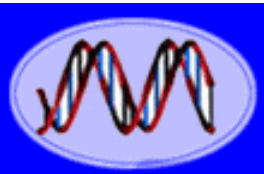
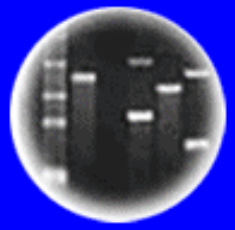


DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

HC70A, PLSS530, & SAS70A
Winter 2014
Genetic Engineering in Medicine, Agriculture, and Law

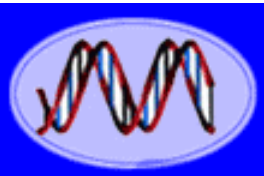
Professors Bob Goldberg, Channapatna Prakash, & John Harada

Lecture 2
The Age of DNA: What Is Genetic Engineering-Part Two

UCLA

TUSKEGEE UNIVERSITY

UC DAVIS
UNIVERSITY OF CALIFORNIA



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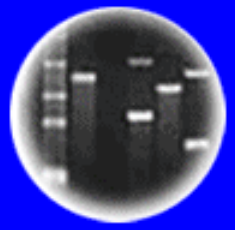

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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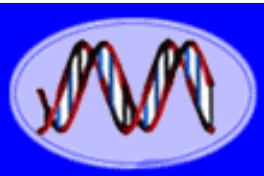
THEMES

1. Spectacular Examples of Genetic Engineering 1.0 - What Can Be Done?
2. What Does Genetic Engineering Tell Us About Basic Genetic Processes in All Organisms?
3. The Future is Here - Genetic Engineering 2.0!
4. Genetic Engineering - Anything New?
5. Are Vegetables Engineered - Demonstration
6. Classical vs. 21st Century Genetic Engineering –
7. Is Science Hocus Pocus or a Precise Process?
8. Understanding Basic Genetic Processes ➡ Understanding How Genetic Engineering Uses Natural Rules of the Cell (i.e., it isn't magic)!



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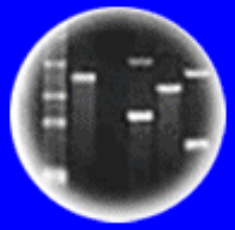


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Last Lecture - Age of DNA & Genetic Engineering: Part One

Today's Class – Age of DNA & Genetic Engineering: Part Two

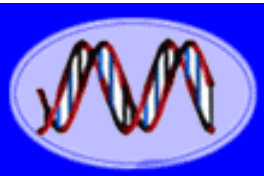
Genetic Engineering – Spectacular Examples

Genetic Engineering - Anything New?

Recall: The Era Of DNA Manipulation Means……

1. Specific DNA Sequence and/or Gene Can Be Isolated From Any Organism
2. DNA Segments of Any Kind From Any Organism Can Be Combined
3. Isolated Genes Can Be Re-Inserted Into the Chromosomes of Any Organism and Made to Work

The Critical Point - There Are No Genetic Limits. All Biological Organisms Use the Same Genetic Rules. The Implications Are Enormous!!



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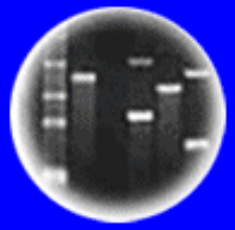


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

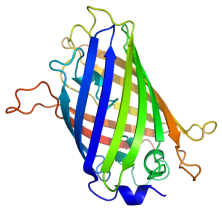
Plants of Tomorrow

What Can Be Done With Genetic Engineering?

A Few Examples of Genetic Engineering 1.0

Using a Jellyfish Gene to Make Bacteria, Animals, and Plants Glow!!!!

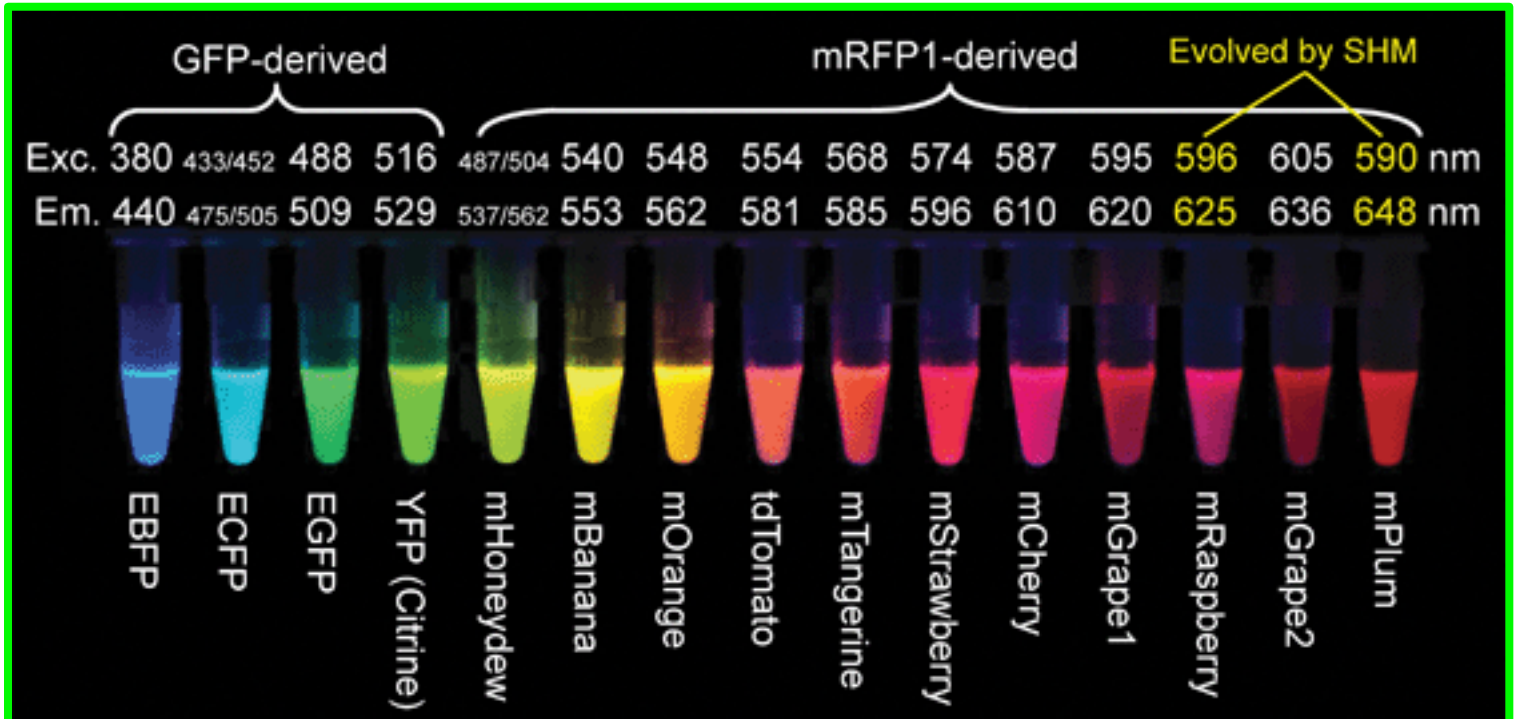
Green Fluorescence Protein (GFP)



(238 amino acids)

Nobel Prize in Chemistry – 2008 – Shimomura, Chalfie, & Tsien

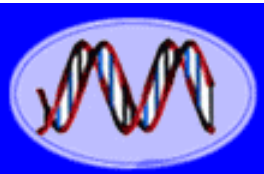
There Are Many Different Kinds of Fluorescing Proteins!



Engineered or From Different Marine Creatures

Nathan Shaner et al (2004) *Nature Biotech.* **22**: 1567-1572
Lei Wang et al (2004) *Proc. Natl. Acad. Sci. USA* **101**: 16745-16749

Nobel Prize in Chemistry – 2008 – Shimomura, Chalfie, & Tsien



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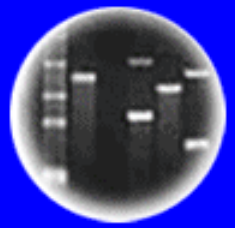
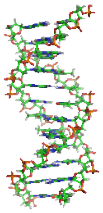


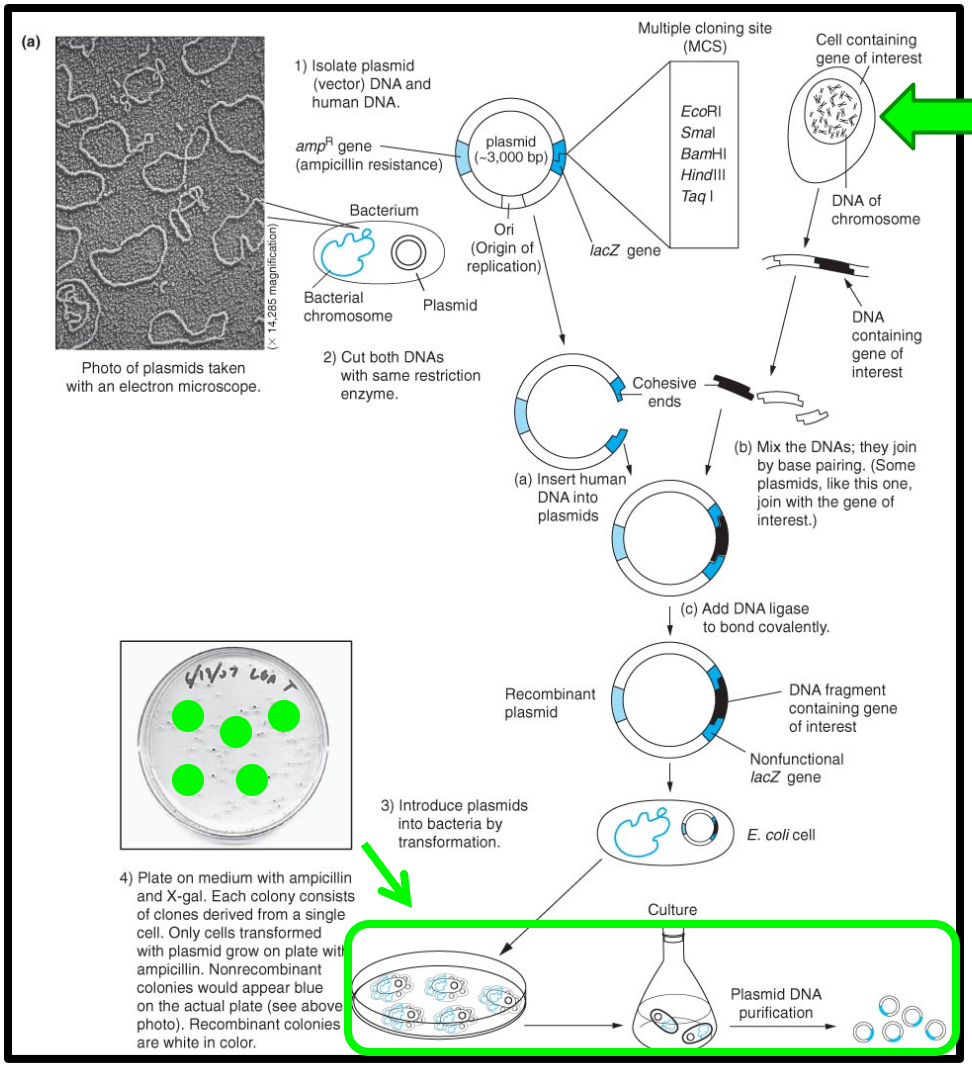
DNA Fingerprinting

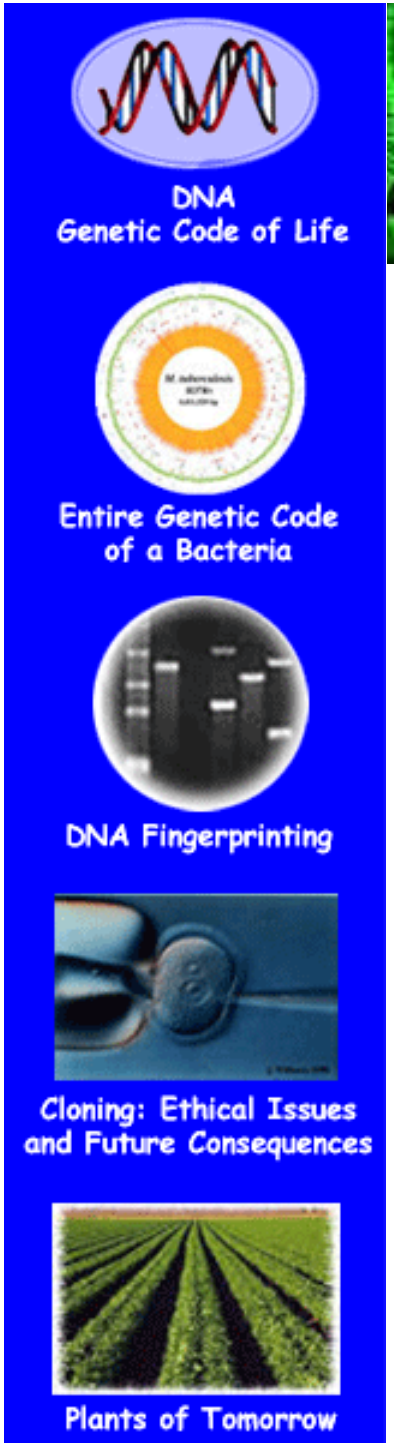
Cloning: Ethical Issues and Future Consequences

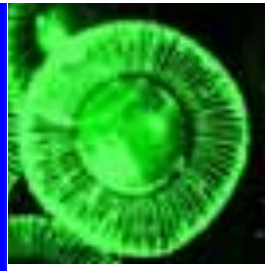
Plants of Tomorrow

Using Recombinant DNA to Clone the Jellyfish GFP Gene

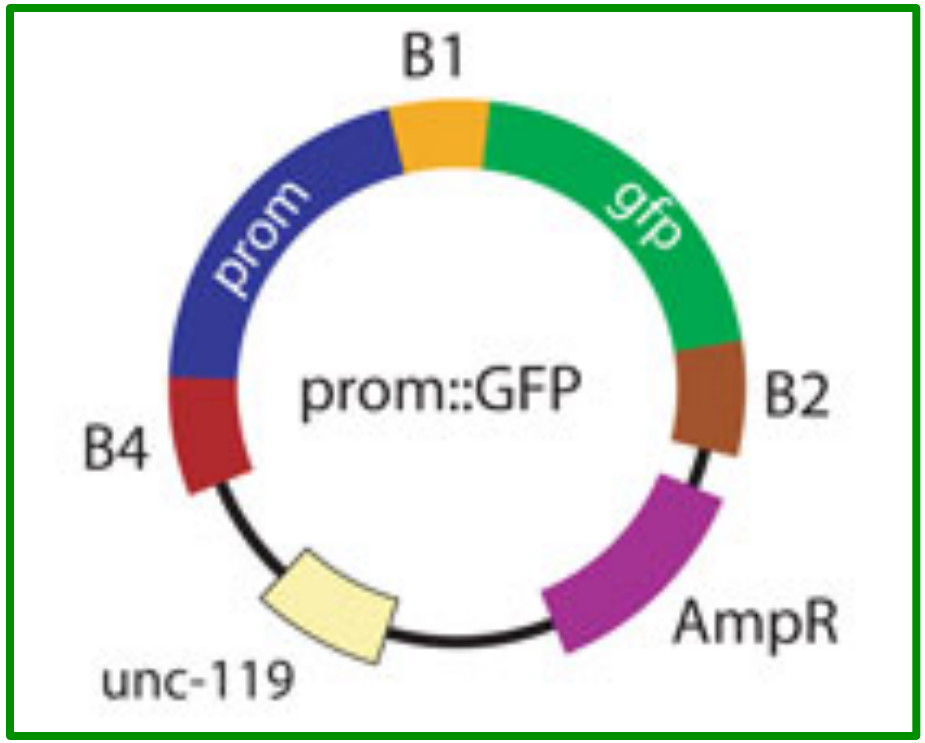


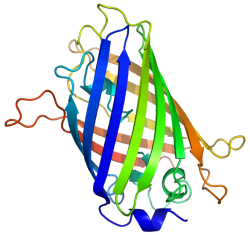


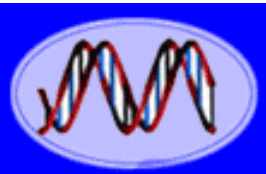




A Recombinant Plasmid Containing the GFP Gene







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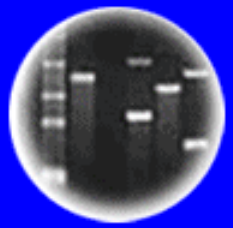
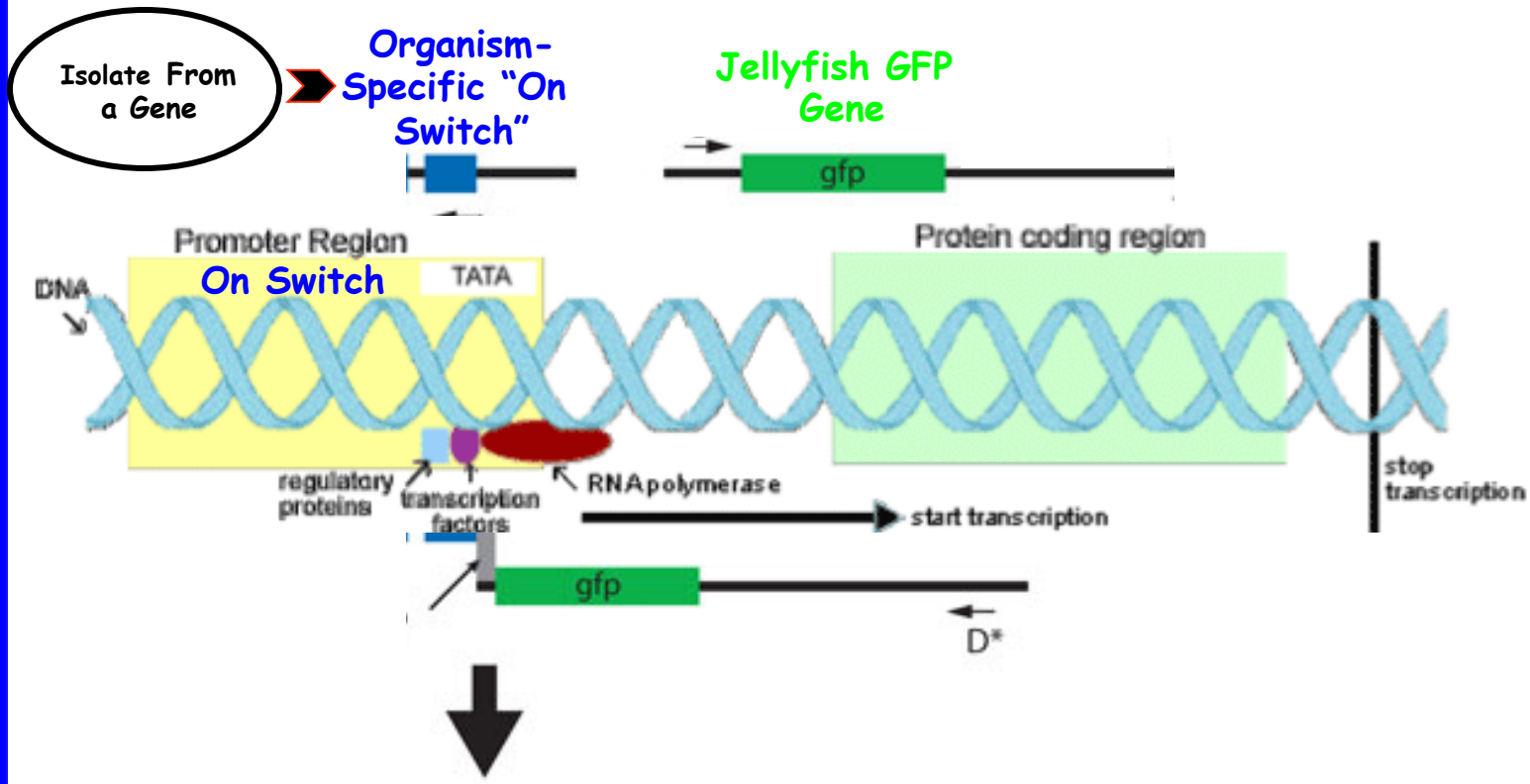


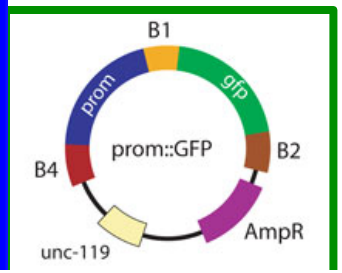
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

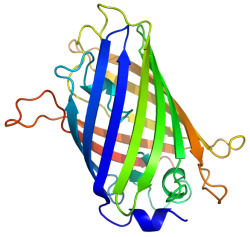
Plants of Tomorrow

Engineering the Jellyfish GFP Gene to Be Active in Different Organisms

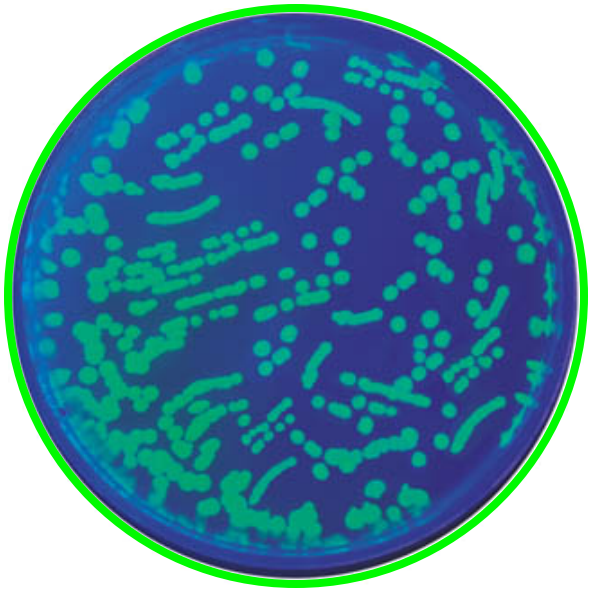


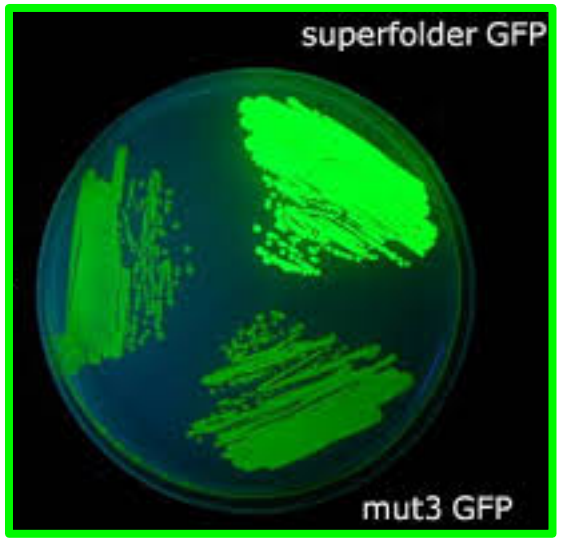


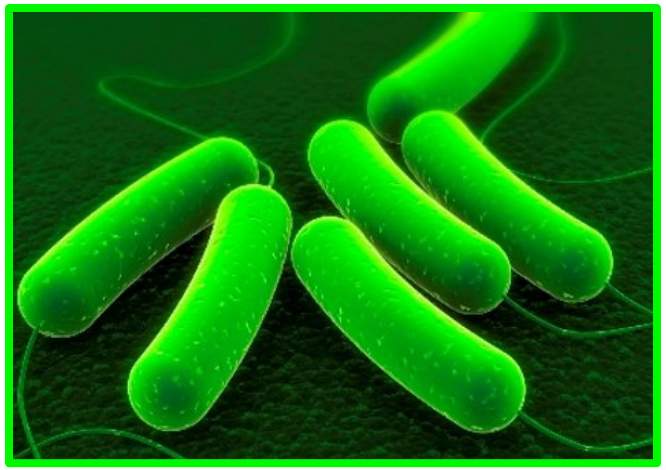
Engineered Chimeric GFP Gene



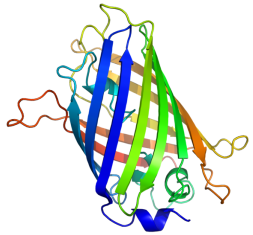
GloColi – *E. coli* Engineered With the Jellyfish GFP Gene!

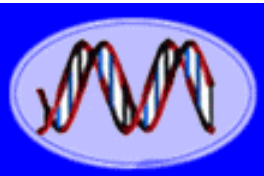






E. Coli Synthesizes GFP Protein!





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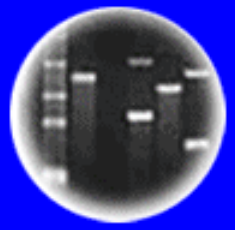


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

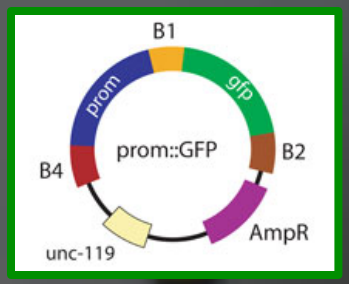
Question One

Engineering *E. coli* to Express a Jellyfish *GFP Gene* Implies That Genetic Processes in Bacteria and Jellyfish Are Similar Even Though They Are Separated By One Billion Years of Evolution!

a. Yes
b. No

Engineering a "GloFish"……

Zebrafish - Danio rerio



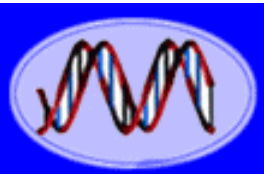
…….Using Genetic Engineering To Insert A Jellyfish Gene into a Zebrafish Egg!

A "GloFish" Embryo!!

Zebrafish *- Danio rerio*

Genetically Engineered "GloFish!!"

Note Different Fluorescing Colors – Due to Different Jellyfish Genes



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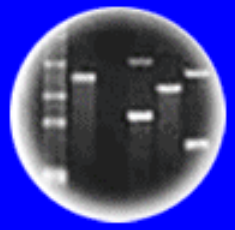

DNA Fingerprinting

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Plants of Tomorrow

GloFish Are Not Sold In California

(& Canada, or Europe)

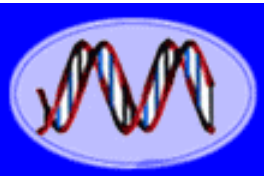
- **Cal. Fish and Game Code § 15007 (2007)**

<u>Regulation</u> Makes it illegal to spawn, cultivate, or incubate any transgenic fish in the state controlled waters of the Pacific Ocean.

- **Title 14, Section 671.1 CA Code of Regulations (2003)**

<u>Regulation</u>. Movement of live transgenic aquatic animals from facilities is prohibited unless specifically permitted by the Department. Release of transgenic aquatic animals or their progeny into waters of the state is prohibited.

Genetic Engineering & the Law!!



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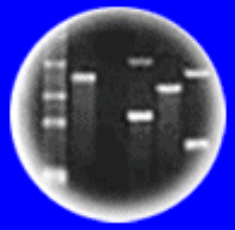

DNA Fingerprinting

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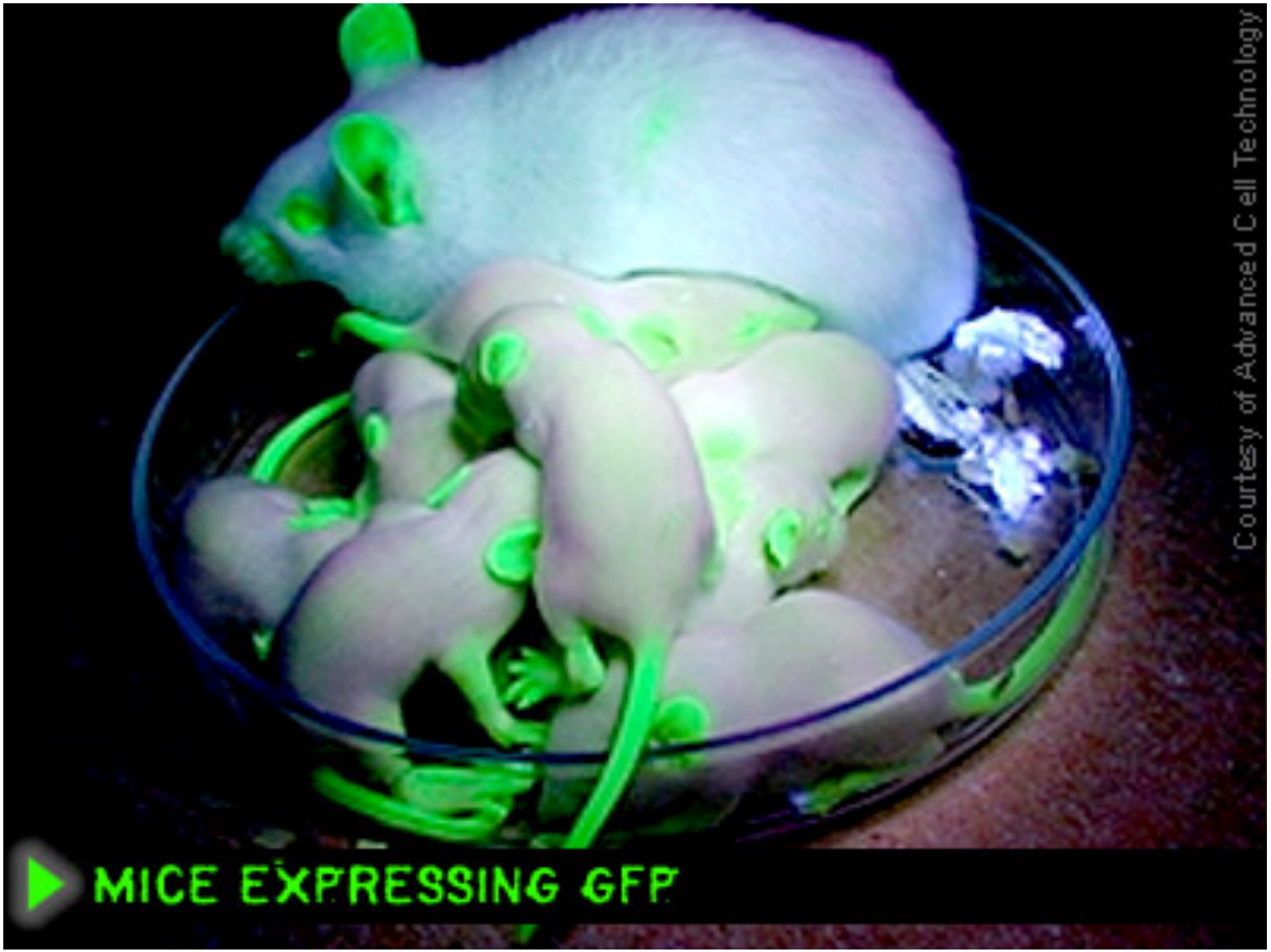
Question Two

Should GloFish Be Sold in California?

a. Yes
b. No

How About a GloFly!

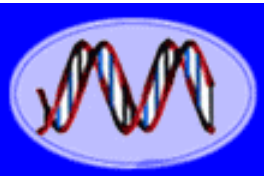
What About "GloMice!!!"



And Glo Monkeys, Cats and Pigs as Well!!

Engineering a GloPlant With the Same Jellyfish Gene!!!

What are the Philosophical and Biological Implications of These Experiments?



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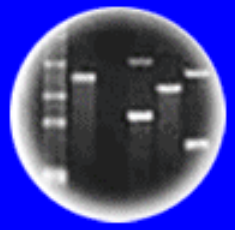

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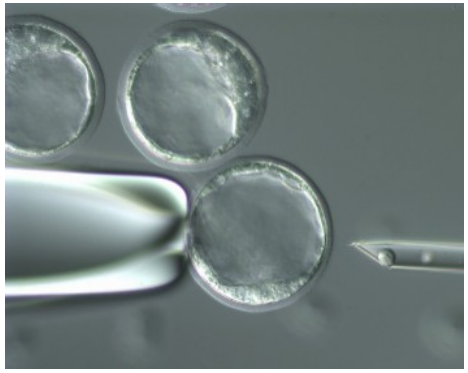
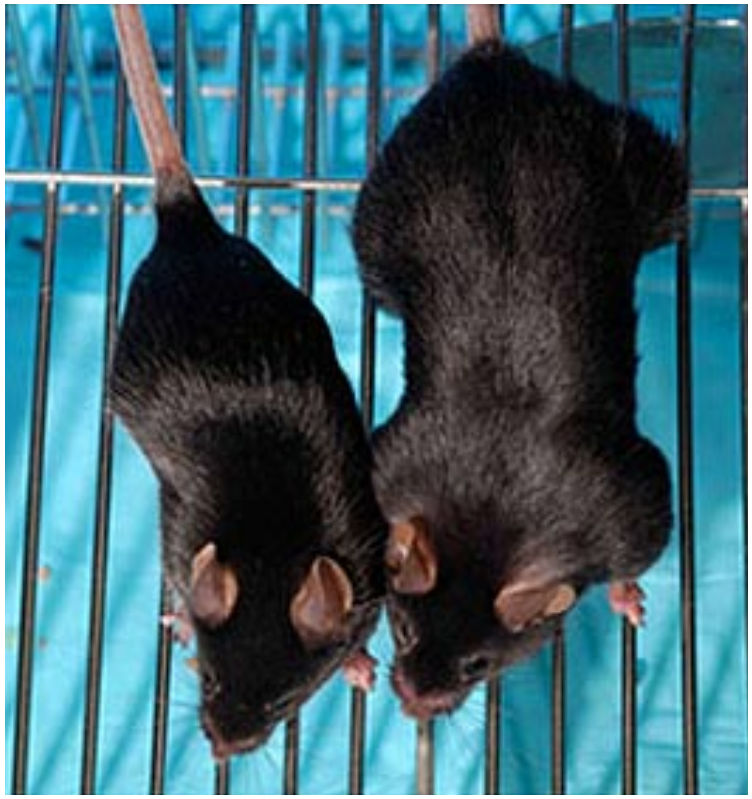
Question Three

Does Engineering Different Organisms With a Chimeric Glofish Gene Indicate that Genes Work Independently of Other Genes?

a. Yes
b. No

Engineering "Mighty Mouse" With a Rat Growth Hormone Gene





How About a Salmon That Grows Faster Using a Fish Growth Hormone Gene?

GENETIC ENGINEERING

Genetically-modified salmon are closer than ever to a dinner plate near you

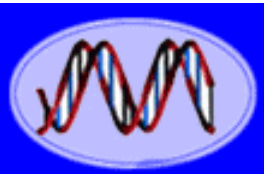
The super salmon are (almost) here. The Food and Drug Administration has reportedly finished its evaluation of the environmental impacts of the first fish genetically engineered (GE) for human consumption.

FDA expected to approve Genetically Modified Salmon

AQUAADVANTAGE SALMON | JANUARY 3, 2013 | BY: MARK WACHTLER | + *Subscribe*

FDA faces opposition over genetically engineered salmon

A group of eight senators is asking the FDA to cease consideration of the fish as food, and is threatening to pull funding for the study if the agency does not comply.



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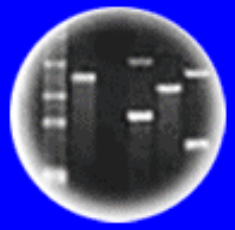

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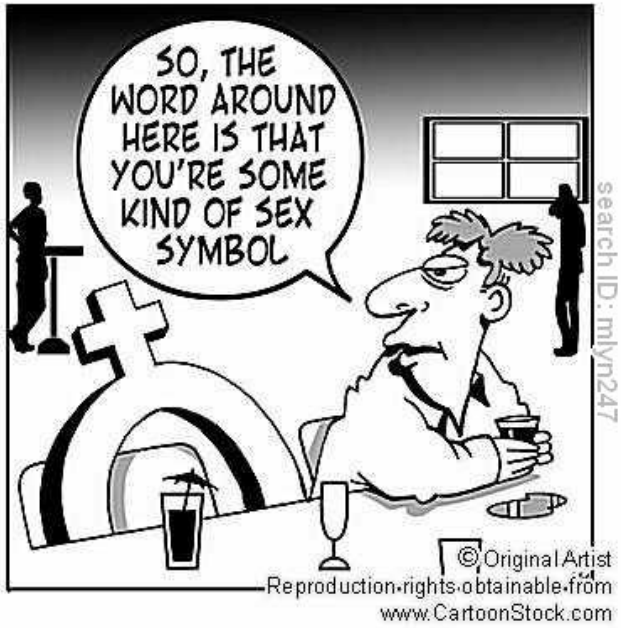
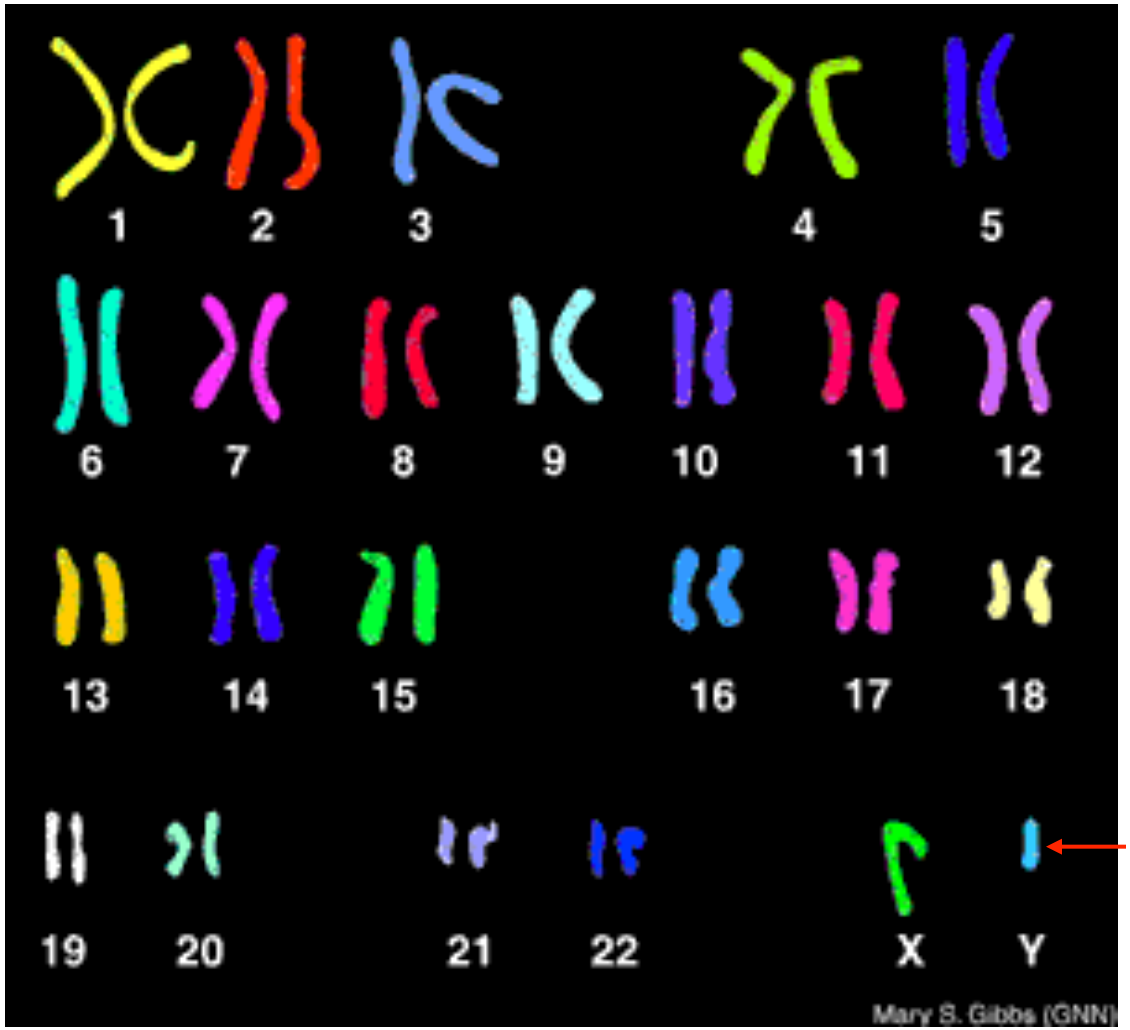
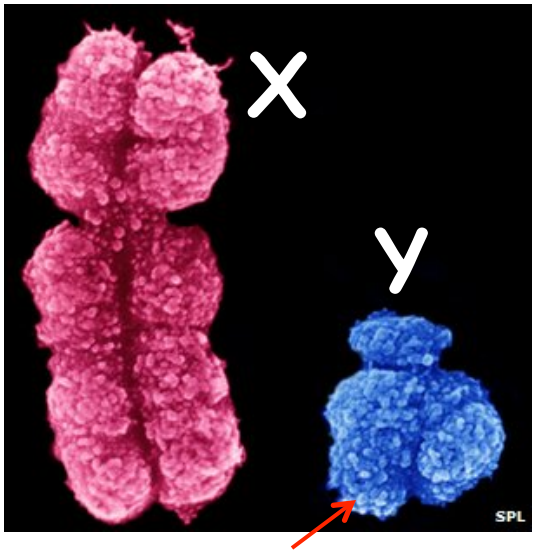
How About Changing The Sex Of An Organism?



Males and Females Differ By the Presence or Absence Of the Y Chromosome (simplistically!!)

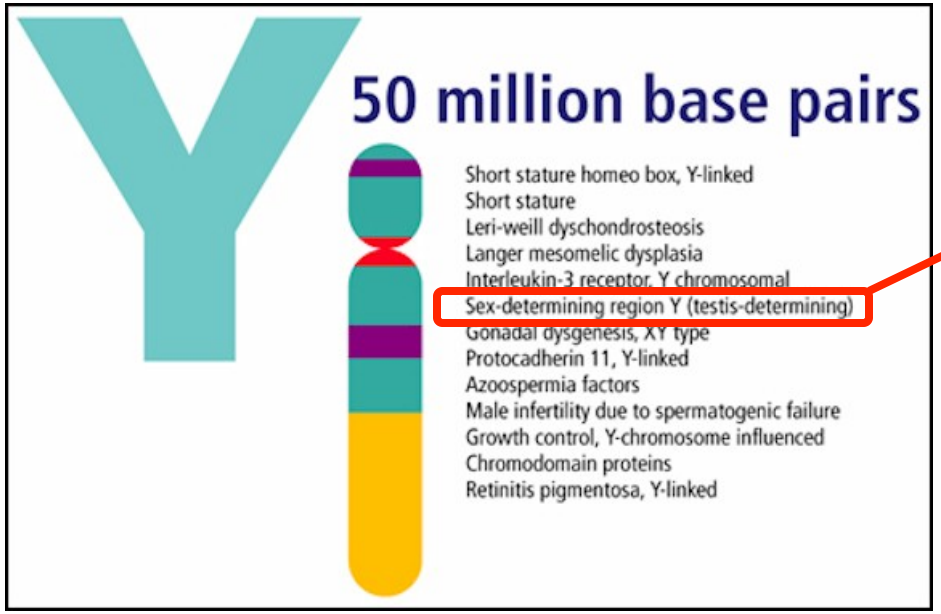


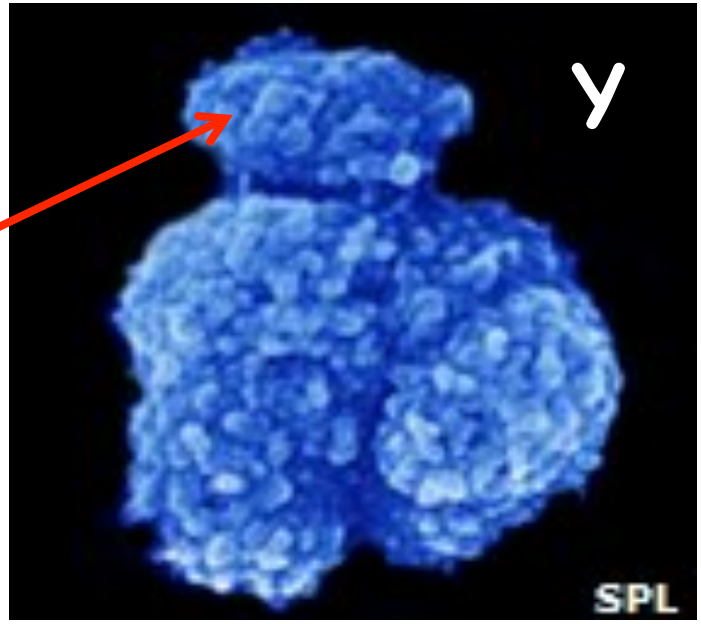


Male SRY Gene
(Sex Determining Region Y)

The Human SRY Gene For Maleness Controls Gender

Y chromosome: Why men contribute so little



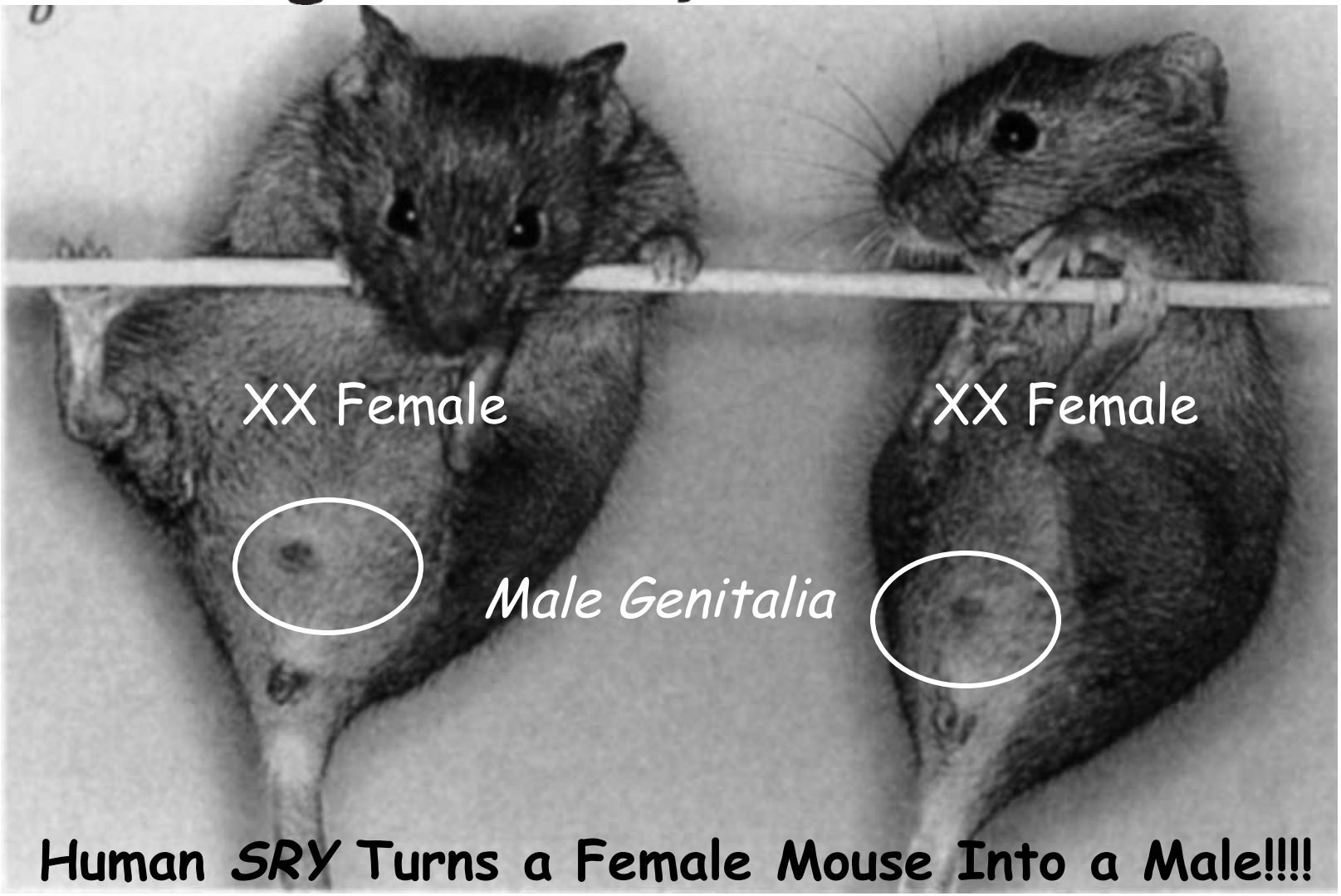


Two Y Genes Can Replace the Entire Y Chromosome for Assisted Reproduction in the Mouse

Science. November 25, 2013

Male development of chromosomally female mice transgenic for *Sry*

Nature, May 9, 1991



Human *SRY* Turns a Female Mouse Into a Male!!!!

Functional Proof That SRY Controls Male Development

What Does This Experiment "Say" About Human & Mice Genes?

What Are the Conclusions of This Experiment?

- *Ground State of Mammalian Development is FEMALE!*

- *ONE Gene Switches Development From Male to Female!*

- *Eve Had a Y Chromosome and LOST the SRY Gene!!*

"So the LORD God caused a deep sleep to fall upon the man, and while he slept took one of his ribs and closed up its place with flesh; and the rib which the LORD God had taken from the man he made into a woman and brought her to the man. Then the man said, "This at last is bone of my bones and flesh of my flesh; she shall be called Woman, because she was taken out of Man." Genesis, Chapter 2

Using Genetic Engineering to Change Body Architecture-Engineering Eyes on a Fly's Leg With a Single Gene

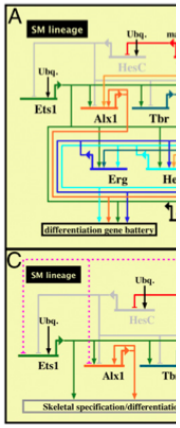
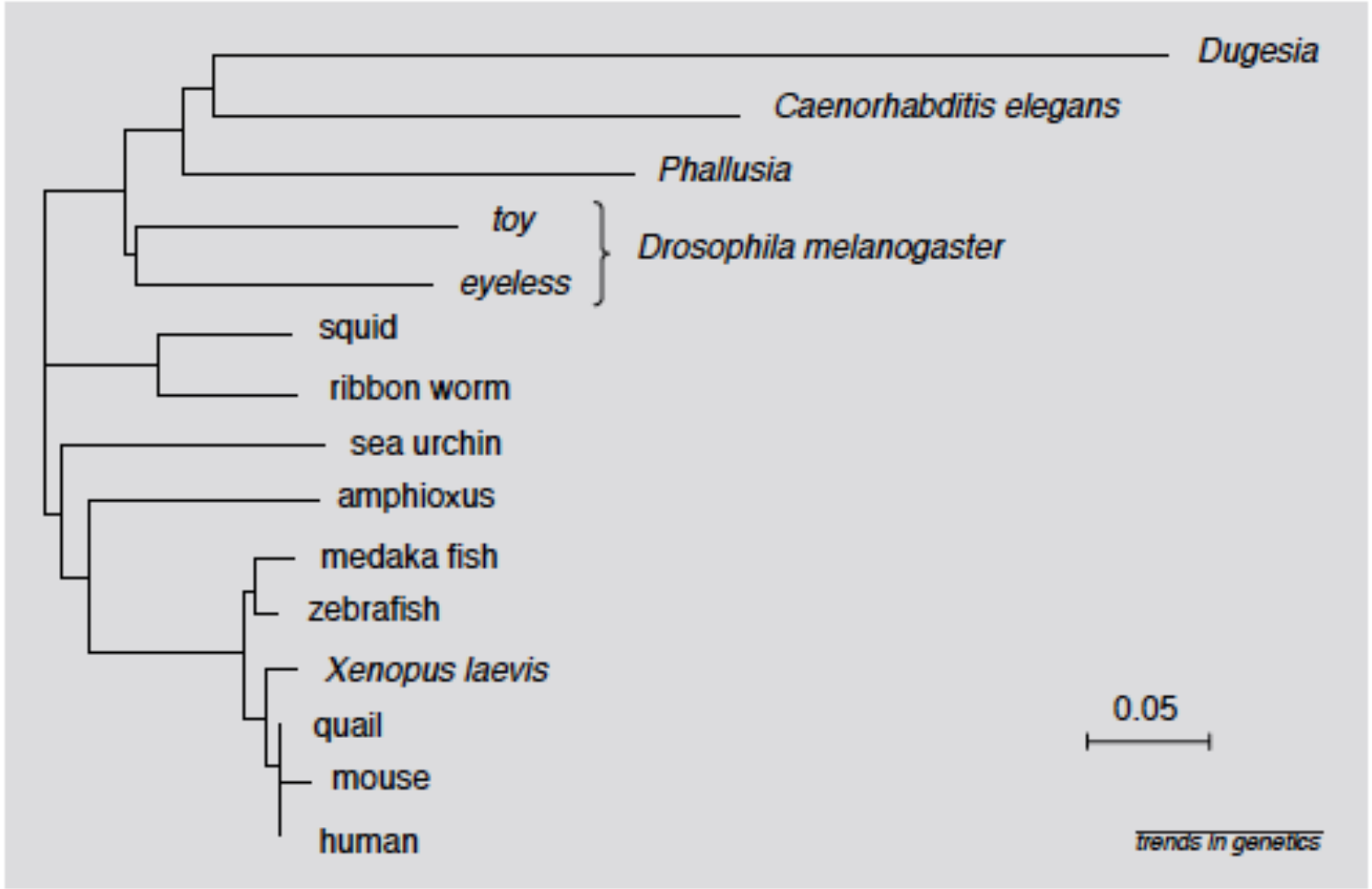


FIGURE 3. Phylogenetic tree of the *Pax 6* genes



Abnormal activity of the eyeless gene has generated an eye on the leg of a fly.

How About Genetically Engineered Humans?

THE CURE

Gene therapy has emerged from exile with breakthrough treatments for blindness, cancer, and the deadly bubble boy disease.
By Jill Neimark

Treatment for Blood Disease Is Gene Therapy Landmark

By NICHOLAS WADE
Published: December 10, 2011

December 9, 2012

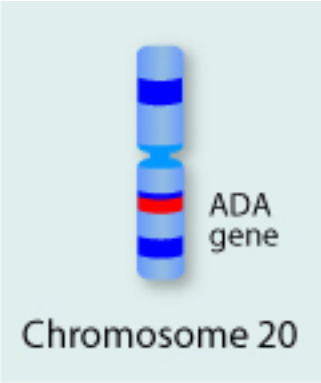
In Girl's Last Hope, Altered Immune Cells Beat Leukemia

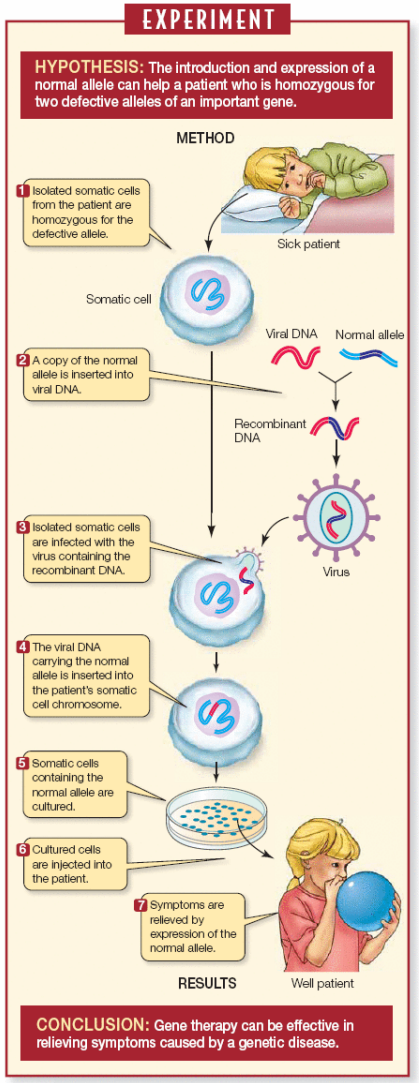
Gene Therapy Helps Blind Children See

By Jocelyn Kaiser
*Science*NOW Daily News
24 October 2009

Humans Have Been Genetically Engineered To Cure a Lethal Genetic Disease (SCID)

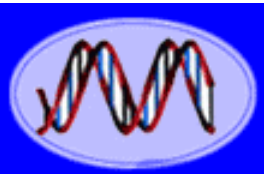
The Age of Human Genetic Engineering Began Almost Twenty Years Ago Treating Severe Combined Immunodeficiency Disease (SCID) With Normal ADA Genes!!!





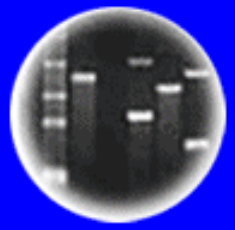
Several Teenagers Are Alive Because They Have Been Engineered With an ADA Gene That They Were Not Born With!!!

Adenosine Deaminase Gene (ADA)



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

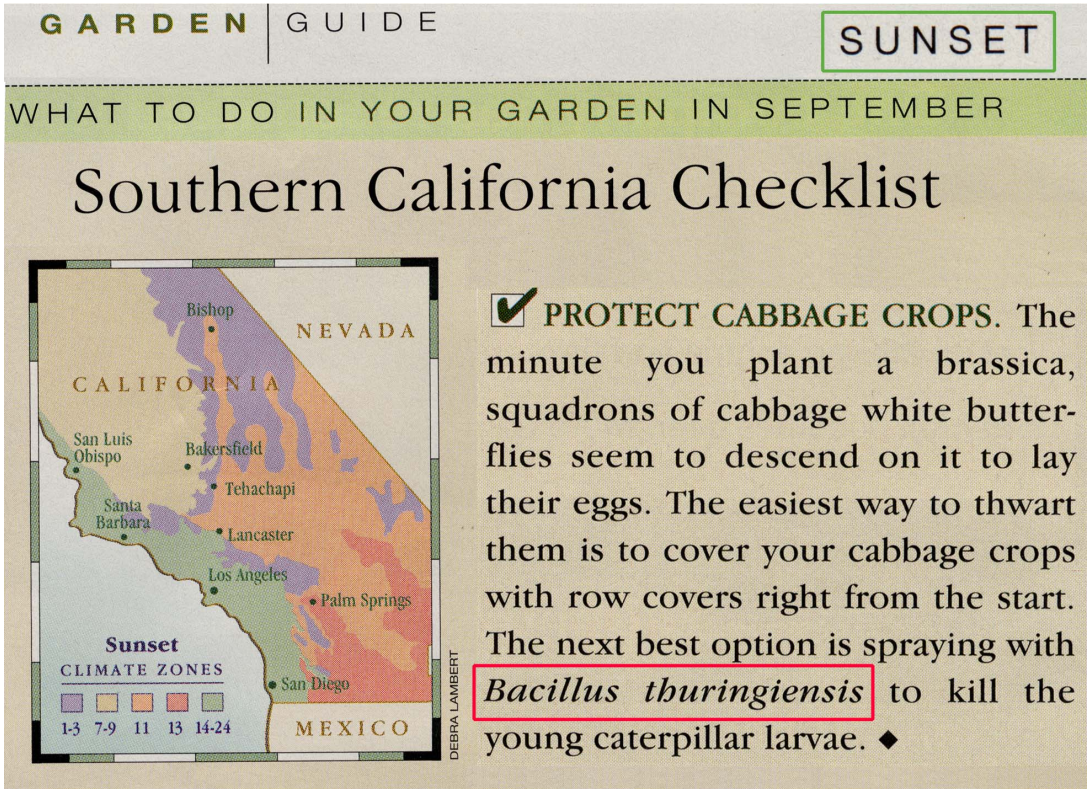


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

What About Inserting Bacterial Genes Into Plants To Produce a Result With Significant Applications??



GARDEN | GUIDE

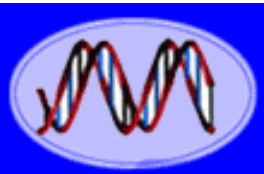
SUNSET

WHAT TO DO IN YOUR GARDEN IN SEPTEMBER

Southern California Checklist

DEBRA LAMBERT

✔ PROTECT CABBAGE CROPS. The minute you plant a brassica, squadrons of cabbage white butterflies seem to descend on it to lay their eggs. The easiest way to thwart them is to cover your cabbage crops with row covers right from the start. The next best option is spraying with *Bacillus thuringiensis* to kill the young caterpillar larvae. ◆



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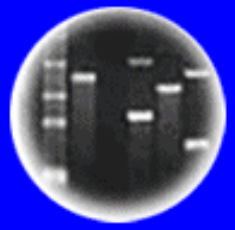

DNA Fingerprinting

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Plants of Tomorrow

How to Use Bt Pesticide as an Organic Pest Control

Learn how to use Bt pesticide to kill cabbage worms, tomato hornworms and other pests in your organic vegetable garden.

By Barbara Pleasant
April 24, 2013

Bt is one of the safest natural pesticides you can use to control caterpillar pests without harming beneficial insects.

Photo Courtesy Safe Brand

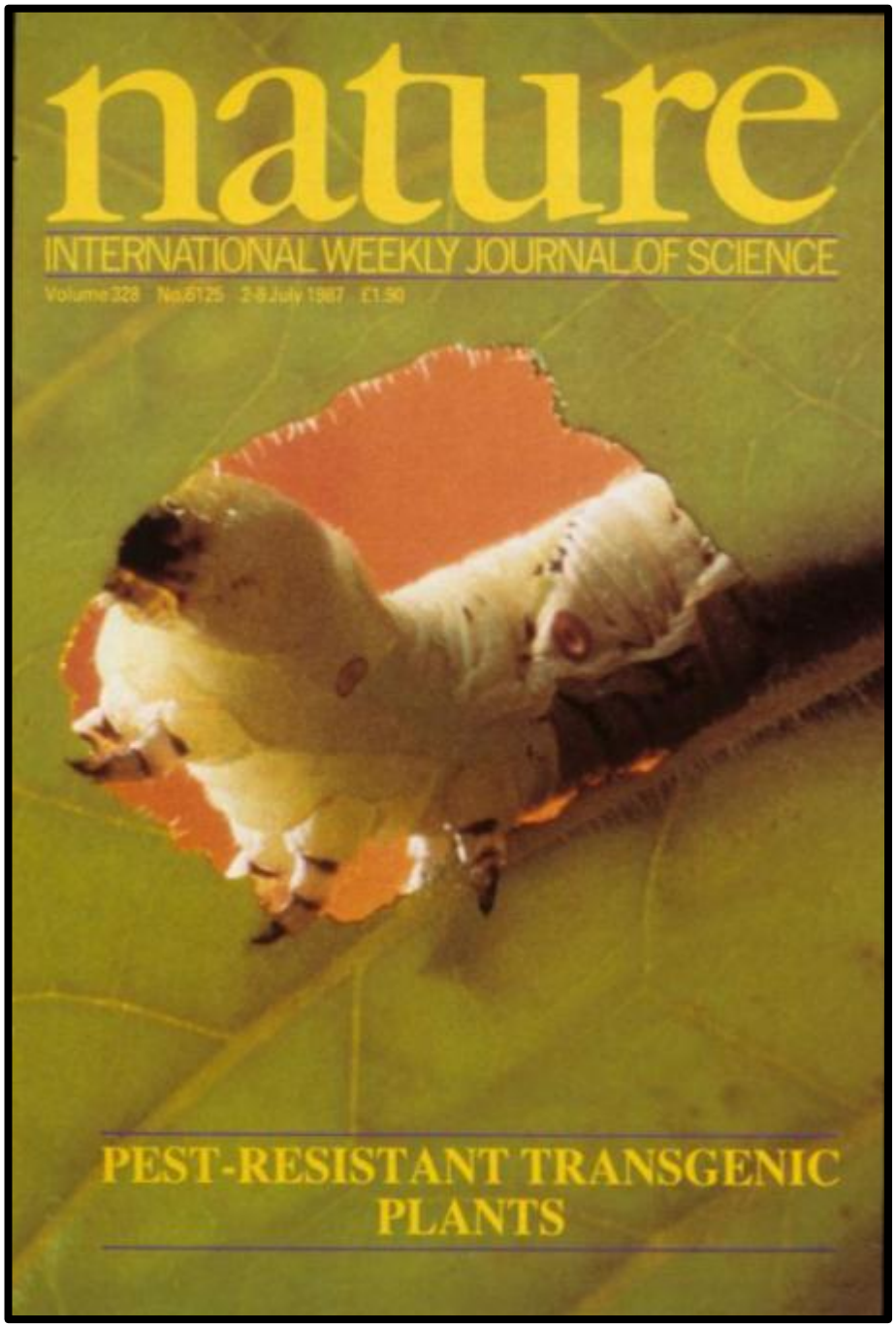
FOR ORGANIC GARDENING

OMRI Listed

Monterey

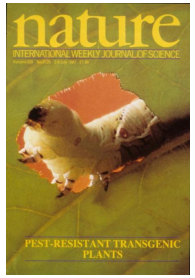
Active Ingredient:
Bacillus thuringiensis subspecies *kurstaki* strain SA-12 solids, spores and Lepidopteran active toxins (At least 6 million viable spores per mg)* 98.35%
Other Ingredients: 1.65%
Total: 100.00%

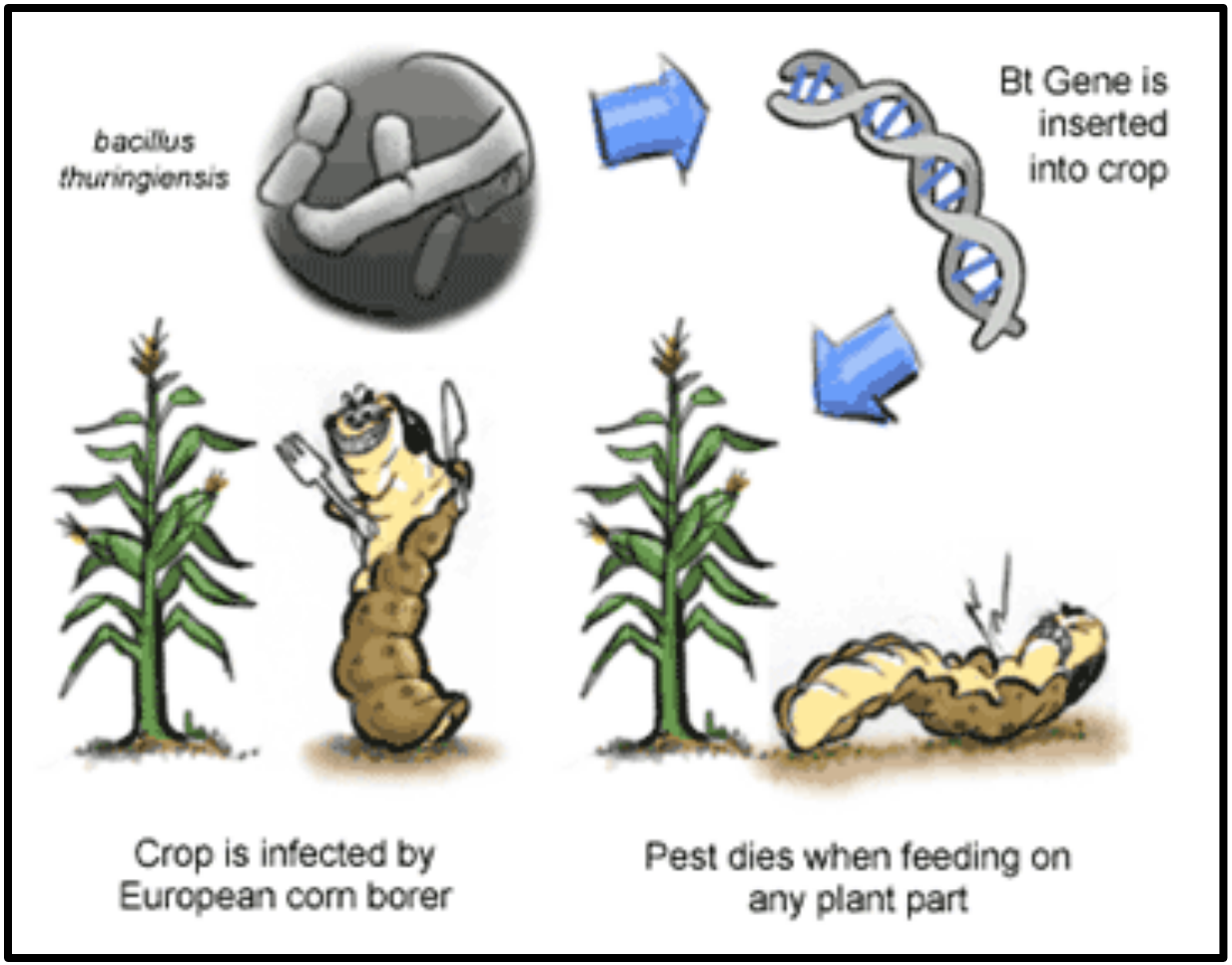
*The percent active ingredient does not indicate product performance and potency measurements are not federally standardized.



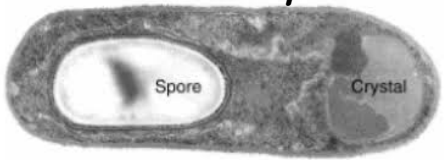
July, 1987
Old Technology!

Crops Can Be Engineered With Bt For Insect Resistance

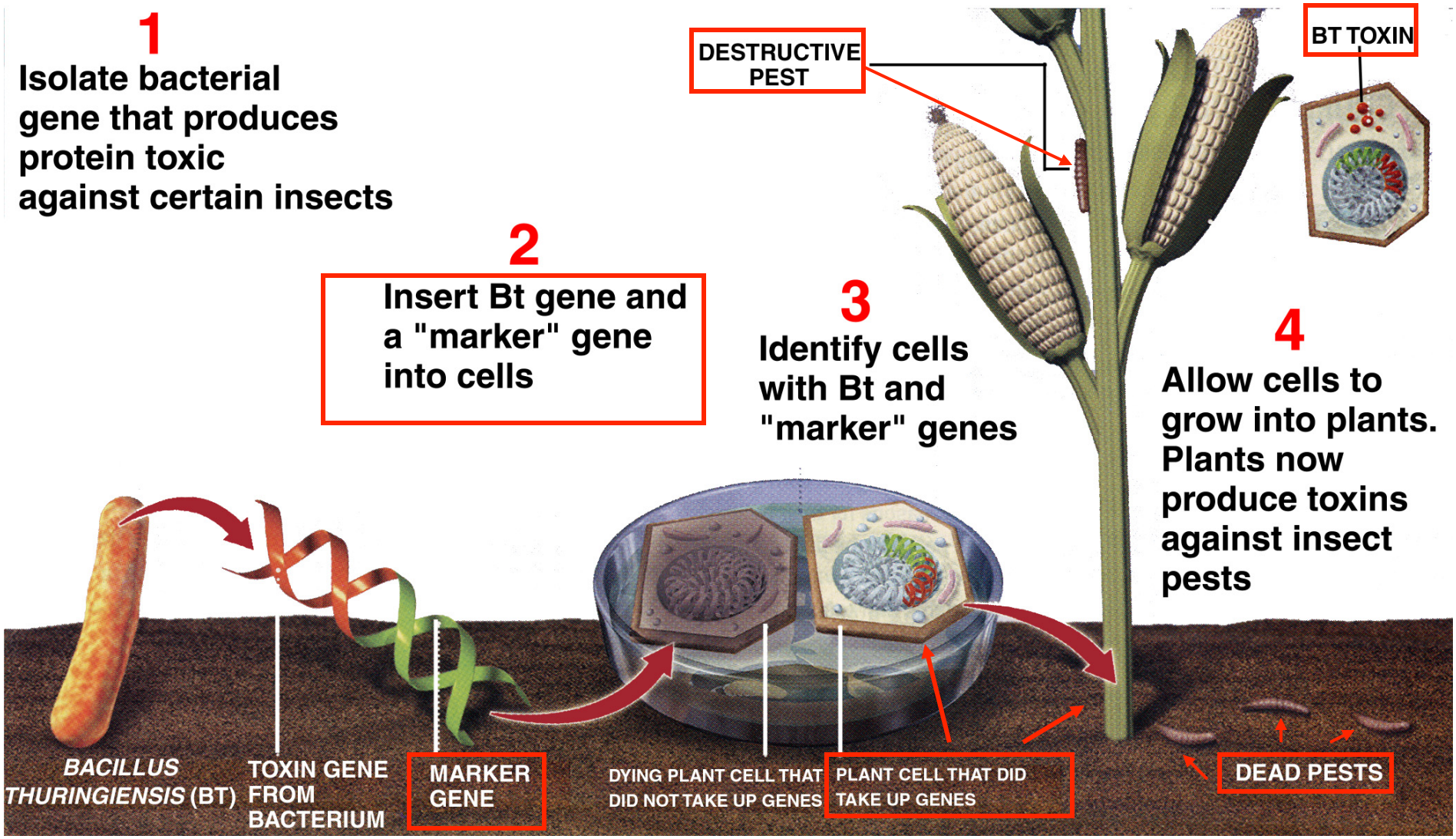


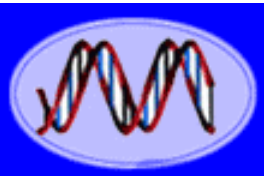


Bt Toxin in Spores



How to Make an Insect-Resistant Plant





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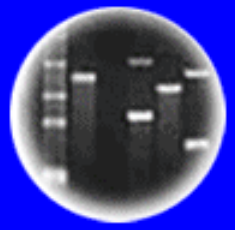
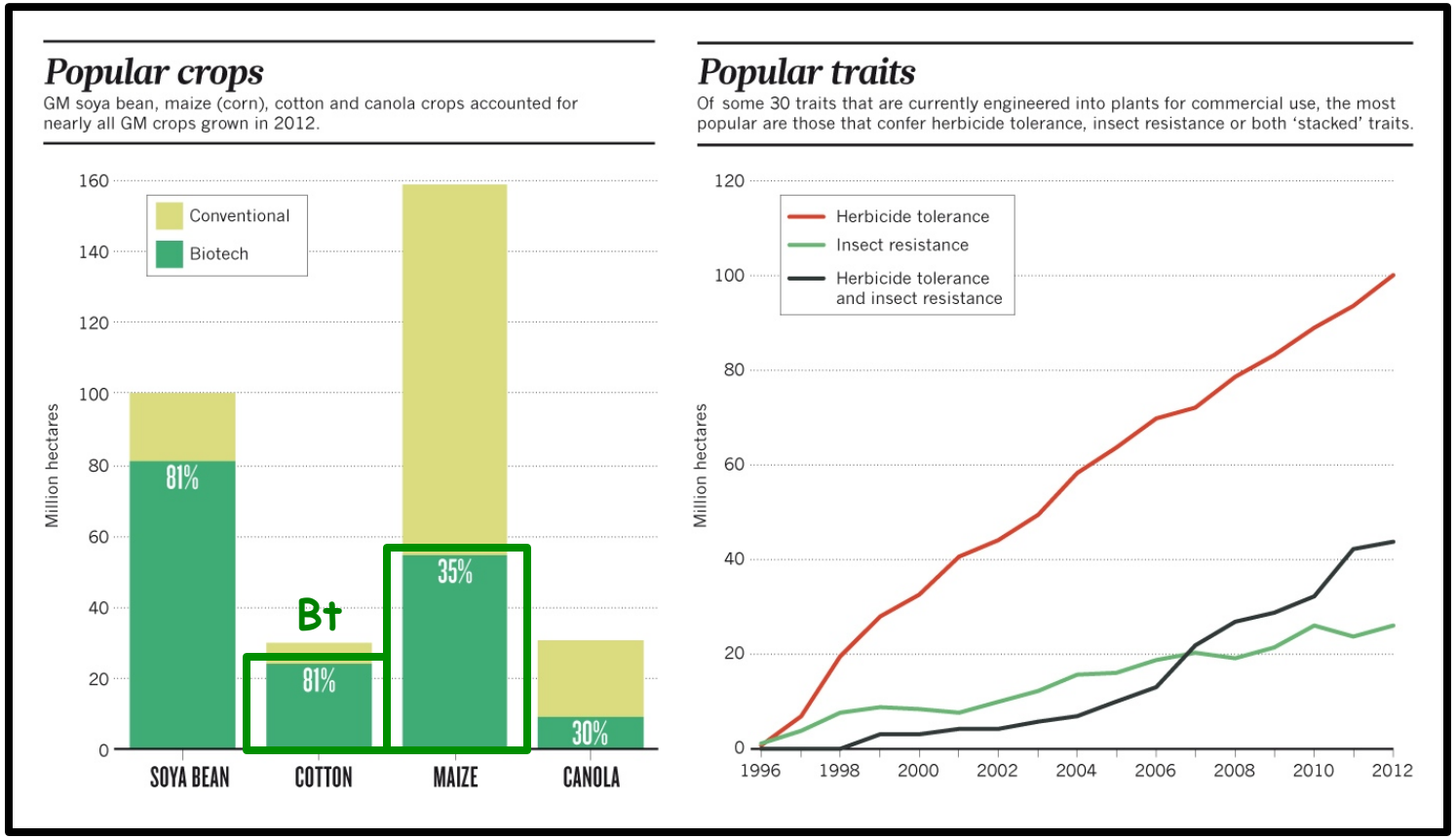


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

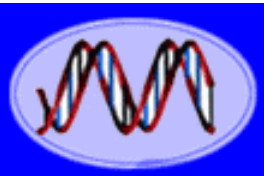
Plants of Tomorrow

The 2013 GMO Landscape



Genetic Engineering a Plant to Resist Worms!

Implications For Agriculture?



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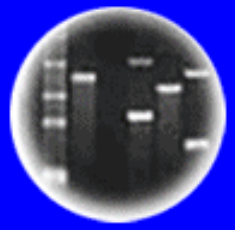


DNA Fingerprinting

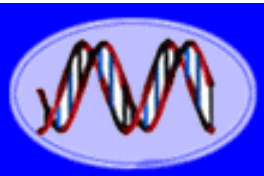
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Question Four

Does the Same Bt Protein in Engineered Crops & Organic Sprays Protect Plants From Insect Damage?

a. Yes
b. No



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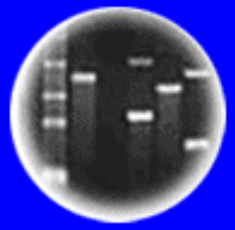


DNA Fingerprinting

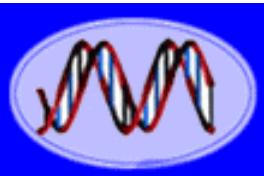
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Question Five

Would you eat food obtained from genetically modified plants and animals?

a. yes
b. no



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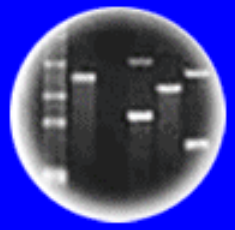


DNA Fingerprinting

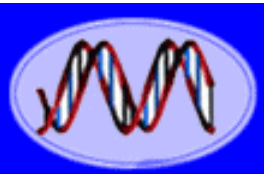
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Question Six

Would You Use a Genetically Engineered Drug?

a. yes
b. no



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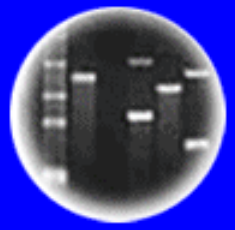
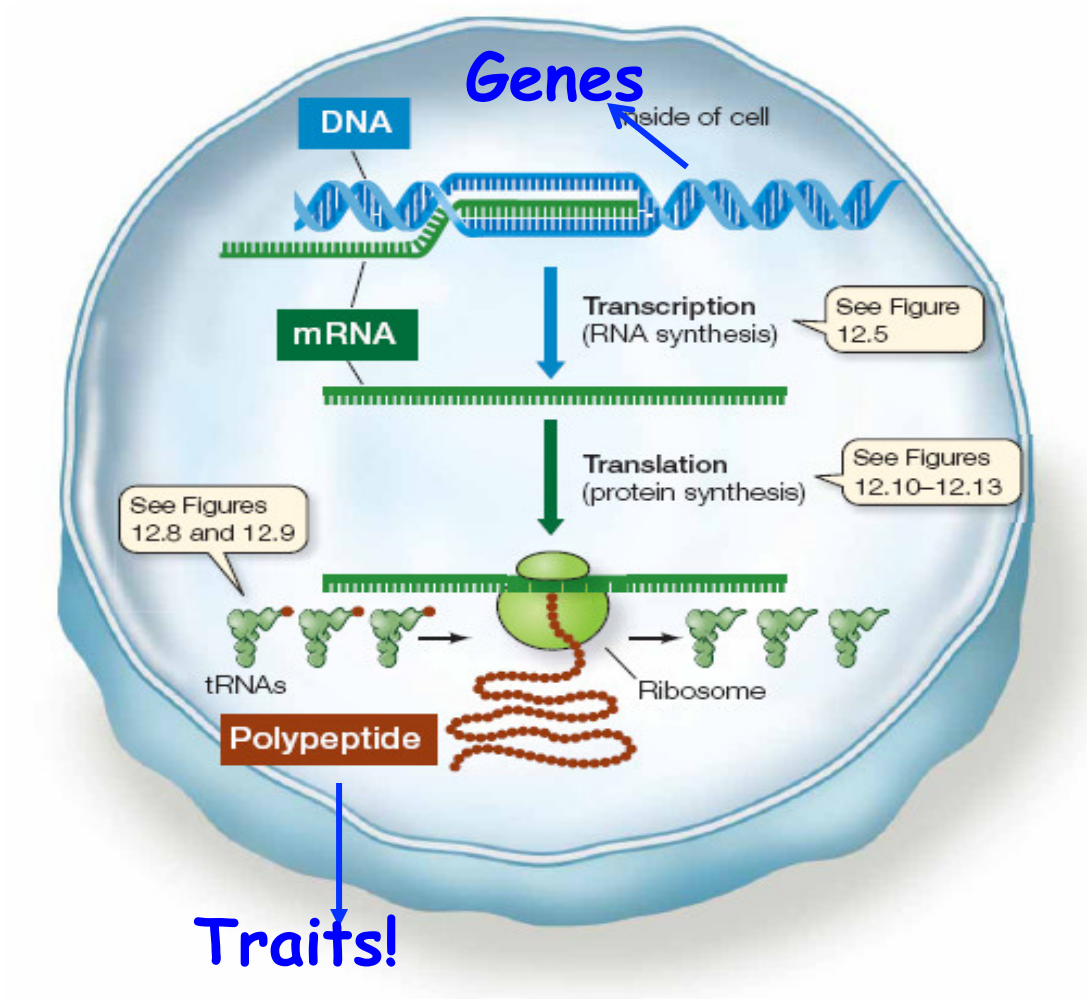


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

What Can We Infer FROM These Genetic Engineering Experiments About How Genes "Work" and Genetic Processes in All Living Organisms?



Observations and Inferences From Genetic Engineering Experiments

1. *Genes Can Work Independently of Each Other -*
   E.G. - The Jellyfish Fluorescence Gene Works Perfectly in a Variety of Organisms

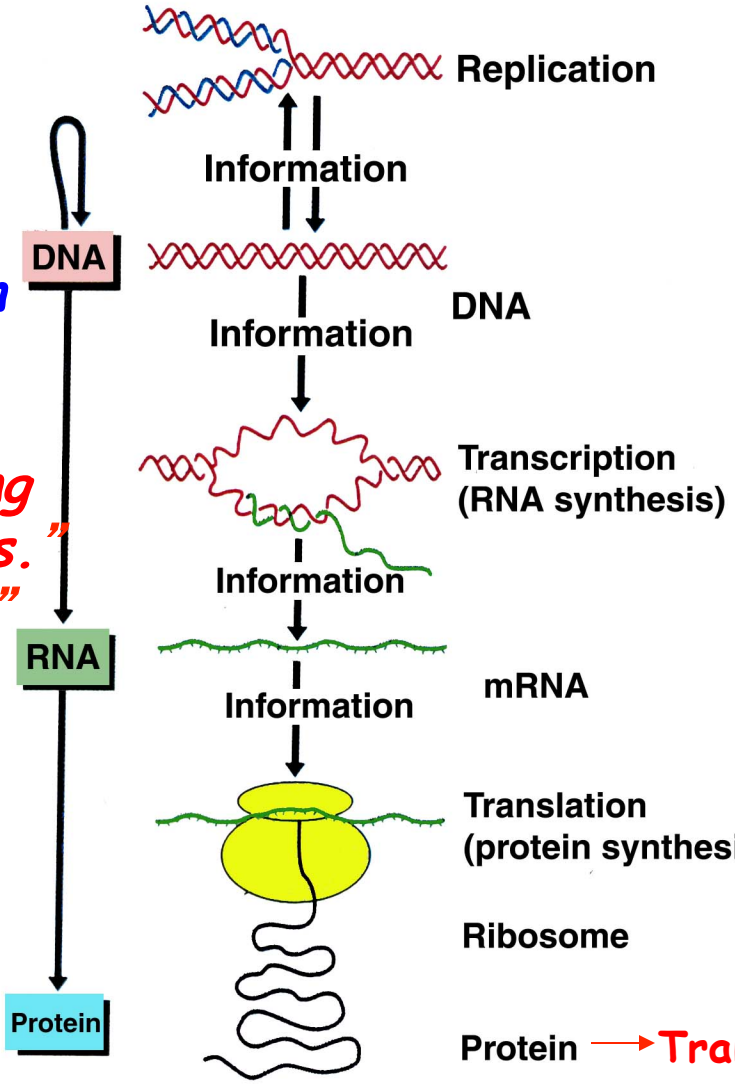
2. *Basic Genetic Processes Are Universal (Replication & DNA to RNA to Protein) –*
   E. G. - The Bt Gene Directs the Production of BT Protein in Crops.

3. *Basic Genetic Processes Can Be Used to Engineer or Transfer Genes From One Organism to Another and Transfer Them Stably Generation After Generation –*
   E.G. - The Chimeric Glofish & Bt Genes Are Inherited Generation After Generation.

Translating The Genetic Code Into Proteins is a Conserved Process

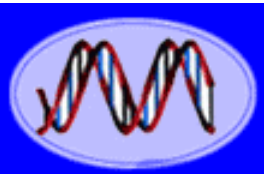
Can Intervene in This Process in Cells

Genetic Engineering Is not "Hocus Pocus." It Uses "Natural" Cell Processes!!!!



Trait (e.g., eye color)

All Organisms Use The SAME Processes And "RULES" to Generate Traits!! And The SAME Molecules & Chemistry!!



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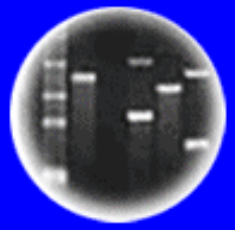


DNA Fingerprinting

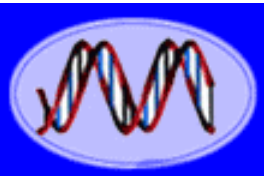
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Summary - Age of DNA - There Are NO Genetic Limitations to What Can Be Done Using Genetic Engineering

- Synthetic Chromosomes & Microbes (GE 2.0)
- Recombinant Plasmids & Bacteria
- GlowFish, GloMice, GloMonkey, GloPlant
- Mighty Mice and Giant Fish
- Insect Resistant Crops
- Novel Fly Body Plans (e.g., eye on leg)
- Engineered Humans

GE 1.0



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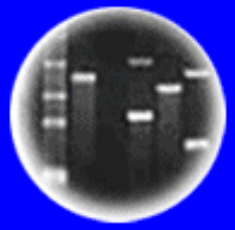

DNA Fingerprinting

Cloning: Ethical Issues
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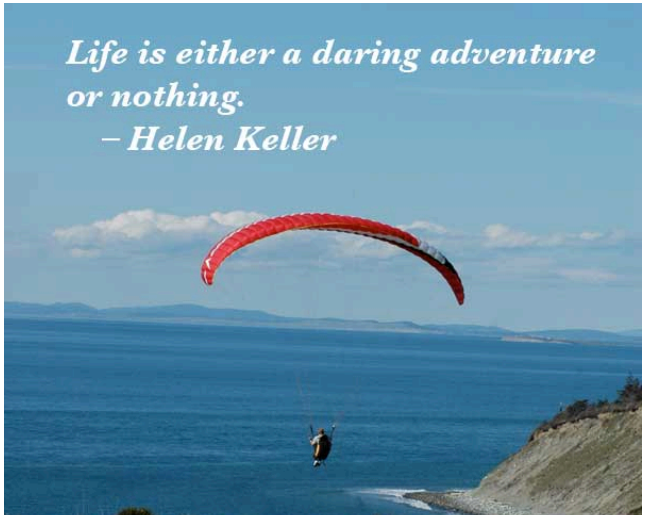
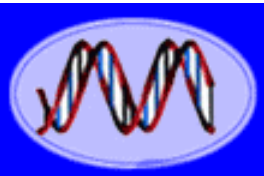
Plants of Tomorrow

We Are Only Limited By Our Ingenuity and Our "Fear" of the Unknown



Creating Life: Synthetic Microbes
J. Craig Venter
Genetic Engineering 2.0

60 Minutes-December 2010



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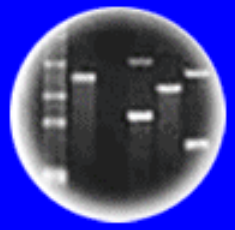

DNA Fingerprinting

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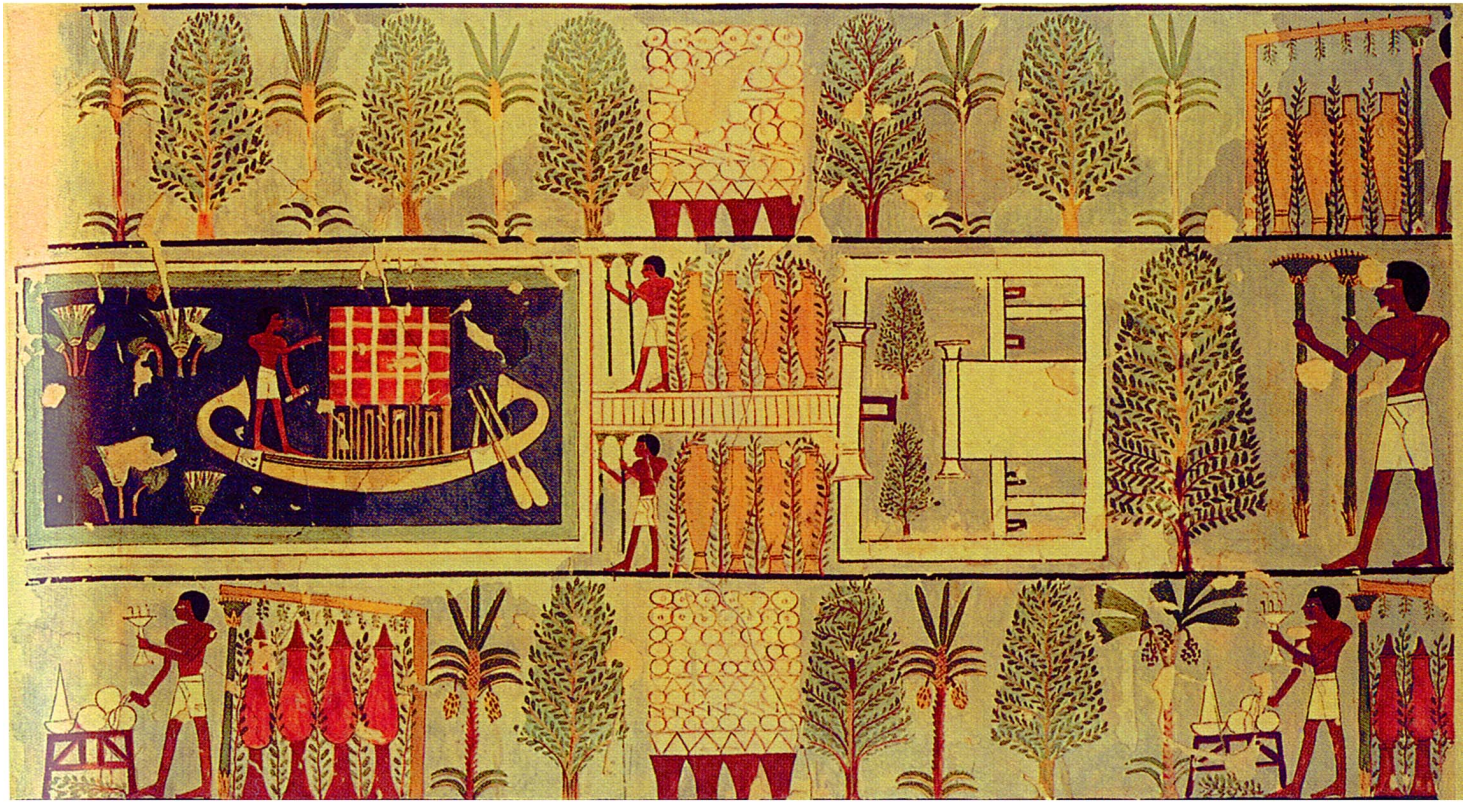
There is Nothing New About Genetic Engineering!

Manipulating Genes IS Manipulating Genes No Matter What <u>Technology or Processes</u> Are Used!!

This is Genetic Engineeering 0.0!!

Breeding And Cultivation Of Plants Have Taken Place Over Thousand Of Years

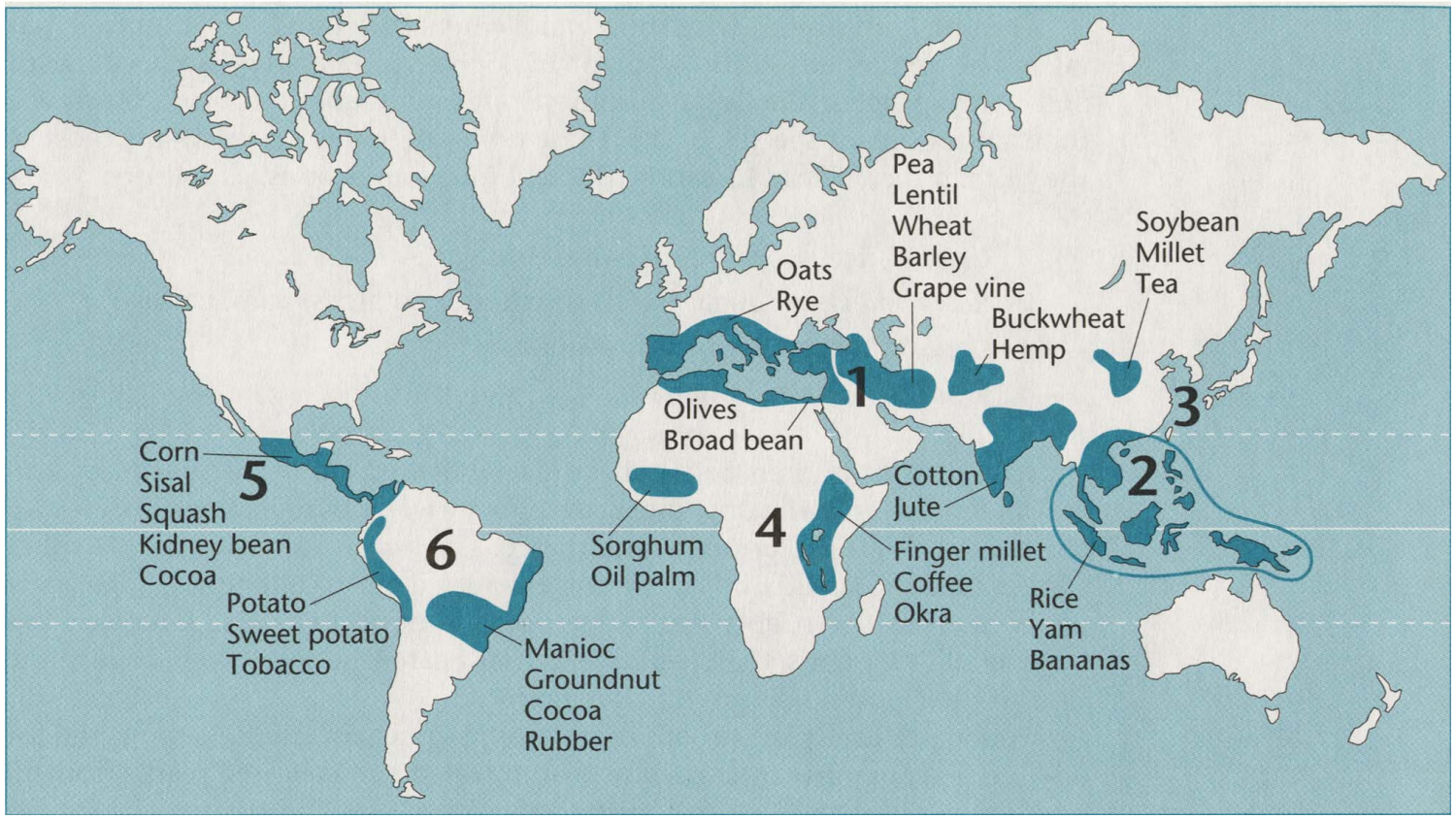


Genetic Engineering is Not New

Crops of Egypt 400 B.C.

Most Major Crops Were Engineered From Wild Relatives by Early "Bioengineers" Over 10,000 Years Ago!!

Regions Where Major Crops Were Established



Breeding Involves Gene Manipulation Using EXISTING Genetic Variability!

Breeding Uses Natural Genetic Variability of Genes As Raw Material - Variability Generated by Mutations

Mutations in a Gene That Change Its Chemical Sequence & Slightly Alters Its Function (e.g., fruit size, color)

Tomatoes Were Engineered From Small Wild Relatives Because of Mutations in Fruit Size Genes!

The Early Tomato "Bioengineers" Selected For Large Fruit Size Because it Provided More Food!

What They Were Selecting Was a Different Form (Allele) of a Fruit Size Gene!

Engineering Teosinte Into Domesticated Corn

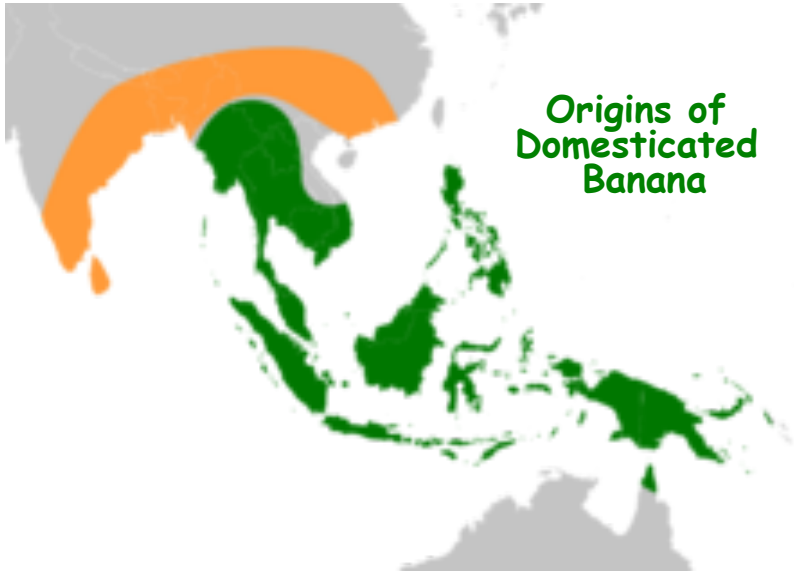
Teosinte | Domesticated corn | Teosinte | Early domesticated corn

Note: Architecture and Fruit (cob) Size

Only Five Genes Cause These Plants to Differ
& We Now Know What They Are

How Does This Differ From Putting an Eye on a Fly's Leg?

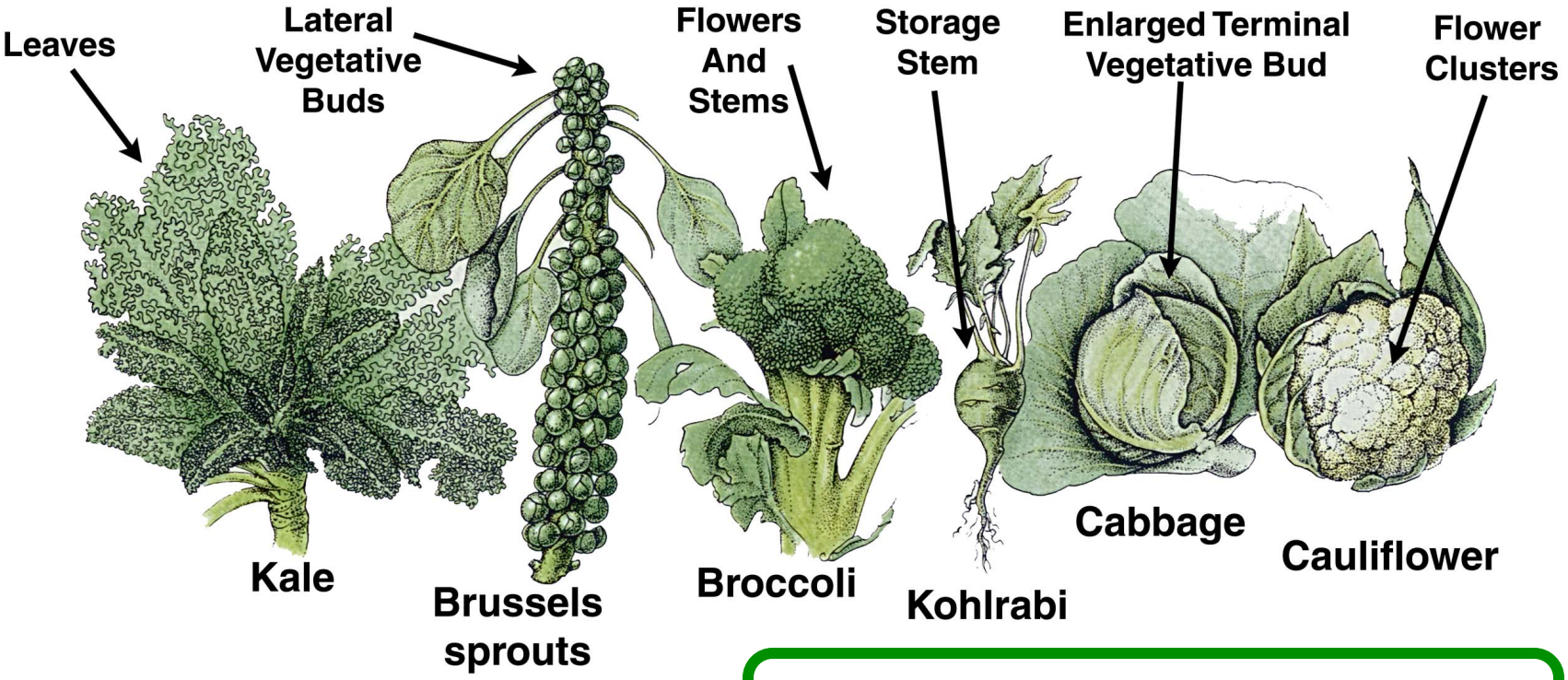
Engineering the Modern Banana



Modern Banana

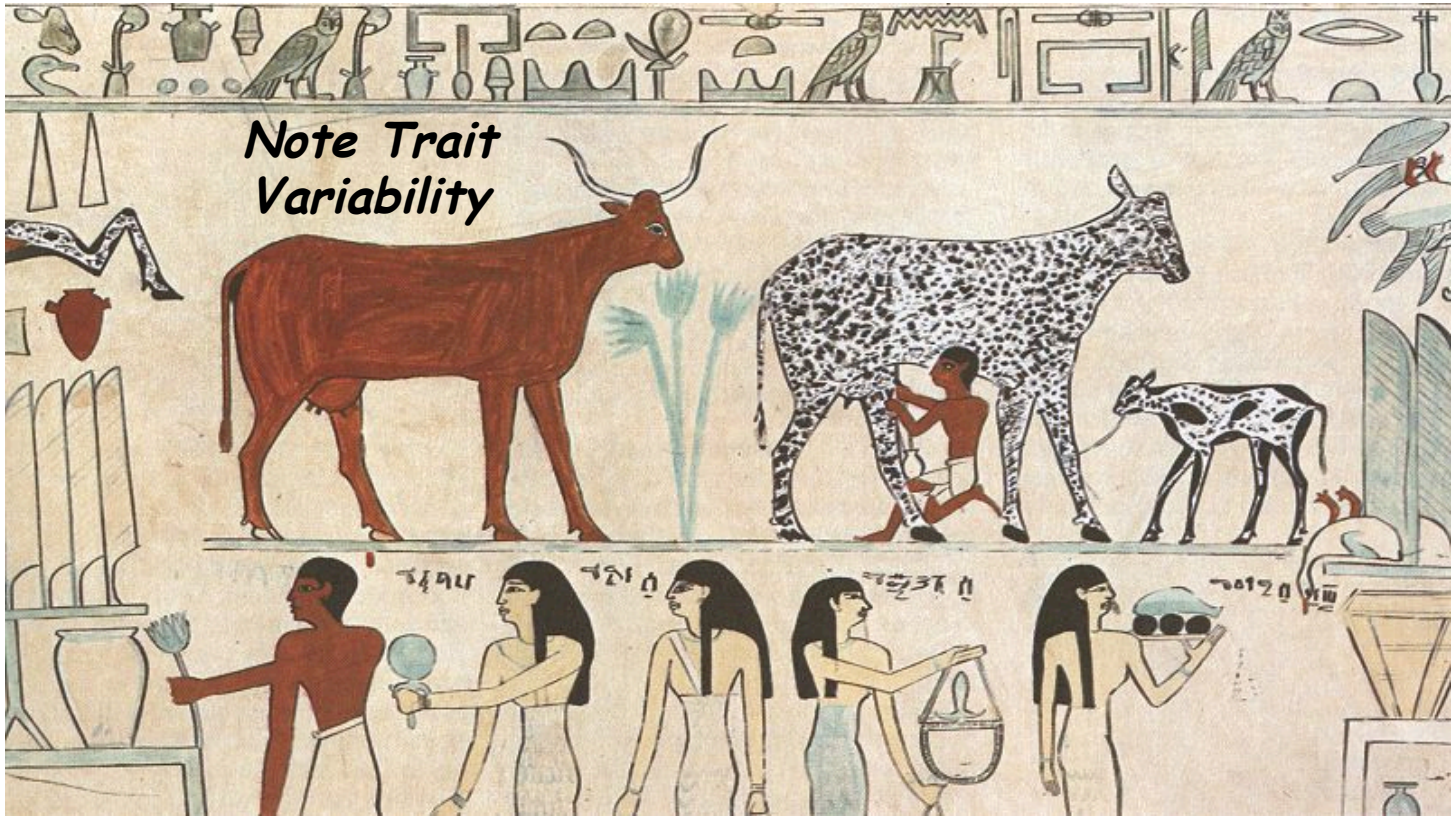
Note: Fruit Architecture and Presence of Seeds

Engineering Vegetables With Different Plant Architectures



How Are These Plants Related?

Farm Animals Were Also "Engineered" By Breeding Wild Relatives
Cattle Breeding in Egypt 4,000 Years Ago!



Manipulating Existing Genetic Variability Brought About By Chance Mutations!

Even Domesticated Pets Were "Engineered" By Breeding Wild Relatives

Vol 438|8 December 2005 nature

Nature, December 2005

NEWS & VIEWS

C. COLLINS/CORBIS

GENOMICS

The dog has its day

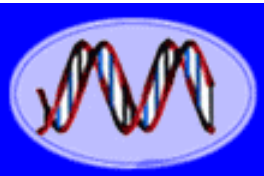
Hans Ellegren

Domestication and selective breeding have transformed wolves into the diversity of dogs we see today. The sequence of the genome of one breed adds to our understanding of mammalian biology and genome evolution.

The Dog Genome Has Been Sequenced!

Canine DNA Forensic Testing


DELL



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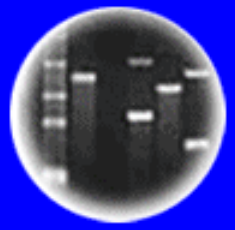

DNA Fingerprinting

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and Future Consequences

Plants of Tomorrow

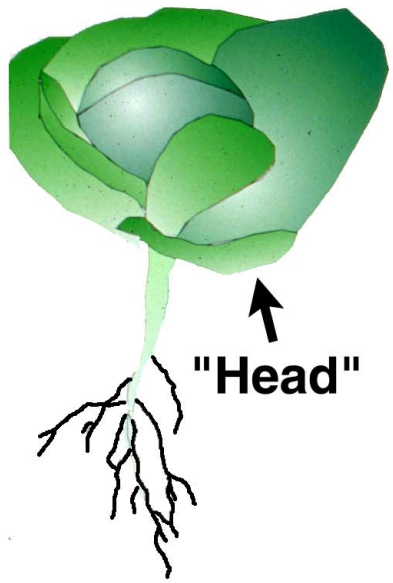
The Problem With Breeding the "Old Fashioned Way"

Cannot Predict Results!

The Problem With Breeding the "Old Fashioned Way"

Engineering A Novel Crop By "Wide" Breeding

Cabbage (*Brassica*) **Radish (*Raphanus*)**

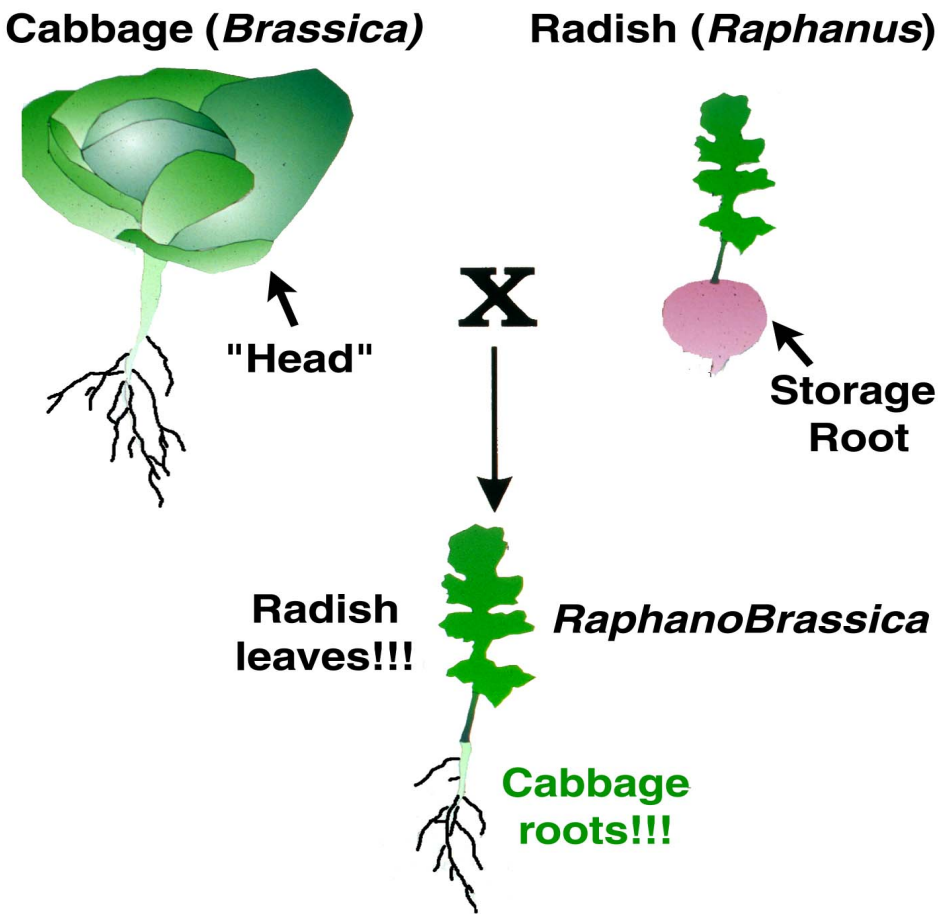


X

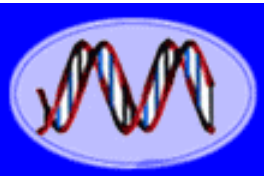
???

Karpechenko, G.D., 1928. *Polyploid hybrids of Raphanus sativus L. X Brassica oleracea L.* Zeitschrift für induktive Abstammungs- und Vererbungslehre 48, 1-85.

Engineering A Novel Crop By "Wide" Breeding

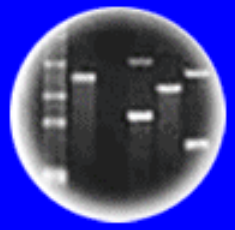


Results Show the Unpredictability of Classical Breeding Approaches!!
Compare With the Modern Genetic Engineering Examples Shown Previously



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

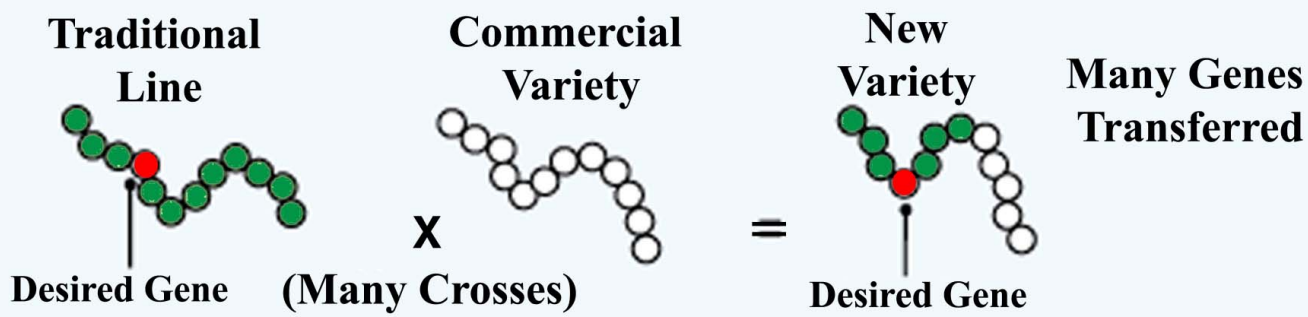
Genetic Engineering is a TECHNIQUE!

How Do Classical Genetic Engineering Methods Differ From Those Using DNA and 21st Century Technologies?

Classical vs. Molecular Genetic Engineering Techniques

TRADITIONAL PLANT BREEDING

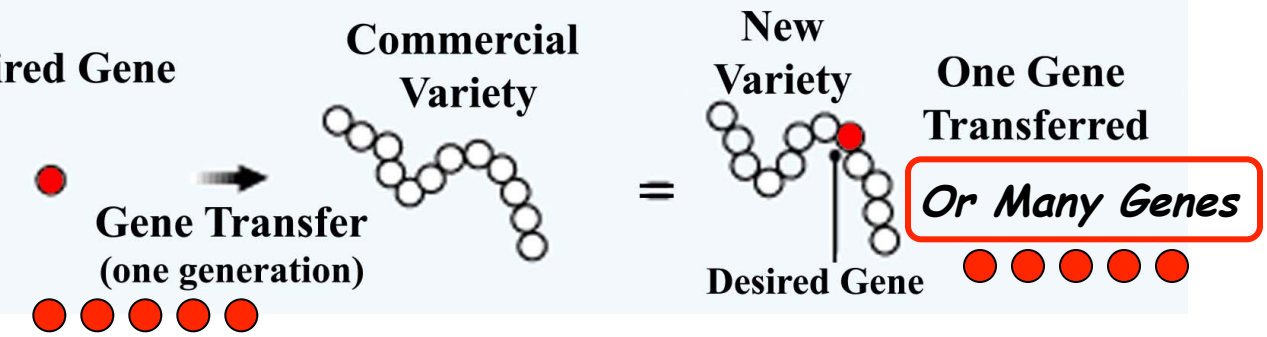
Plant Breeding Combines Many Genes At Once



PLANT BIOTECHNOLOGY

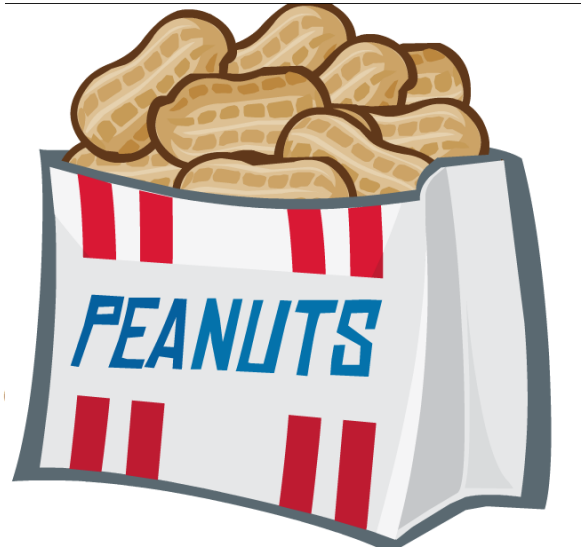
Biotechnology Adds A Single Gene

Or Many Cloned or Synthesized Genes



Both Manipulate Genes – But in Different Ways!!

Classical vs. Molecular Genetic Engineering



What Are The Limitations of Classical Breeding/Genetic Engineering?

1. Limited To Genes of Interbreeding Organisms and, Clearly, Severe Ethical Issues With Humans (eugenics)

2. Only Can Make New Combinations of EXISTING Genes - Genes Created By "Natural" Mutations

3. Can't Make Existing Genes "Better" - Just Better or More Useful Combinations of Existing Genes and/or Alleles

4. Takes Time - Limited To Generation Time of Organism - Decades For Some Crop Plants

5. Only Useful For "Obvious" Traits - One's That Can Be Observed or Followed

6. Unpredictable Outcomes (Bringing in Thousands of Genes at Once – Some With Deleterious Consequences)

What Are The Advantages of Using 21st Century Genetic Engineering Methods?

1. Any Gene From Any Organism Can Be Used In Any Organism - There Are No Breeding Barriers (e.g., genes of all sequenced genomes)

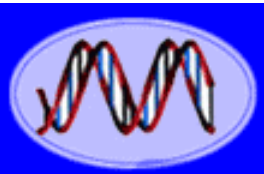
2. New Genes Can Be Engineered - Genes That Work Better and/or Produce New Proteins (i.e., create new genetic variability and/or alleles)

3. Existing Genes Can Be Engineered to be Switched On in "Places" That They Are Normally Off - Gene Control or Regulation Altered (e.g., fly eye on leg)

4. Speed - Can Engineer a New Organism in a Generation

5. Can Change, Alter, Manipulate, Synthesize and/or Control the Genetic Blueprint of Any Organism

6. Very Precise (Working With Known Genes & Proteins)



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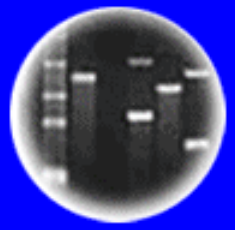

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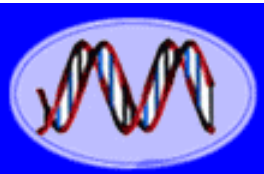
HOW IS SCIENCE CARRIED OUT?

SCIENTIFIC KNOWLEDGE IS OBTAINED BY A PRECISE & SPECIFIC PROCESS



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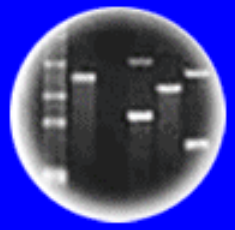

DNA Fingerprinting

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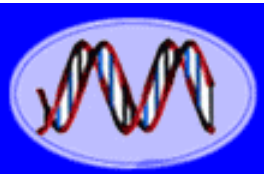
Plants of Tomorrow

Science is NOT "Hocus Pocus" or Based on Opinions and Beliefs

- Science is Based on Observation, Hypothesis Testing, Rigorous Experimentation, and Verification

- Technology, or the Application of Scientific Knowledge, Has Transformed Dramatically Our Lives and How We Live

What Are the Data!!!!!



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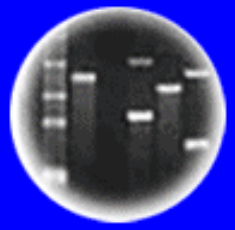

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Plants of Tomorrow

It Has Lead to Civilization and Culture as We Know It!

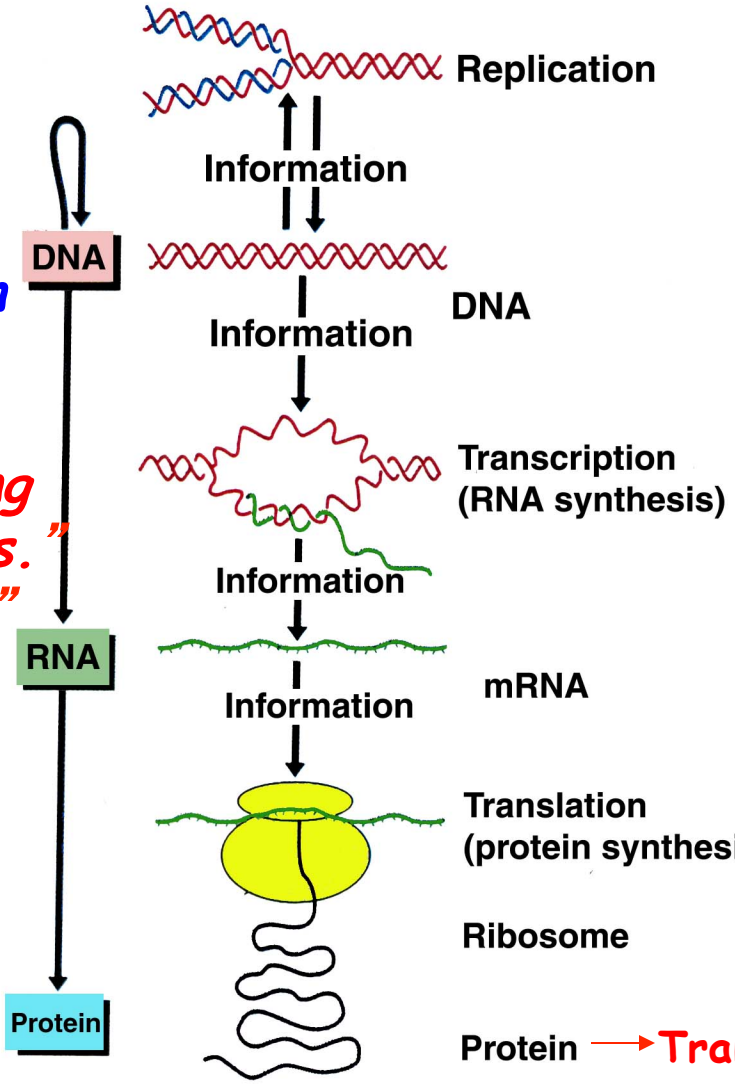
- Agriculture
- Medicine
- Computers and Automation
- Airplanes, Cars, and Satellites
- Countries and Cities
- Political Systems
- Art and Literature
- Etc., Etc., Etc.

Simply Put:Our Way of Life!

Translating The Genetic Code Into Proteins is a Conserved Process

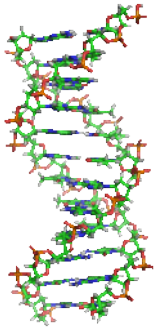
Can Intervene in This Process in Cells

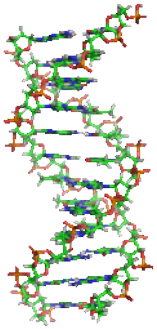
Genetic Engineering Is not "Hocus Pocus." It Uses "Natural" Cell Processes!!!!



Trait (e.g., eye color)

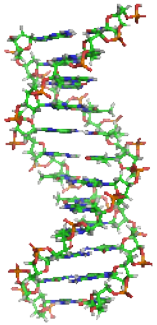
All Organisms Use The SAME Processes And "RULES" to Generate Traits!! And The SAME Molecules & Chemistry!!





We Live in the
The Age of DNA!

Genetic Engineering Is
Manipulating DNA Either Classically or By
Exciting Modern Approaches (GE 1.0 and 2.0)!
It's a Scientific Process
Not Hocus Pocus



<u>Understanding Genetic Engineering</u>
Requires a Basic Understanding of Genes
And How They Work