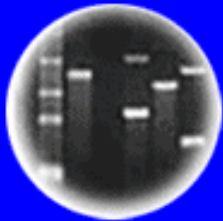


DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

HC70A & SAS70A Winter 2019 Genetic Engineering in Medicine, Agriculture, and Law

**Professors Bob Goldberg,
John Harada, and Channapatna Prakash**

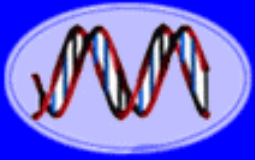
Lecture 2 The Age of DNA: What Is Genetic Engineering-Part Two

UCLA

TUSKEGEE
UNIVERSITY

UC DAVIS
UNIVERSITY OF CALIFORNIA

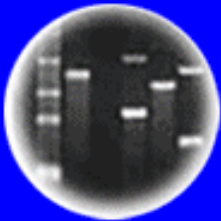
THEMES



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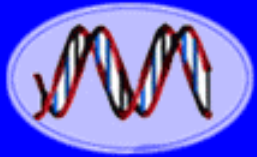


Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

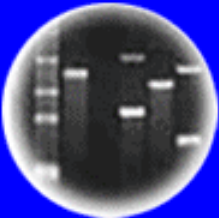
1. The Significance of Genetic Engineering
2. What Are the Tools of Genetic Engineering?
3. What Can Be Done With Genetic Engineering- Some Examples
4. What Does Genetic Engineering Tell Us About Basic Genetic Processes in All Organisms?
5. Genetic Engineering - Anything New?
6. Are Vegetables Engineered - Demonstration
7. Classical vs. 21st Century Genetic Engineering
8. Synthetic Genomes



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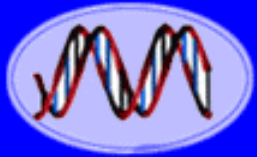
Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

Last Week's Lecture...a Reminder

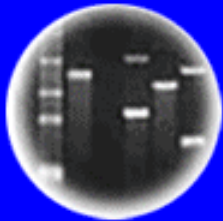




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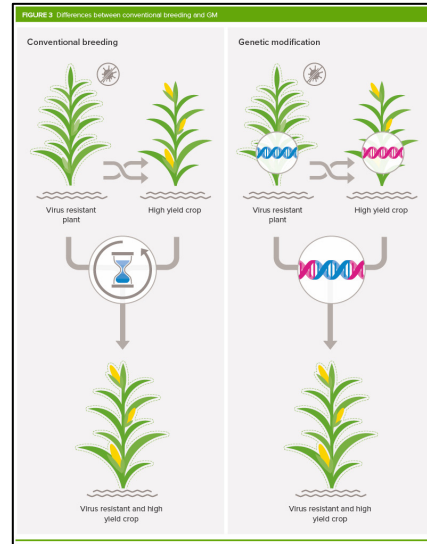
Cloning: Ethical Issues
and Future Consequences



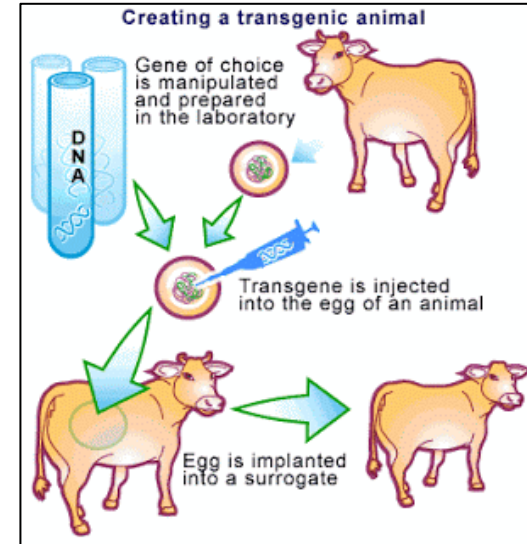
Plants of Tomorrow

Three Genetic Engineering Techniques That Generate GMOs!!!

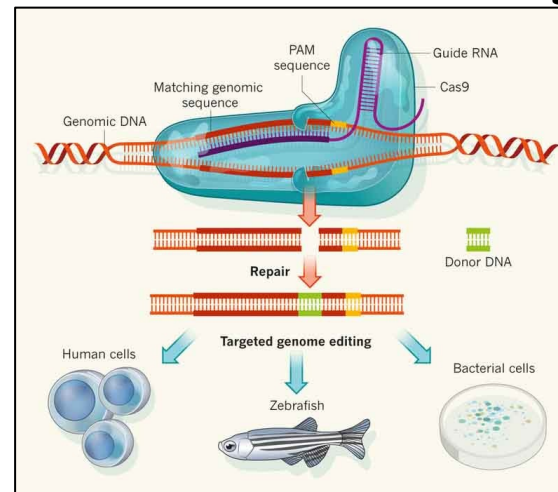
1. Classical Breeding



2. Transgenic Organism

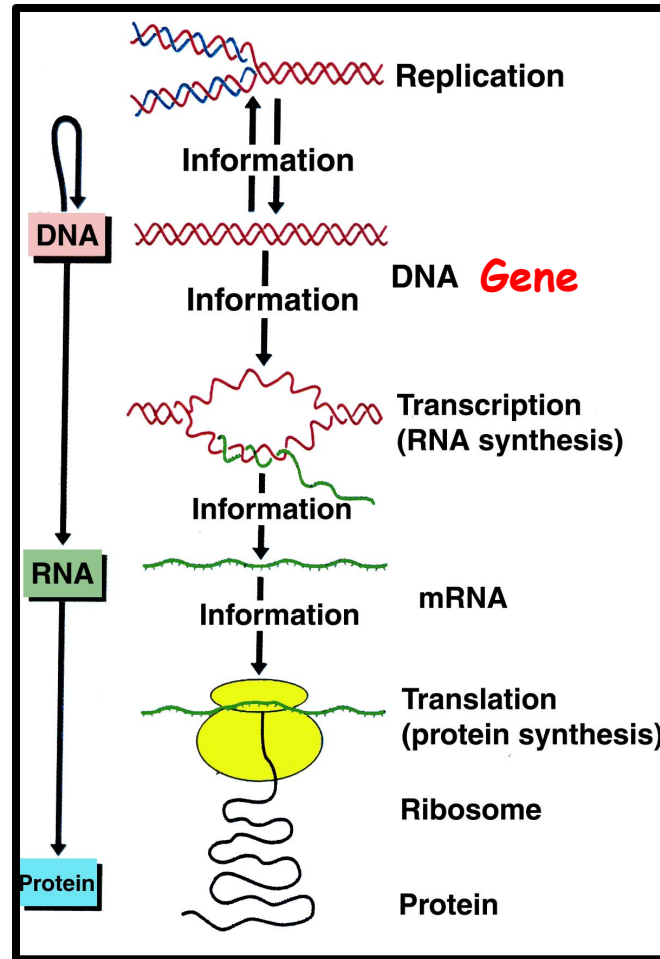


3. CRISPR Gene Editing

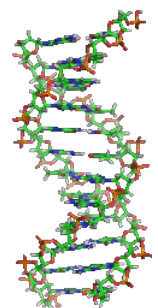
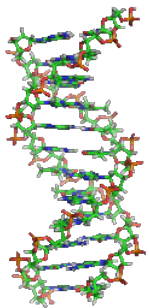


*Genes & DNA Obey the Same Rules Using **Either** Classical or Modern DNA Engineering Approaches!! **BOTH Produce GMOs!!!!!!***

1. Can Intervene in Cellular Genetic Processes - DNA to RNA to Protein (Trait)

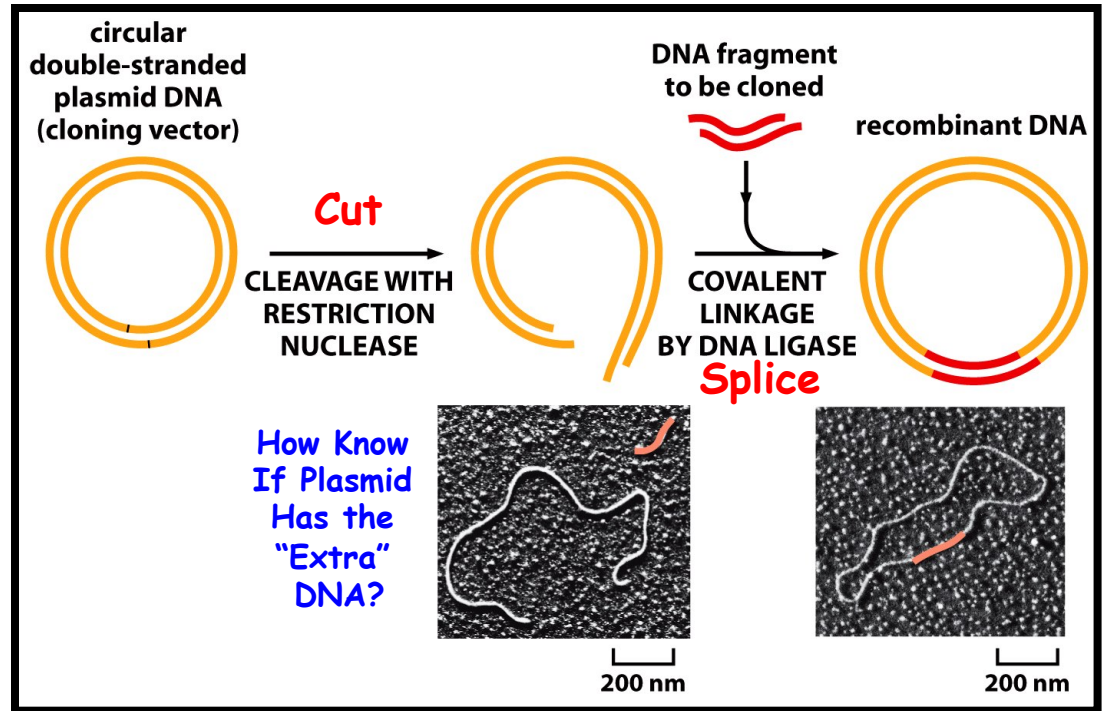
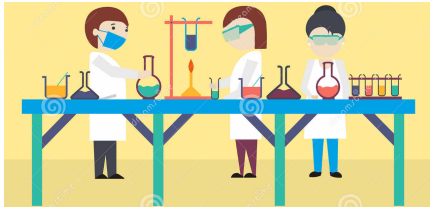


*2. All Organisms Use The **SAME** Processes And "RULES" to Generate Traits!!*



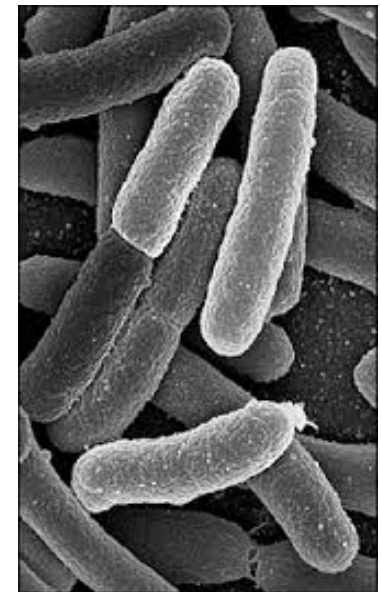
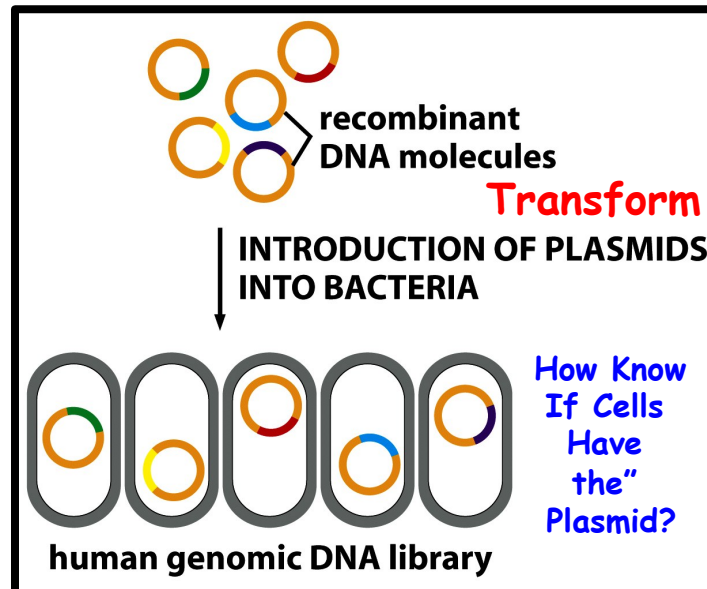
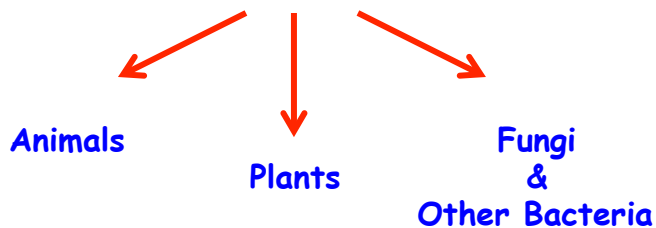


What "Tools" Are Needed For Genetic Engineering?



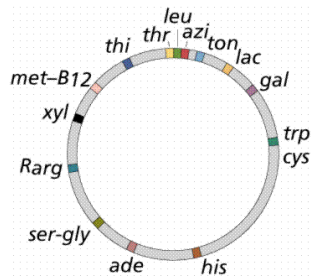
1. DNA
2. Vector (e.g., Plasmids)
3. Enzymes
 1. Restriction Enzymes (Cut & Create Sticky Ends)
 2. Ligase Enzyme (Splice)
 3. Terminal Transferase (Sticky Ends)
4. Cells (*E. coli*)

Lab Engineered DNA



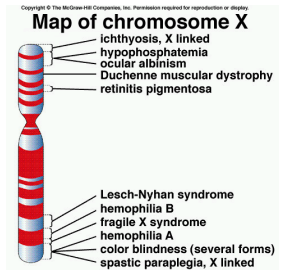
“Why” Clone Genes From An Organism’s Genome?

An Essential HC70A Concept!



50 million bases

Cocci and adenovirus receptor	Myeloproliferative syndrome, transient
Amlyoidosis, cerebral cortical, Dutch type	Leukemia, transient, of Down syndrome
Alzheimer disease, APP-related	Enterokinase deficiency
Schizophrenia, chronic	Multiple carboxylase deficiency
Usher syndrome, autosomal recessive	T-cell lymphoma, immunoblastic, metastatic
Ankyrotic lateral sclerosis	Mycobacterial infection, atypical
Oligomycin sensitivity	Down syndrome (critical region)
Jervell and Lange-Nielsen syndrome	Autoimmune polyglandular disease, type I
Lung Q1 syndrome	Bethlem myopathy
Down syndrome cell adhesion molecule	Epilepsy, progressive myoclonic
Homocystinuria	Holoprosencephaly, alobar
Strabismic, congenital, autosomal dominant	Krabbe's syndrome
Deafness, autosomal recessive	Hemolytic anemia
Myxovirus (influenza) resistance	Breast cancer
Leukemia, acute myeloid	Platelet disorder, with myeloid malignancy

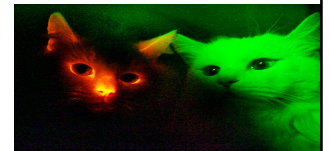


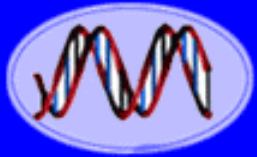
1. **PURIFY** Individual Genes From the Genome (e.g., One of 25,000 Human Genes)
2. **AMPLIFY** The Gene Using Plasmids in Bacterial Cells to Obtain Enough DNA For Study
3. **USE** the Cloned Gene To:
 1. Study Gene Structure & Function (THE Major Use!)
 2. Use to Convert Cells Into Factories To Make Drugs and Pharmaceuticals
 3. Use to Diagnose Genetic Diseases
 4. Use to Identify Individuals (e.g., paternity, forensics)
 5. Use to Correct Genetic Disease
 6. Use to Engineer New Crops and Farm Animals
 7. Synthesize New Genomes and Many Other Uses

What Is the Significance of Genetic Engineering?

1. Specific DNA Sequences and Genes Can Be Isolated From Any Organism
2. DNA Segments of Any Kind From Any Organism Can Be Combined (Genetic Engineering!!!!!!!)
3. Isolated Genes Can Be Engineered and Re-Inserted Into the Chromosomes of Any Organism and Made to Work
4. Genes and Genomes Can Be Synthesized, Edited, and Made To Work in Any Organism

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

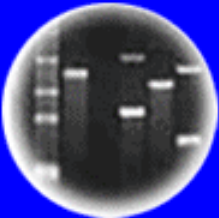




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DNA Fingerprinting



Cloning: Ethical Issues
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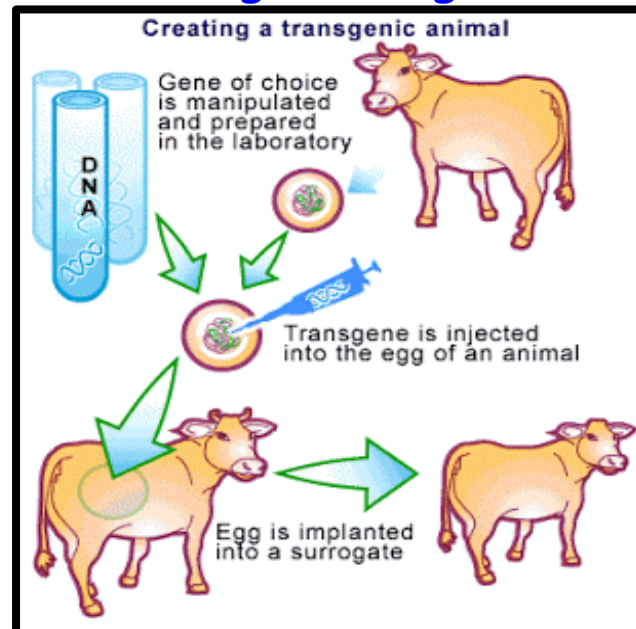


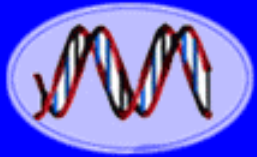
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What Can Be Done With Modern Genetic Engineering?

Some Examples

2. Transgenic Organisms

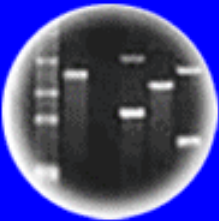




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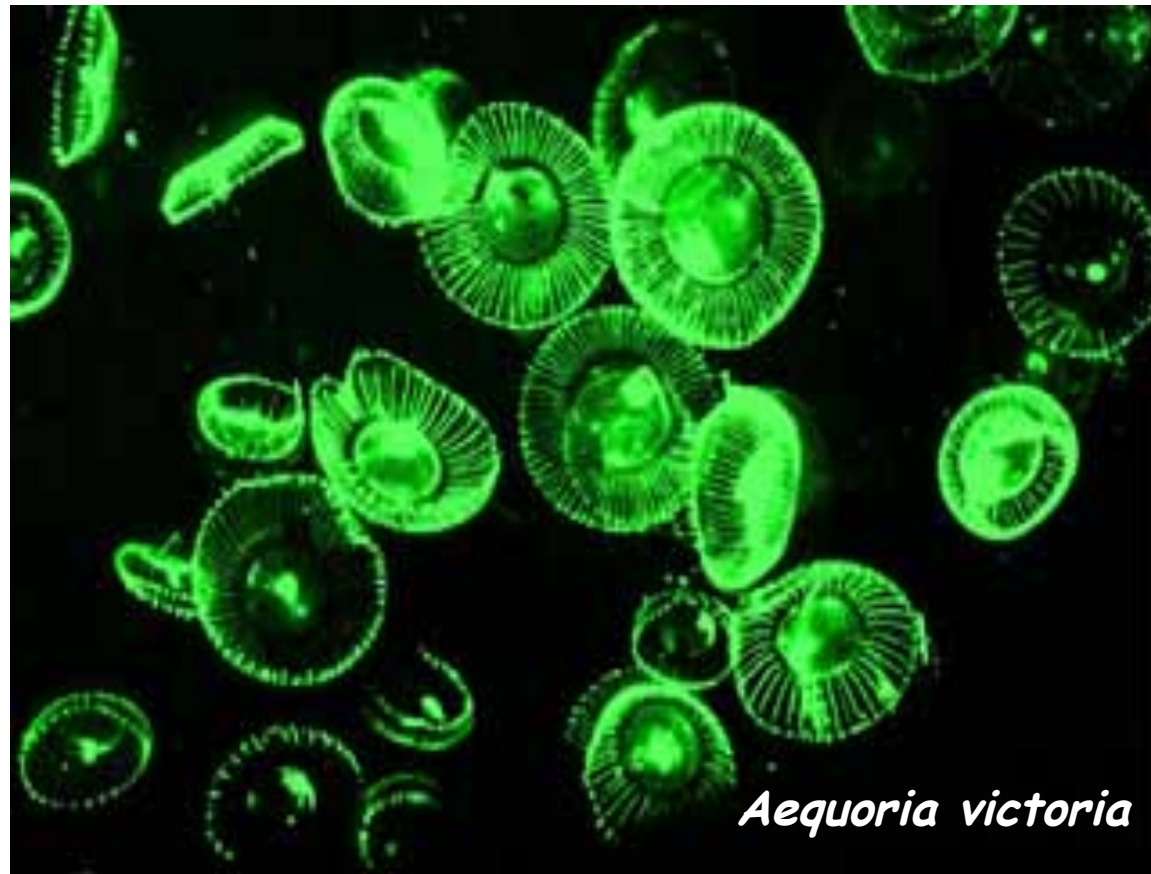


Cloning: Ethical Issues
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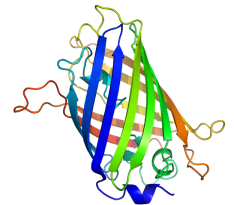
Plants of Tomorrow

Using a Jellyfish Gene to Engineer *Glowing* Bacteria, Animals, and Plants!!!

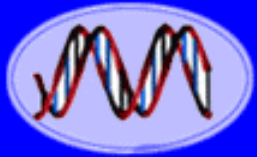


Aequoria victoria

Green Fluorescence Protein (GFP)
(238 amino acids)



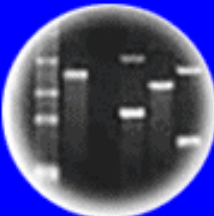
Nobel Prize in Chemistry - 2008 - Shimomura, Chalfie, & Tsien



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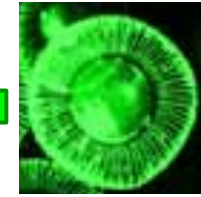
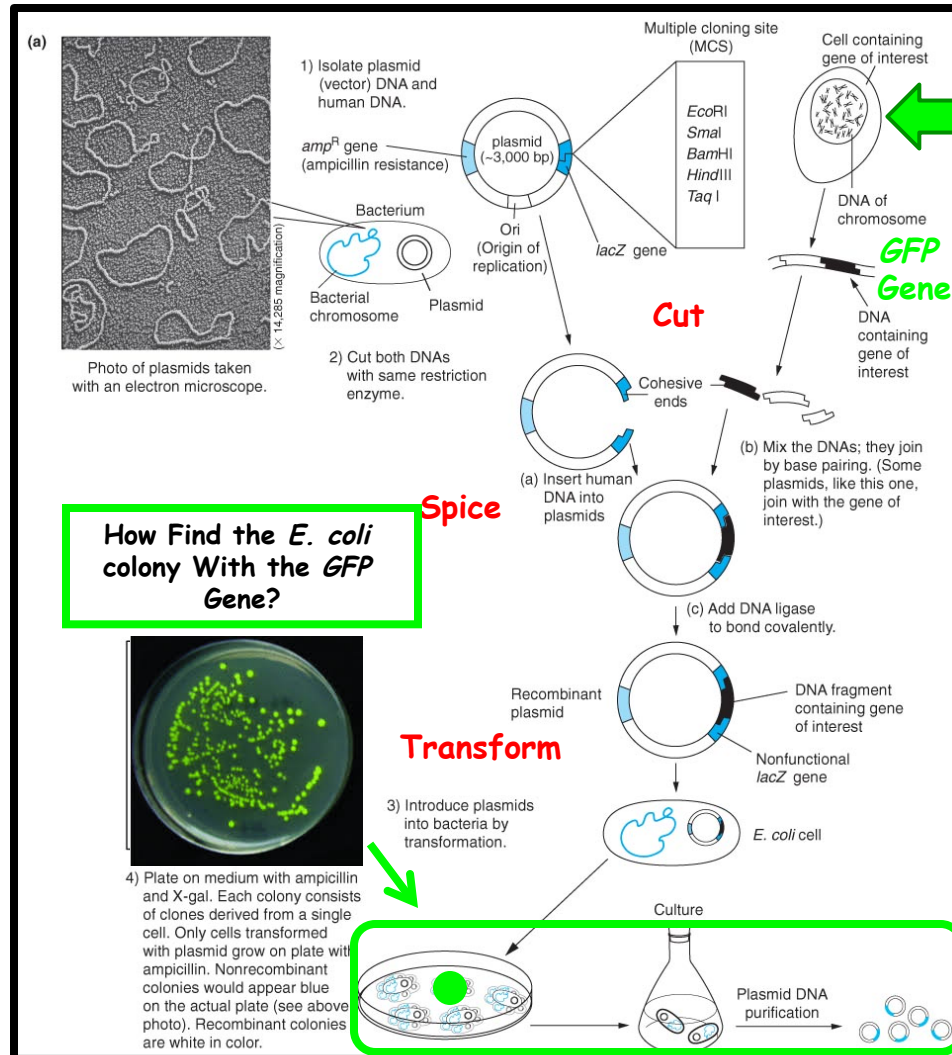
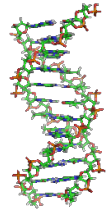


Cloning: Ethical Issues
and Future Consequences



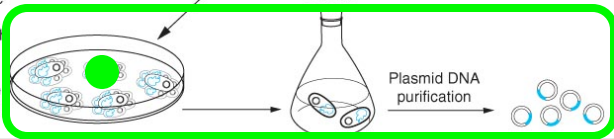
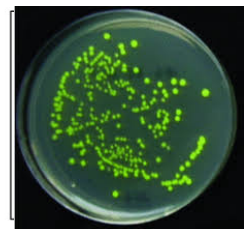
Plants of Tomorrow

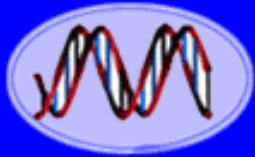
Using Recombinant DNA to Isolate the Jellyfish **GFP** Gene



- Clone Jelly Fish GFP Gene
- Insert GFP Gene Into Plasmid Vector "Behind" a Specific "Switch"
- Transform Into *E. coli* and Select Cells With Recombinant Plasmid
- Determine If GFP Gene Is Active

How Find the *E. coli* colony With the GFP Gene?

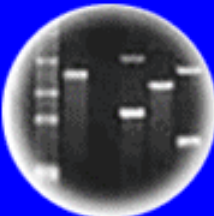




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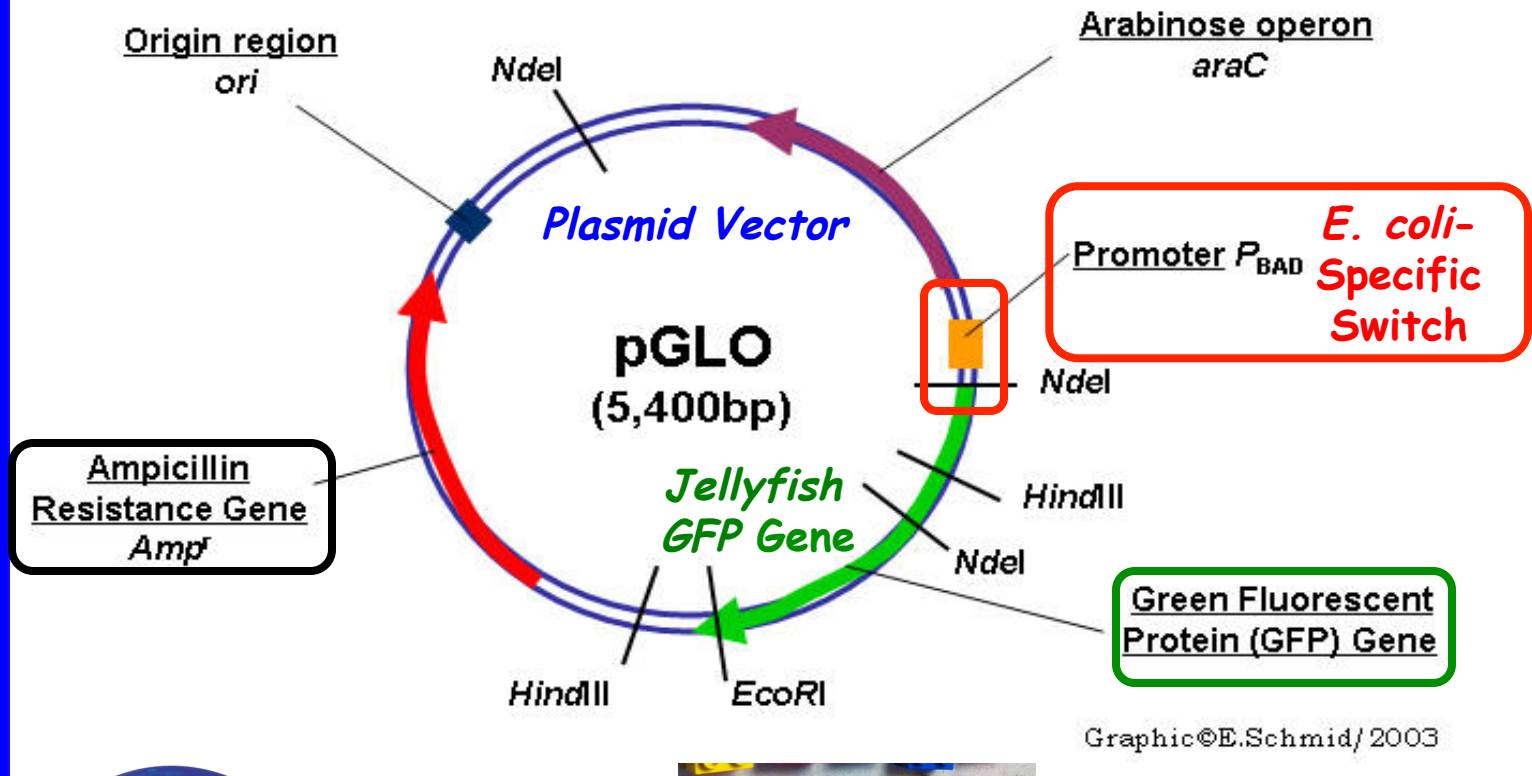


Plants of Tomorrow

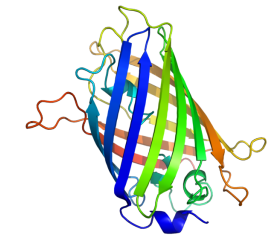


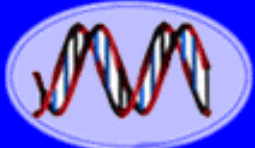
A Recombinant Plasmid Containing the GFP Gene

How Make it Active in Living Cells?



Need a Species-Specific Switch to
Allow a Gene To Function in a
Specific Organism

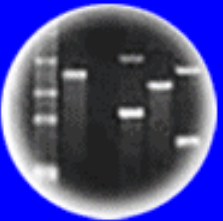




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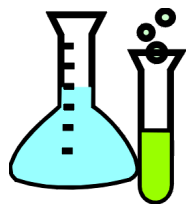


Cloning: Ethical Issues and Future Consequences



Plants of Tomorrow

Engineering the Jellyfish GFP Gene to Be Active in Different Organisms



Isolate From a Gene "Cut"

Species-Specific "On Switch"

Jellyfish GFP Gene

Anneal & Splice

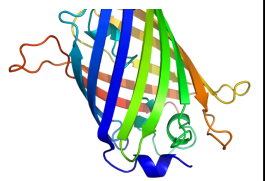
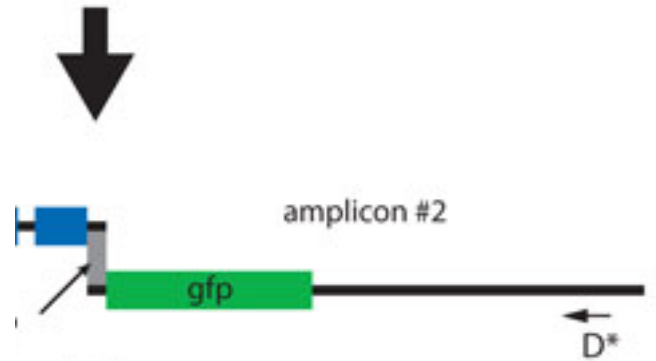
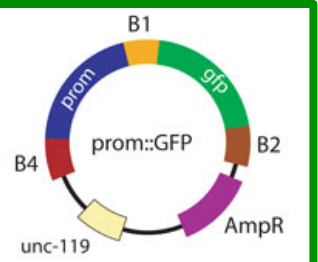
Engineered Chimeric GFP Gene

Transform

Animals

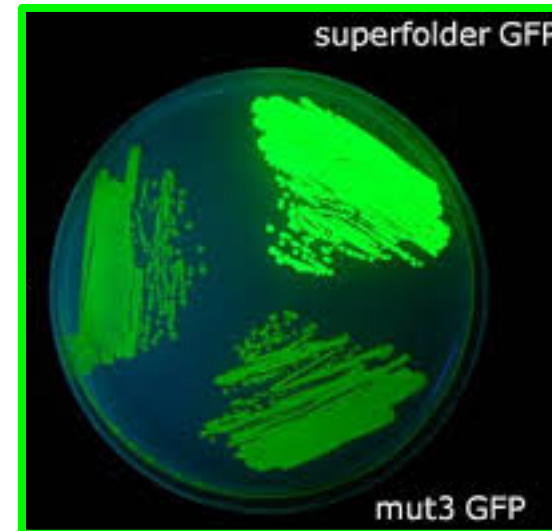
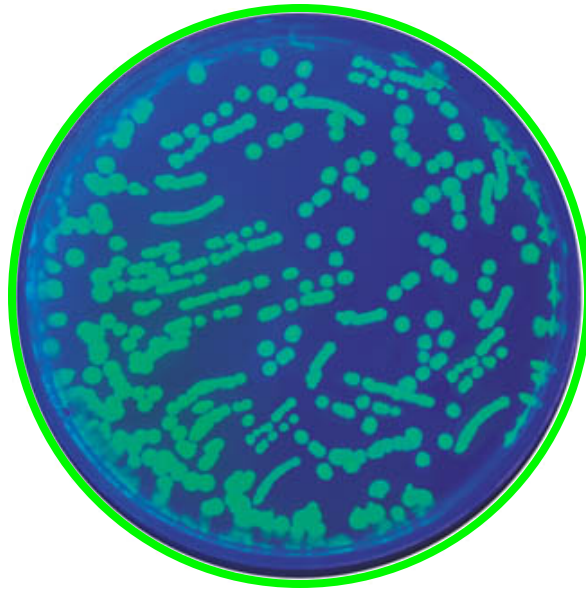
Plants

Bacteria

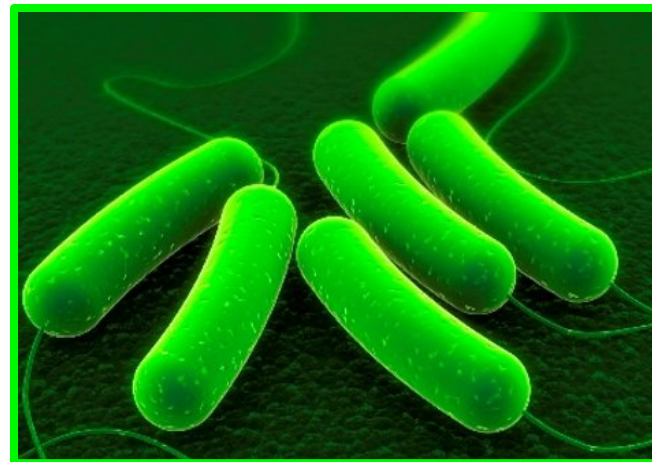


GloColi - Engineering *E. coli* With the Jellyfish GFP Gene!

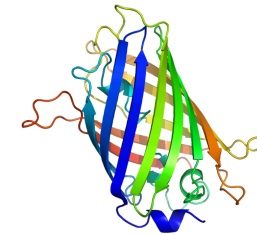
What Are the Conceptual Implications of This Experiment?



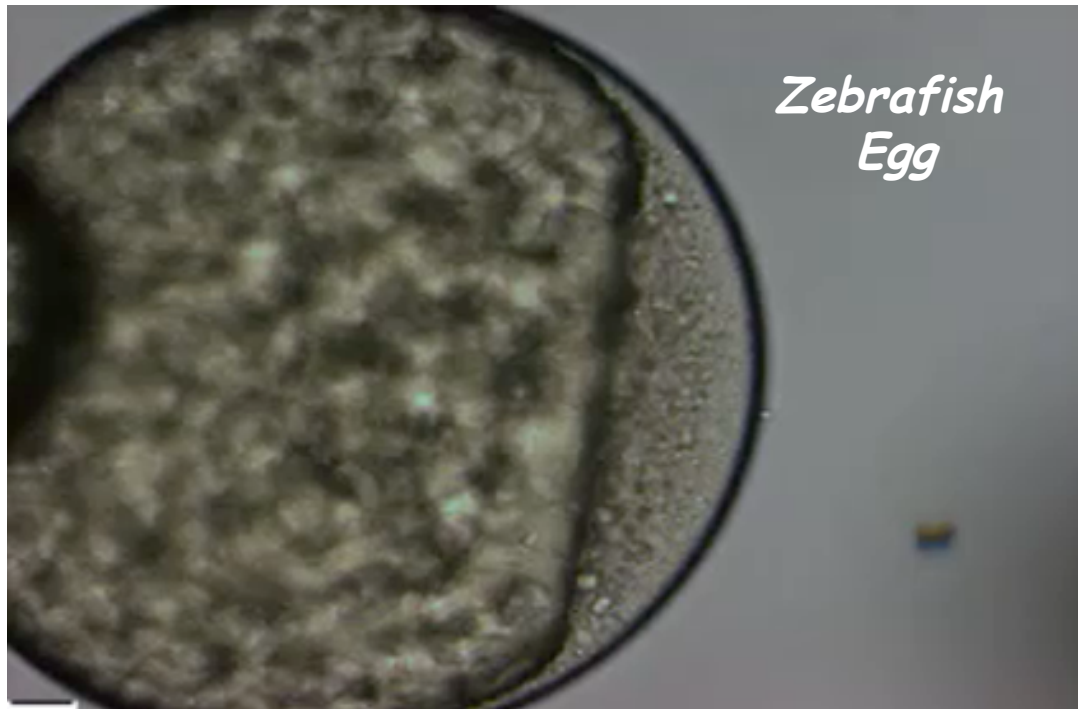
E. Coli Switch + Jellyfish GFP Gene



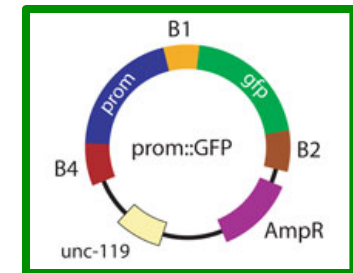
E. Coli Synthesizes GFP Protein!



Engineering a “GloFish”



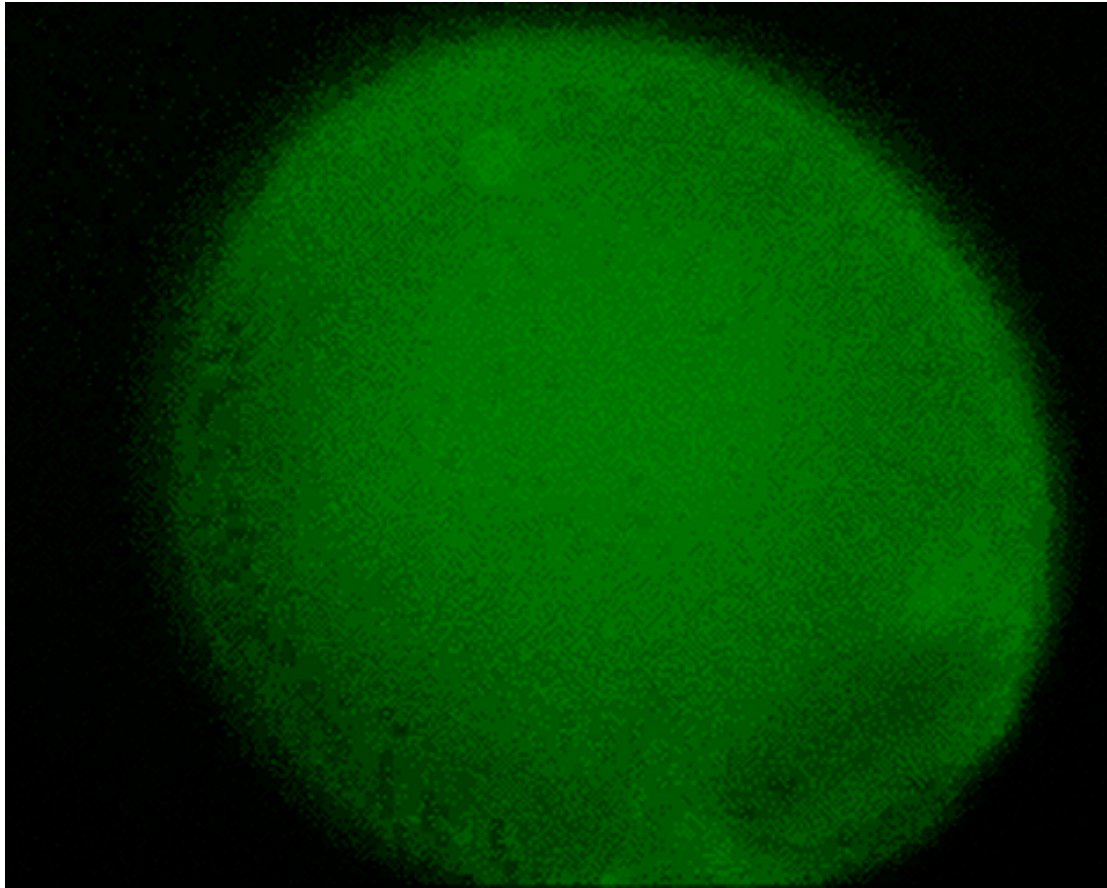
Zebrafish
Danio rerio



Using Genetic Engineering To Insert An Engineered Jellyfish GFP Gene into a Zebrafish Egg!

What Switch Used?

A "GloFish" Embryo!!

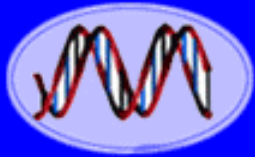


Zebrafish - Danio rerio

Genetically Engineered "GloFish!!"



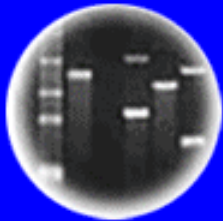
**Note Different Fluorescing Colors - Due to
Different Engineered Jellyfish Genes**



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Plants of Tomorrow

Can GloFish Can Be Purchased In California?

- **Cal. Depart. of Fish and Game Code § 15007 (2007) Regulation** Makes it illegal to spawn, cultivate, or incubate any transgenic fish in the state controlled waters of the Pacific Ocean.
- **Cal. Depart. of Fish and Game Code Ruling (2015)**
The Dept. of Fish and Game will propose the addition of an **exception** to Section 1.92 that would allow the sale of transgenic tropical aquarium fish that the Dept. has determined pose no foreseeable risk or harm to native fish or wildlife.

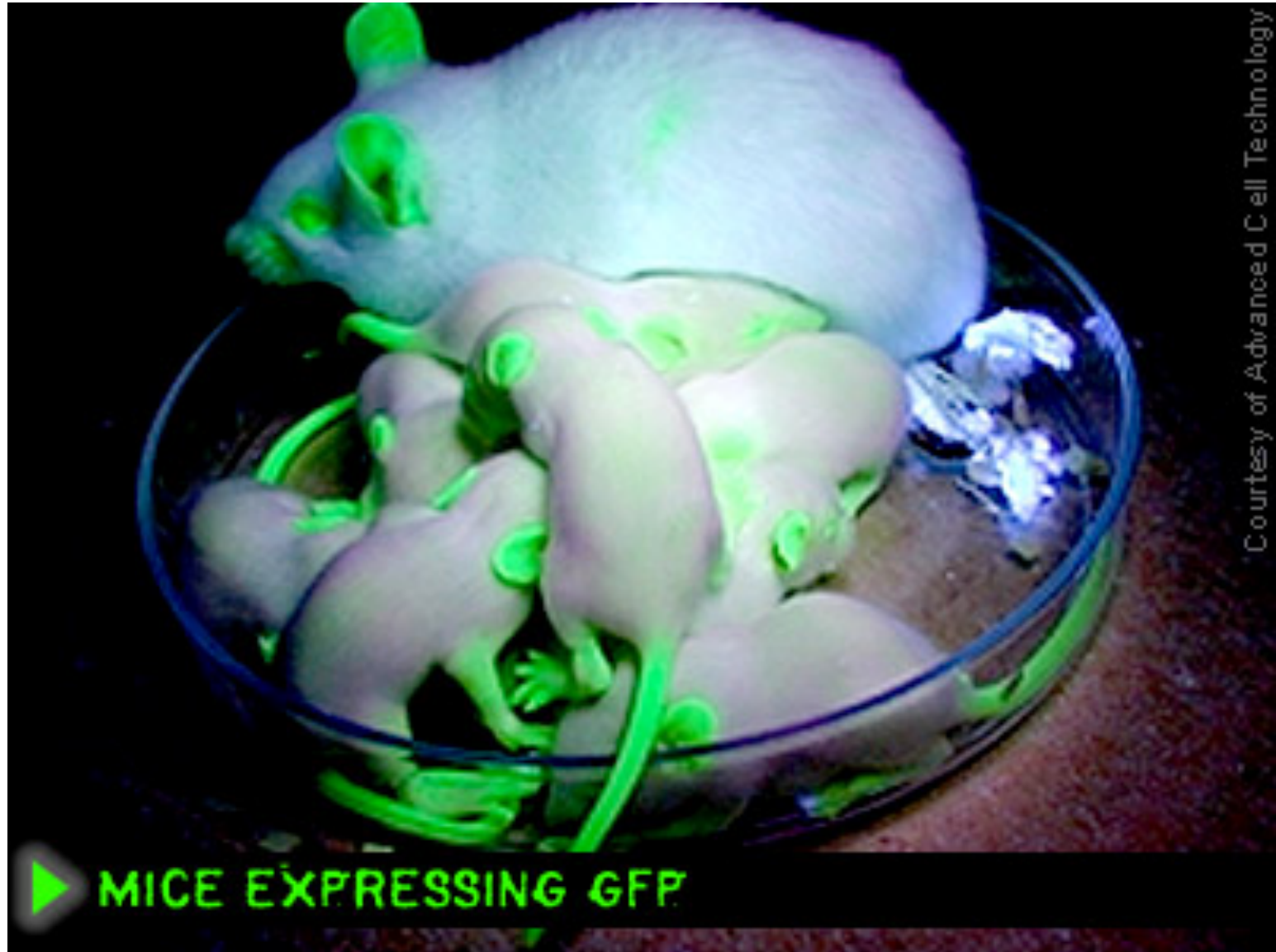
Genetic Engineering & The Law!!



How About a Glo Fly!



What About “Glo Mice!!!”



And Glo Monkeys, Cats and Pigs as Well!!



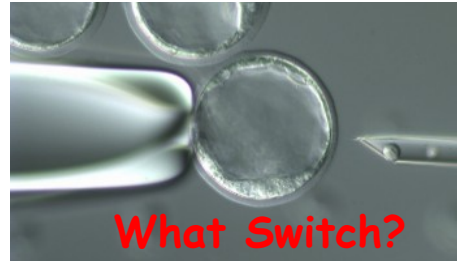
*Engineering a Glo Plant With the
Same Jellyfish Gene!!!*



*What are the Biological Implications of
These Experiments?*

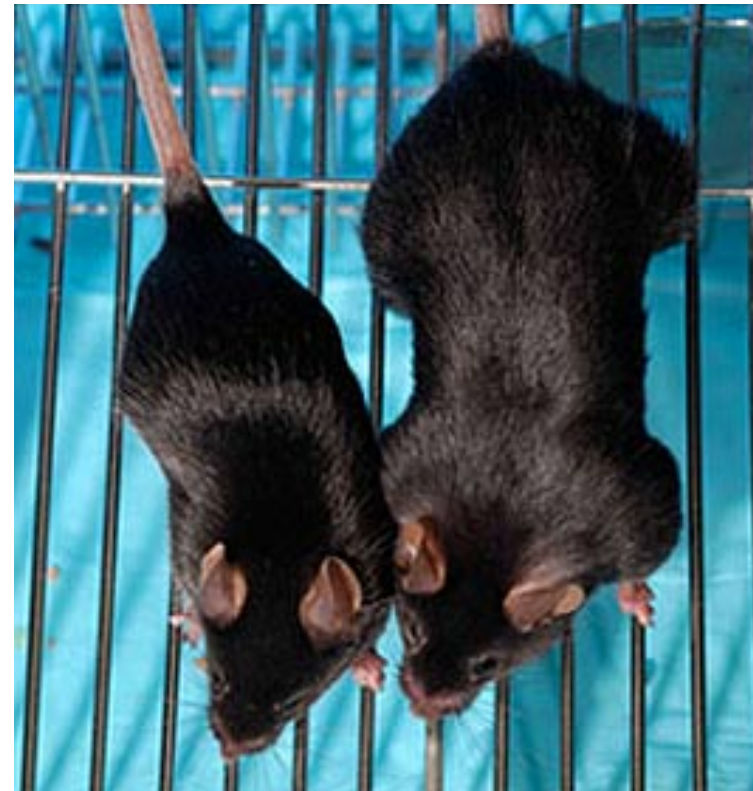
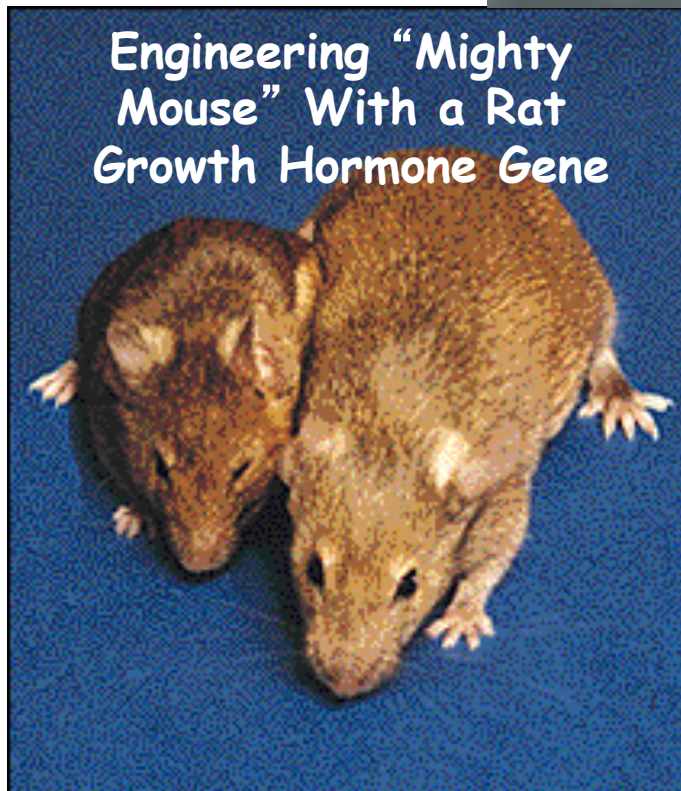
Dramatic growth of mice that develop from eggs microinjected with metallothionein–growth hormone fusion genes

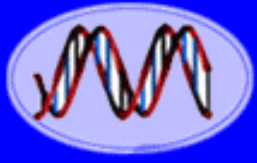
Richard D. Palmiter^{*}, Ralph L. Brinster[†], Robert E. Hammer[†],
Myrna E. Trumbauer[†], Michael G. Rosenfeld[‡], Neal C. Birnberg[§]
& Ronald M. Evans[§]



What Switch?

Nature, December,
1982
37 Years Ago!!!!!!!

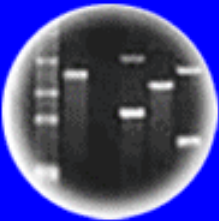




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Plants of Tomorrow

Genetic Engineering Faster Growing Salmon For More Productive Aquafarms!



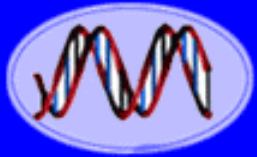
GMO salmon caught in U.S. regulatory net, but Canadians have eaten 5 tons

FDA Approves Application for AquaBounty Salmon Facility in Indiana



Genetic Engineering & the Law!!

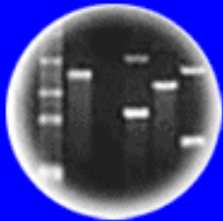
***The Fiscal Year (FY) 2018 Consolidated Appropriations Act covering the funding of the federal government during fiscal year 2018 (FY18) was signed into law by the President on March 23, 2018 becoming Public Law 115-141. In part, this law directs that during FY18 the FDA shall not allow the introduction or delivery for introduction into interstate commerce of any food that contains genetically engineered salmon, until FDA publishes final labeling guidelines for informing consumers of such content. This language was also found in the FY16 and FY17 Omnibus Appropriations Acts. The



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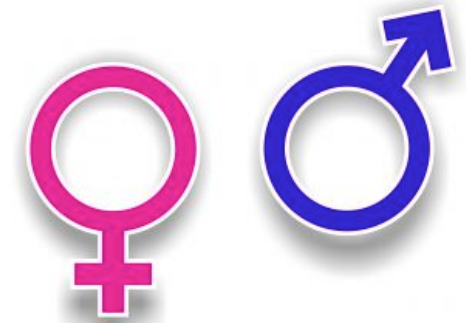
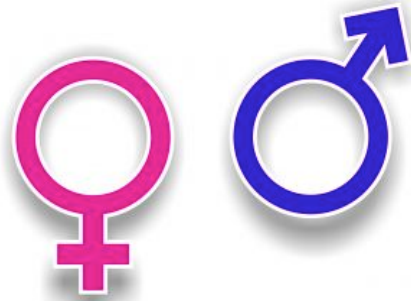
DNA Fingerprinting



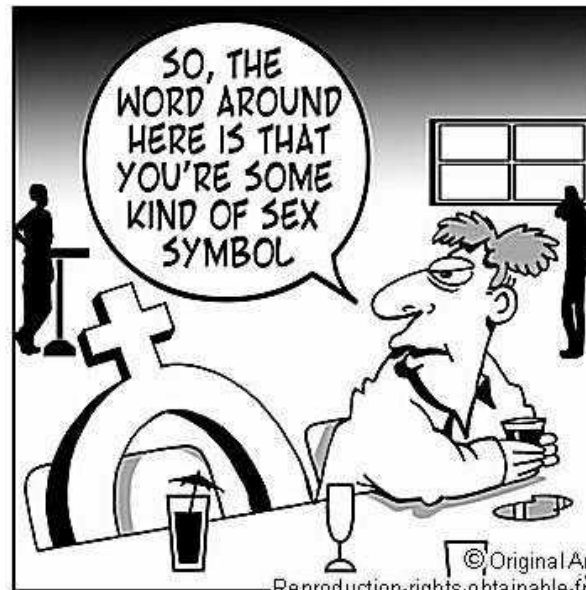
Cloning: Ethical Issues
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Plants of Tomorrow



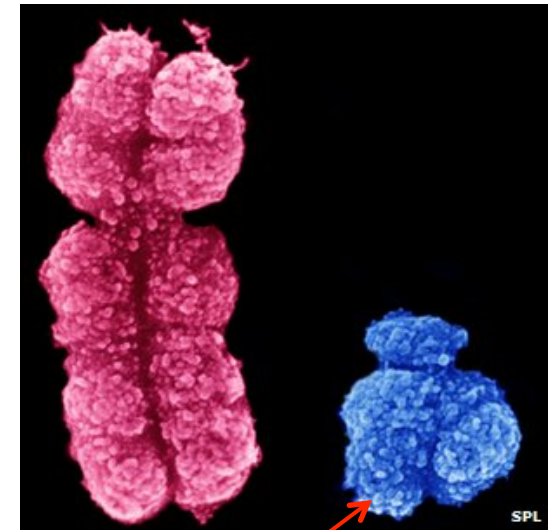
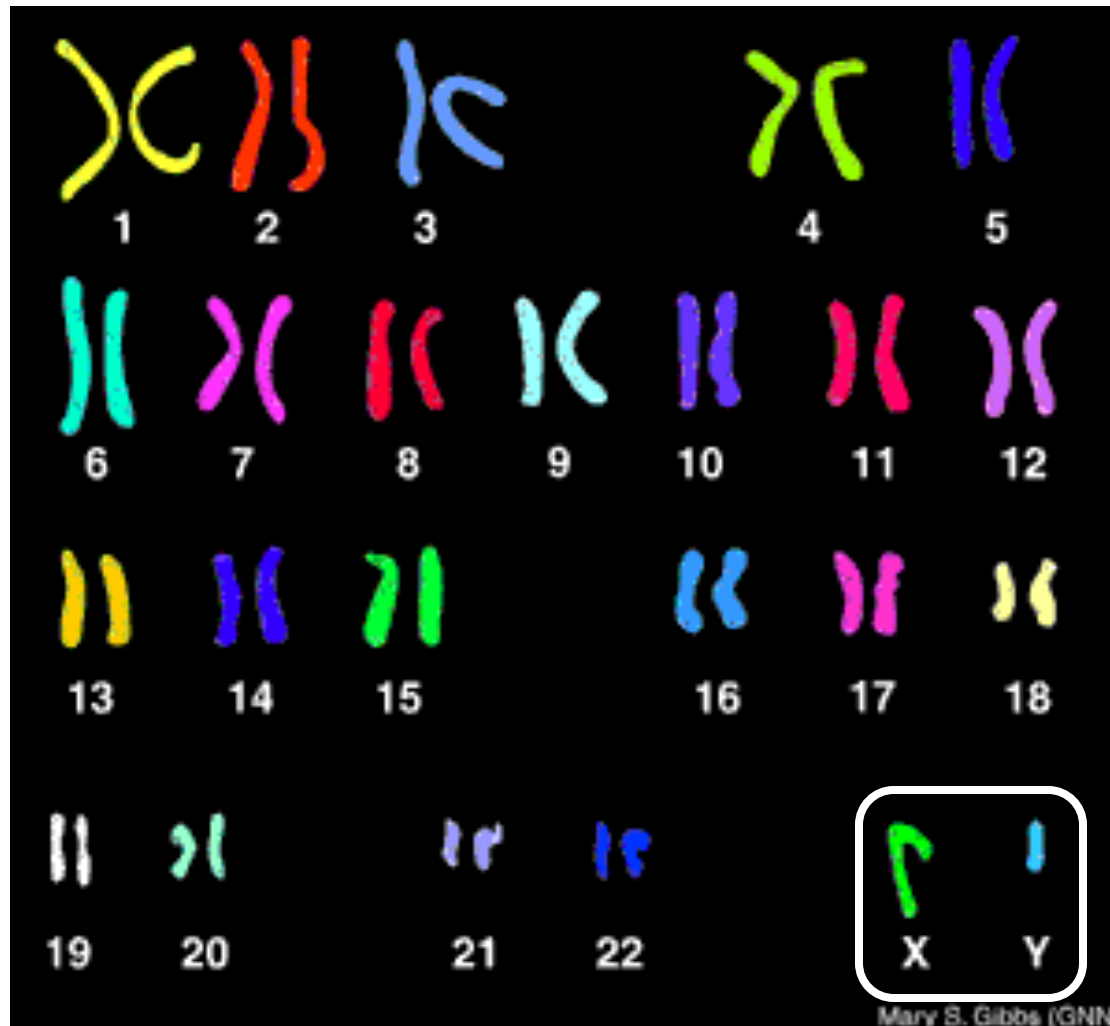
How About Engineering The Sex Of An Organism?



search ID: mlYn247

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www.CartoonStock.com

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

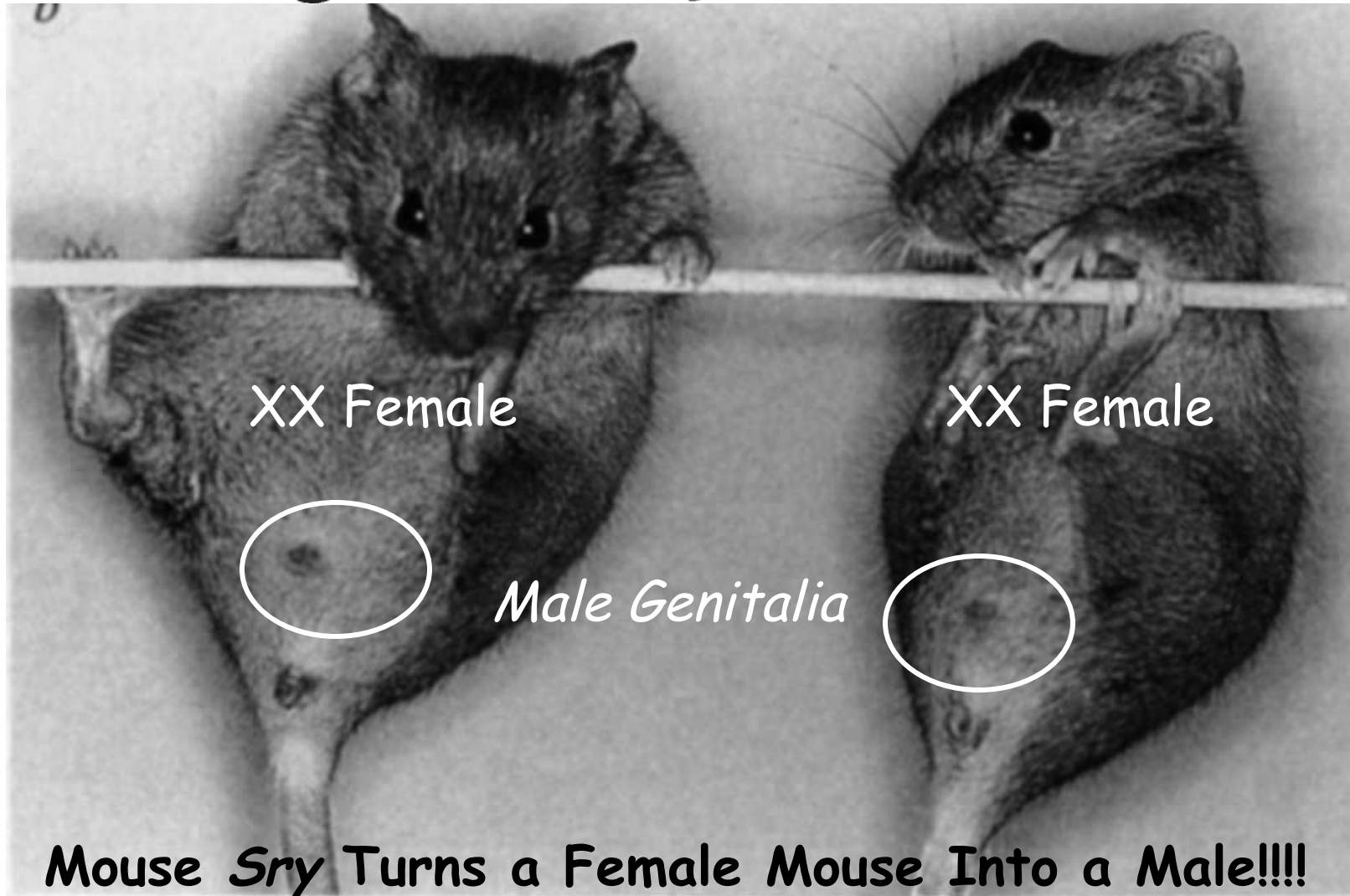


Male SRY Gene
(Sex Determining Region Y)
Regulates Other Genes
Turns on Switches

The Human SRY Gene For Maleness Controls Gender


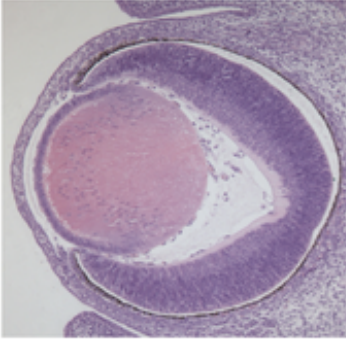
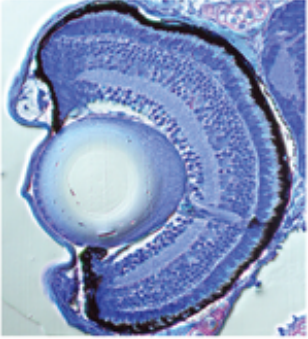

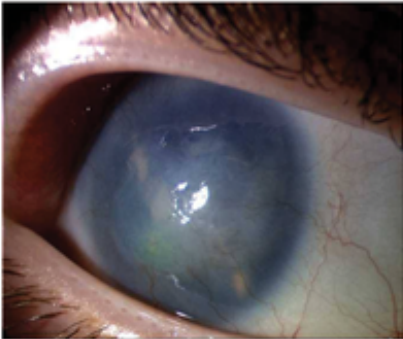
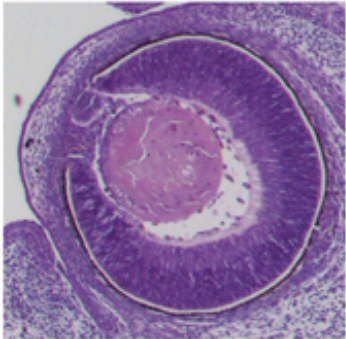
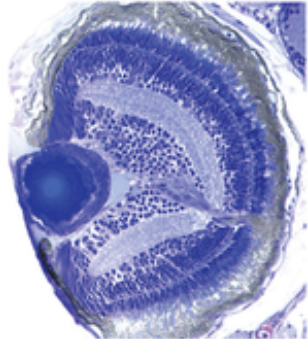
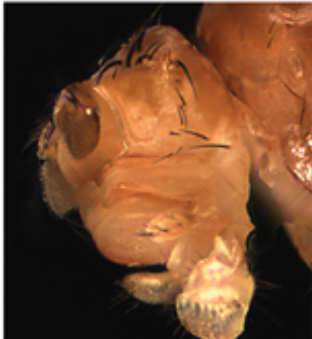
Male development of chromosomally female mice transgenic for *Sry*

Nature, May 9, 1991



Functional Proof That Sry Controls Male Development

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

	Human	Mouse	Zebrafish	<i>Drosophila</i>
WT				
mut				
	PAX6^{+/-}	Pax6^{-/-}	pax6b^{-/-}	ey^{-/-}
EQs	cornea opaque <u>iris absent</u> retina degenerate lens opaque aqueous humor of eyeball increased pressure	eye decreased size lens fused_to cornea iris morphology anterior chamber absent	eye decreased size lens decreased size retina malformed	eye absent

Induction of Ectopic Eyes by Targeted Expression of the *eyeless* Gene in *Drosophila*

Science 267, 1788, 1995



Mouse PAX-6 Gene (eyeless in flies) Engineered To Work

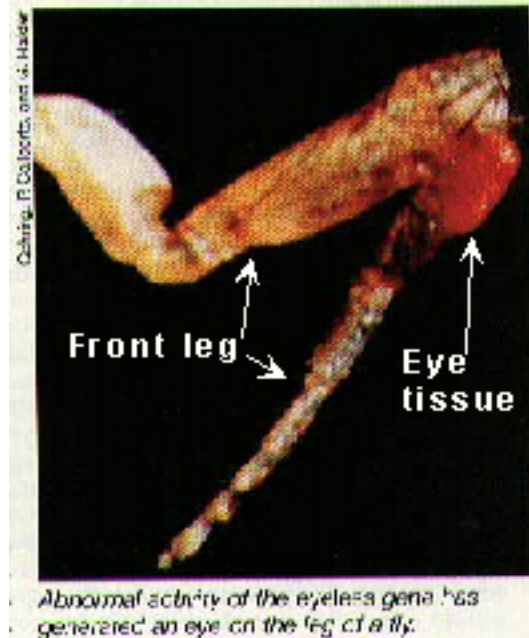


Different Fly Regions

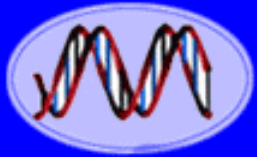
Fly Leg Switch + Mouse Eye Gene



What Does This Experiment Tell Us About Mouse & Fly Eye Genes?



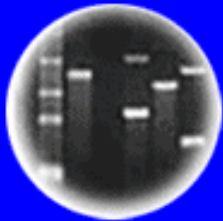
Abnormal activity of the *eyeless* gene has generated an eye on the leg of a fly.



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 Agricultural Application?

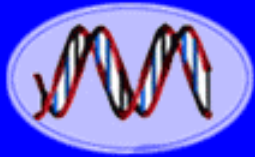
GARDEN GUIDE SUNSET

WHAT TO DO IN YOUR GARDEN IN SEPTEMBER

Southern California Checklist

☑ 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. ♦

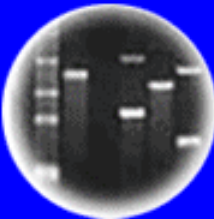
DEBRA LAMBERT



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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

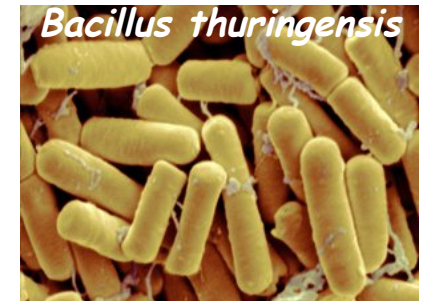
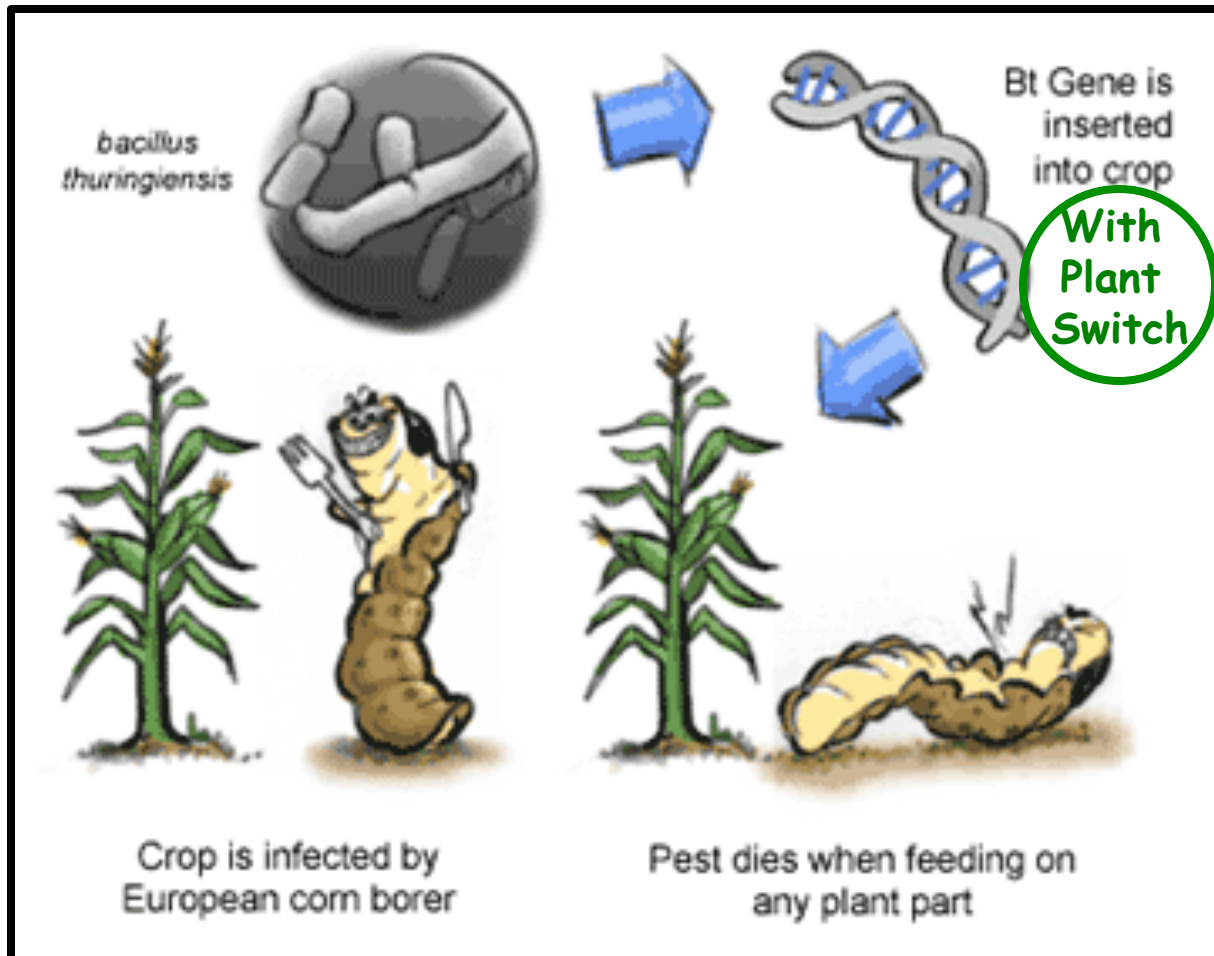
Active Ingredient:
Bacillus thuringiensis subspecies *kurstaki* strain SA-12 solids, spores and Lepidopteran active toxins (At least 6 million viable spores per mg)* 98.35%

Other ingredients: 1.65%

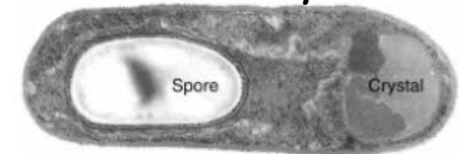
Total: 100.00%

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

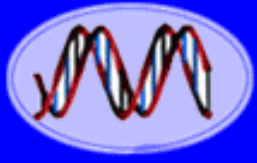
Crops Can Be Engineered With Bt For Insect Resistance



Bt Toxin in Spores



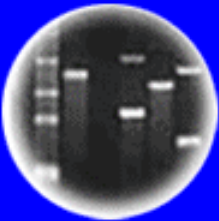
Hornworm



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DNA Fingerprinting



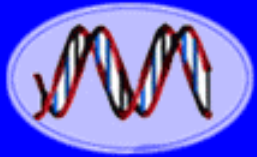
Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

Genetic Engineering a Plant to Resist Worms! Implications For Agriculture

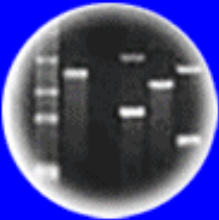




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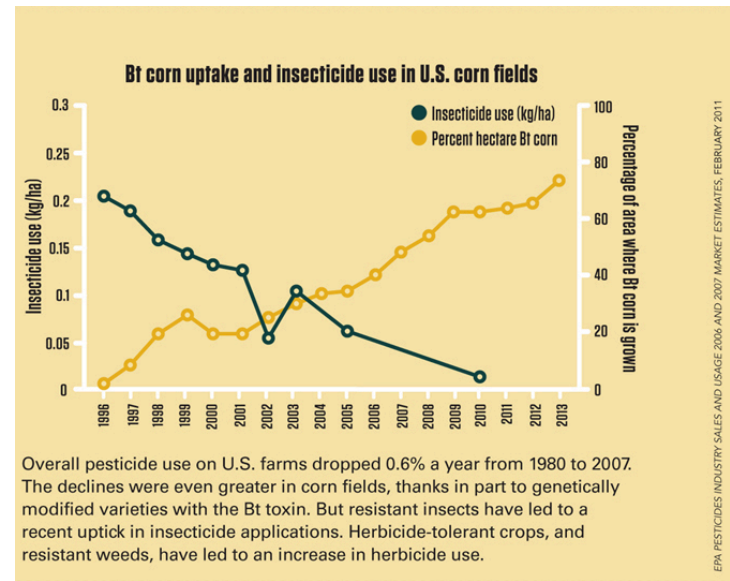
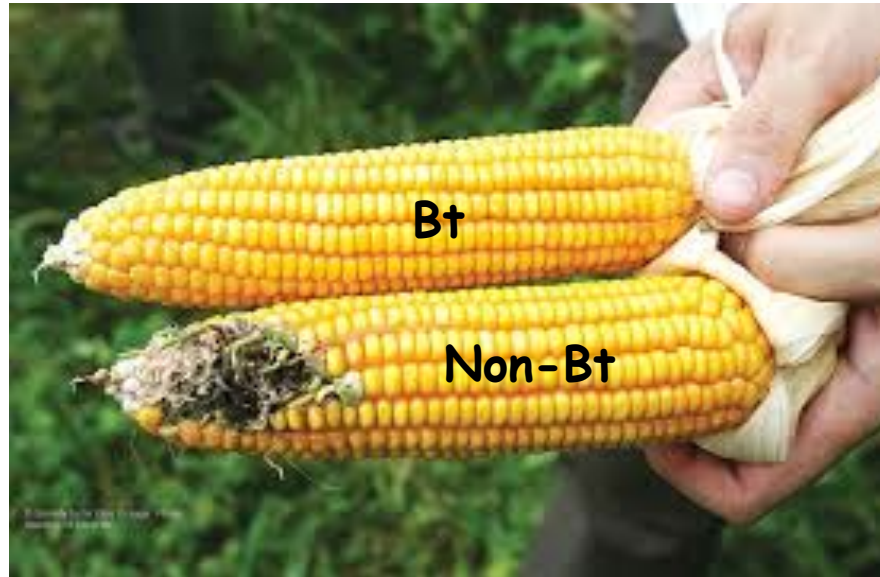


Cloning: Ethical Issues
and Future Consequences



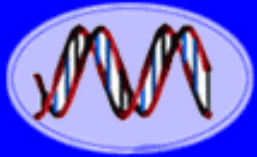
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Adoption of Bt Corn By US Farmers Has Reduced the Use of Pesticides!!!!



Overall pesticide use on U.S. farms dropped 0.6% a year from 1980 to 2007. The declines were even greater in corn fields, thanks in part to genetically modified varieties with the Bt toxin. But resistant insects have led to a recent uptick in insecticide applications. Herbicide-tolerant crops, and resistant weeds, have led to an increase in herbicide use.

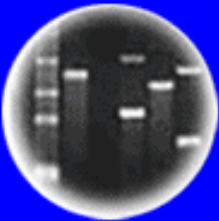
What Can We Infer FROM These Genetic Engineering Experiments About How Genes “Work” and Genetic Processes in All Living Organisms?



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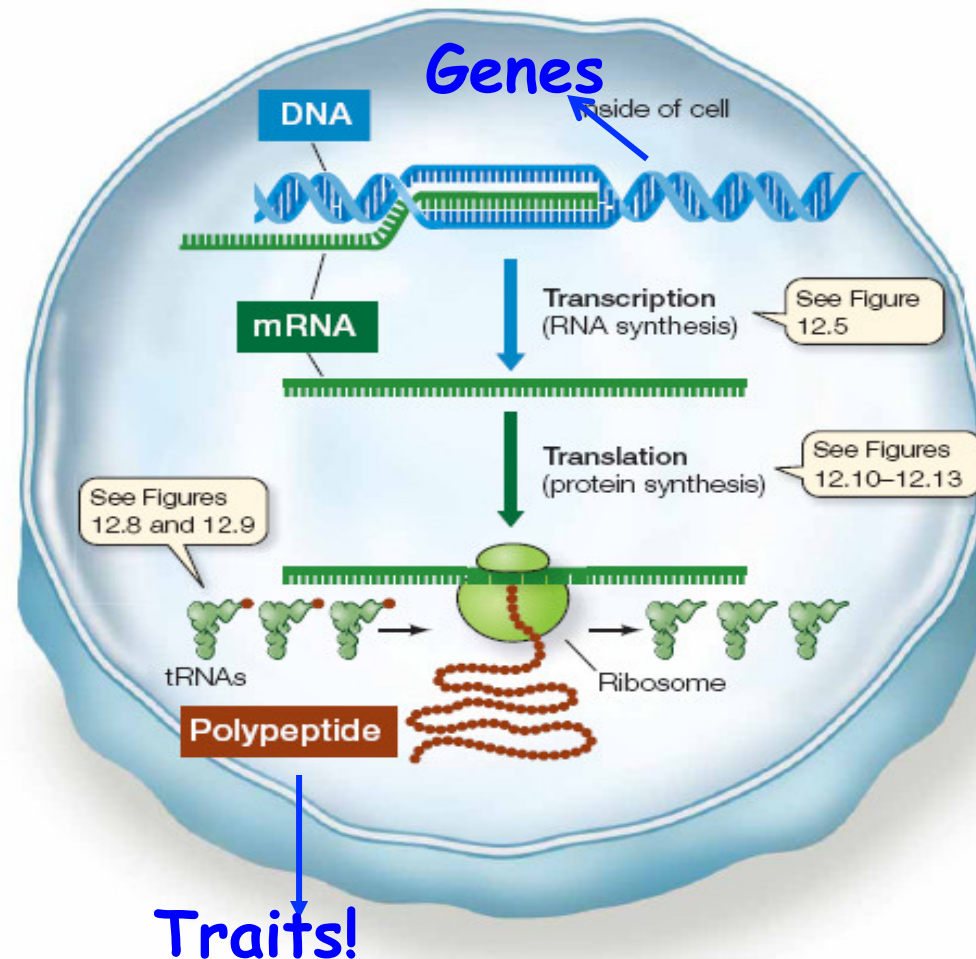
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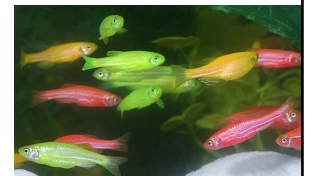


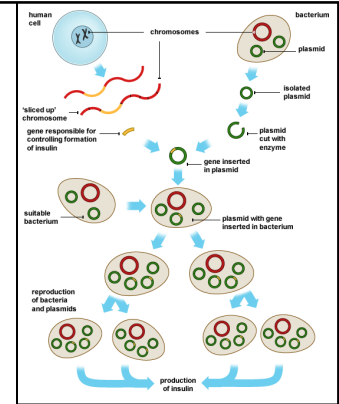
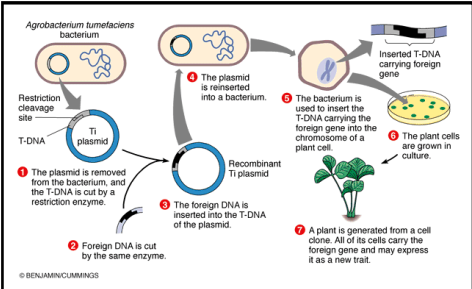
Plants of Tomorrow



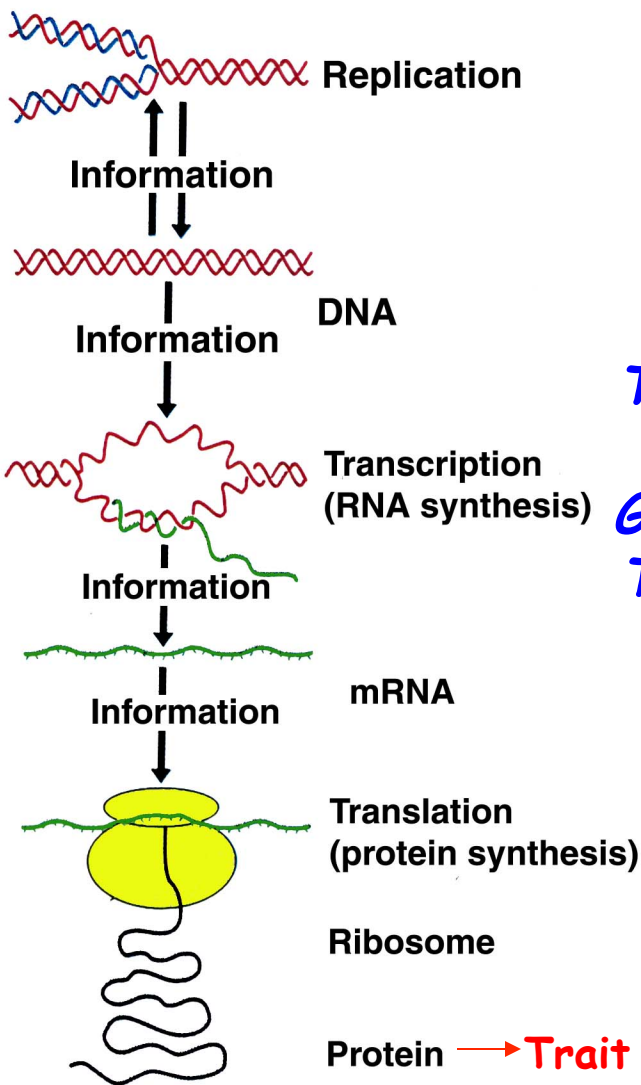
Observations and Inferences From Genetic Engineering Experiments

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 Glo Fish & Bt Genes Are Inherited Generation After Generation.





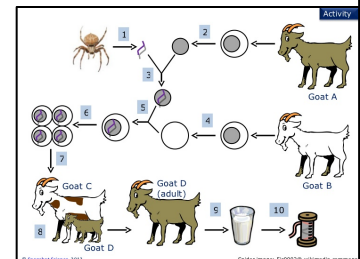
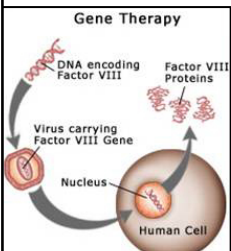
THE FACTS ARE:

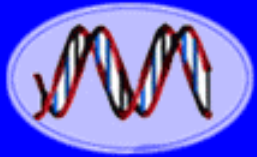


Can Intervene in This Process in Cells

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

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

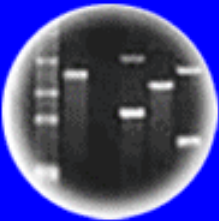




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Entire Genetic Code
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DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

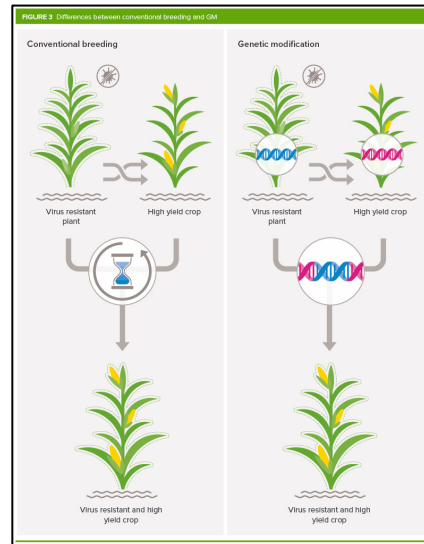


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There is Nothing New About Genetic Engineering!

**Manipulating Genes IS
Manipulating Genes No Matter
What Technology or Techniques
Are Used!!**

1. Classical Breeding

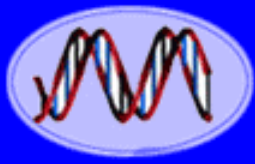


**All Major Crops Were Engineered From Wild Relatives
by Early "Bioengineers" Over 10,000 Years Ago!!**

Regions Where Major Crops Were Established



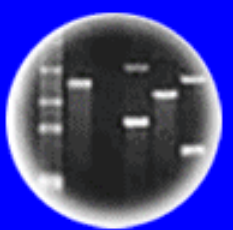
Breeding Involves Gene Manipulation Using EXISTING Genetic Variability!



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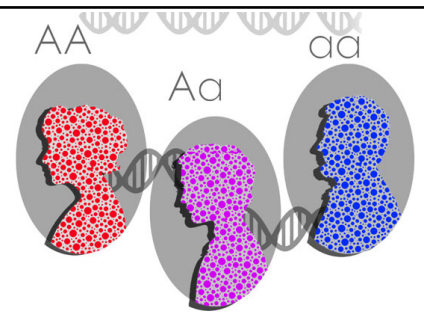
DNA Fingerprinting



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Populations of All Organisms Contain Genetic Variability



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

Tomato Genetic Diversity



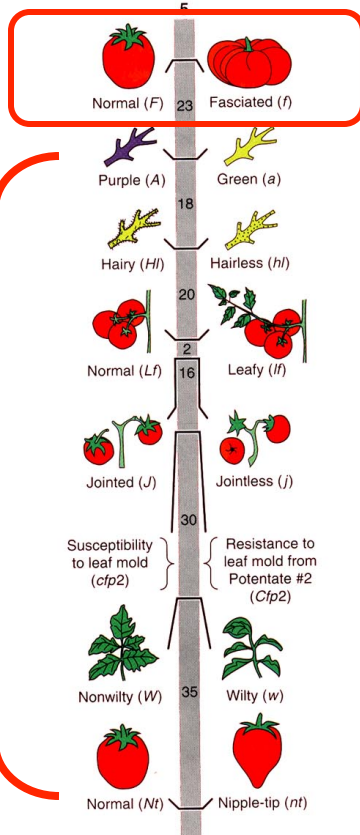
**Alleles Are
Different Forms of
the Same Gene!**

**Mutations in a Gene That Change Its Chemical Sequence
& Slightly Alters Its Function (e.g., fruit size, color) and Produce
Allelic Forms & Genetic Variability**

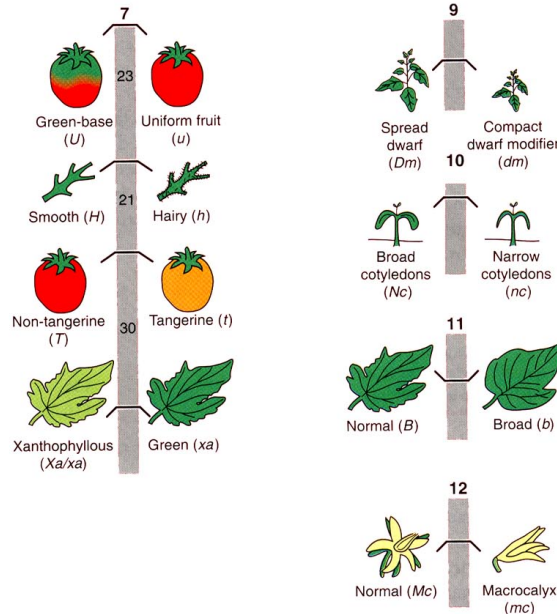
Alleles Reside at the Same Position on a Chromosome Because They Represent the **SAME** Gene

Alleles

Allele Phenotypes Specify Markers For Each Gene Location!

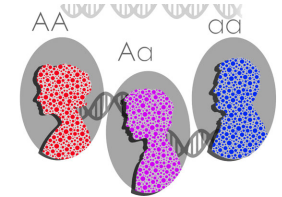


Different Genes



Gene Engineering Can Generate New Forms of Alleles of a Gene and, Therefore, Results in More Genetic Diversity

mutations result in genetic diversity!!!



Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

Gene	Alternative Alleles
 Eye colour	 Brown Blue Emerald Grey
 Hair colour	 Blonde Red Brown Black

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



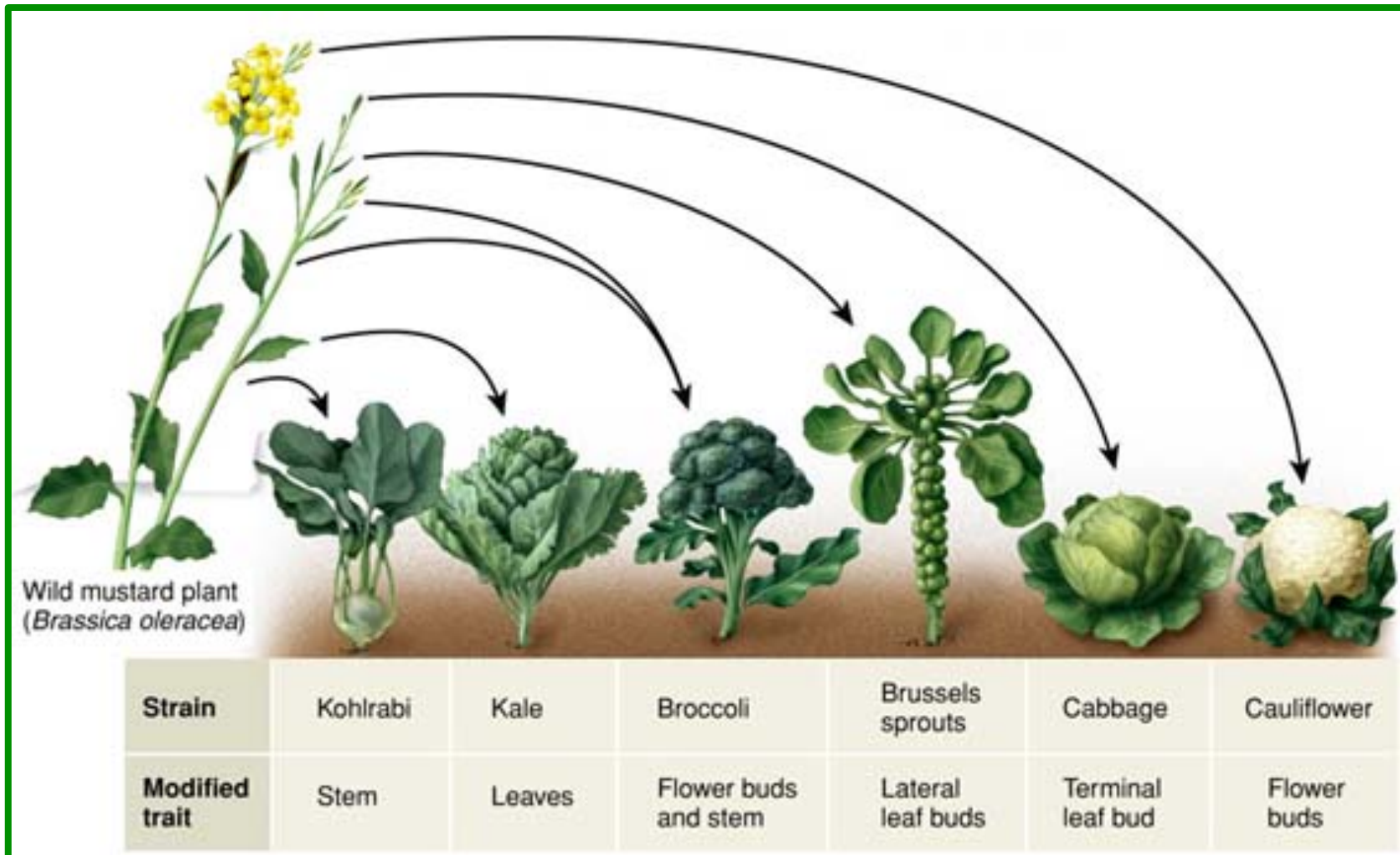
*The Early Tomato “Bioengineers” Selected For Large
Fruit Size Because it Provided More Food!*

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

al·lele ə'lēl/ Noun GENETICS plural noun: alleles

one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.

Engineering Brassica Vegetables From Wild Mustard They Are *GMOs* as *Genes* Were Manipulated By Breeding!!!!!!

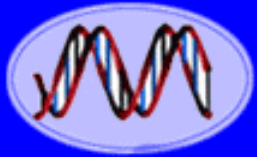


Mutations in *Genes* Controlling Different Plant Organs - e.g., Flowers, Leaves

Domesticated Animals Were Also “Engineered” By Breeding Wild Relatives



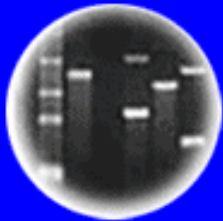
*Manipulating Existing Genetic Variability
Brought About By Chance Mutations!*



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DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

The Problem With Breeding the “Old Fashioned Way”

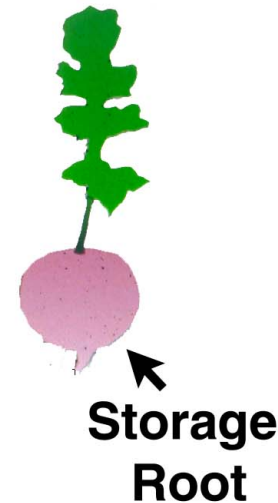
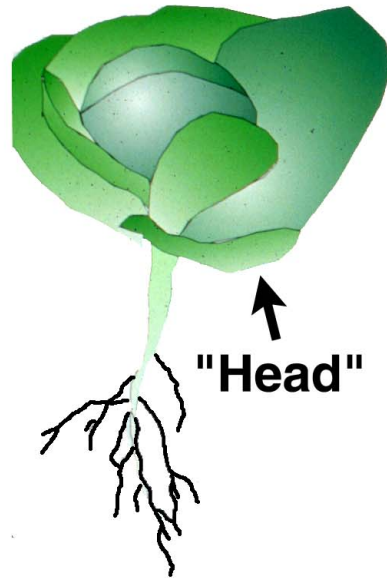
Cannot Predict Results!



The Problem With Breeding the "Old Fashioned Way"

Engineering A Novel Crop By "Wide" Breeding

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

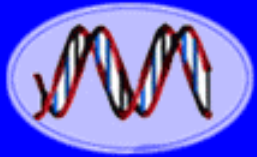


X

???

iko

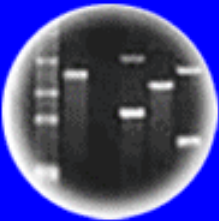
Karpechenko, G.D., 1928. *Polyloid hybrids of Raphanus sativus L. X Brassica oleracea L.* Zeitschrift für induktive Abstammungs- und Vererbungslehre 48, 1-85.



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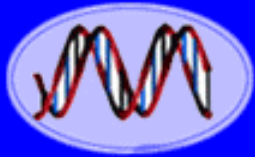
DNA Fingerprinting



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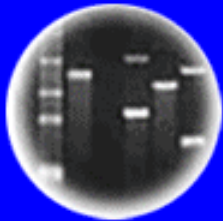
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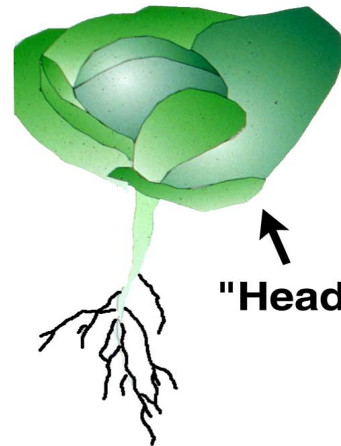


Plants of Tomorrow

Engineering A Novel Crop By "Wide" Breeding

Cabbage (*Brassica*)

Radish (*Raphanus*)



"Head"



Storage
Root

X

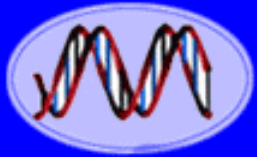


Radish
leaves!!!

RaphanoBrassica

Cabbage
roots!!!

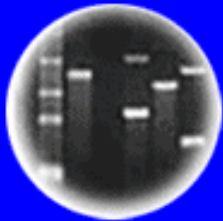
*Results Show the Unpredictability of Classical Breeding Approaches!!
Compare With the Modern Genetic Engineering Examples Shown
Previously*



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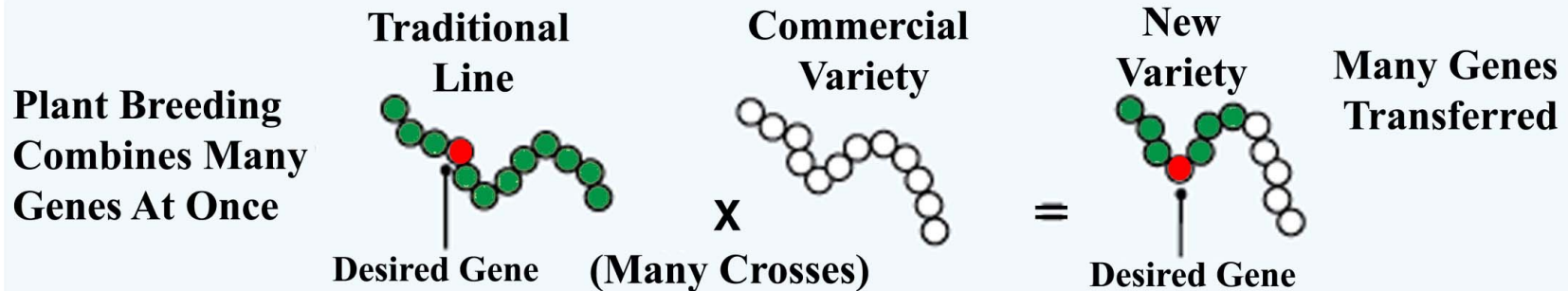
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Genetic Engineering is a TECHNIQUE!

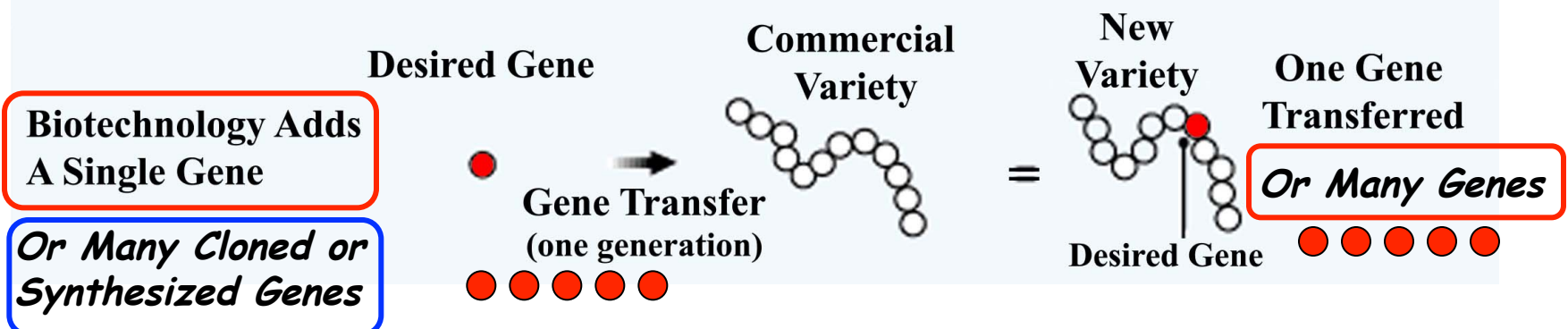
*How Do Classical Genetic Engineering
Methods Differ From Those Using
DNA and 21st Century Technologies?*

Classical vs. DNA or Molecular Genetic Engineering Techniques

TRADITIONAL PLANT BREEDING



PLANT BIOTECHNOLOGY



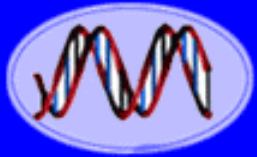
All Manipulate Genes - But in Different Ways!!

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?

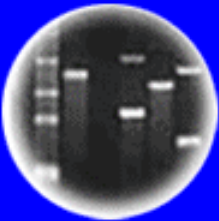
1. Any Gene From Any Organism Can Be Used In Any Organism - There Are No Breeding Barriers (e.g., genes of all sequenced genomes)
2. New Genes Can Be Engineered - Genes That Work Better and/or Produce New Proteins (i.e., create new genetic variability and/or alleles)
3. Existing Genes Can Be Engineered 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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The "Ultimate" in Genetics Engineering is to Synthesize a Genome

