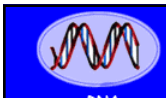

DNA
Genetic Code of Life


Entire Genetic Code of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

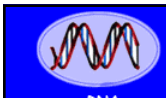
# HC70A, SAS70A, & PLSS599
# Winter 2022
# Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, John Harada, & Channapatna Prakash

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

UCLA TUSKEGEE UNIVERSITY UCDAVIS UNIVERSITY OF CALIFORNIA

1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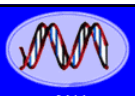
# THEMES

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

2



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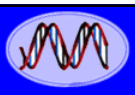
## Last Week's Lecture…a Reminder


3



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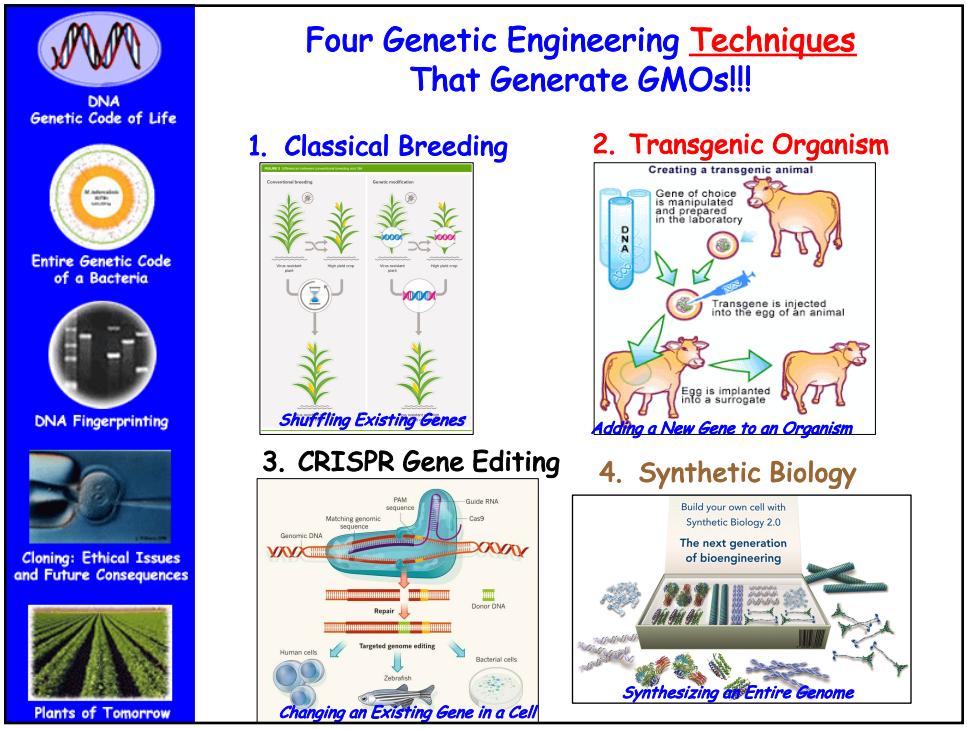
## Four Genetic Engineering Techniques That Generate GMOs!!!

4

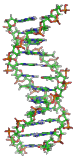


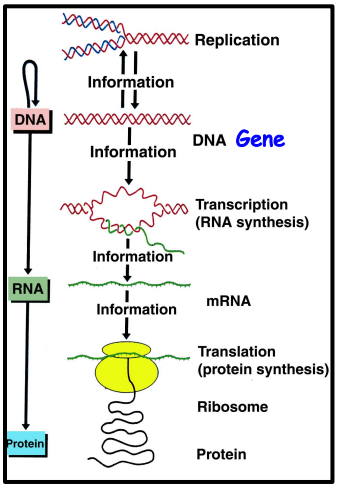

5

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

1. DNA is DNA is DNA Regardless of Source (Berg, Cohen,, & Boyer)

2. Can Intervene in Cellular Genetic Processes – DNA to RNA to Protein (Trait)



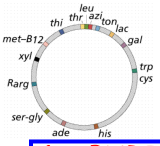




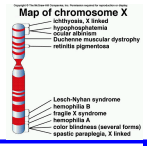
3. All Organisms Use The SAME Processes. "RULES", & Genetic Code to Generate Traits!!

6

# "Why" Clone Genes From An Organism's Genome?
## An Essential HC70A Concept!








1. PURIFY *Individual* Genes From the Genome ( e.g.,One of 25,000 Human Genes – Globin, Insulin, Growth Hormone)
2. AMPLIFY The Gene Using Plasmids in Bacterial Cells to Obtain Large Amounts of Specific DNA Fragments 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
    8. And More……

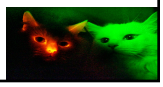
7

# 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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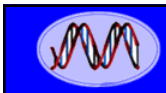
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Entire Genetic Code of a Bacteria


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Plants of Tomorrow

# The Scientific Method

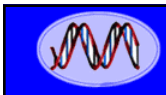


**Scientific Knowledge is Based on Observation, Hypothesis Testing, Rigorous Experimentation, Results, Facts, and Verification**

*What Are the Data?*
*What Is the Evidence?*

Science is NOT "Hocus Pocus" or Based on Opinions and Beliefs
*Uncovers Objective Truths*

9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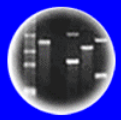
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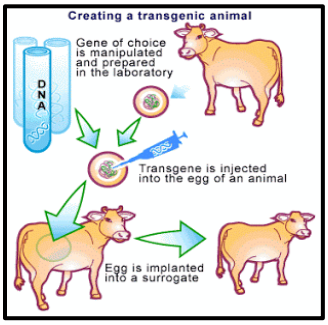
# What Can Be Done With Modern Genetic Engineering?

## Some Examples

### Transgenic Organisms

Adding a New Gene To an Organism's Chromosomes (Genome)

*Method Two*




10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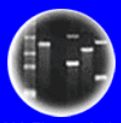
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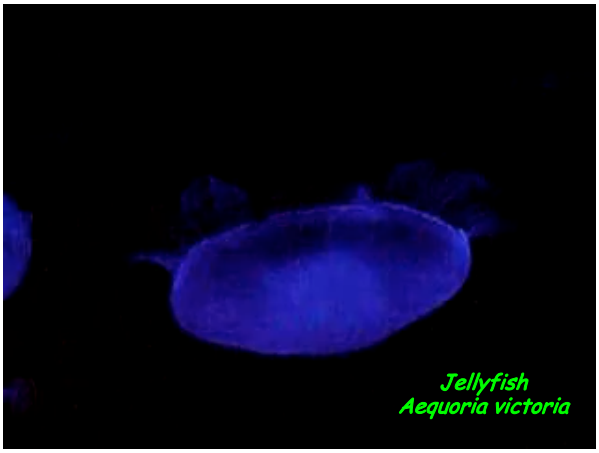
# Using a Jellyfish Gene to Engineer *Glowing* Bacteria, Animals, and Plants!!!!

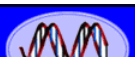



## *Green Fluorescence Protein (GFP)*

(238 amino acids)

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

11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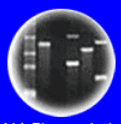
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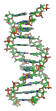
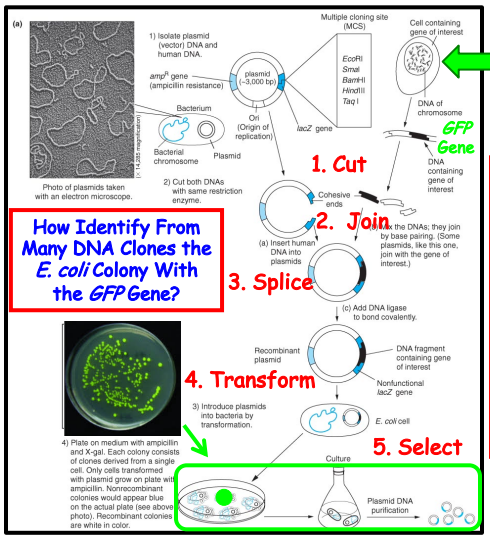
# Using Recombinant DNA to *Clone & Find* the Jellyfish GFP Gene






How Identify From Many DNA Clones the *E. coli* Colony With the *GFP* Gene?

1. Clone Jelly Fish *GFP* Gene
2. Insert *GFP* Gene Into Plasmid Vector "Behind" a Specific "Switch"
3. Transform Into *E. coli*
4. Select Cells With Recombinant Plasmid Determine if *GFP* Gene is Active

## *E. coli* GFP GMO!!!!

12

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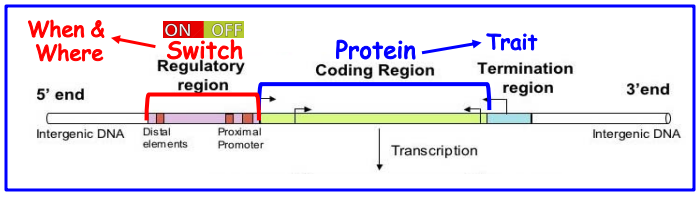
# Anatomy of a Typical Gene



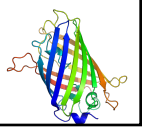

Can Engineer Genes Like *Lego Pieces*

Specific DNA Sequences Perform Specific Functions

Essential HC70A Concept

Bacterial Switch

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



13

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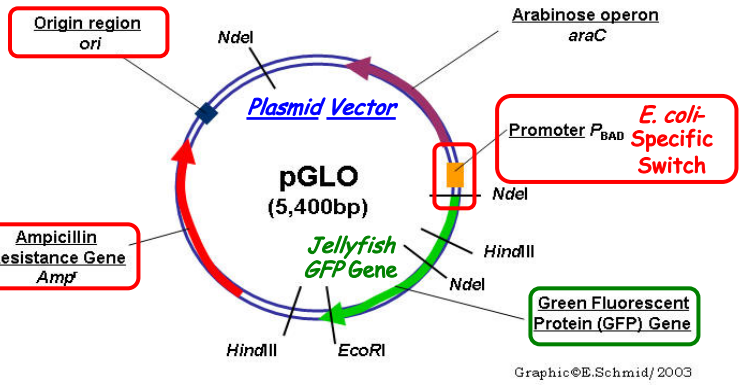
Plants of Tomorrow

# A Recombinant Plasmid Containing the GFP Gene

*How Make it Active in Living Cells?*

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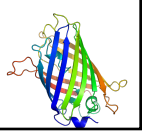
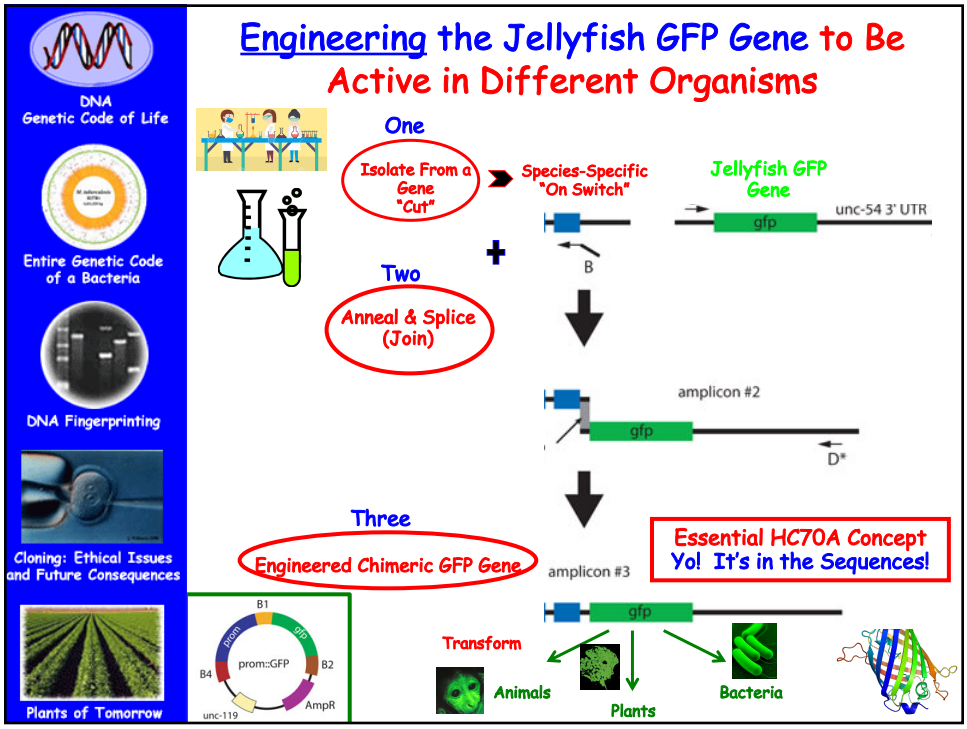


14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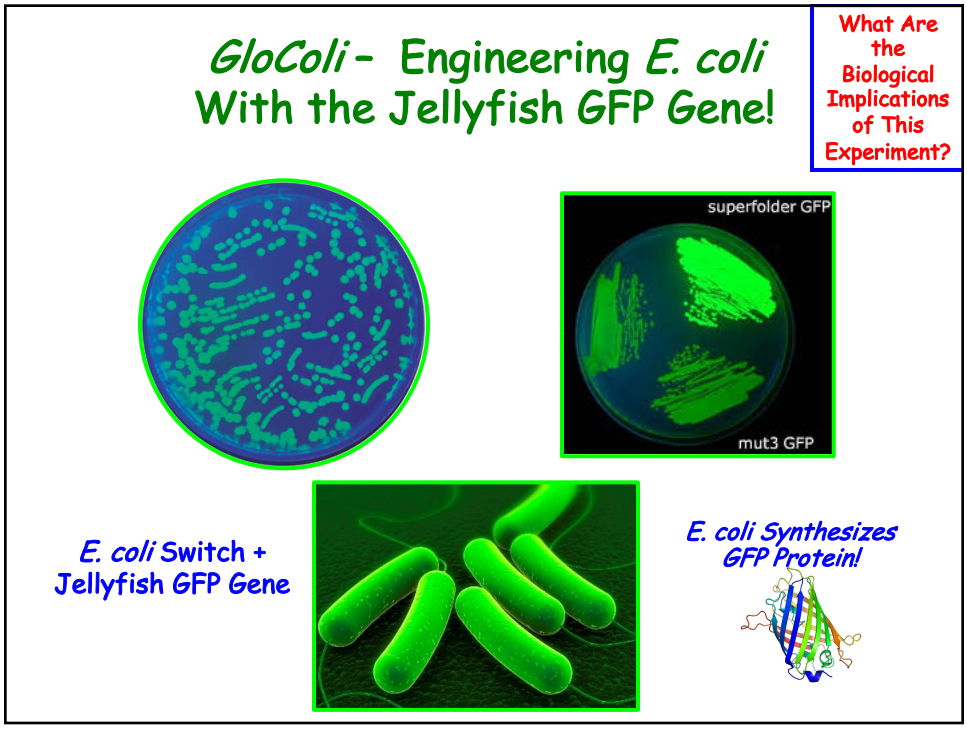
Engineering the Jellyfish GFP Gene to Be Active in Different Organisms
One
Isolate From a Gene "Cut"
Species-Specific "On Switch"
Jellyfish GFP Gene
gfp
unc-54 3' UTR
B
Two
Anneal & Splice (Join)
amplicon #2
gfp
D*
Three
Engineered Chimeric GFP Gene
amplicon #3
gfp
Essential HC70A Concept
Yo! It's in the Sequences!
prom::GFP
B1
B2
B3
B4
AmpR
unc-119
Transform
Animals
Plants
Bacteria

15


GloColi - Engineering E. coli With the Jellyfish GFP Gene!
What Are the Biological Implications of This Experiment?
superfolder GFP
mut3 GFP
E. coli Switch + Jellyfish GFP Gene
E. coli Synthesizes GFP Protein!

16

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

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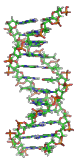
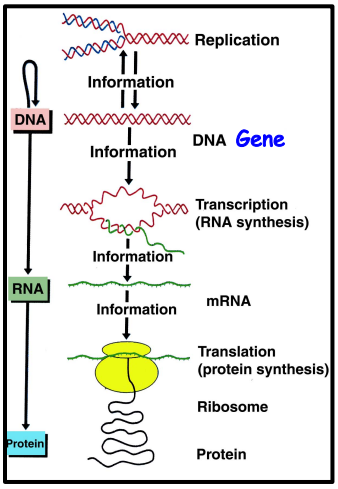
2. Can Intervene in Cellular Genetic Processes – DNA to RNA to Protein (Trait)



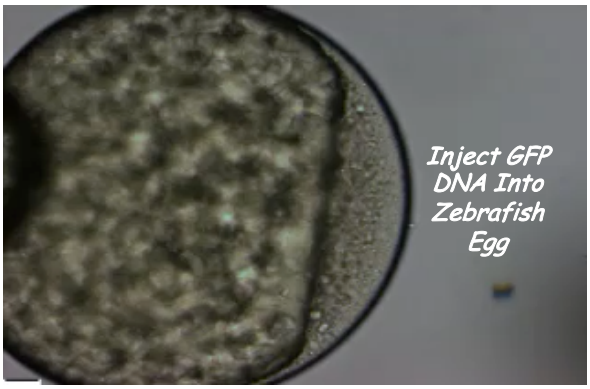




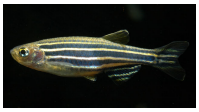
3. All Organisms Use The SAME Processes. "RULES", & Genetic Code to Generate Traits!!

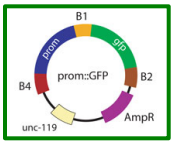
17

## Engineering a "GloFish"




Zebrafish
Danio rerio

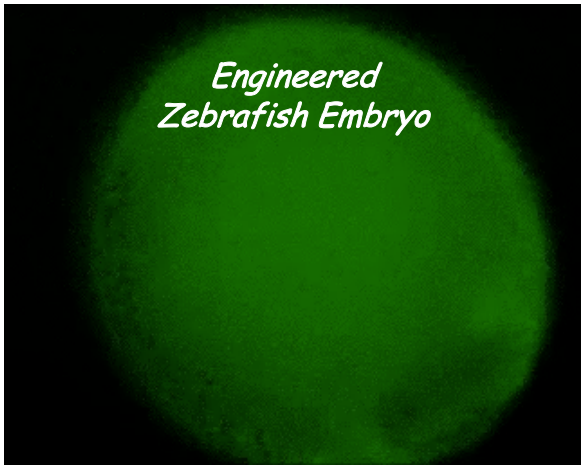





Using Genetic Engineering To Insert An Engineered Jellyfish GFP Gene into a Zebrafish Egg!
What Switch Used?

18

# A "GloFish"Embryo!!




Zebrafish - Danio rerio

19

# Adult Genetically Engineered "GloFish!!"

Note Different Fluorescing Colors – Due to Different Engineered Jellyfish Genes

20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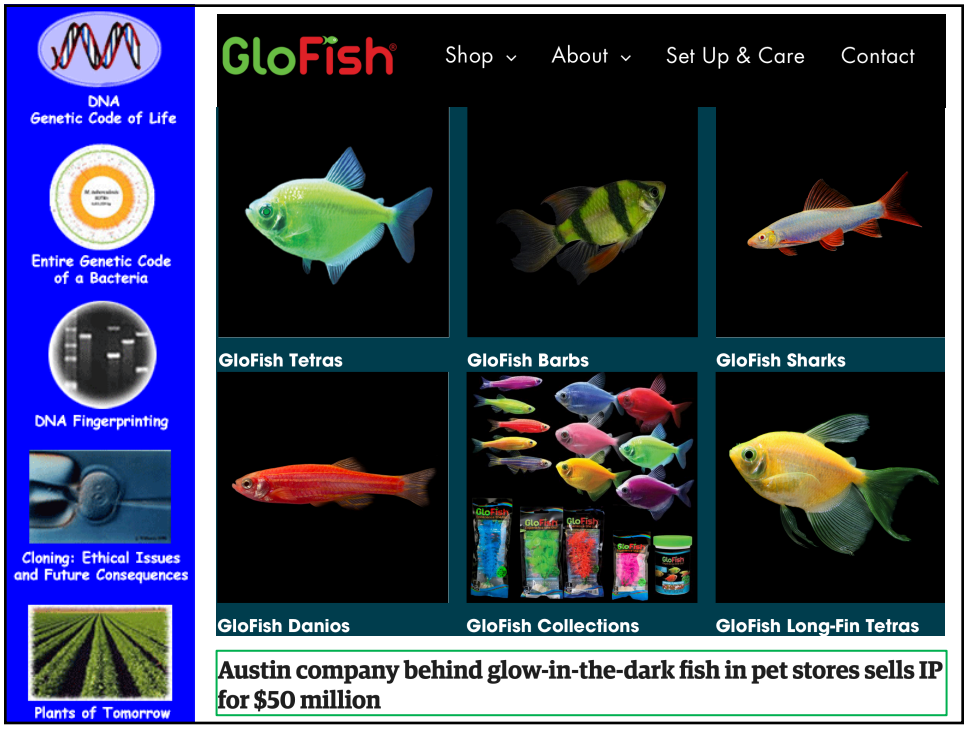
Plants of Tomorrow
Can GloFish Can Be Purchased In California?
• Cal. Depart. of Fish and Game Code § 15007 (2007)
Regulation Makes it illegal to spawn, cultivate, or incubate any transgenic fish in the state controlled waters of the Pacific Ocean.
• Cal. Depart. of Fish and Game Code Ruling (2015)
The Dept. of Fish and Game made an exception to Section 1.92 that allows 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!!
GloFish
petco
GloFish

21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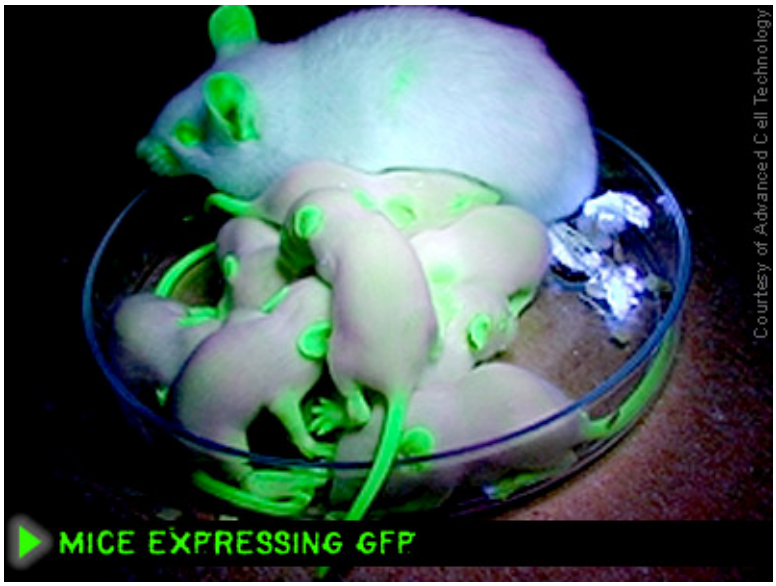
GloFish
Shop
About
Set Up & Care
Contact
GloFish Tetras
GloFish Barbs
GloFish Sharks
GloFish Danios
GloFish Collections
GloFish Long-Fin Tetras
Austin company behind glow-in-the-dark fish in pet stores sells IP for $50 million

22

## How About a Glo Fly!

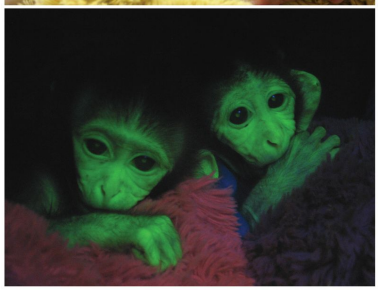
23

## What About "Glo Mice!!!"




24

## And Glo Monkeys, Cats and Pigs as Well!!!



*Humans?*

25

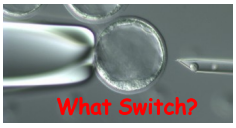
## Engineering a Glo Plant With the <u>Same</u> Jellyfish Gene!!!

*What Are the Biological Implications of This Experiment?*

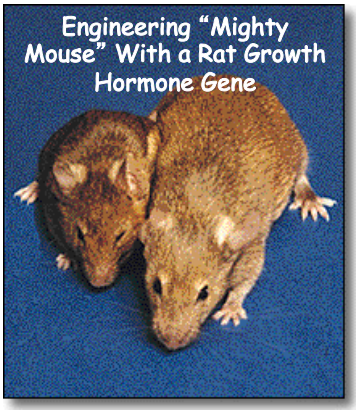
26

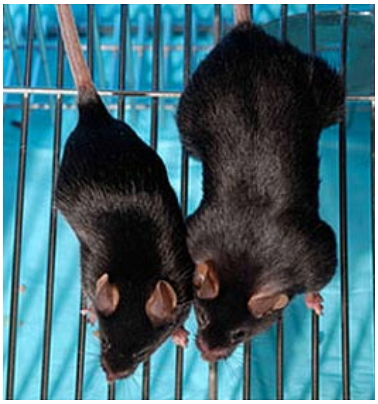
## Dramatic growth of mice that develop from eggs microinjected with metallothionein–growth hormone fusion genes

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

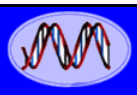



Nature, December, 1982
40 Years Ago!!!!!!!!



27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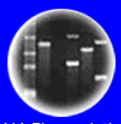
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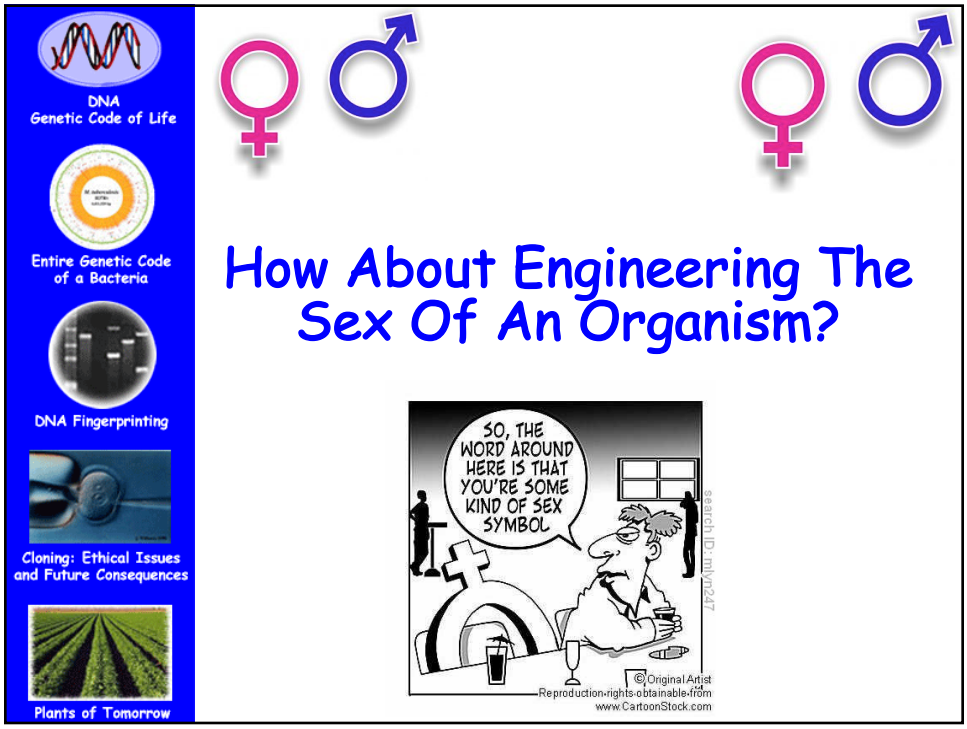
## *Genetic Engineering Faster Growing Salmon For More Productive Aquafarms!*


FDA Approves Application for AquaBounty Salmon Facility in Indiana

**GMO salmon gets FDA green light to be sold in the US**

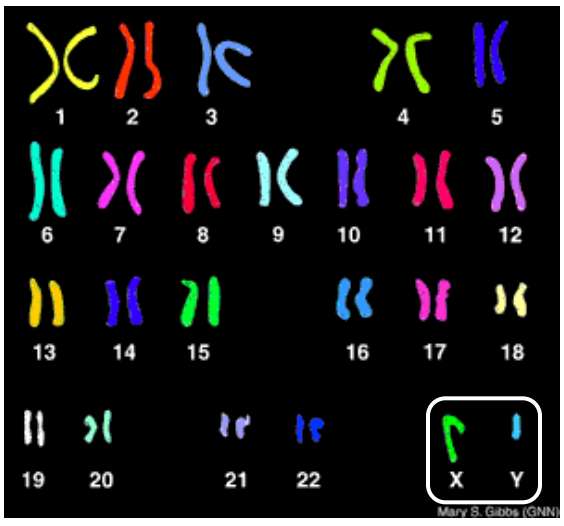
U.S. FOOD & DRUG ADMINISTRATION

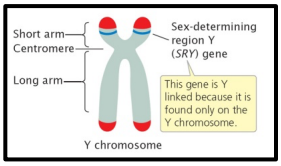
28

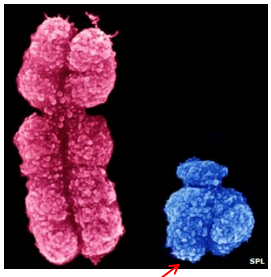



29

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



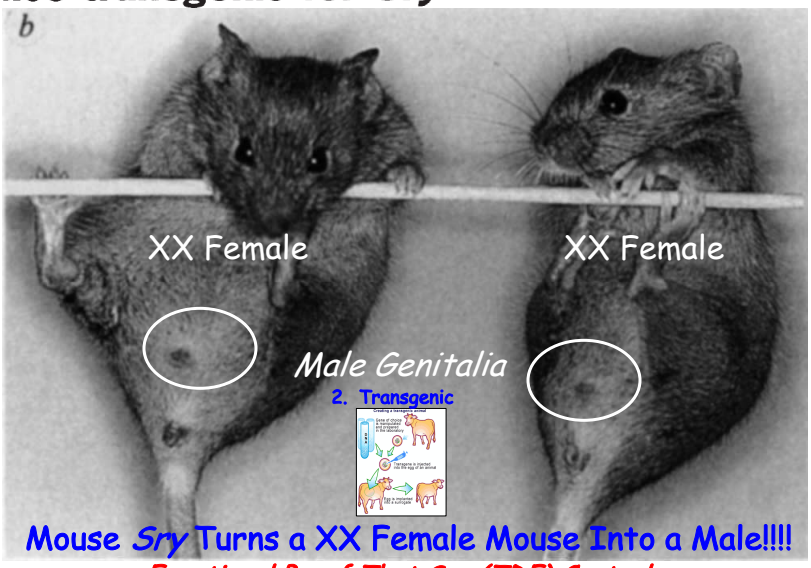

*Male SRY Gene*
*(Sex Determining Region Y)*
Regulates Other Genes
Turns on Switches

*The Human SRY (Testes Determining Factor) Gene Controls Male Sex Development*

30

# Male development of chromosomally female mice transgenic for *Sry*

Nature, May 9, 1991




2. Transgenic



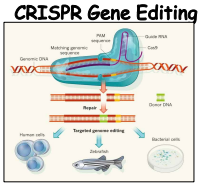
**Mouse *Sry* Turns a XX Female Mouse Into a Male!!!!**

***Functional Proof That Sry (TDF) Controls Male Sex Development***

31

# Engineering Male XY Pigs Into Female Pigs

3. CRISPR Gene Editing

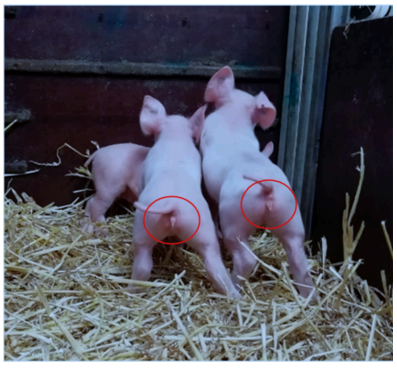


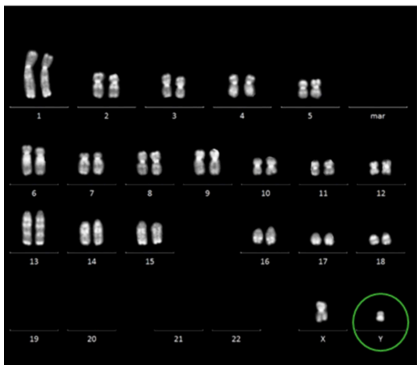
**Use *Gene Editing* to Mutate the SRY Gene**

Knockout of the HMG domain of the porcine SRY gene causes sex reversal in gene-edited pigs PNAS, December, 2020




**30 years later!!!!!!**

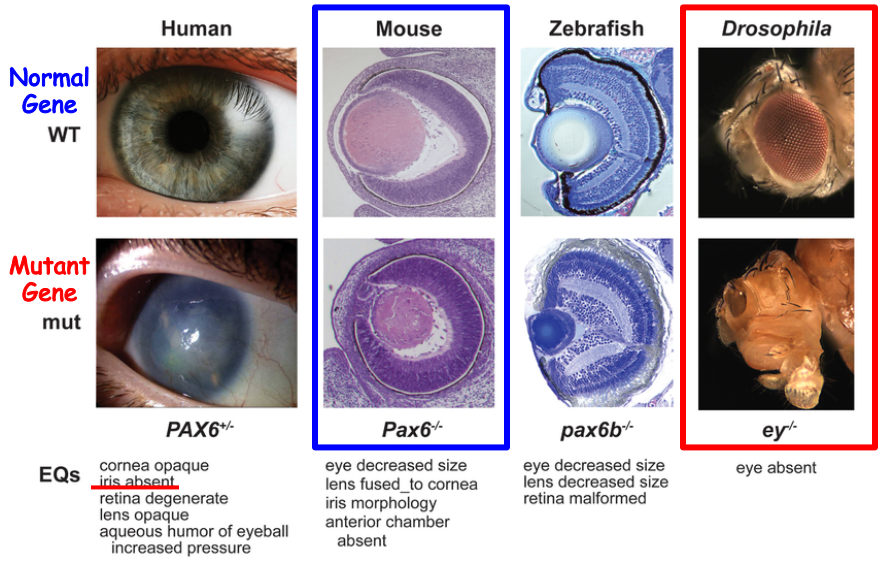




32


33

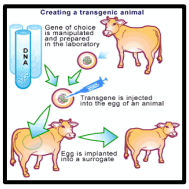
## Using Genetic Engineering to Change Body Architecture-Engineering Eyes on a Fly's Leg With a Single Gene – The *eyeless* Gene!




34

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

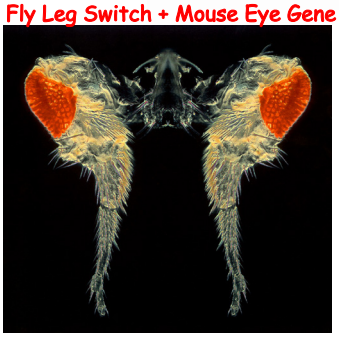
*Science 267, 1788, 1995*



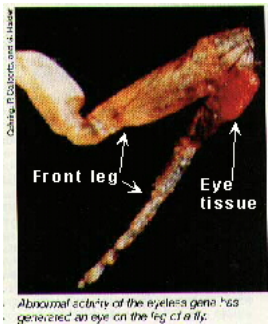

*Mouse PAX-6 Gene (eyeless in flies) Engineered To Work*

*Different Fly Regions*

Fly Leg Switch + Mouse Eye Gene

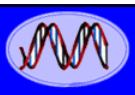


What Does This Experiment Tell Us About Mouse & Fly Eye Genes?



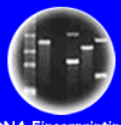

Abnormal activity of the eyeless gene has generated an eye on the leg of a fly.

35



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

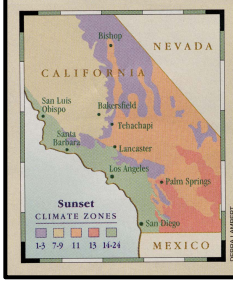
Plants of Tomorrow

## 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. ◆

36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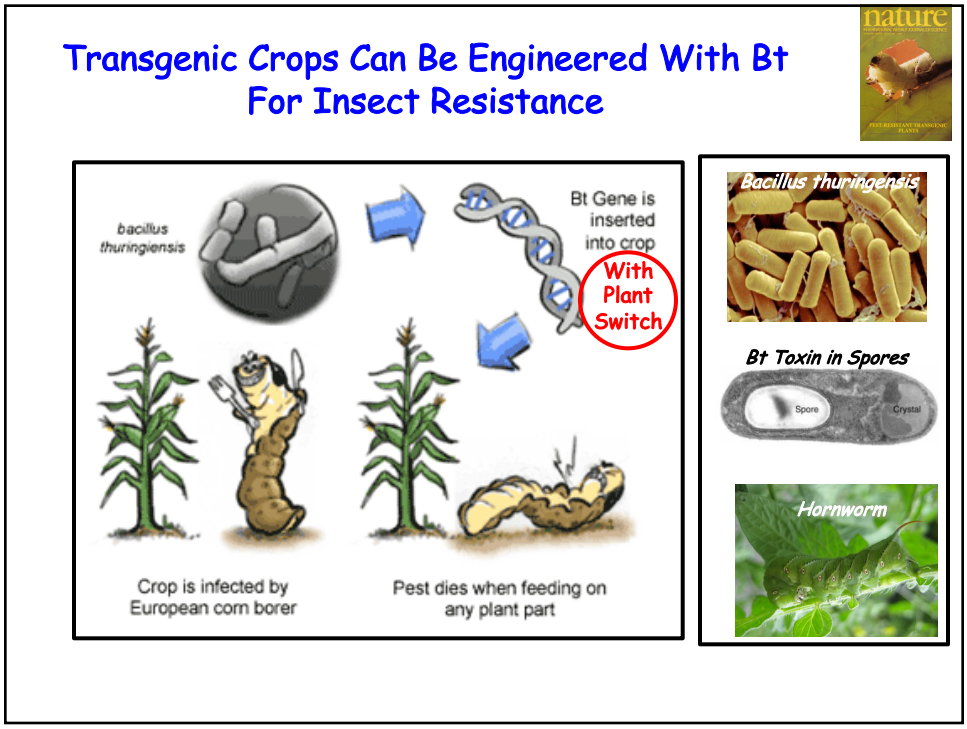
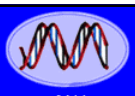
How to Use Bt Pesticide as an Organic Pest Control
Learn how to use Bt pesticide to kill cabbage worms, tomato hornworms and other pests in your organic vegetable garden.
By Barbara Pleasant
April 24, 2013
Bt is one of the safest natural pesticides you can use to control caterpillar pests without harming beneficial insects.
Photo Courtesy Safe Brand
Safer
Caterpillar Killer
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%
Total: . . . . . 100.00%
*The percent active ingredient does not indicate product performance and potency measurements are not federally standardized.

37


Transgenic Crops Can Be Engineered With Bt For Insect Resistance
nature
bacillus thuringiensis
Bt Gene is inserted into crop
With Plant Switch
Crop is infected by European corn borer
Pest dies when feeding on any plant part
Bacillus thuringiensis
Bt Toxin in Spores
Spore
Crystal
Hornworm

38



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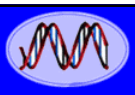
## Genetic Engineering a Plant to Resist Worms! Implications For Agriculture


39



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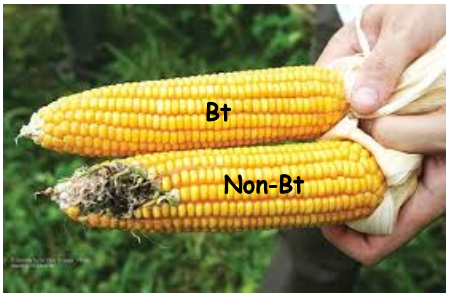
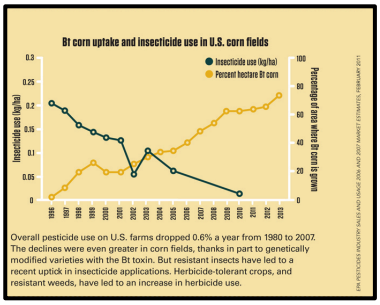
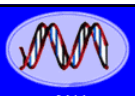
## Adoption of Bt Corn By US Farmers Has Reduced the Use of Pesticides!!!!




40



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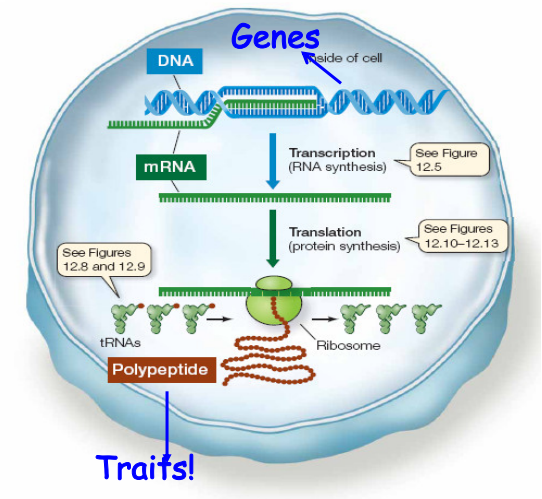
## What Can We Infer FROM These Genetic Engineering Experiments About How Genes "Work" and Genetic Processes in All Living Organisms?



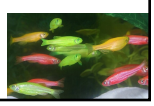

41

## Observations and Inferences From Genetic Engineering Experiments

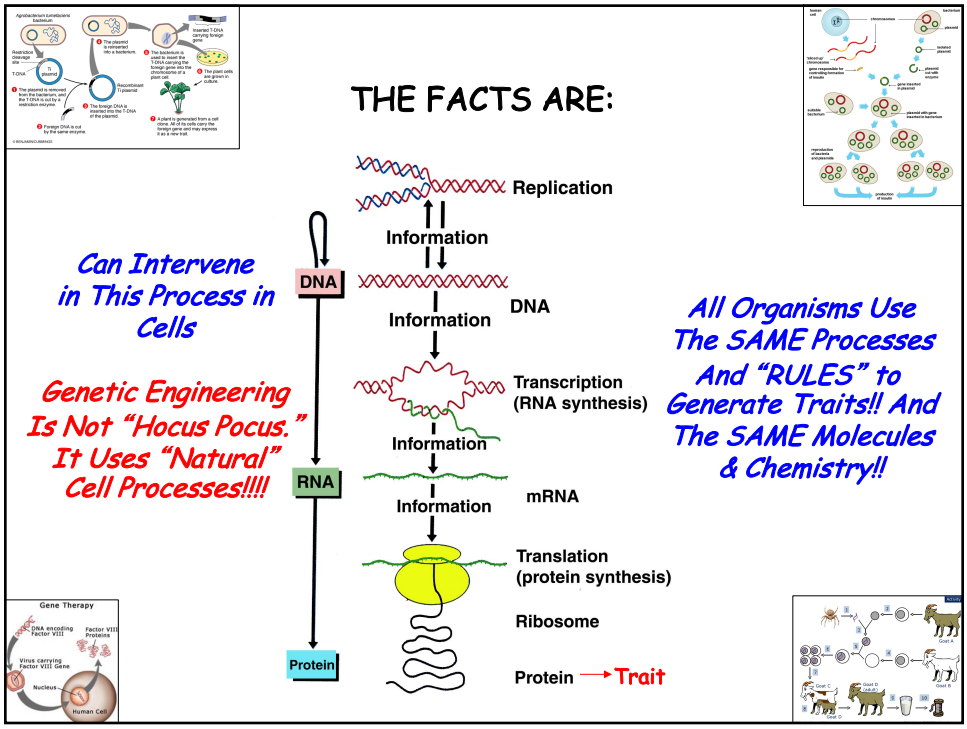
1. ***Genes Can Work Independently of Each Other*** *E.G. - The Jellyfish Fluorescence Gene Works Perfectly in a Variety of Organisms*

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.*



42


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!!
DNA
RNA
Protein
Replication
Information
DNA
Transcription (RNA synthesis)
mRNA
Translation (protein synthesis)
Ribosome
Protein → Trait
Gene Therapy

43


DNA
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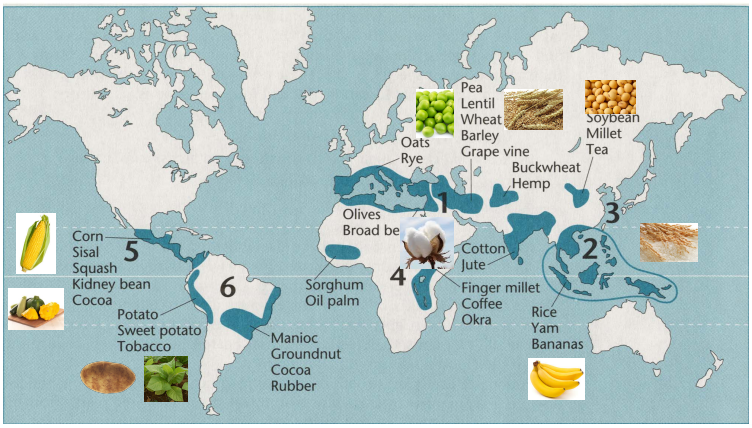
There is Nothing New About Genetic Engineering!
Manipulating Genes IS Manipulating Genes No Matter What Technology or Techniques Are Used!!
1. Classical Breeding
New Gene Combinations

44

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

**Regions Where Major Crops Were Established**



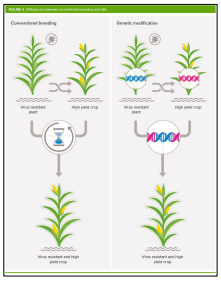

*Breeding Involves Gene Manipulation Using EXISTING Genetic Variability!*

45


46

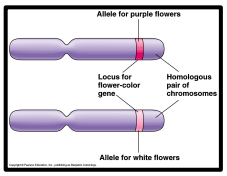
## Breeding Uses Natural Genetic Variability of Genes As Raw Material - *Variability Generated by Mutations*

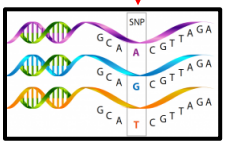
**Classical Breeding – Method One**



**Alleles Are Different Forms of the Same Gene Generated By Spontaneous Mutations!**

**Tomato Genetic Diversity**


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

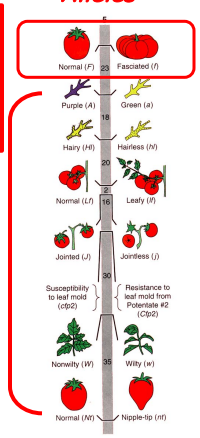
47

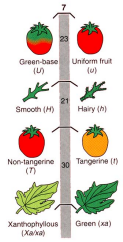
## *Alleles Reside at the Same Position on a Chromosome Because They Represent the SAME Gene*

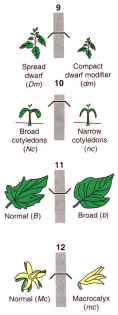
*Alleles*

**Allele Phenotypes Specify Markers For Each Gene Location!**

*Different Genes*



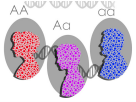



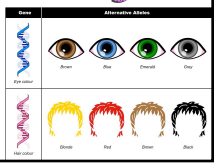




*mutations result in genetic diversity!!!*

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

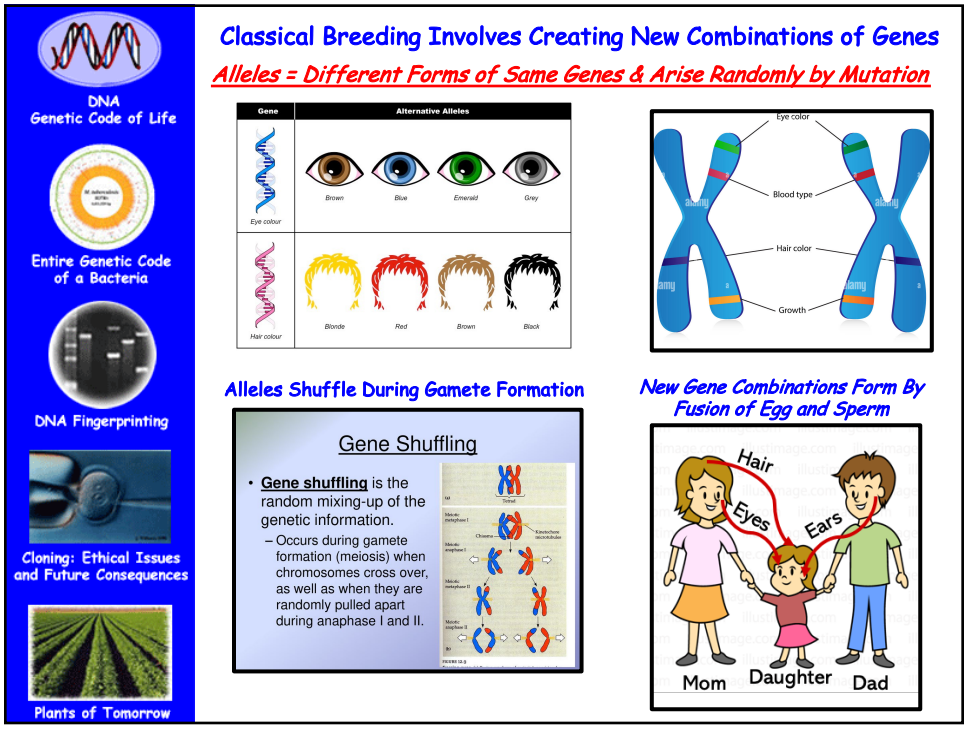
**Human Eye Colors**



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

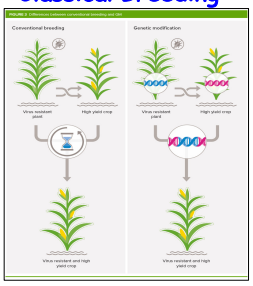
48




49

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

1. Classical Breeding



New Allele Combinations

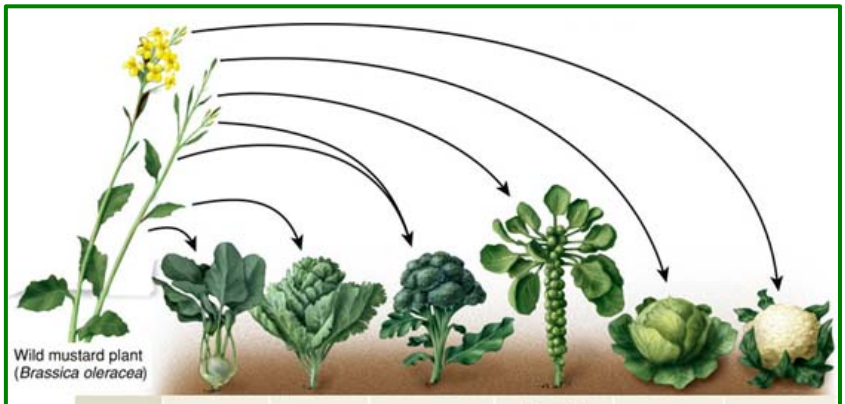
*The Early Tomato "Bioengineers" Selected For Large Fruit Size Because it Provided More Food!*

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

*al·lele ə ˈlēl/ 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.*

50

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




| Strain | Kohlrabi | Kale | Broccoli | Brussels sprouts | Cabbage | Cauliflower |
|---|---|---|---|---|---|---|
| Modified trait | Stem | Leaves | Flower buds and stem | Lateral leaf buds | Terminal leaf bud | Flower buds |

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

51

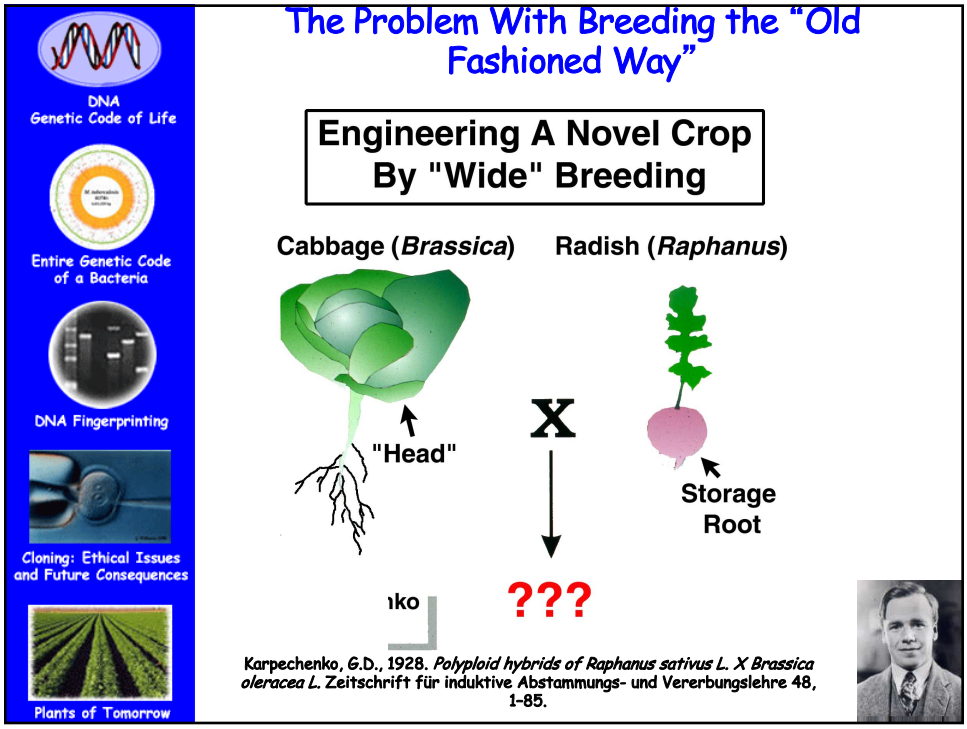
## Domesticated Animals Were Also "Engineered" By Breeding Wild Relatives

*Manipulating Existing Genetic Variability Brought About By Chance Mutations to Make New Allele Combinations That Don't Exist Naturally*

52


DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
The Problem With Breeding the "Old Fashioned Way"
Cannot Predict Results!

53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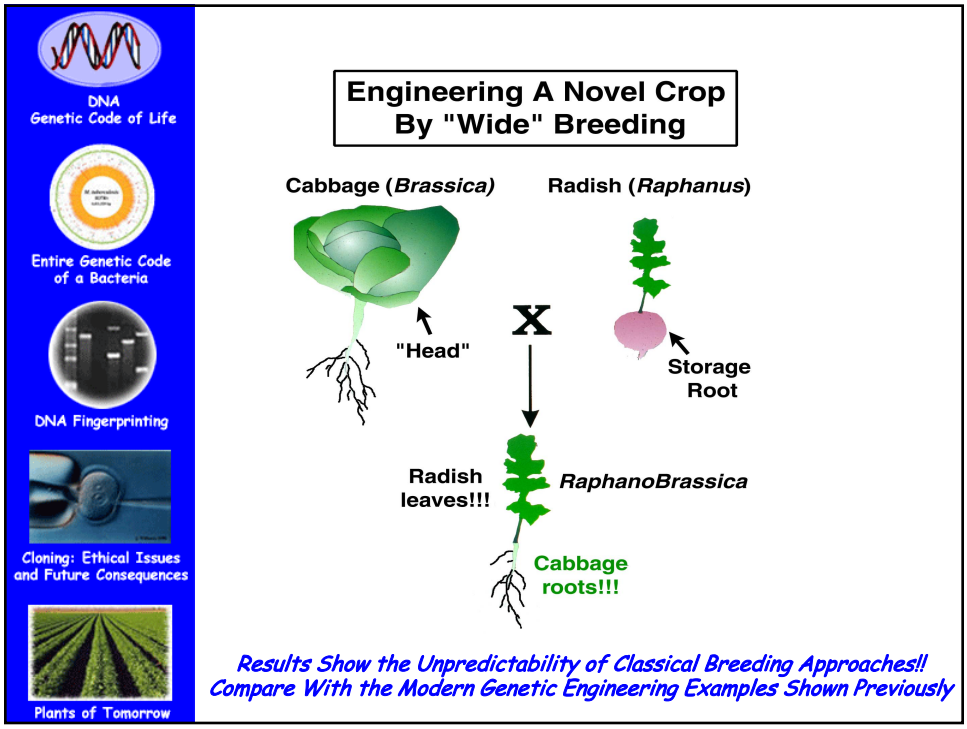
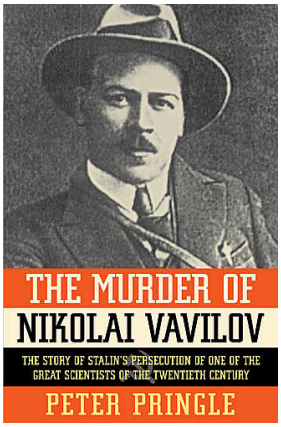
The Problem With Breeding the "Old Fashioned Way"
Engineering A Novel Crop By "Wide" Breeding
Cabbage (Brassica)
Radish (Raphanus)
"Head"
X
Storage Root
???
Karpechenko, G.D., 1928. Polyploid hybrids of Raphanus sativus L. X Brassica oleracea L. Zeitschrift für induktive Abstammungs- und Vererbungslehre 48, 1-85.

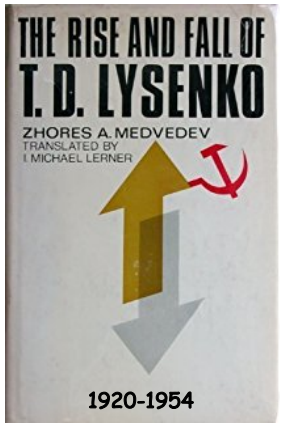
54



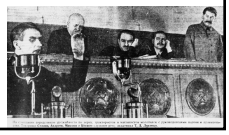

55

## How Ideology Destroys Science & Leads to Horrific Tragedy

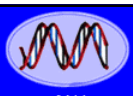






56



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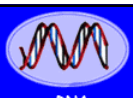
## Karpechenko's Dream Come True!!!!

*Grafting Potato and Tomato Plants!*

57



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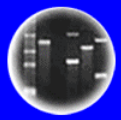

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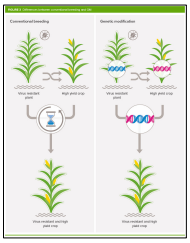
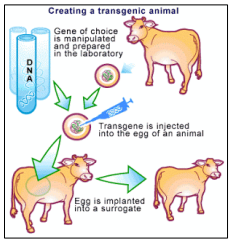
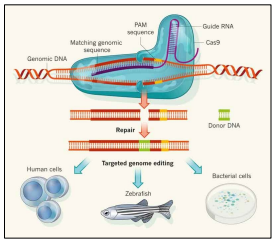
Plants of Tomorrow

## Genetic Engineering is a TECHNIQUE!

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





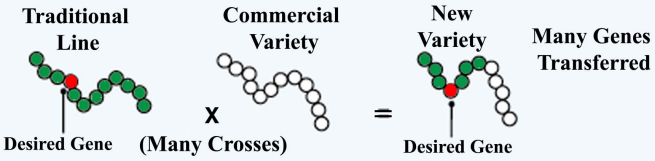




58

# Classical vs. DNA or Molecular Genetic Engineering Techniques

## TRADITIONAL PLANT BREEDING

Plant Breeding Combines Many Genes At Once

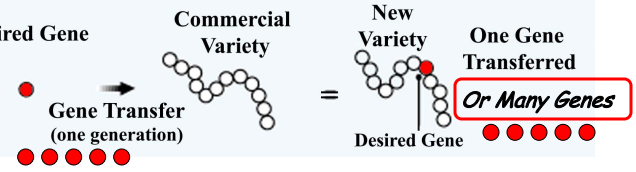



## PLANT BIOTECHNOLOGY

Biotechnology Adds A Single Gene

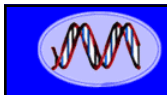
*Or Many Cloned or Synthesized Genes*

*Or Edit One or Many Endogenous Genes!!!*

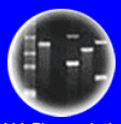



*All Manipulate Genes – But in Different Ways!!*

59


DNA
Genetic Code of Life

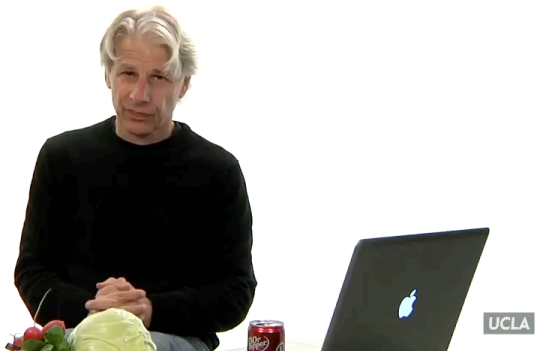

Entire Genetic Code of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences

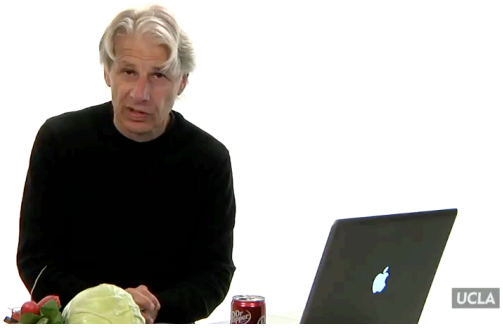

Plants of Tomorrow

## Classical Breeding Moves Thousands of Genes At Once With Unpredictable Outcomes




60


## Genetic Engineering With DNA Transfers One Gene at a Time, or Edits One Gene, With Predictable Outcomes




61

## 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)

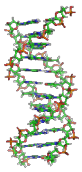
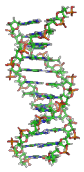
62

## 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)

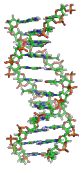
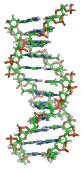
There Are No Genetic Limits!

63

## We Live in The Age of DNA & Genetic Engineering!

Understanding Genetic Engineering
*Requires a Basic Understanding of Genes And How They Work*

64