




DNA
Genetic Code of Life




Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences





Plants of Tomorrow

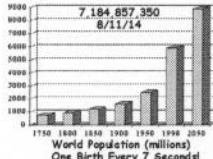
HC70AL Summer 2014 Gene Discovery Laboratory

What Are the Genes Required to Make a Seed?

8/11/14





We Face Major Challenges in Agriculture





7,184,857,350
8/11/14

World Population (millions)
One Birth Every 7 Seconds


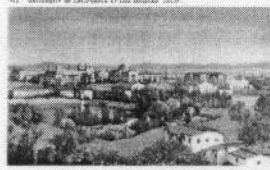



OVER THE NEXT 50 YEARS WE WILL NEED TO PRODUCE
MORE FOOD THAN IN THE WHOLE OF HUMAN HISTORY
AND DO IT WITH **FEWER INPUTS** ON LESS ARABLE LAND!!!
CROP YIELDS NEED TO BE INCREASED SIGNIFICANTLY!!




3,000 Acres/Day of Productive Farmland is Lost to Development Each Day in the United States

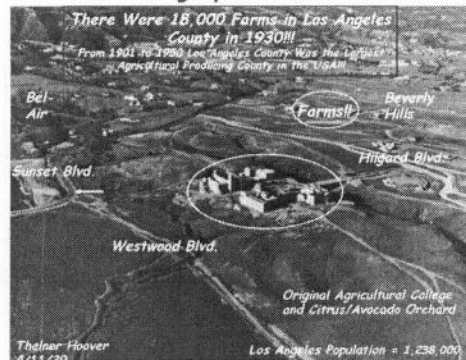
"Major Land Uses Overview." USDA, Economic Research Service, Web, April 3, 2013.

An Example From UCLA & Los Angeles History...

Aerial Photograph of UCLA in 1929



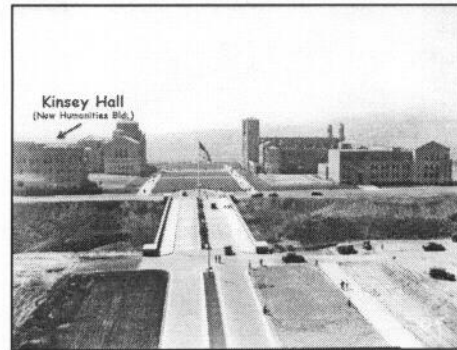
There Were 18,000 Farms in Los Angeles County in 1930!!!
From 1901 to 1990 Los Angeles County Was the Largest Agricultural Producing County in the U.S.A!!

Bel Air Farm Hills Beverly Hills Hilgard Blvd. Sunset Blvd. Westwood Blvd.

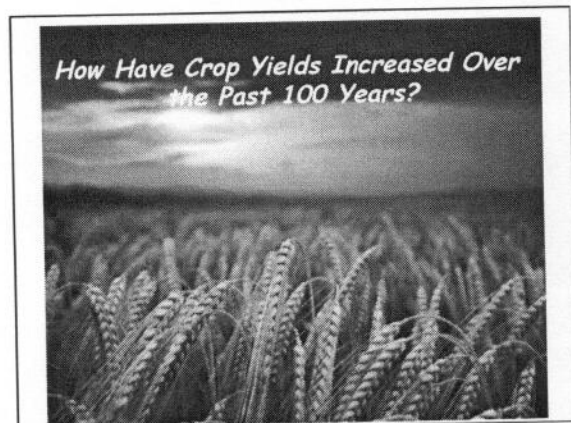
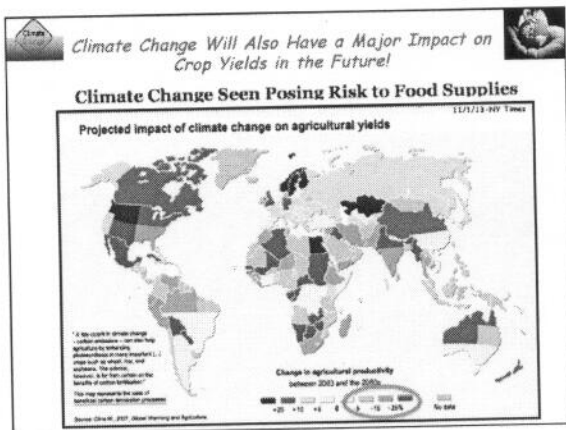
Original Agricultural College and Citrus/Avocado Orchard

Thelmer Hoover 4/11/29 Los Angeles Population = 1,238,000

Original UCLA College of Agriculture-1930



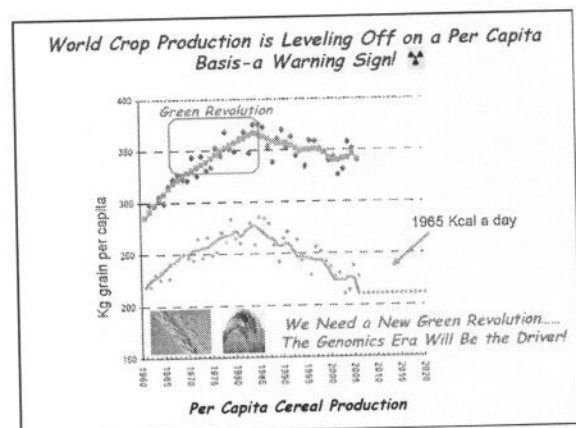
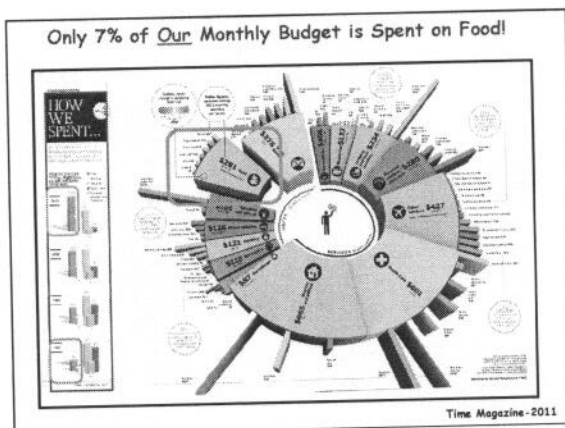
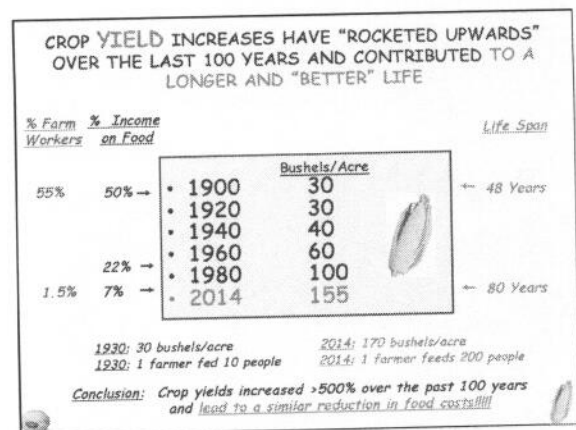
Kinsey Hall (New Humanities Bld.)

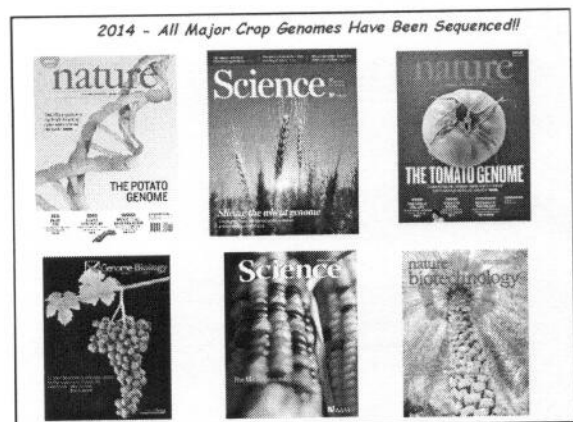
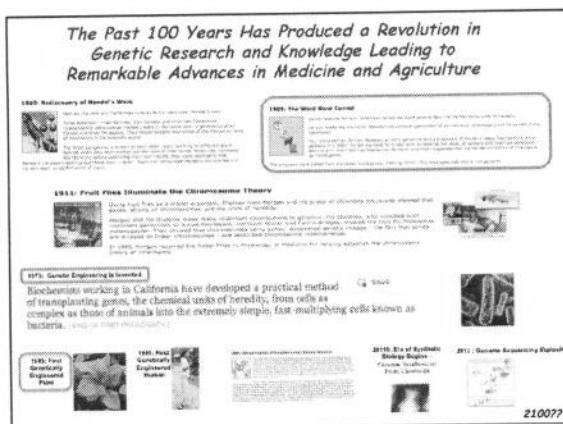
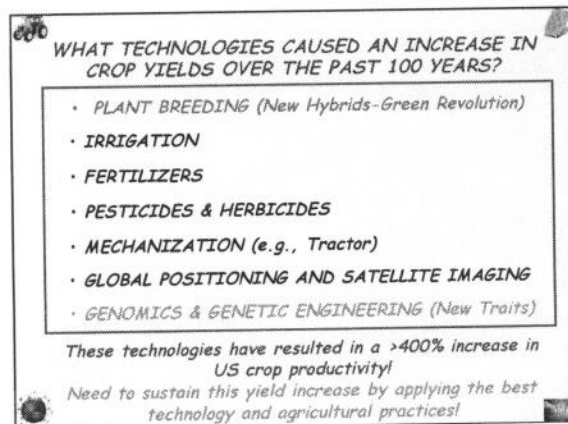
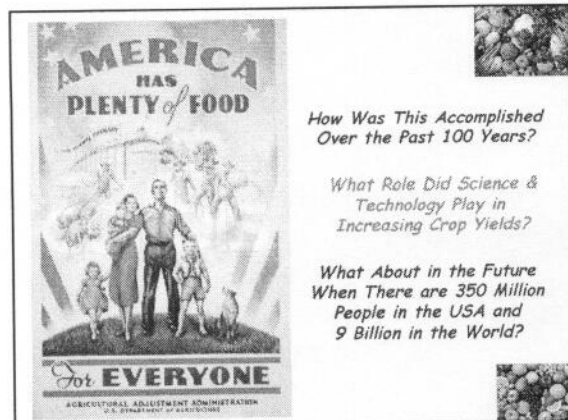
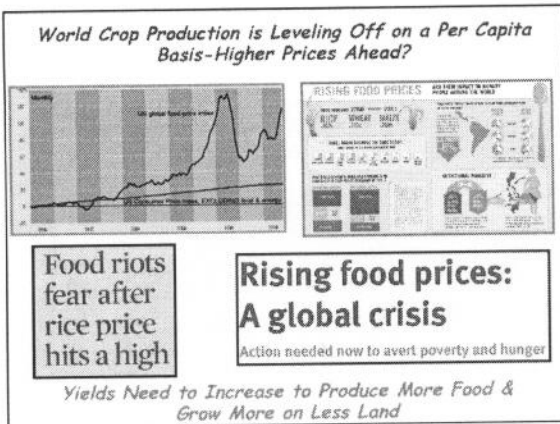


THE ADMINISTRATION'S PROMISES HAVE BEEN KEPT

Big Changes in the US Over The Past 100 Years
"We've Come a Long Way Baby"

	1900	2014
Life Expectancy	48 (women)	81 (women)
Average Family Income (20114 Dollars)	\$8,000	\$50,000
Gasoline Use Per Capita	34 gallons	1,100 gallons
Flush Toilets Per Housing Unit	10%	99%
High School Grads	13%	90%
Farm Workers	55%	1.5%





How Will Crop Yields Be Increased in the Future?



...By Using a Variety of Approaches to Identify Genes and Processes That Will Help Increase Crop Yields and Food Production Significantly in the 21st Century...

Yield (Developmental Traits)

- Seed Number
- Seed Size
- Growth Rate
- Organ Size (More Seeds)
- Plant Architecture
- Flowering Time
- Senescence
- Maturity
- Stature



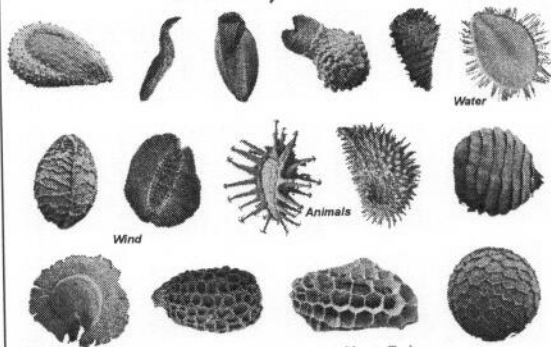
Yield (Stress Traits)

- Nutrient Uptake
- Drought Resistance
- Heat Resistance
- Cold Tolerance
- Salt Tolerance
- Shade Tolerance
- Disease Resistance



.....And by Using Genomics, Breeding, and Genetic Engineering to Introduce These "Yield" Genes Into Crops (One thing we can be sure of-we can't predict what new technology will be the driver 10-25 years out!)

So.....Why Seeds??



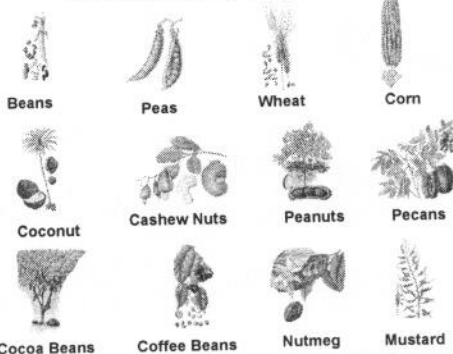
Seeds Protect and Disperse Plant Embryos and Come in Many Shapes and Sizes!

SEEDS

- 22,300 Seed-Bearing Plant Species
 - 90% of all known plants
 - 1,000 species produce "naked" seeds (gymnosperms)
 - Remainder are from flowering plants
- 2,938 Seed Species Stored in the Svalbard Seed Vault
- Global Seed Vault in Norway
 - 320,552 seed samples from around the world
 - Bank can hold 2.25 billion seeds!
- 55 Pounds is the Weight of the Largest Seed - Fruit of the Double Coconut Palm
- Smallest Seeds Come From Orchids and Weigh ~10 Billionths of an Ounce
- 385 Million Years is the Age of the Oldest Seed Fossil
- 2,000 Year-Old Date is the Oldest Viable Seed
- 150 Miles per hour is the Speed of Seeds Released by the Tropical Sand Box Tree
- \$36.5 Billion Dollars is the Value of the World Seed Market (2008)

Reference: Discover Magazine, April, 2009

Seeds Are Used in Many Ways as Food, Beverages, Spices, and Fuels!



Most Importantly.... Our Food is Derived From Fifteen Crops & Over Half Produce Seeds For Human and Animal Consumption

Seed Crops

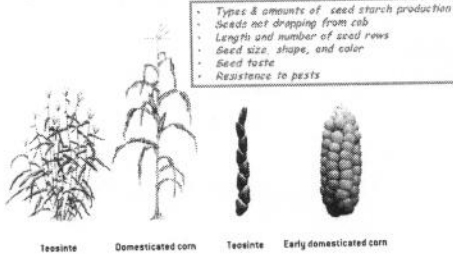
- Wheat
- Rice
- Corn
- Barley
- Sorghum
- Soybean
- Common Bean
- Coconut
- Canola

Non-Seed Crops

- Potato
- Sweet Potato
- Cassava
- Sugar Beet
- Sugar Cane
- Banana

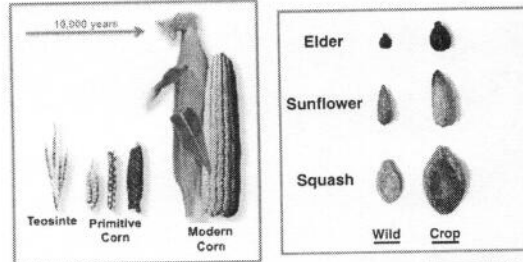
In Some World Populations 75% of Calories Are Derived From Seeds!

All Crops Have Been Engineered - Turning Wild Teosinte Into Domesticated Corn 10,000 Years Ago - Seed & Plant Engineering!!



*Note: Architecture and Fruit (cob) Size
Only Five Genes Cause These Plants to Differ
& We Now Know What They Are*

"Manipulating" Plants to Increase Seed Yield Is Not New..... Seed Size!



All Vegetables in Grocery Store are "GMOs!!"

Goldberg Lab Research

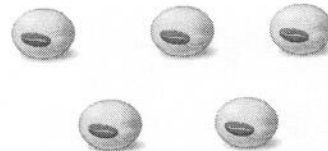
Diversity of Oil Seed Plants

Soybean - A Reminder

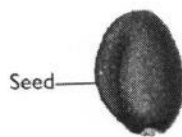
- **Second Major US Crop**
- **Total Crop Value \$32 Billion**
(50% Value Exported)
- **Important Biofuel Source**
(Biodiesel - 20% of US Soybean Oil Production)
- **Excellent Model Plant**
(Transformation, Knockdowns, Genetics)
- **Genome Sequenced**
- **Major Food Source**



How Is a Seed Formed?

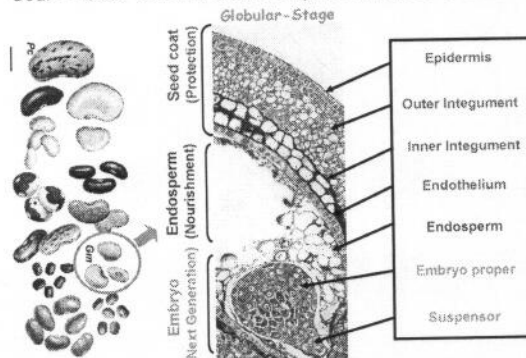


In the Beginning....



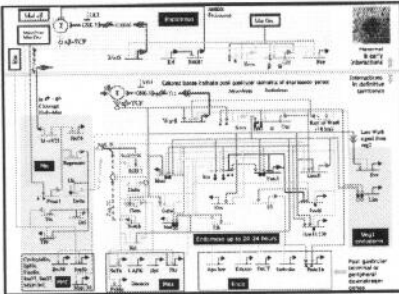
<http://estdb.biology.ucla.edu/seed/presentation>

Goal - What Are the Genes Required to Make a Seed?



And How Are They Wired in a Plant Genome?

Ultimate Goal.....To Uncover Regulatory Genes and Circuits Driving Seed Differentiation and Development Using Genomics



Learn How To Make a Seed!

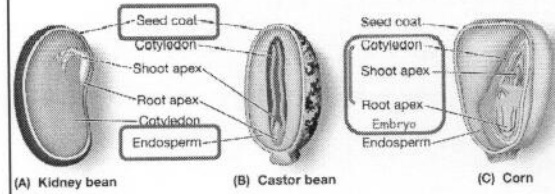


- Big Seeds
- More Seeds
- More Yield
- Increased Food and Fuel

Eric Davidson et al. Science, 2007

Knowledge of Cell-Specific TF mRNAs and Knock-Down Effects On Embryo Phenotype and TF mRNAs

What is a Seed?



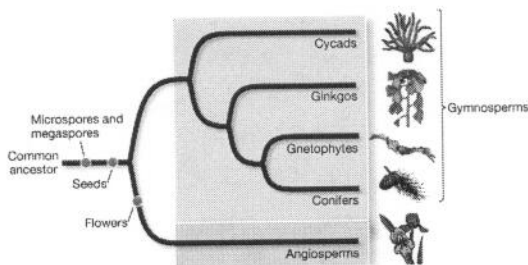
A Seed Consists of Three Parts With Distinct Genetic Origins

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

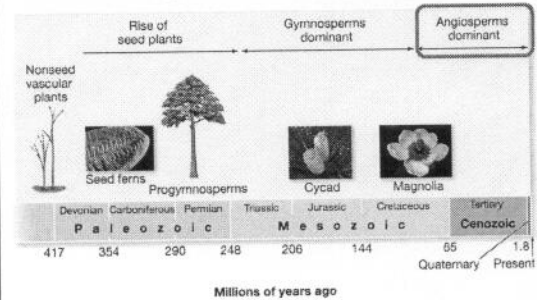
The Embryo Becomes the Next Plant Generation

20.2 The Major Groups of Living Seed Plants

The Plant Kingdom



Seed Producing Plants Are ~300 Million Years Old

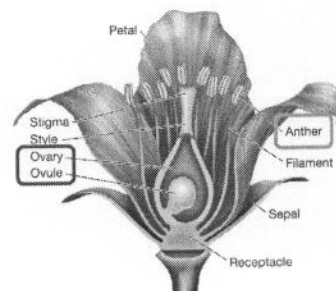


Major Crops (Soybean and Corn) Are Flowering Plants

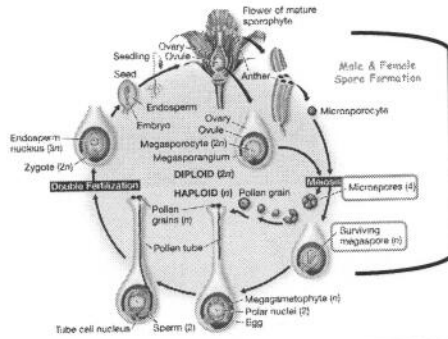
Major Characteristics of Plants

1. Alternation of Spore and Gamete-Producing Generations
2. Meiotic Products Are Spores
3. Gametes Produced By Mitosis
4. Double Fertilization (Two Sperm Cells)
5. Cellulose Cell Walls
6. Autotrophic-Covert Light Energy to Chemical Energy by Photosynthesis (Ultimate Source of Food on Earth)
7. Morphogenesis in Absence of Cell Movement

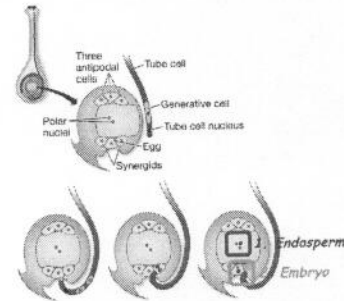
The Plant Life Cycle Begins in the Flower



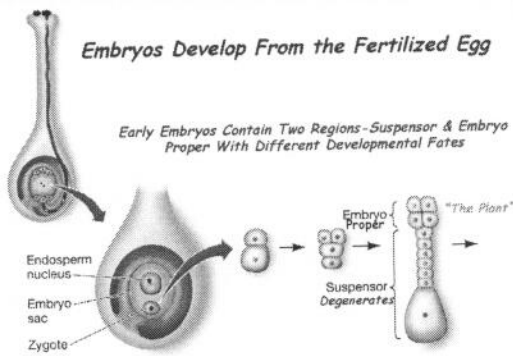
Life Cycle of a Flowering Plant



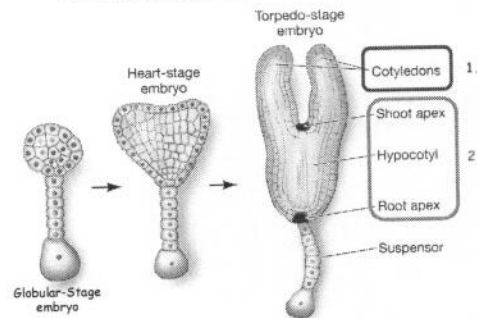
Double Fertilization Leads to the Three Parts of the Seed



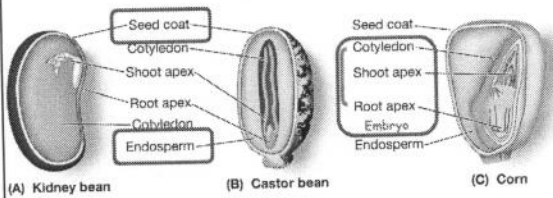
Embryos Develop From the Fertilized Egg



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



The "End Product" is Dormant Seed With a Mature Embryo?

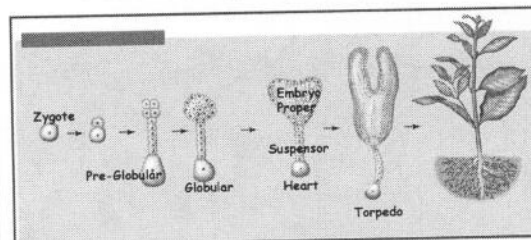


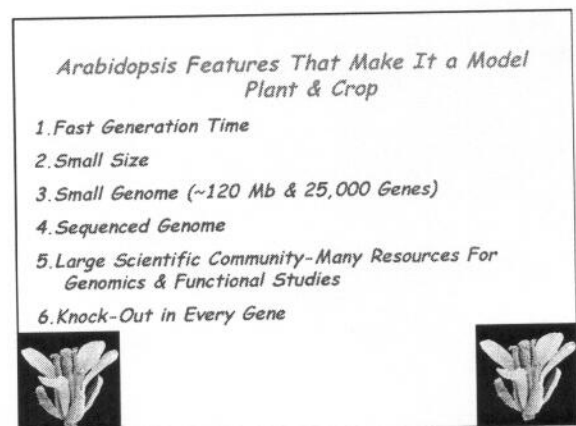
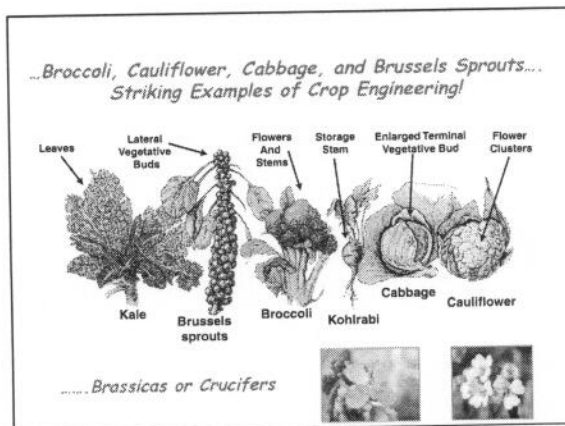
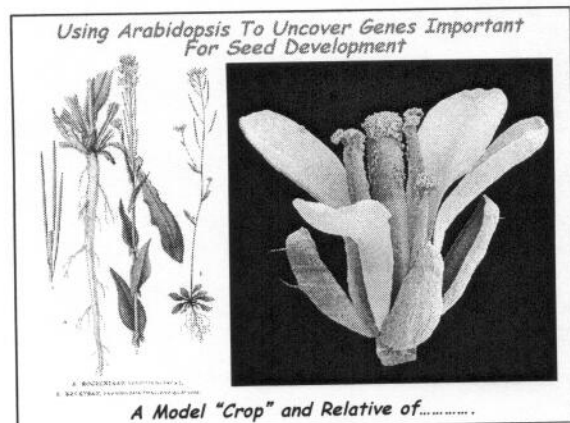
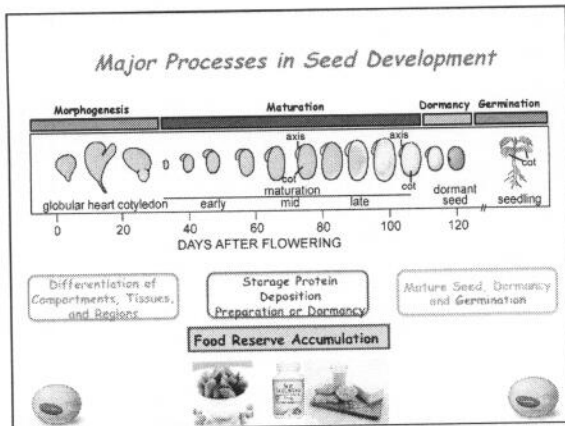
A Seed Consists of Three Parts With Distinct Genetic Origins

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.

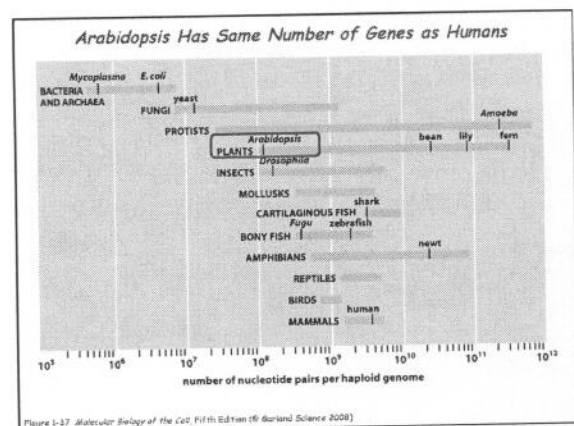
Plant Embryo Development

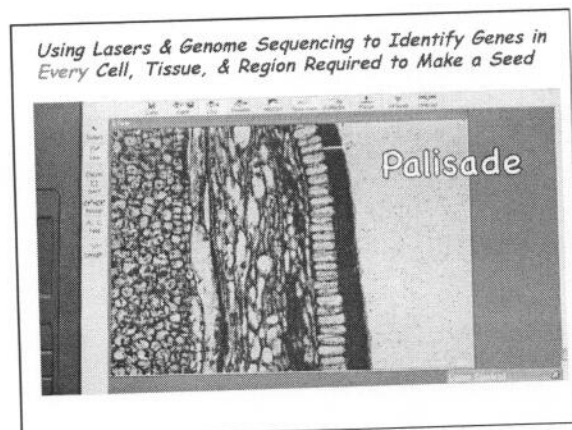
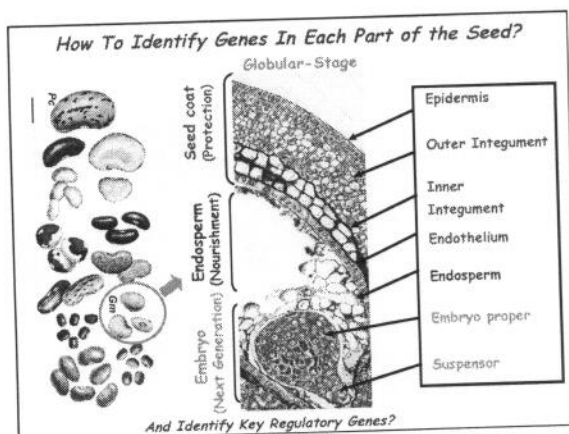
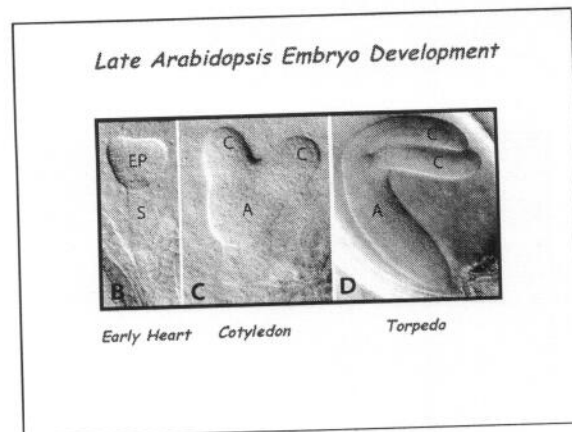
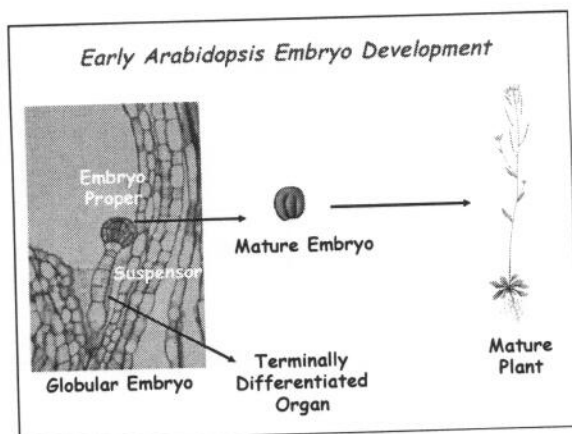
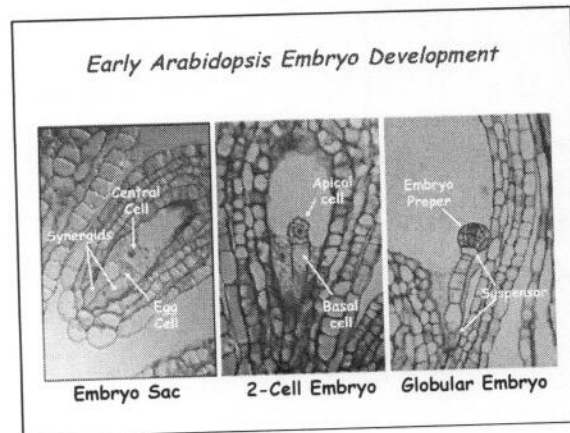
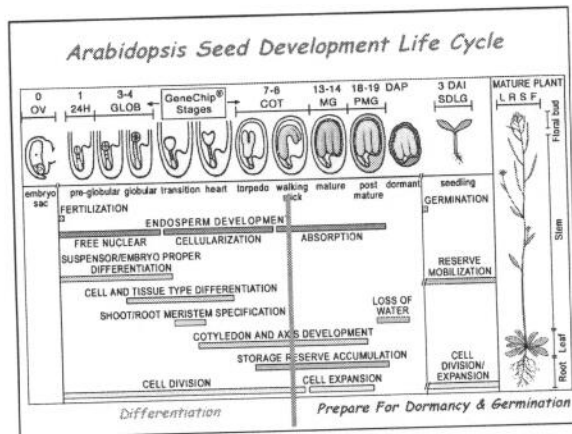




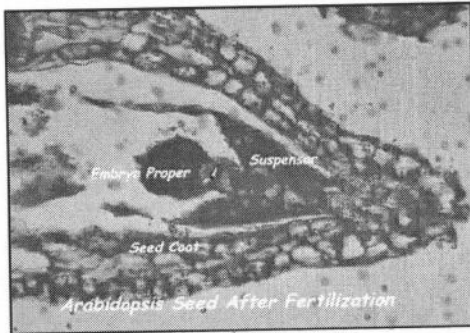
The Arabidopsis Genome

- High quality sequence with few gaps
- TIGR did initial genome annotation
- TAIR took over responsibility in 2005
- Current stats:
27,379 protein coding genes
4827 pseudogenes or transposable elements
1312 ncRNAs

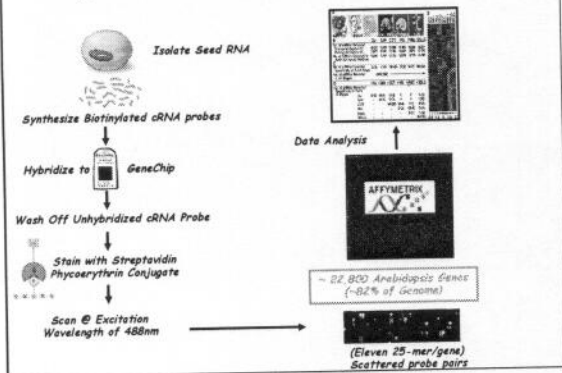




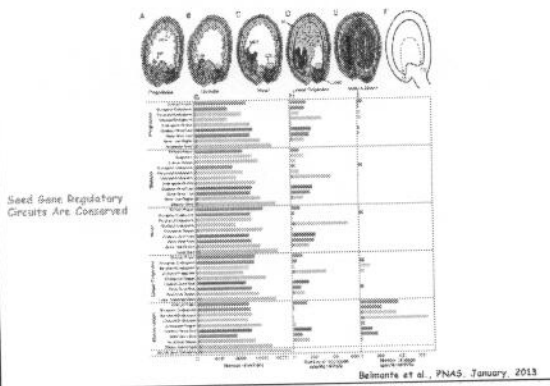
Using Laser Capture Microdissection (LCM) to Isolate Specific Seed Tissues and Compartments



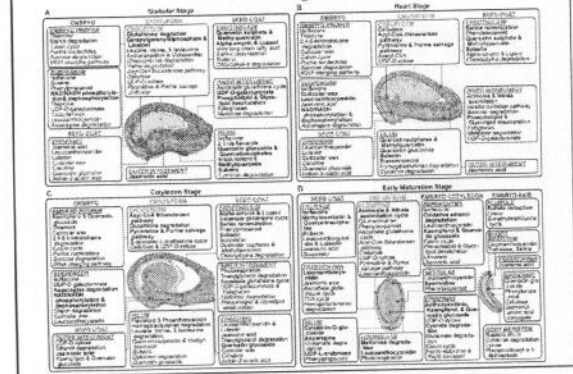
Using Genomics to Analyze Seed mRNA Populations



What Are the Genes Required to Make an Arabidopsis Seed?

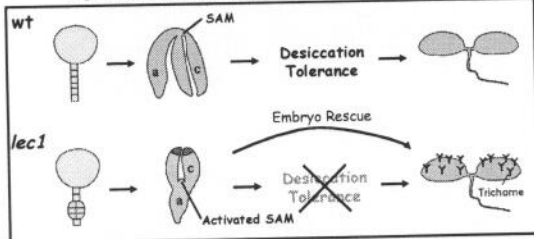


What Metabolic Pathways Are Prevalent in Different Seed Regions?



How to Identify the Functions of Seed Transcription Factor Genes?

(e.g. leafy cotyledon1 (lec1) Mutants Disrupt Seed Development)



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

Letan et al., Cell, 1998

Examples of Arabidopsis Mutants

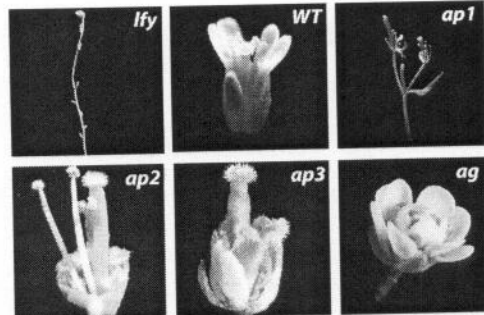


Figure 3-1a
Arabidopsis Genetics: Analysis, Ninth Edition
© 2008 Sinauer Associates, Inc. and W. H. Freeman & Co.

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

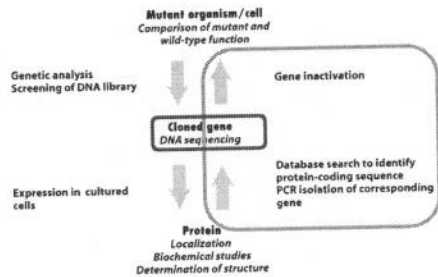


Figure 8-4 Molecular Biology of the Cell, Sixth Edition © 2008 W. H. Freeman and Company

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

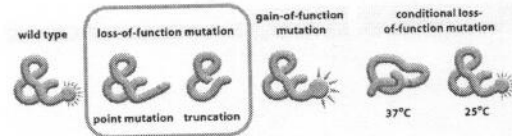


Figure 8-16 Molecular Biology of the Cell © Garland Science 2008

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

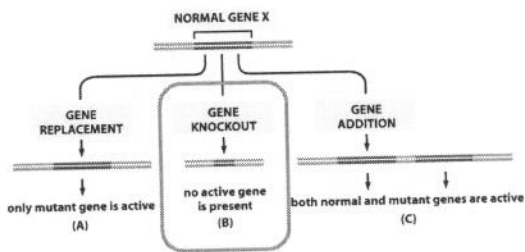


Figure 8-64 Molecular Biology of the Cell © Garland Science 2008

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

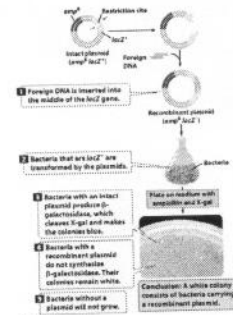


Figure 8-6 Genetic Analysis: From Molecules to Cells © Garland Science 2008

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

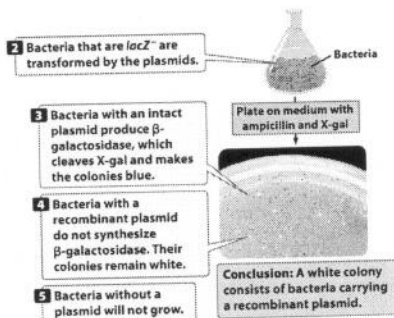


Figure 18-8 part 2 Genetic Analysis: From Molecules to Cells © Garland Science 2008

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

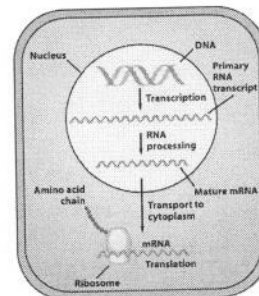


Figure 1-1 Molecular Biology of the Cell, Sixth Edition © Garland Science 2008

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
© 2004 W. H. Freeman and Company

Chromosomes & Alleles...a Review

Genes exist in different versions called alleles.

One allele encodes round seeds...

Allele *R*

...and a different allele encodes wrinkled seeds.

Allele *r*

Different alleles for a particular gene occupy the same locus on homologous chromosomes.

Figure 3.5
Genetics: A Conceptual Approach, Third Edition
© 2004 W. H. Freeman and Company

Simple Mendelian Genetics...a Review

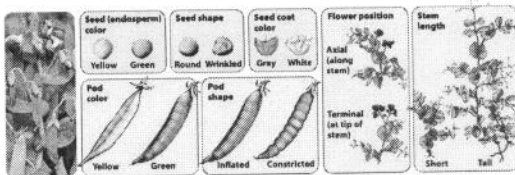
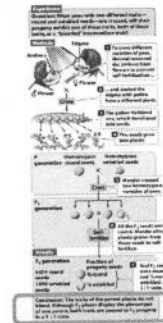
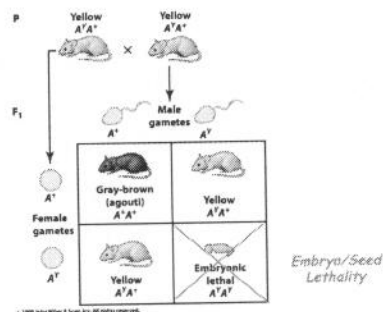


Figure 3-1
Genetics: A Conceptual Approach, Third Edition
© 2004 W. H. Freeman and Company

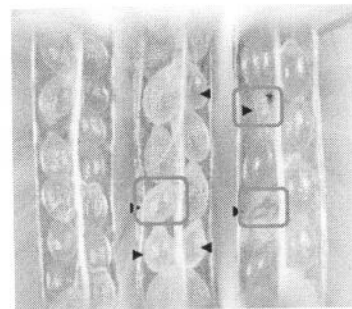
Simple Mendelian Genetics...a Review



What Happens if the KO Results in a Lethal Phenotype?

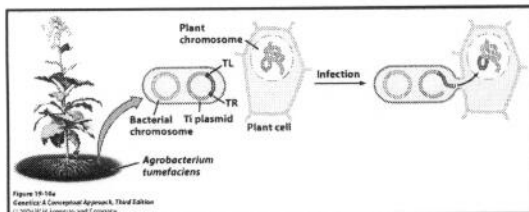


Note Segregation of Wt and Mutant Seeds



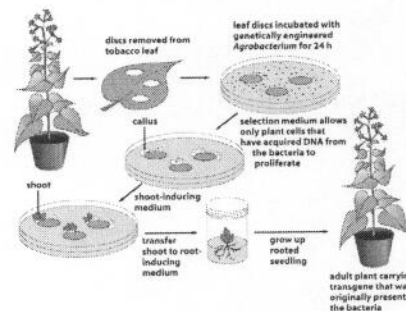
Arabidopsis Siliques and Developing Seeds

Using Agrobacterium T-DNA as a Mutagen

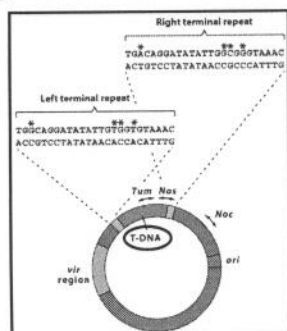


T-DNA Inserts Randomly Into Plant Genome

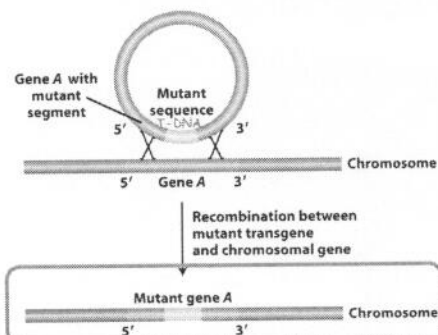
Transforming Plants With T-DNA



Agrobacterium Ti Plasmid



Using Agrobacterium T-DNA as a Mutagen

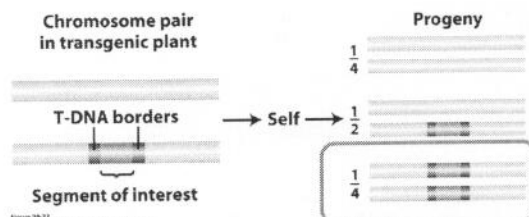


Segregation of T-DNA Inserts in Knock-Out Lines

		Male Gametes	
		T-DNA Allele	Wt Allele
Female Gametes	T-DNA Allele	T-DNA/T-DNA	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!*

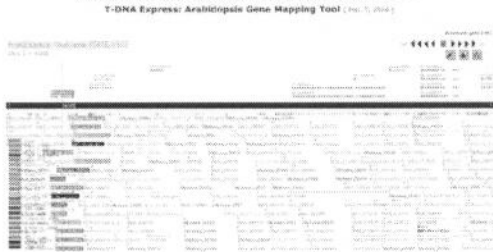
Inheritance of the T-DNA Inserts



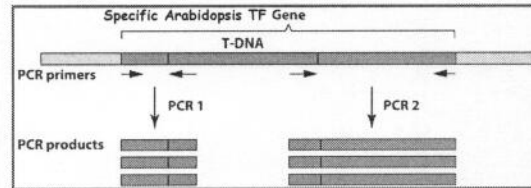
*If Seed Lethal This Class
Does Not Appear*

Arabidopsis Plants Have Been Generated With T-DNA Insertions in Every Gene!

Shenoy
T-DNA Express: Arabidopsis Gene Mapping Tool (v1.0.0)



Using Reverse Genetics To Identify T-DNA Mutations in Specific Arabidopsis TF Genes



DNA Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

HC70AL Summer 2014 Gene Discovery Laboratory

What Are the Genes Required to Make a Seed?

Approach?

Gene To Mutant To Phenotype!!

DNA Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

HC70AL Summer 2014 Gene Discovery Laboratory

What Will You Do This Quarter?

1. Study One Arabidopsis TF Gene
2. Look For Mutants in KO of This Genes
3. Determine if Mutant Affects Seed Development