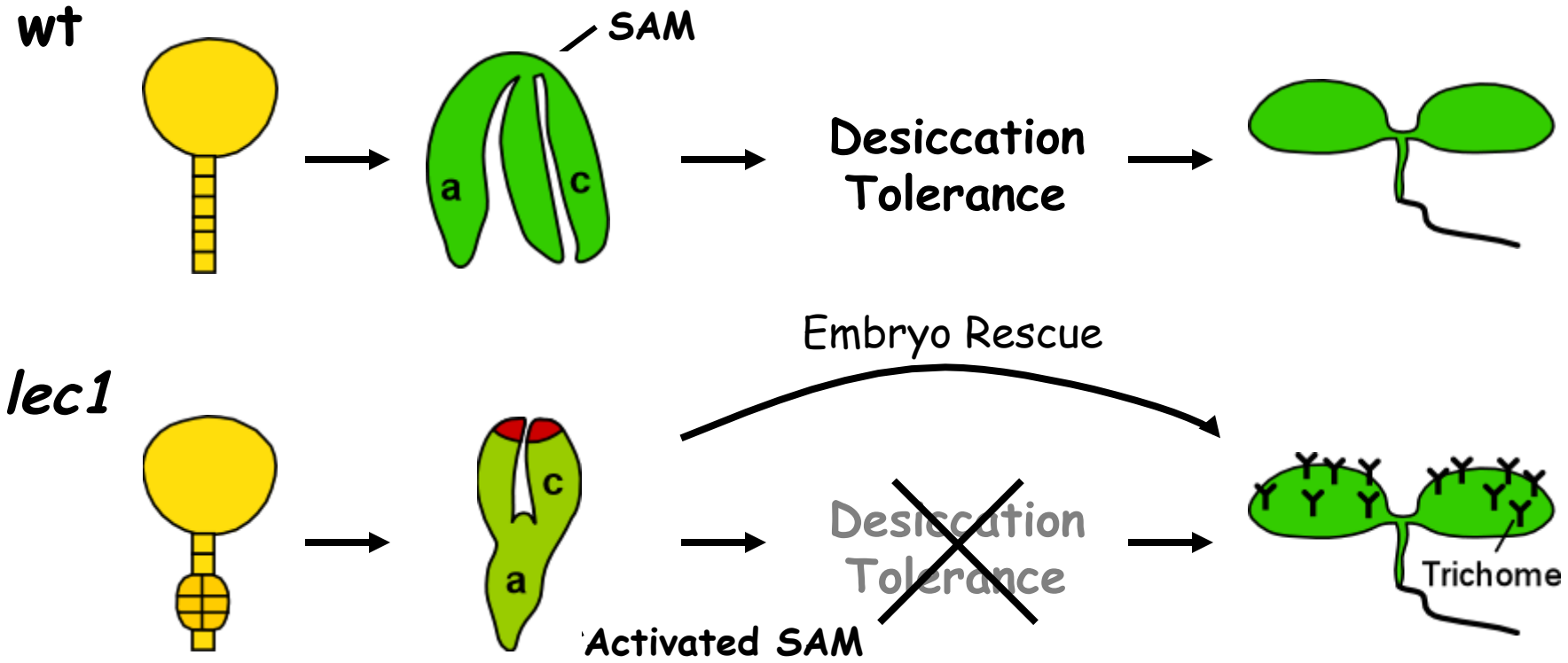
1. A Seed Coat From Maternal Floral Tissue
2. An Endosperm From Fertilization of Embryo Sac Central Cell
3. An Embryo From Fertilization of the Egg

The Embryo Becomes the Next Plant Generation

Recall…Genome-Wide Profiling of mRNAs During Arabidopsis Seed Development & Plant Life Cycle



Searching For Functions of Seed Transcription Gactor Genes Using Reverse Genetics (e.g., lec1 Mutants Disrupt Seed Development)



- *Suppression of Suspensor Embryonic Potential*
- *Development of Cotyledon Identity*
- *Initiation and Maintenance of Seed Maturation*
- *Inhibition Germination*

Lotan et al., Cell, 1998

Examples of Arabidopsis Mutants

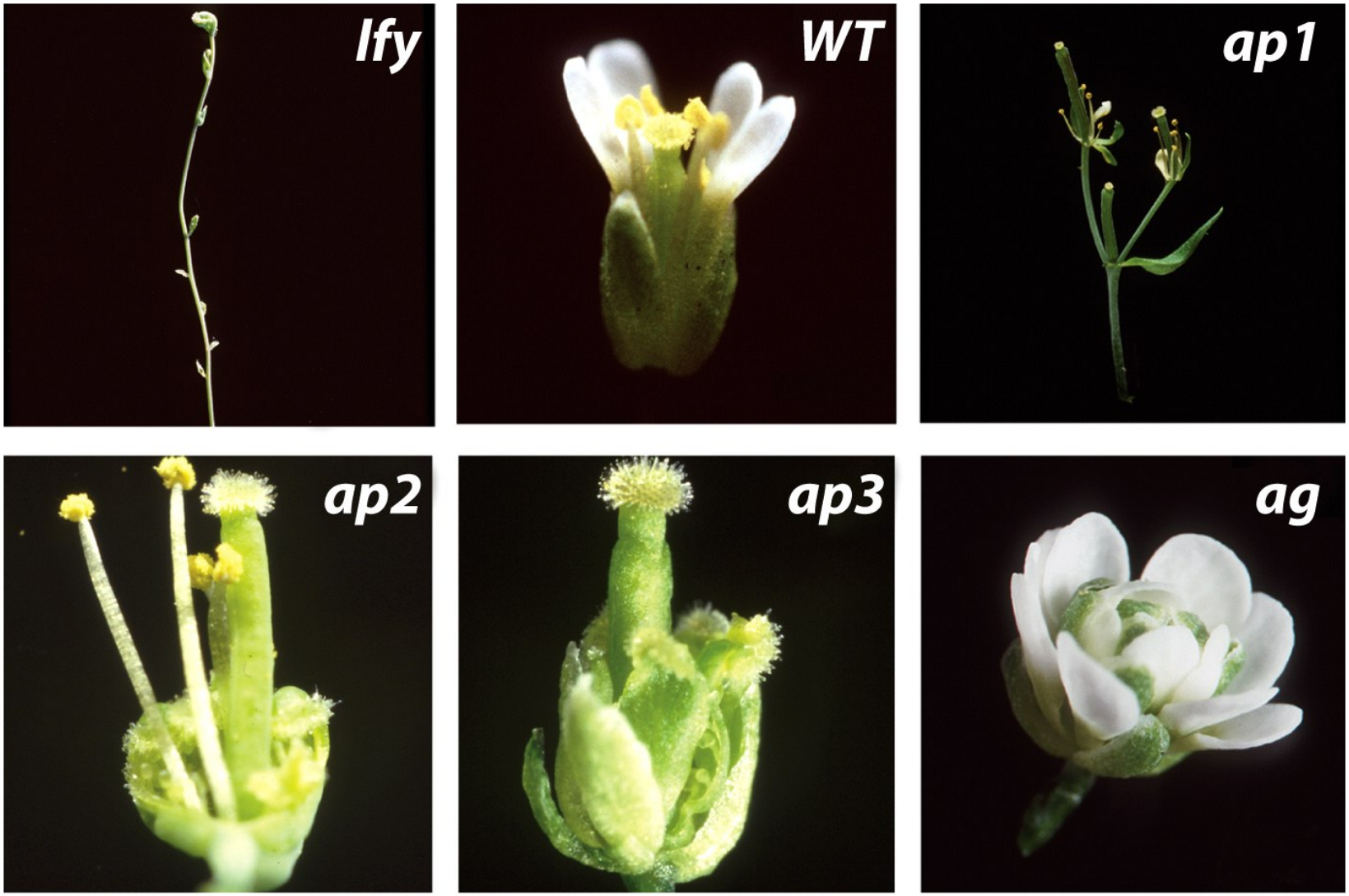


Figure 2-1a
Introduction to Genetic Analysis, Ninth Edition

Reverse Genetics Starts With Gene Sequence and Searches For a Mutant Phenotype (i.e., Function)

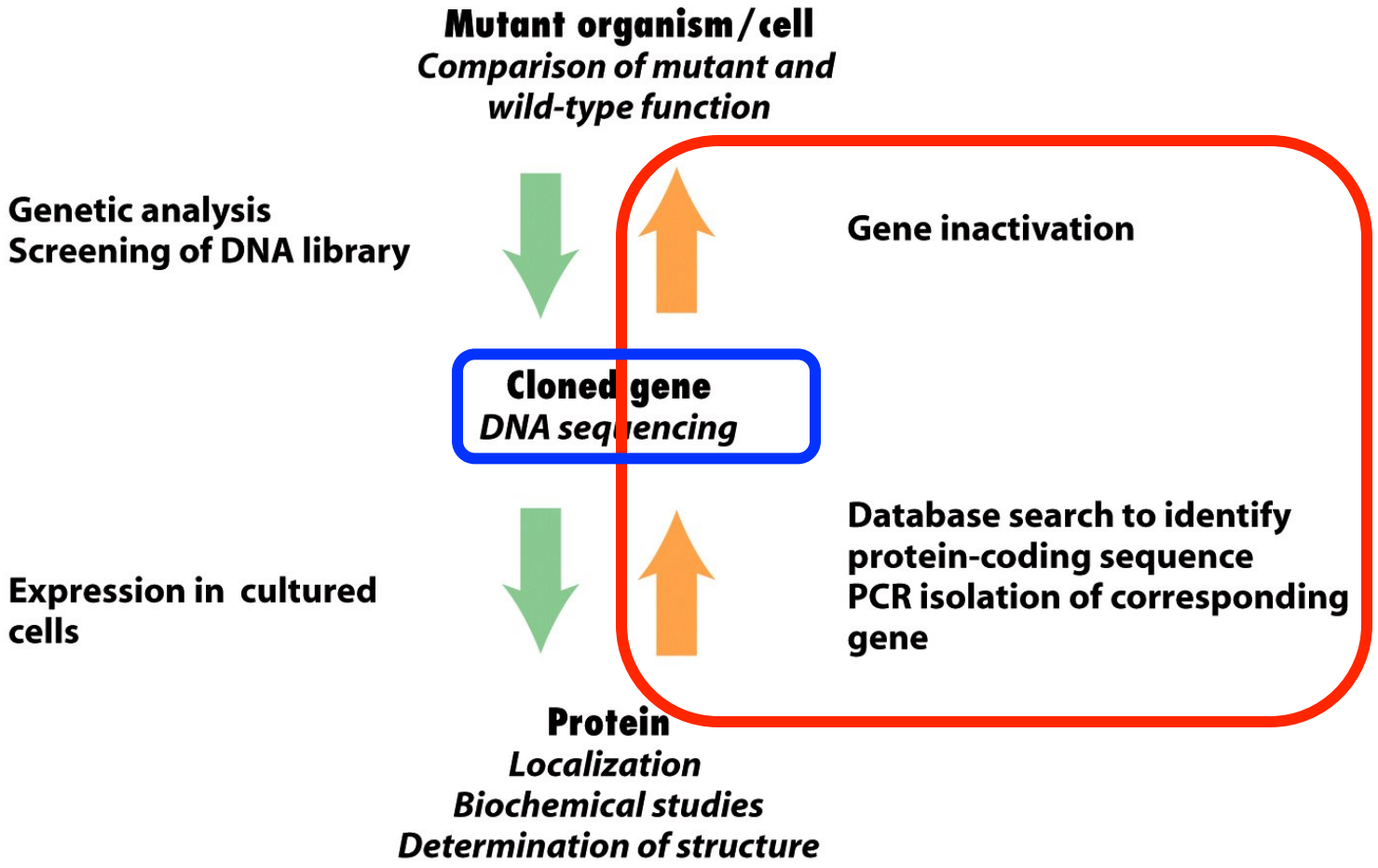


Figure 5-1
Molecular Cell Biology, Sixth Edition

Knock-Out Mutations Are Loss-of-Function or Null Mutations

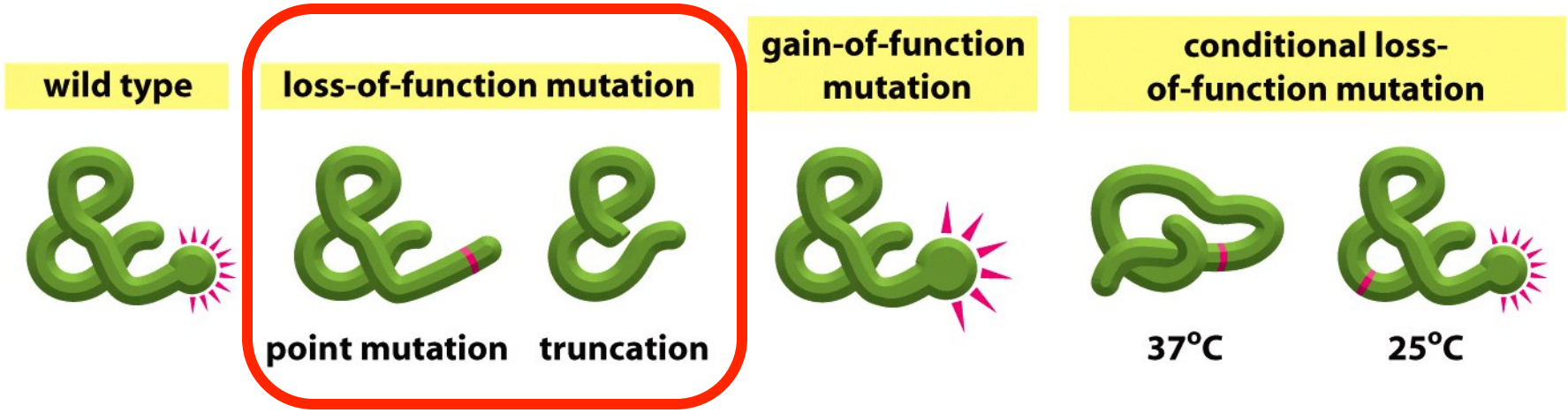


Figure 8-56 *Molecular Biology of the Cell* (© Garland Science 2008)

Knock-Out Mutations Are Loss-of-Function or Null Mutations

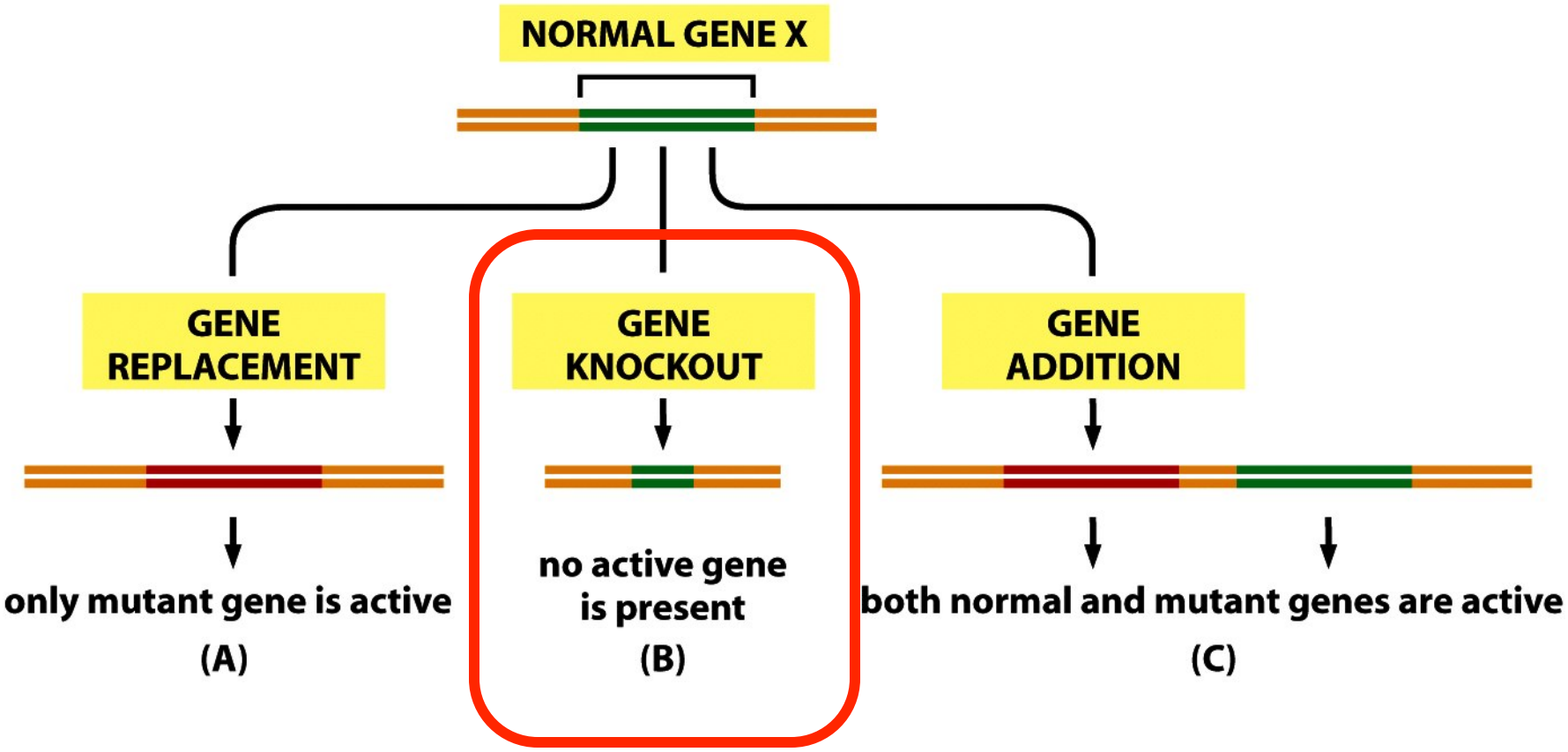


Figure 8-64 *Molecular Biology of the Cell* (© Garland Science 2008)

Inserting Foreign DNA Into the LacZ Gene is a Knock-Out Mutation

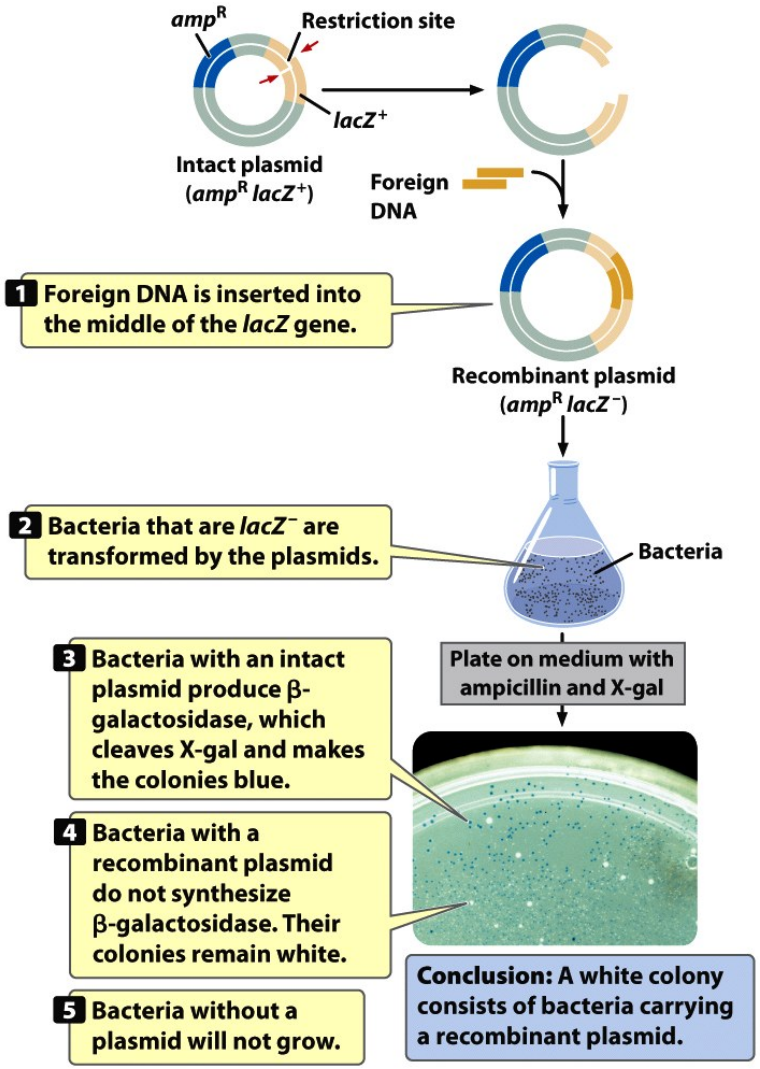


Figure 19-8
Genetics: A Conceptual Approach, Third Edition

Inserting Foreign DNA Into the LacZ Gene is a Knock-Out Mutation

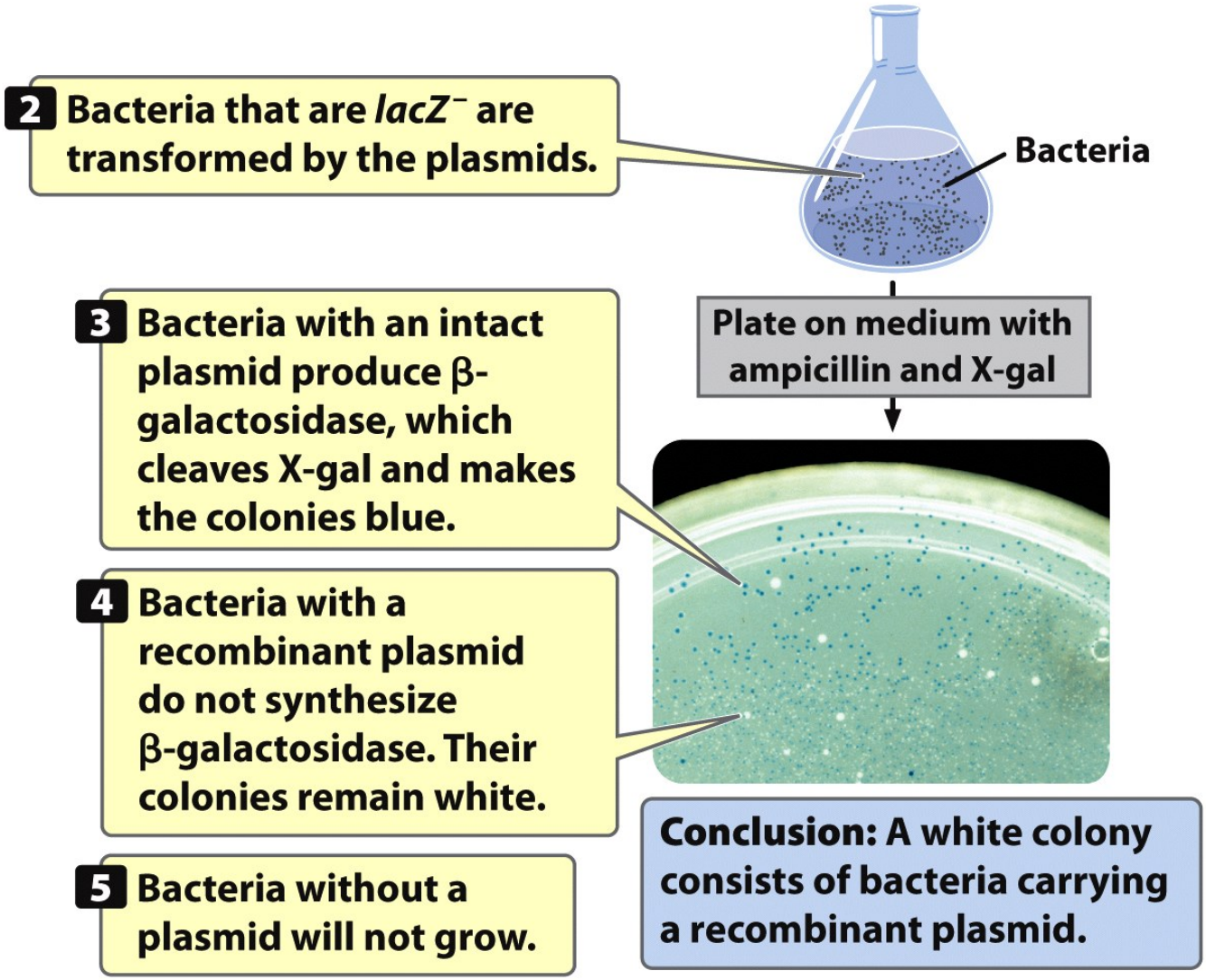


Figure 19-8 part 2
Genetics: A Conceptual Approach, Third Edition

A Null Mutation Can Affect Any Part of the Flow From DNA to Protein

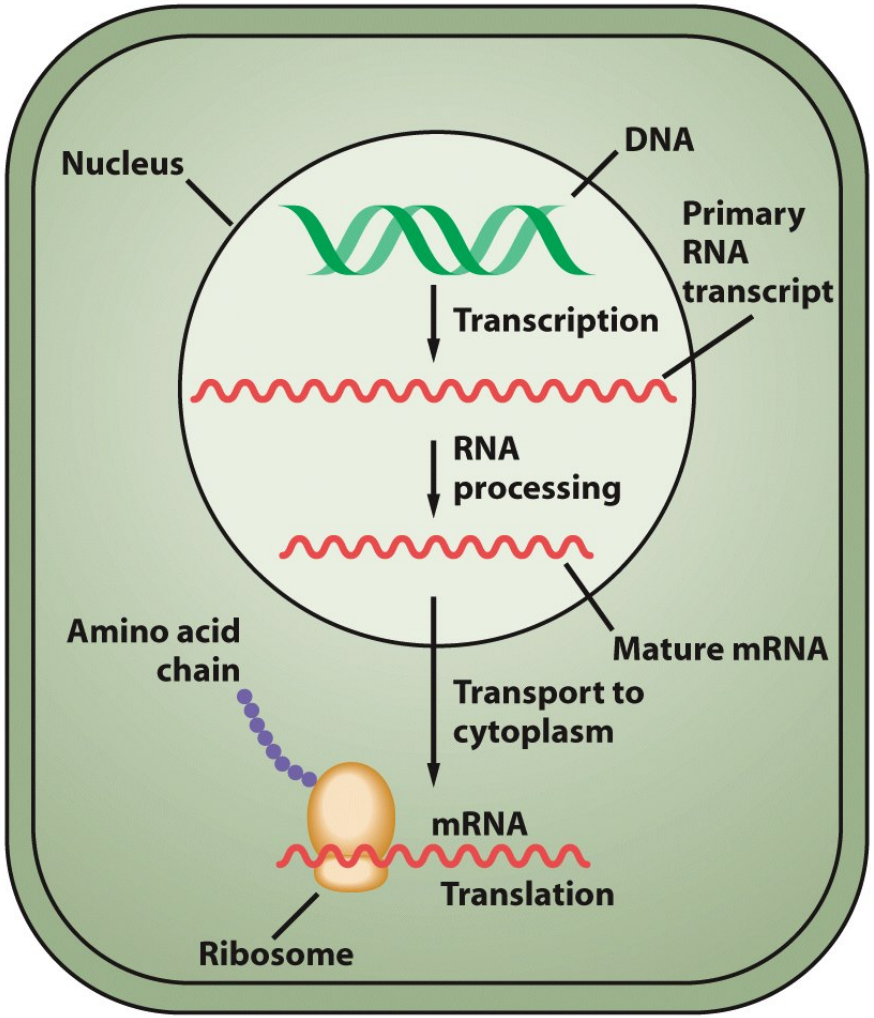


Figure 1-5
Introduction to Genetic Analysis, Ninth Edition

Simple Mendelian Genetics…a Review

Table 3.1 Summary of important genetic terms

Term	Definition
Gene	A genetic factor (region of DNA) that helps determine a characteristic
Allele	One of two or more alternate forms of a gene
Locus	Specific place on a chromosome occupied by an allele
Genotype	Set of alleles possessed by an individual organism
Heterozygote	An individual organism possessing two different alleles at a locus
Homozygote	An individual organism possessing two of the same alleles at a locus
Phenotype or trait	The appearance or manifestation of a character
Character or characteristic	An attribute or feature

Table 3-1
Genetics: A Conceptual Approach, Third Edition

Chromosomes & Alleles…a Review

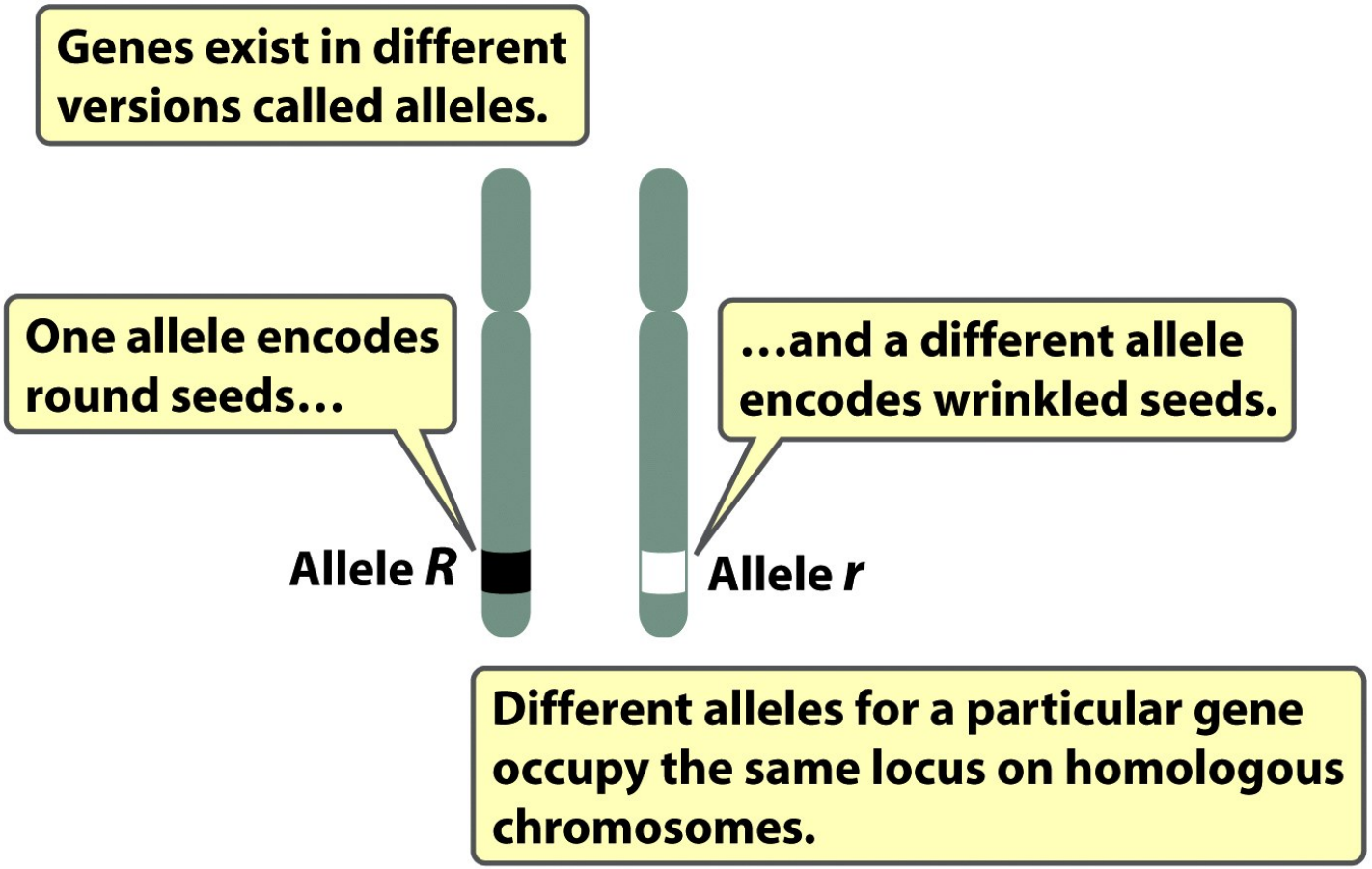


Figure 3-2
Genetics: A Conceptual Approach, Third Edition

Simple Mendelian Genetics…a Review

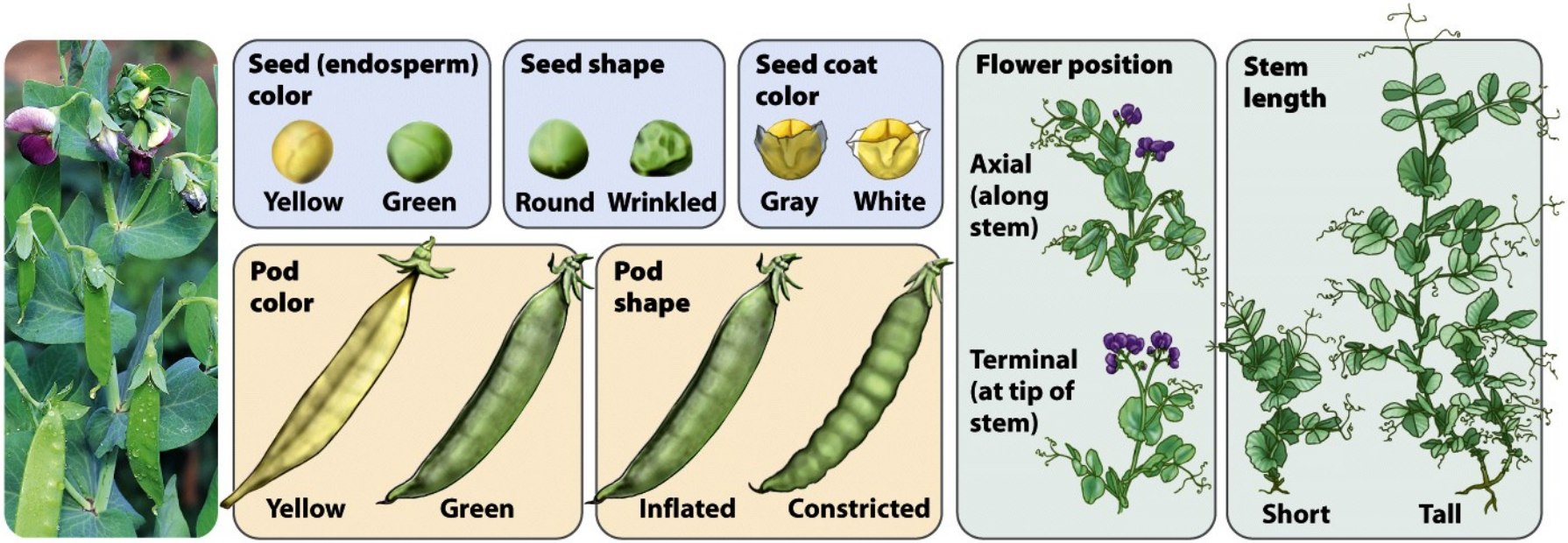
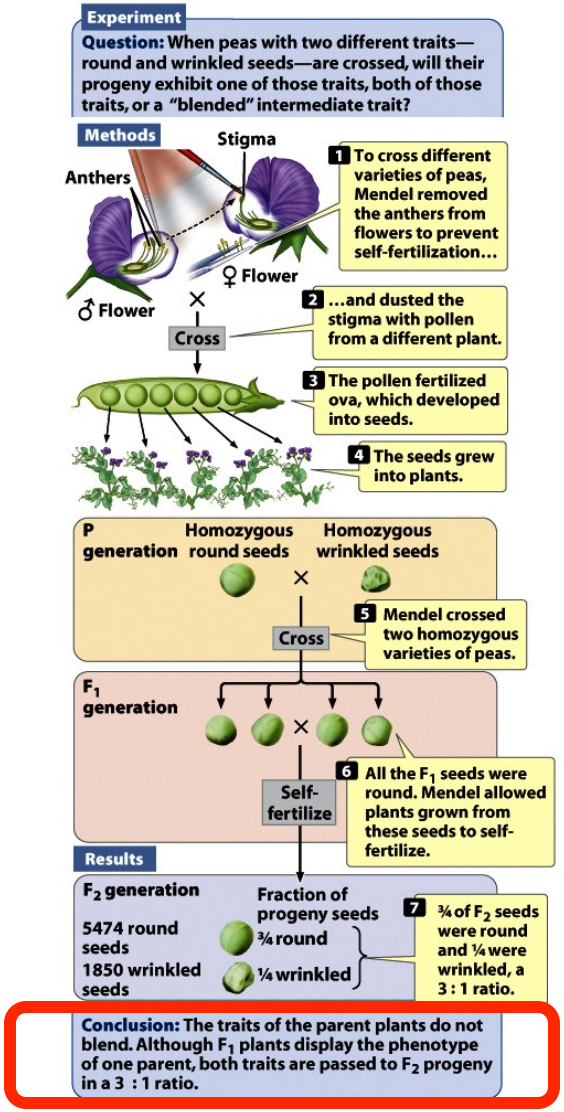
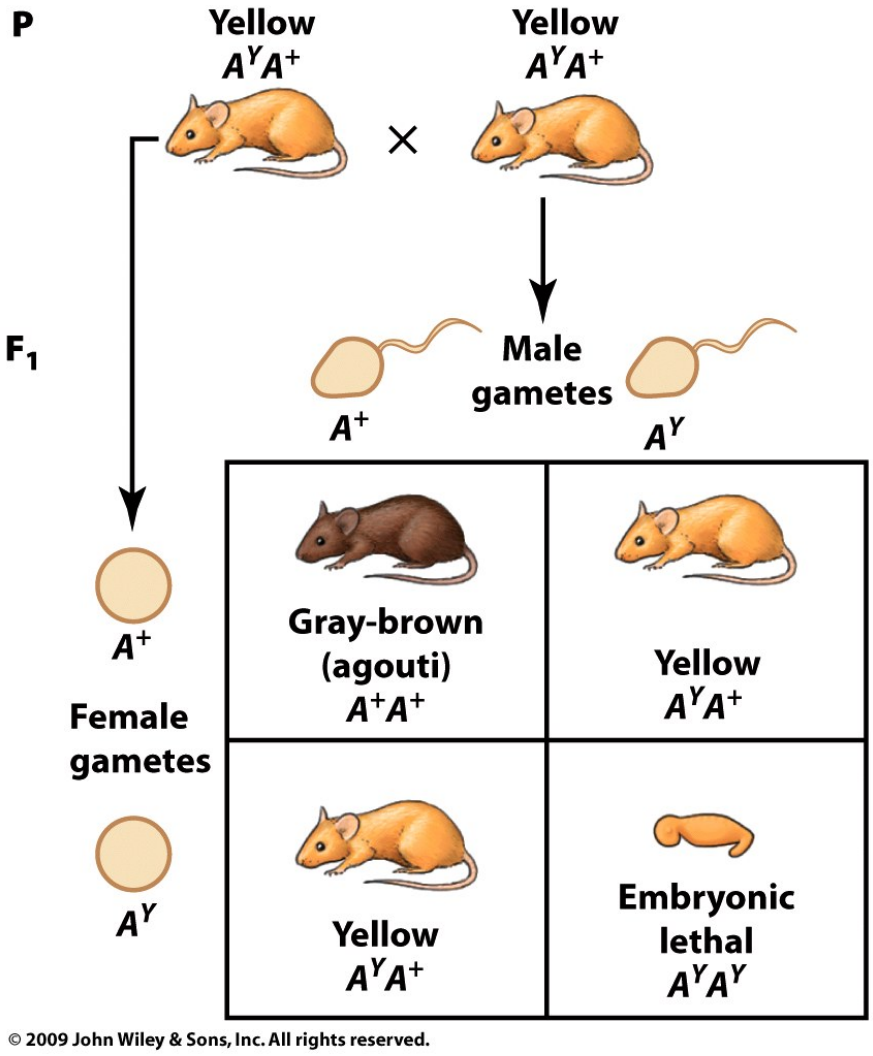


Figure 3-1
Genetics: A Conceptual Approach, Third Edition

Simple Mendelian Genetics…a Review

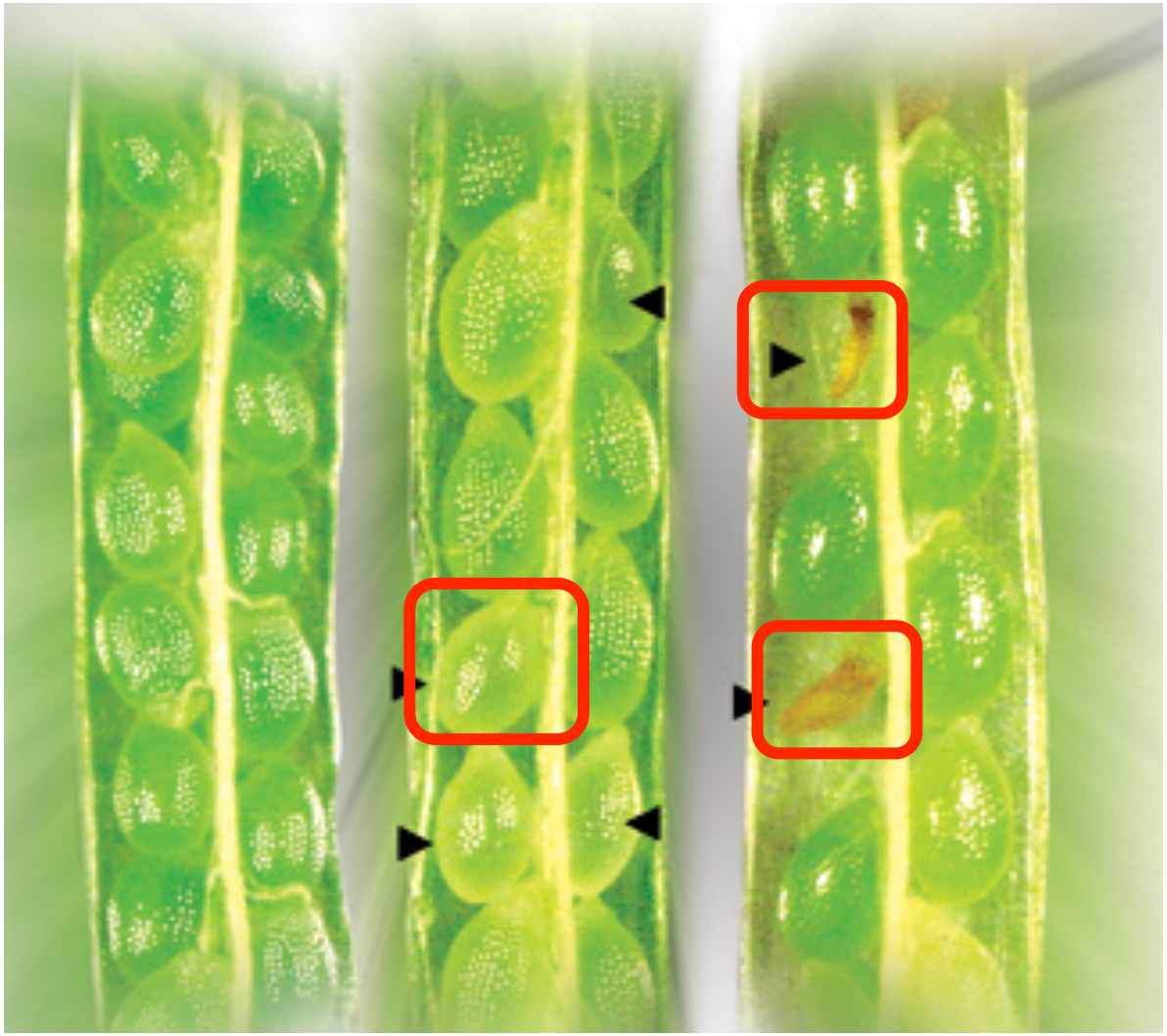


What Happens if the KO Results in a Lethal Phenotype?



Embryo/See Lethality

Note Segregation of Wt and Mutant Seeds



Arabidopsis Siliques and Developing Seeds

Using Agrobacterium T-DNA as a Mutagen

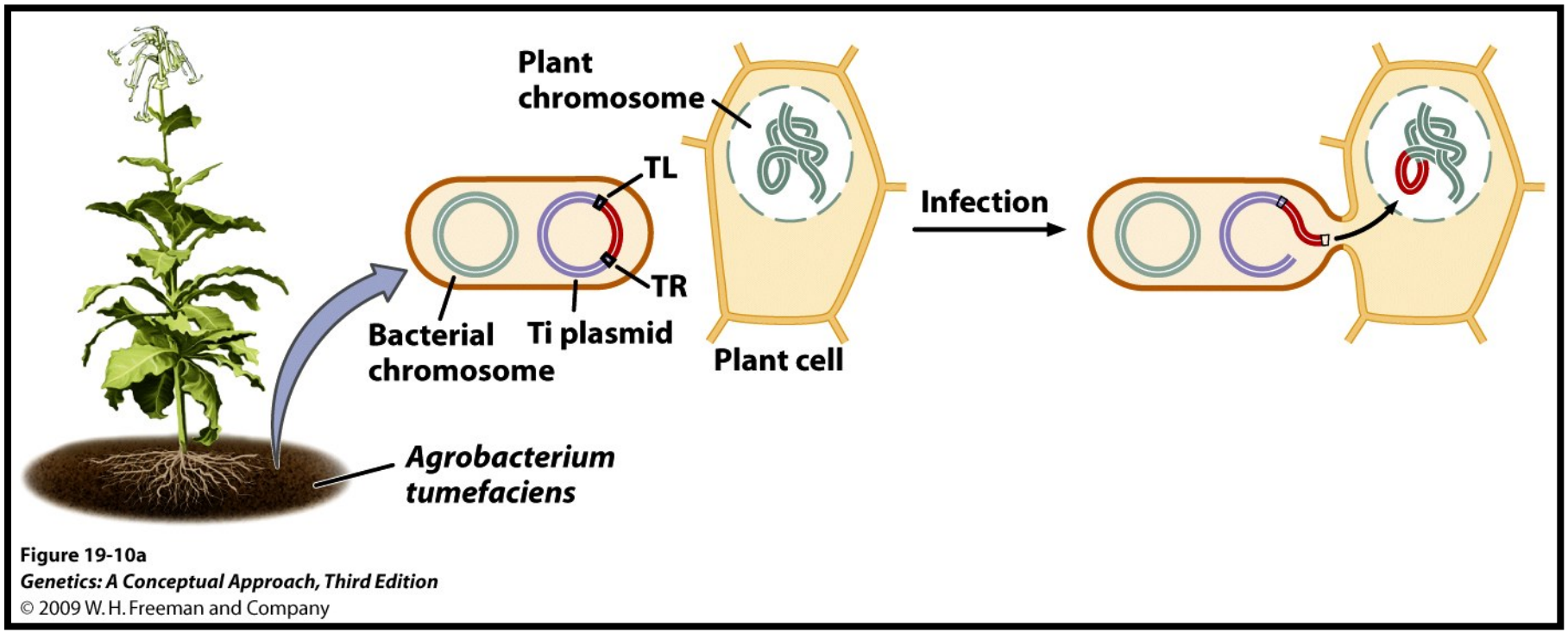


Figure 19-10a
Genetics: A Conceptual Approach, Third Edition

T-DNA Inserts Randomly Into Plant Genome

Transforming Plants With T-DNA

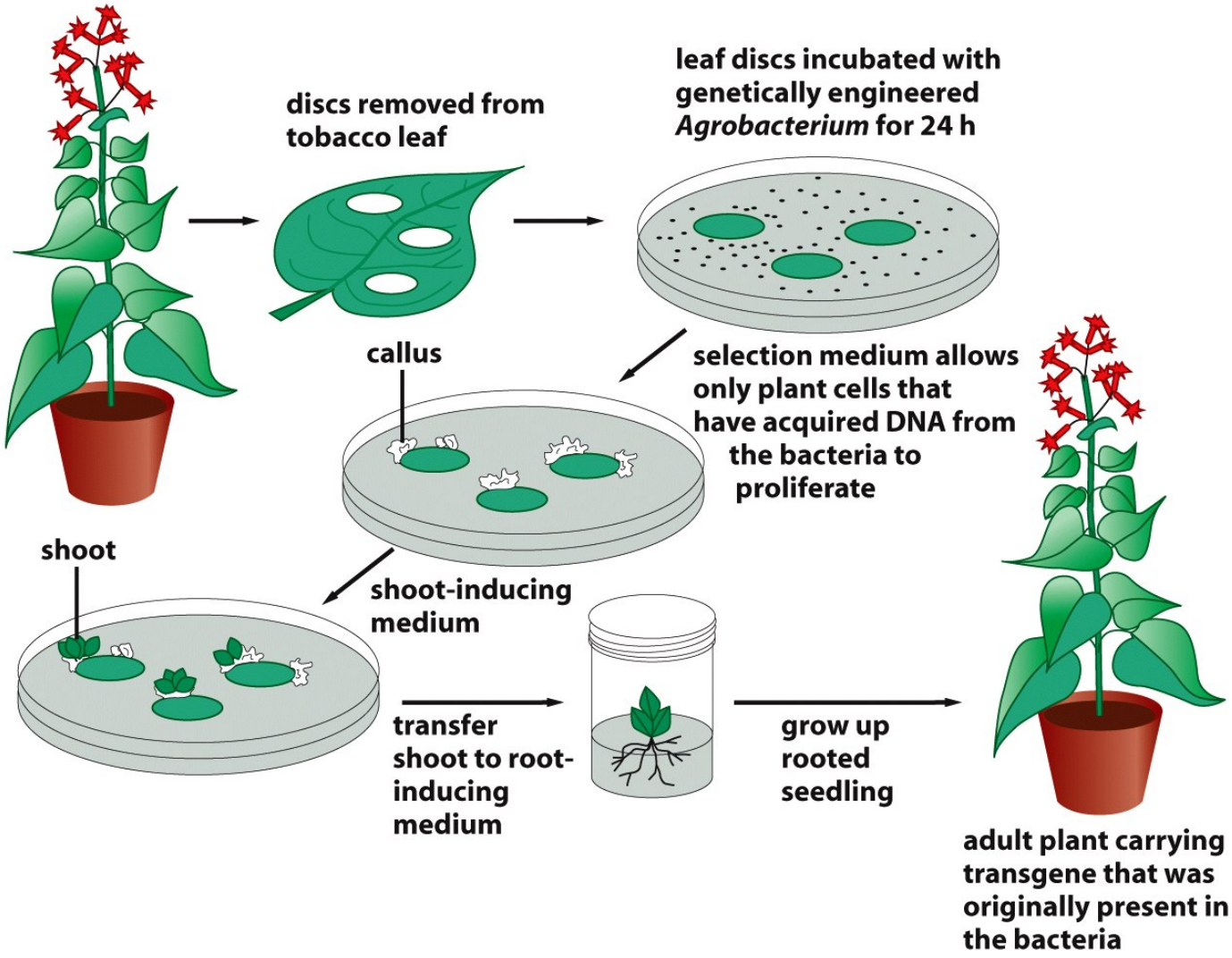
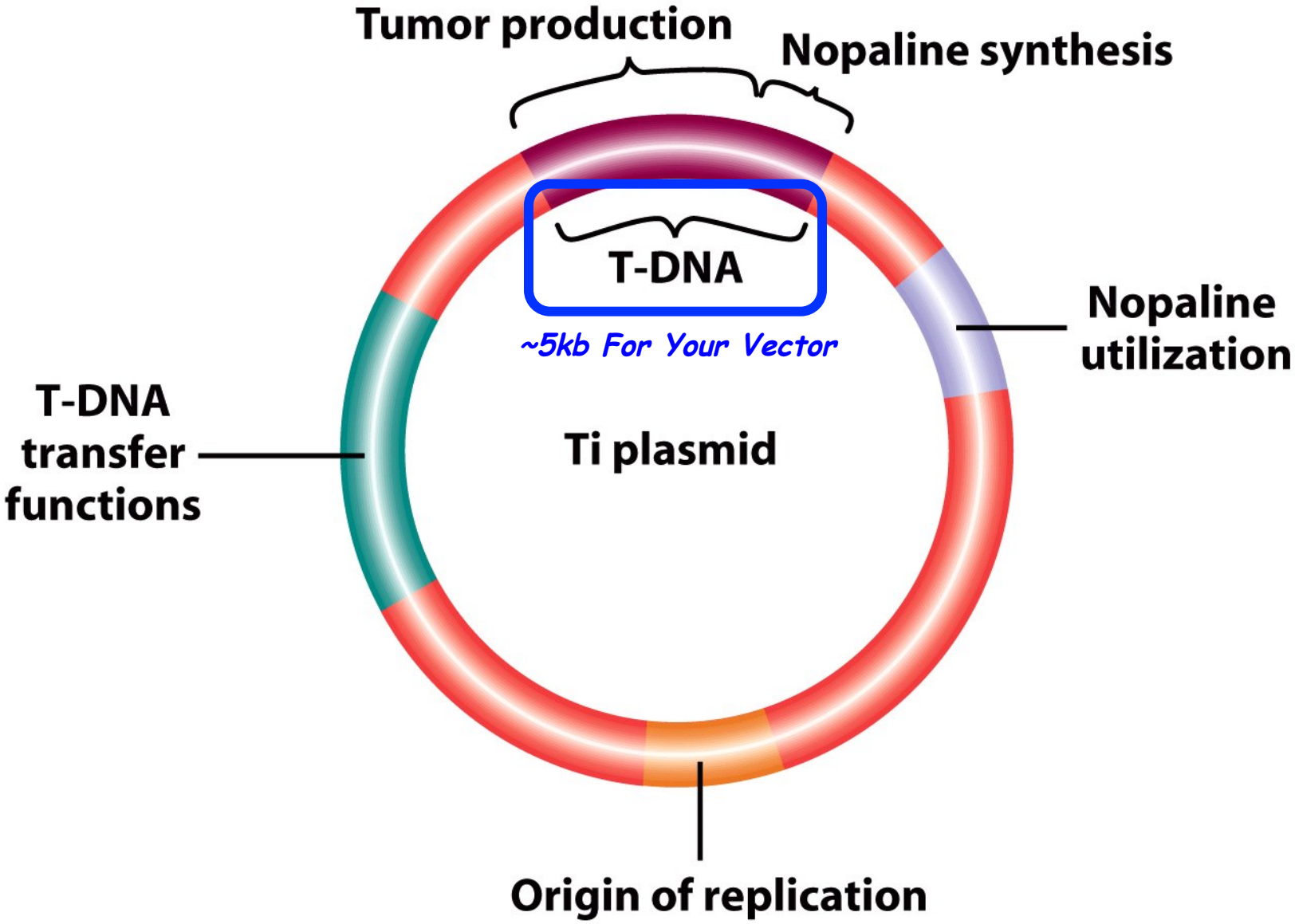
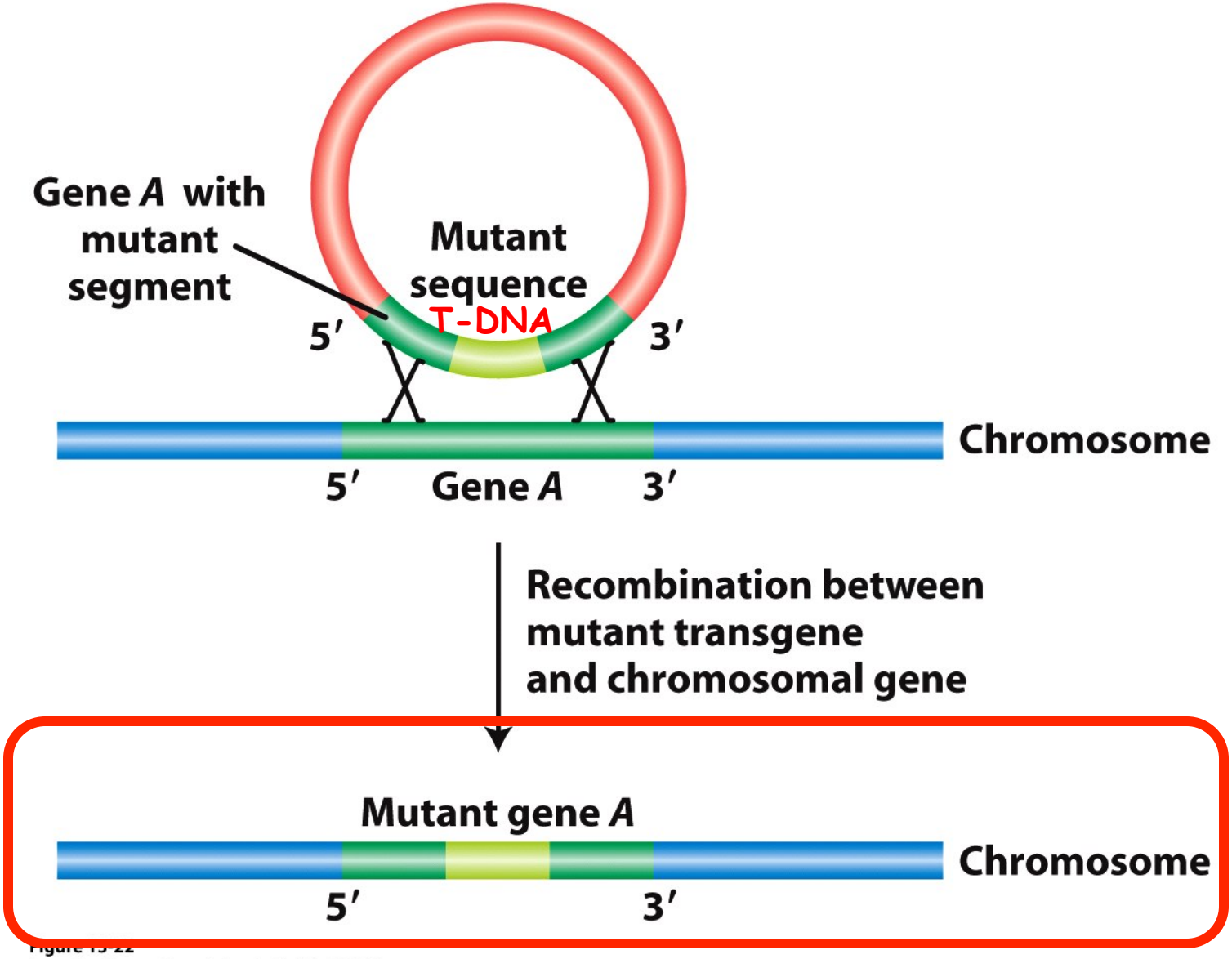


Figure 8-67a *Molecular Biology of the Cell* (© Garland Science 2008)

Agrobacterium Ti-Plasmid



Using Agrobacterium T-DNA as a Mutagen



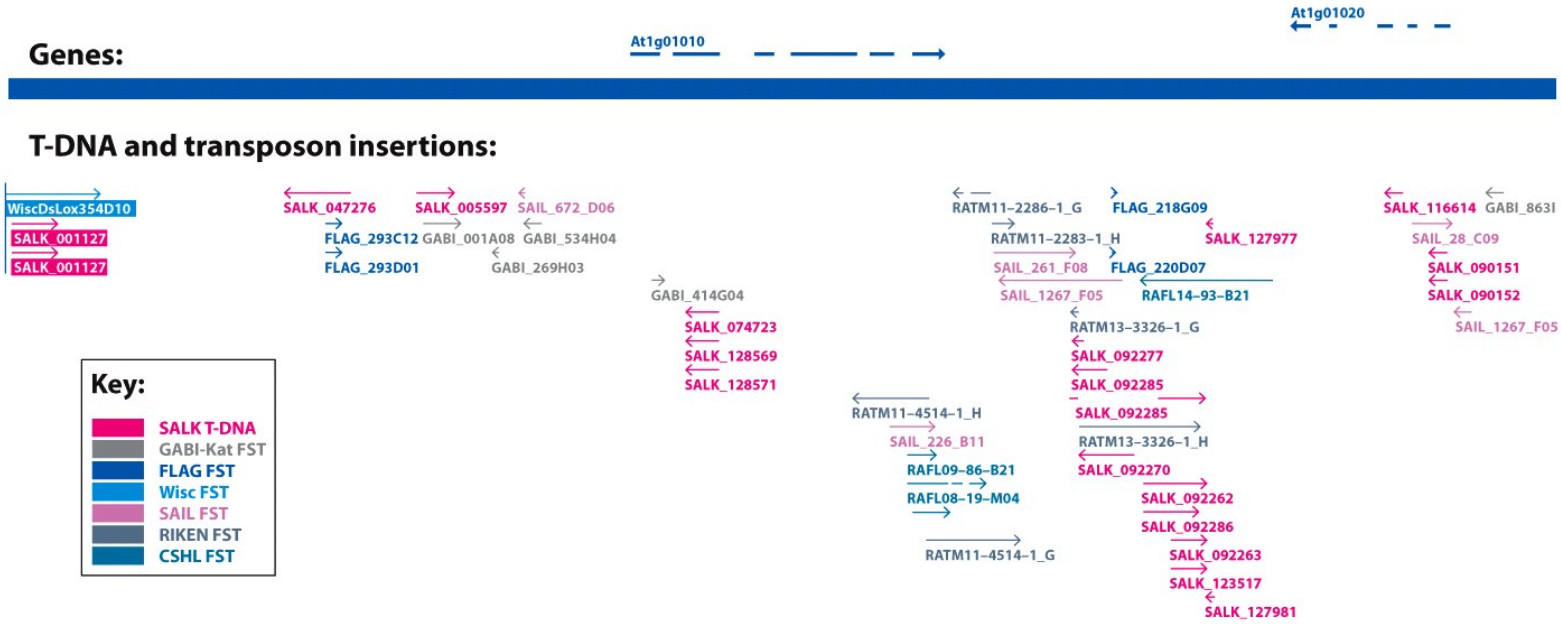
Introduction to Genetic Analysis, Ninth Edition

A Collection of Arabidopsis T-DNA Mutants Exists

SIGnAL Salk Institute Genomic Analysis Laboratory — Funded by the National Science Foundation

SIGnAL "T-DNA Express" Arabidopsis Gene Mapping Tool (Dec.20, 2004)

Arabidopsis thaliana **chromosome 1, nucleotide pairs 1 through 10,001.**



Courtesy National Science Foundation

Segregation of T-DNA Inserts in Knock-Out Lines

Male Gametes

Female Gametes	T-DNA Allele	Wt Allele
T-DNA Allele	T-DNA/TDNA	Wt/T-DNA
Wt Allele	T-DNA/Wt	Wt/Wt

If Gene Is Critical For Controlling Seed Development
The T-DNA/T-DNA Class Does Not Appear!

Inheritance of the T-DNA Inserts

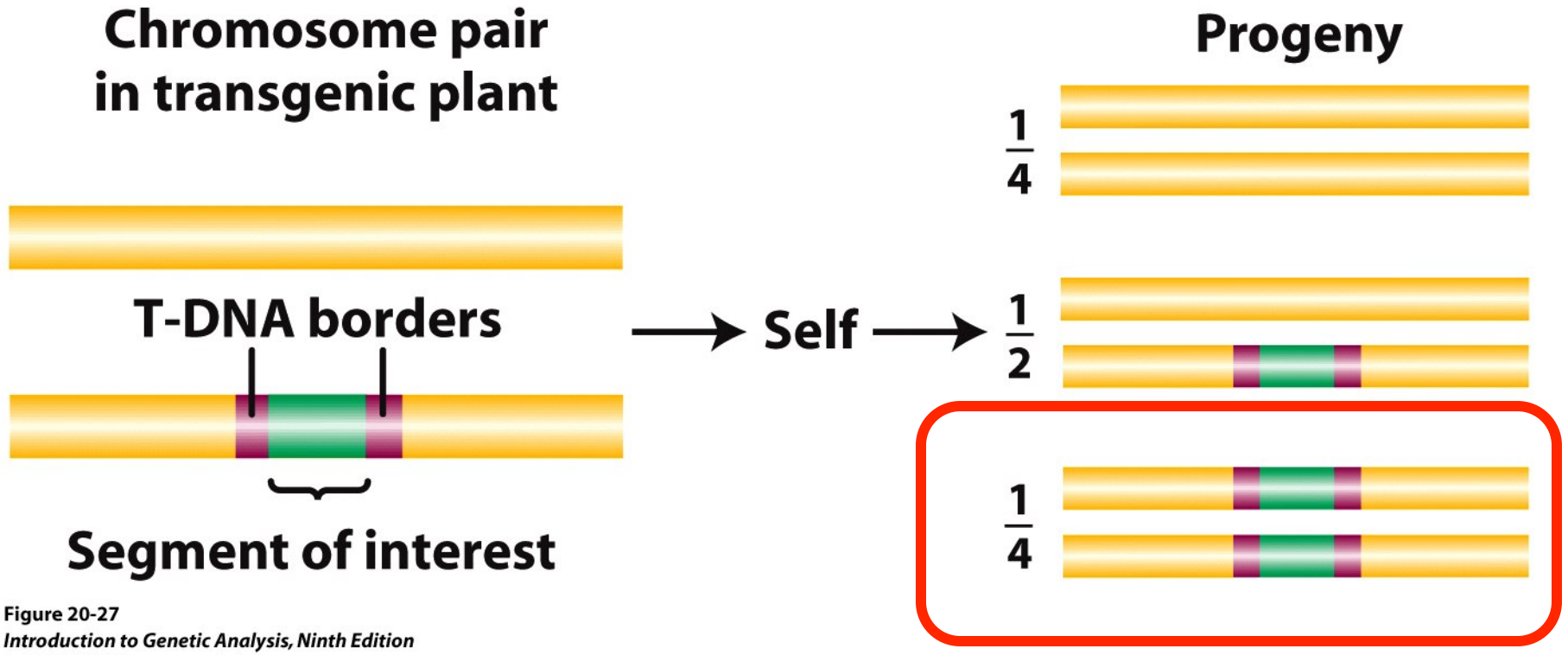
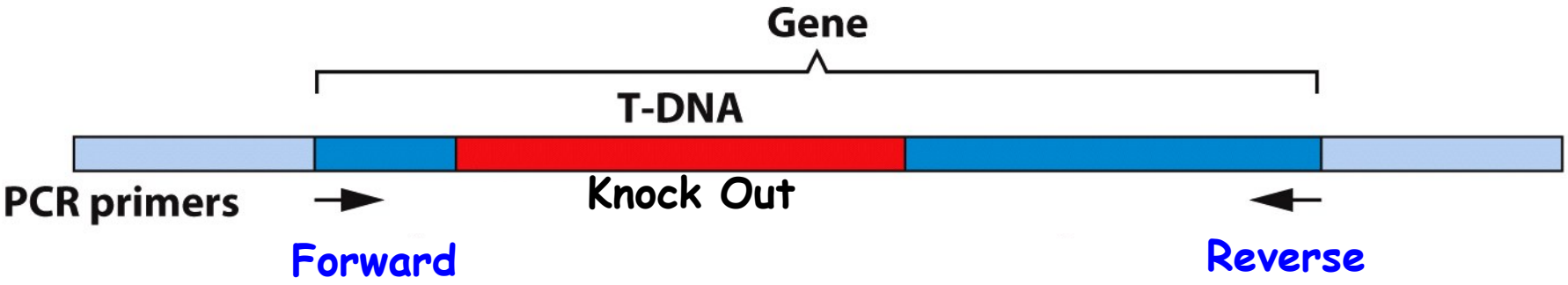


Figure 20-27
Introduction to Genetic Analysis, Ninth Edition

If Seed Lethal This Class Does Not Appear

Using PCR to Genotype Segregating Plants



What Do You Expect If Using Only Gene Primers?

1. *Homozygous Wt Plant?*
2. *Heterozygous Plant?*
3. *Homozygous T-DNA Plant?*
    1. *If Lethal?*
    2. *If Not Lethal?*