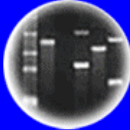


DNA  
Genetic Code of Life



Entire Genetic Code  
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues  
and Future Consequences



Plants of Tomorrow

# HC70A & SAS70A Winter 2018 Genetic Engineering in Medicine, Agriculture, and Law

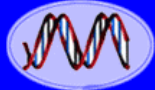
**Professors Bob Goldberg,  
John Harada, and Channapatna Prakash**

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

**UCLA**

**TUSKEGEE**  
UNIVERSITY

**UC DAVIS**  
UNIVERSITY OF CALIFORNIA



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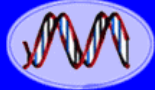
Cloning: Ethical Issues  
and Future Consequences



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## **THEMES**

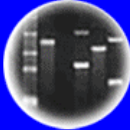
1. The Scientific Process Revisted
2. The Significance of Genetic Engineering
3. What Are the Tools of Genetic Engineering?
4. What Can Be Done With Genetic Engineering-  
Some Examples
5. What Does Genetic Engineering Tell Us About  
Basic Genetic Processes in All Organisms?
6. Genetic Engineering - Anything New?
7. Are Vegetables Engineered - Demonstration
8. Science & Ideology - A Tragic Combination
9. Classical vs. 21st Century Genetic Engineering
10. Understanding How Genetic Engineering Uses  
Natural Rules of the Cell (i.e., It Isn't  
Magic)!



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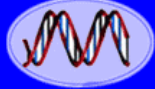


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# WHAT IS SCIENCE?

**WEBSTER:** Knowledge about, or study of, the natural world based on facts learned through experiments and observation.

Technology, like Genetic Engineering, is the application of science knowledge



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# HOW IS SCIENCE CARRIED OUT?



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

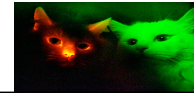
*What Are the Data?  
What Is the Evidence?*

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

# 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!!*



  
DNA  
Genetic Code of Life

  
Entire Genetic Code  
of a Bacteria

  
DNA Fingerprinting

  
Cloning: Ethical Issues  
and Future Consequences




  
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## What "Tools" Do You Need For Genetic Engineering?



# What "Tools" Are Needed For Genetic Engineering?

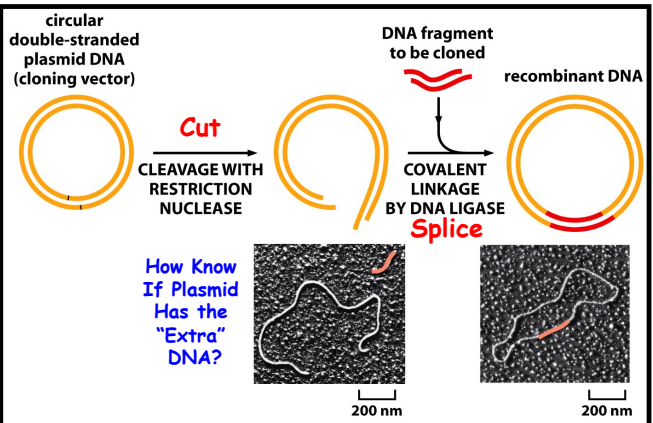
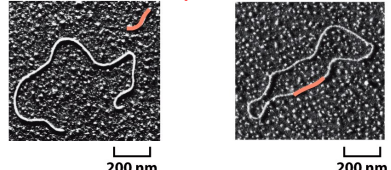


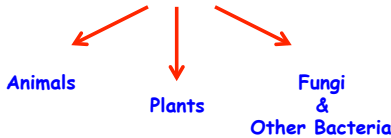
Diagram illustrating the cloning process:

- Start with a **circular double-stranded plasmid DNA (cloning vector)**.
- Perform a **Cut** using **CLEAVAGE WITH RESTRICTION NUCLEASE**.
- Insert a **DNA fragment to be cloned**.
- Perform **COVALENT LINKAGE BY DNA LIGASE** (Splice) to create **recombinant DNA**.

How Know If Plasmid Has the "Extra" DNA? 

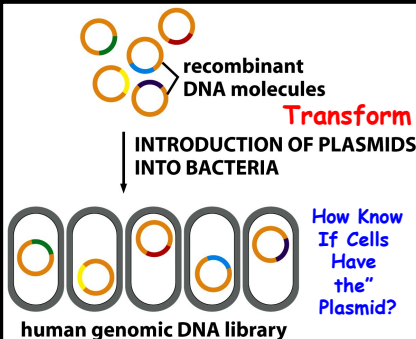
1. DNA
2. Vector (e.g., Plasmids)
3. Enzymes
  1. Restriction Enzymes (Cut & Create Sticky Ends)
  2. Ligase Enzyme (Splice)
  3. Terminal Transferase (Sticky Ends)
4. Cells (*E. coli*)

Lab Engineered DNA



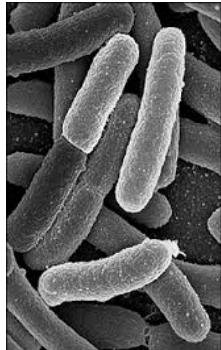
**Transform**

INTRODUCTION OF PLASMIDS INTO BACTERIA



human genomic DNA library

How Know If Cells Have the "Plasmid?"





DNA  
Genetic Code of Life



Entire Genetic Code of a Bacteria



DNA Fingerprinting



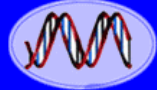
Cloning: Ethical Issues and Future Consequences



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## What Can Be Done With Genetic Engineering?

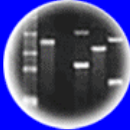
### Some Examples



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Entire Genetic Code  
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues  
and Future Consequences



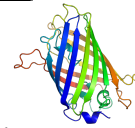
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# Using a Jellyfish Gene to Engineer Glowing Bacteria, Animals, and Plants!!!!



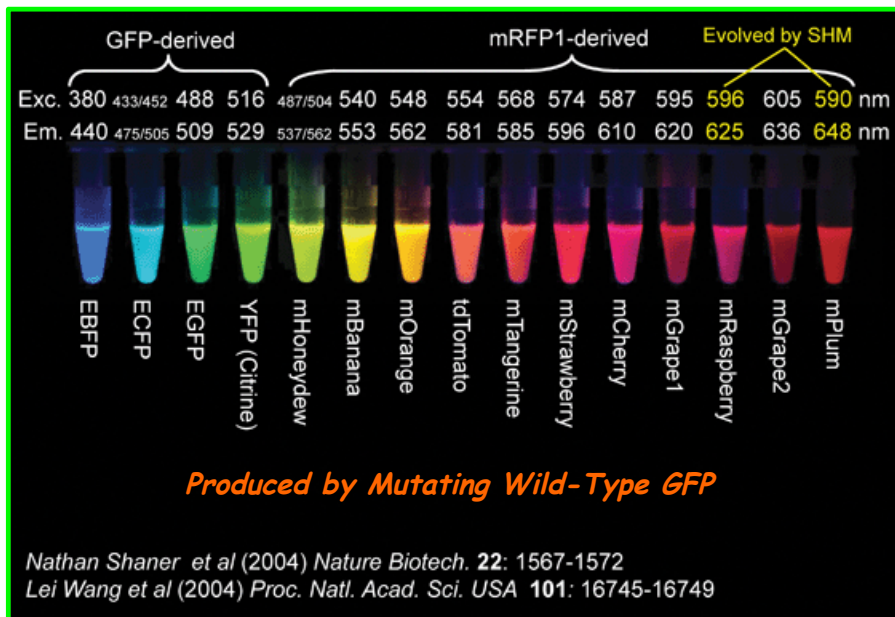
*Aequoria victoria*

**Green Fluorescence Protein (GFP)**  
(238 amino acids)



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

## There Are Many Different Kinds of Fluorescing Proteins!



**DNA Genetic Code of Life**

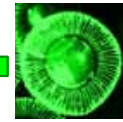
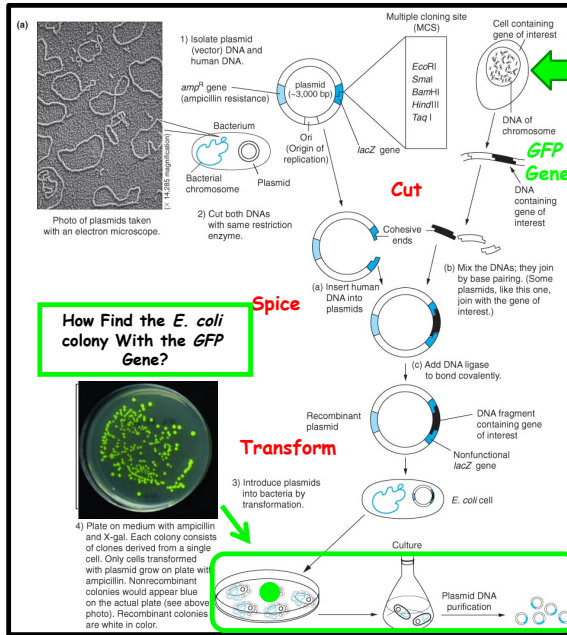
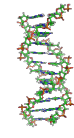
**Entire Genetic Code of a Bacteria**

**DNA Fingerprinting**

**Cloning: Ethical Issues and Future Consequences**

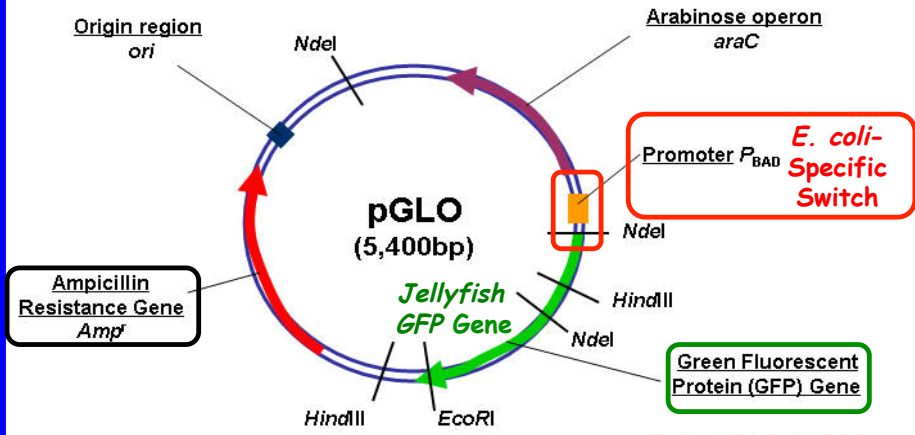
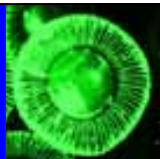
**Plants of Tomorrow**

# Using Recombinant DNA to Isolate the Jellyfish GFP Gene



# A Recombinant Plasmid Containing the GFP Gene

*How Make it Active in Living Cells?*



**DNA Genetic Code of Life**

**Entire Genetic Code of a Bacteria**

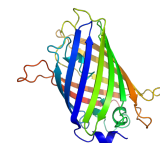
**DNA Fingerprinting**

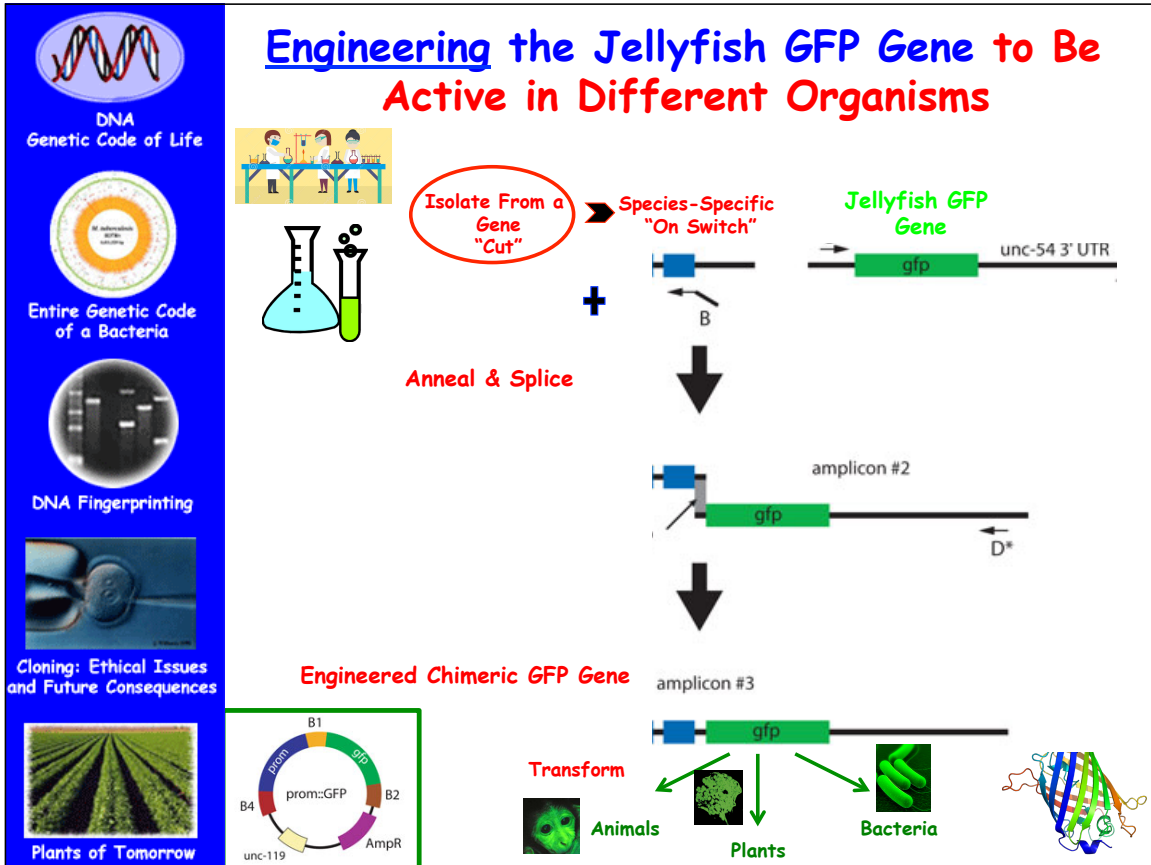
**Cloning: Ethical Issues and Future Consequences**

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





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

What Are the Conceptual Implications of This Experiment?

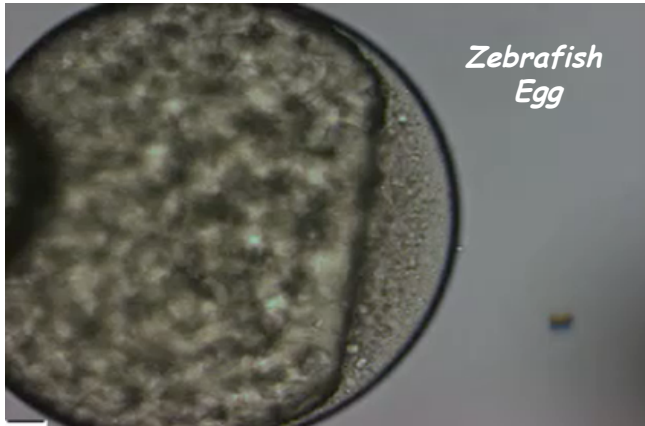
*E. coli* Switch + Jellyfish GFP Gene

superfolder GFP

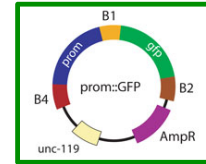
mut3 GFP

*E. coli* Synthesizes GFP Protein!

# Engineering a "GloFish"

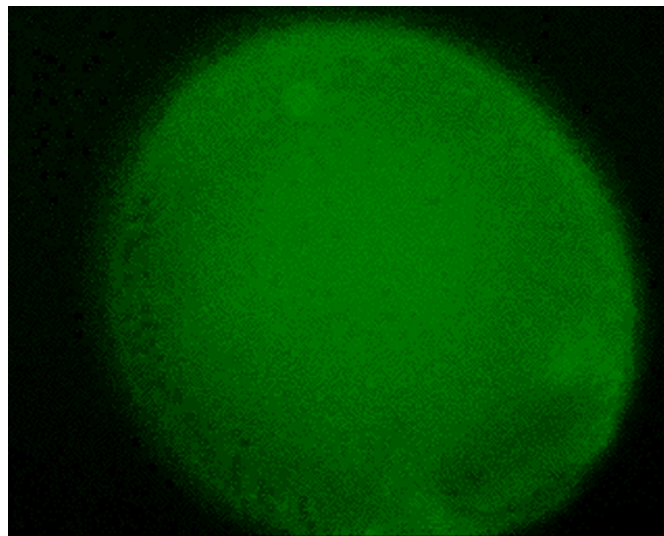


Zebrafish  
*Danio rerio*



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

**Can GloFish Can Be Sold In California?**

- **Cal. Dept. 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. Dept. 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!!**

**DNA Genetic Code of Life**

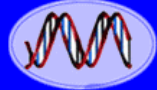
**Entire Genetic Code of a Bacteria**

**DNA Fingerprinting**

**Cloning: Ethical Issues and Future Consequences**

**Plants of Tomorrow**

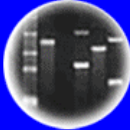




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State of California – Natural Resources Agency  
DEPARTMENT OF FISH AND WILDLIFE  
Fisheries Branch  
830 S Street  
Sacramento, CA 95811  
(916) 327-8840  
[www.wildlife.ca.gov](http://www.wildlife.ca.gov)

EDMUND G. BROWN JR., Governor  
CHARLTON H. BONHAM, Director



January 20, 2016

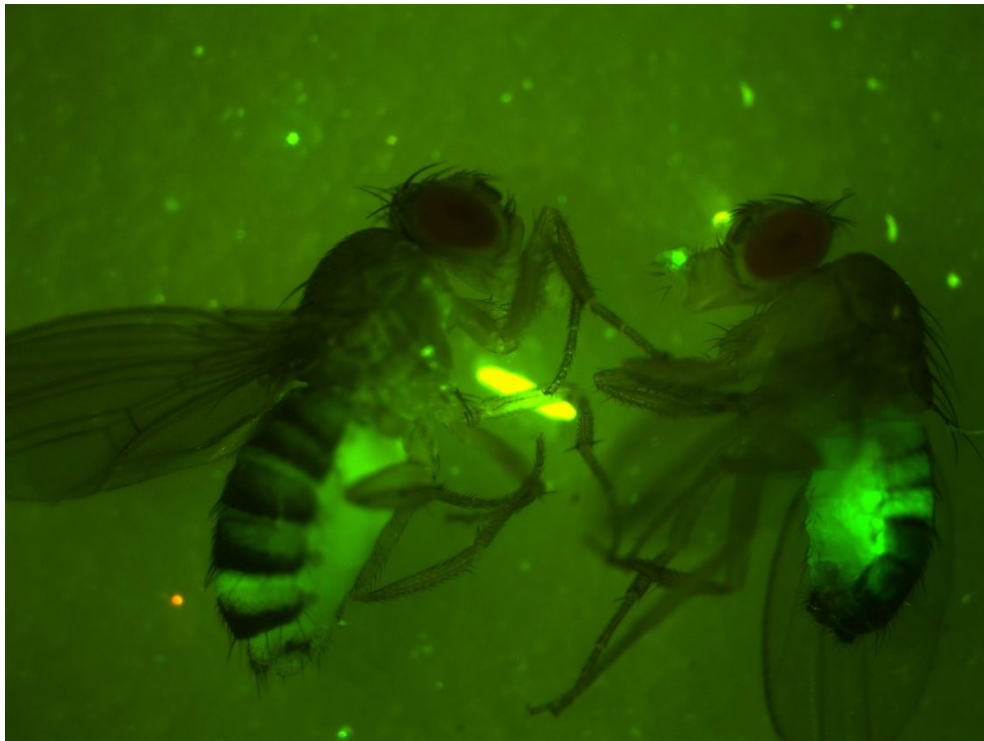
Alan Blake  
Chief Executive Officer  
Yorktown Technologies, L.P.  
P.O. Box 200753  
Austin, Texas, 78720

**Determination that the Proposed Transgenic Aquatic Animals Are Not Detrimental to Native Fish, Wildlife, or Plants in California**

In accordance with Section 703(A), Title 14, California Code of Regulations (CCR), Yorktown has provided all the required information needed for CDFW to make a determination as to whether or not the presence of the proposed transgenic aquatic animals within California is detrimental to native fish, wildlife or plants. Based on the information provided by Yorktown, including species information, scientific reviews, and risk assessments, CDFW has determined that fluorescent zebrafish, white tetra, and tiger barb are not detrimental and pose no reasonably foreseeable risk to California's native fish, wildlife, or plants. Therefore, these transgenic aquatic animals are not subject to regulation under Section 671 and subsection 671(a)(8), Title 14, CCR.

Effective immediately, Yorktown Technologies, L.P. or its authorized agent may import, possess, distribute, and sell fluorescent zebrafish, white tetra, and tiger barb within California for the aquarium fish trade. Yorktown and its authorized agent must possess and provide within three business days, upon request by CDFW, a copy of this determination. Additionally, any wholesaler or retailer purchasing the approved fluorescent fish species from Yorktown or its authorized agent may also import, possess, distribute, and sell the approved species provided that the wholesaler or retailer possesses and provides within three business days, upon request by CDFW,

## 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 Biological Implications of These Experiments?*

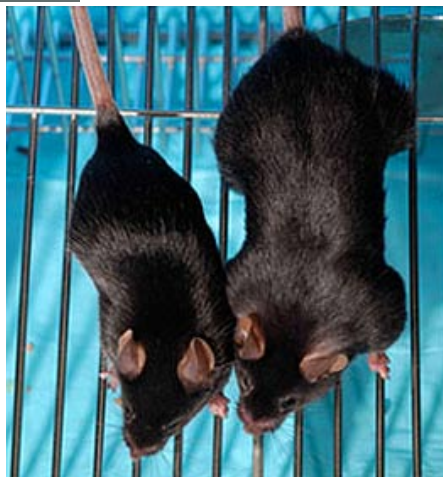
## **Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes**

**Richard D. Palmiter<sup>1</sup>, Ralph L. Brinster<sup>1</sup>, Robert E. Hammer<sup>1</sup>, Myrna E. Trumbauer<sup>1</sup>, Michael G. Rosenfeld<sup>2</sup>, Neal C. Birnberg<sup>3</sup> & Ronald M. Evans<sup>1</sup>**



**Nature, December, 1982  
36 Years Ago!!!!!!!**

**Engineering "Mighty Mouse" With a Rat Growth Hormone Gene**






DNA  
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Entire Genetic Code  
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues  
and Future Consequences



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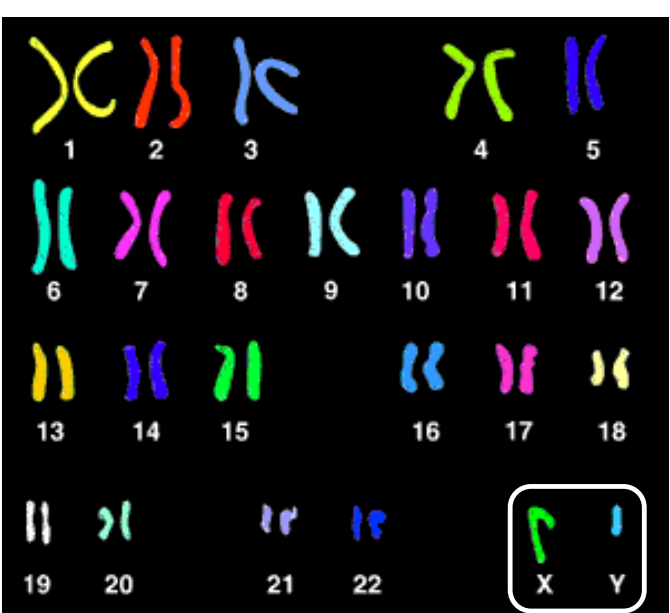



# How About Engineering The Sex Of An Organism?

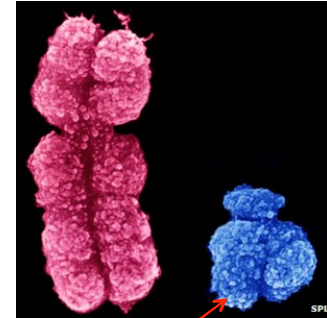


©Original Artist  
Reproduction rights obtainable from  
www.CartoonStock.com

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



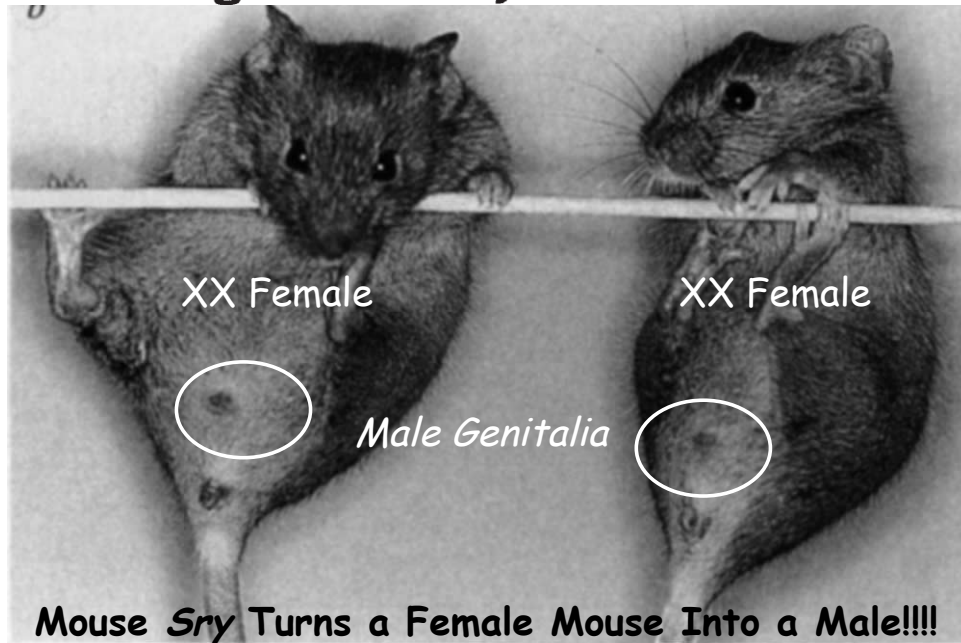
Mary S. Gibbs (GNN)



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



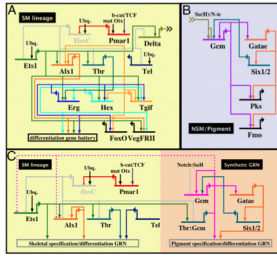
*Functional Proof That Sry Controls Male Development*

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

	Human	Mouse	Zebrafish	<i>Drosophila</i>
WT				
mut				
	<i>PAX6</i> <sup>-/-</sup>	<i>Pax6</i> <sup>-/-</sup>	<i>pax6b</i> <sup>-/-</sup>	<i>ey</i> <sup>-/-</sup>
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

**Regulatory Gene - Turns on Gene Switches**

# 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

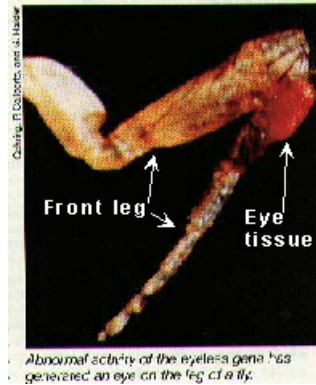


Different Fly Regions

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

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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. ♦

  
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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

 FOR ORGANIC GARDENING

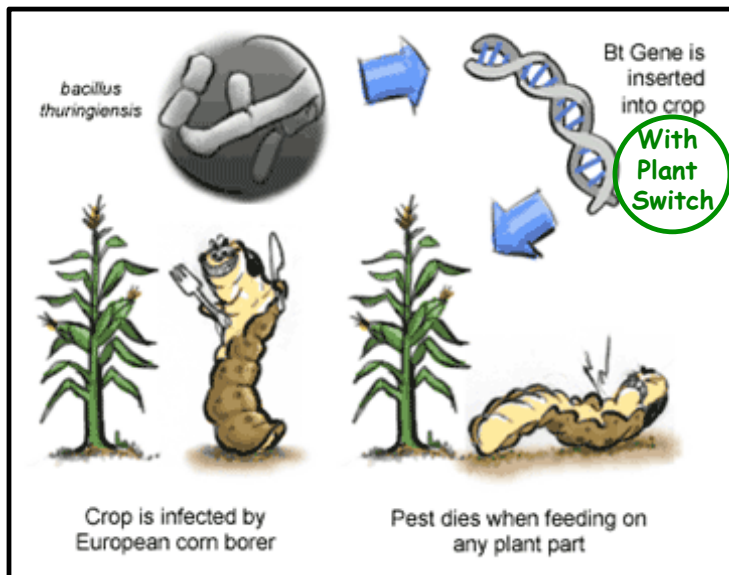


Active Ingredient:  
*Bacillus thuringiensis* subspecies *kurstaki* strain SA-12 solids, spores and Lepidopteran active toxins (At least 6 million viable spores per mg) . . . . . 98.35%

Other ingredients . . . . . 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



*Bacillus thuringiensis*



**Bt Toxin in Spores**

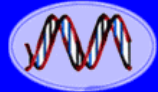


Spore Crystal

*Hornworm*



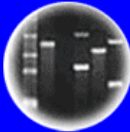




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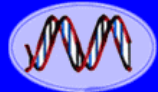


Cloning: Ethical Issues  
and Future Consequences



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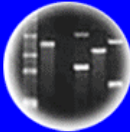
## Genetic Engineering a Plant to Resist Worms! Implications For Agriculture



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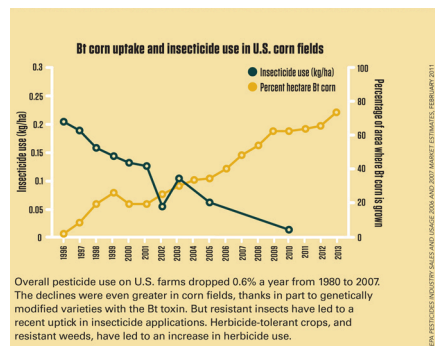
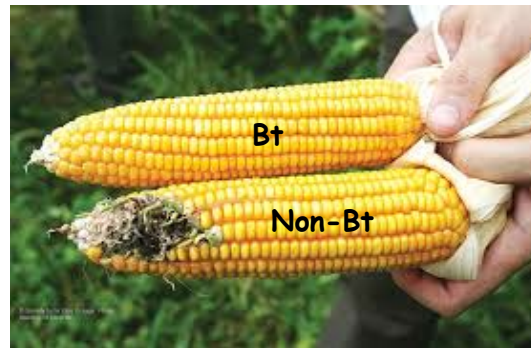


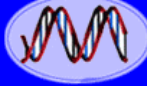
Cloning: Ethical Issues  
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## Adoption of Bt Corn By US Farmers Has Reduced the Use of Pesticides!!!!

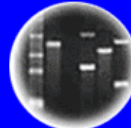





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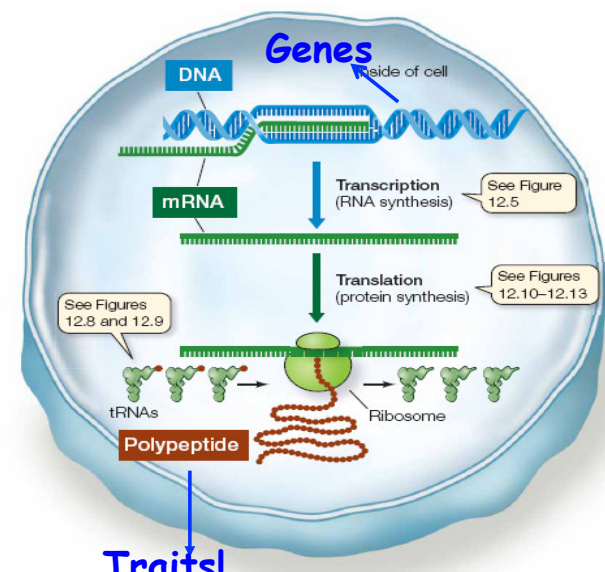


Cloning: Ethical Issues  
and Future Consequences



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

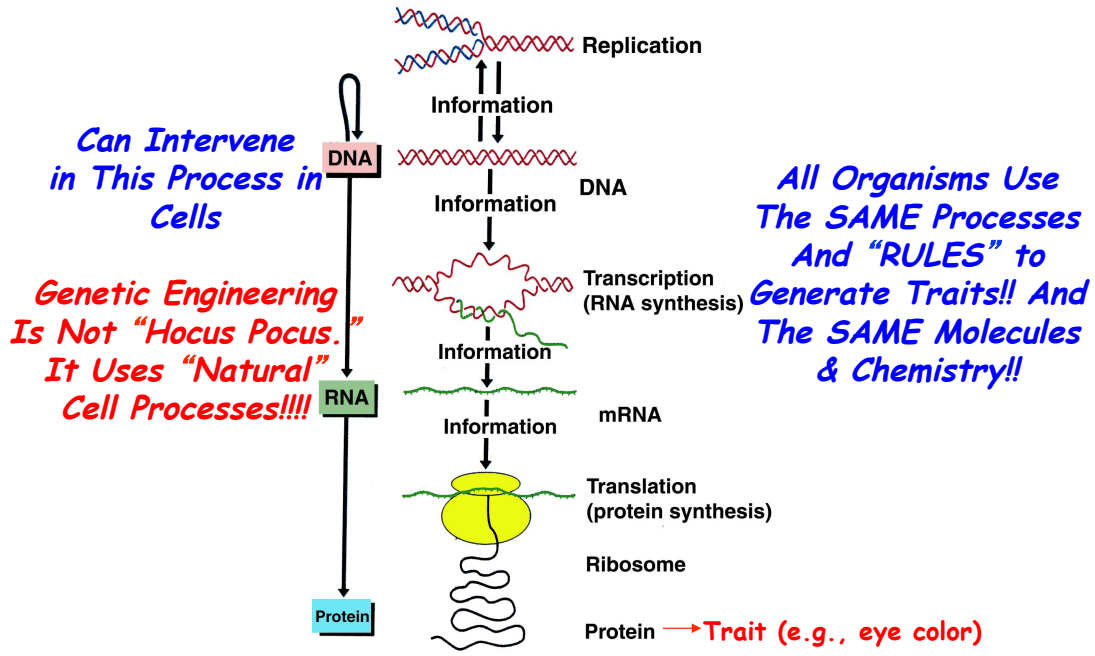


## Observations and Inferences From Genetic Engineering Experiments

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**



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**There is Nothing New About Genetic Engineering!**

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

***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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**Populations of All Organisms Contain 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!*

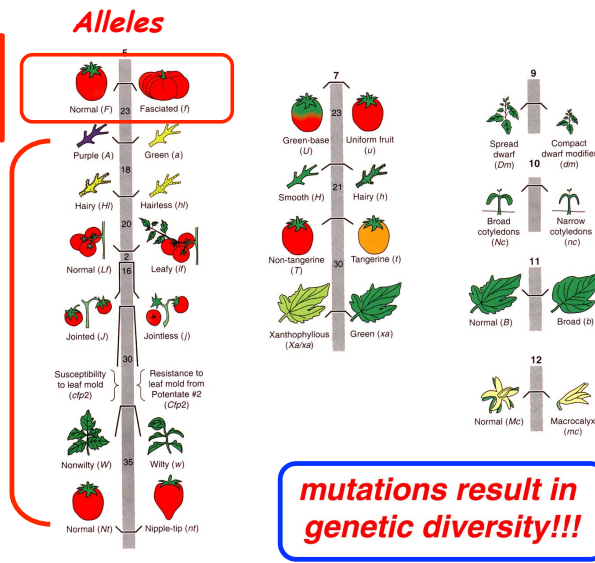
*What They Were Selecting Was a Different Form (Allele) of a Fruit Size Gene.*

al·lele e'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.

## Alleles Reside at the Same Position on a Chromosome

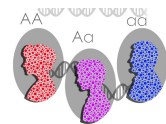
Allele Phenotypes Specify Markers For Each Gene Location!

Different Genes

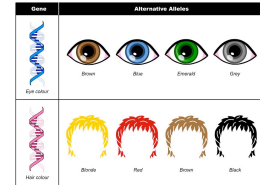


mutations result in genetic diversity!!!

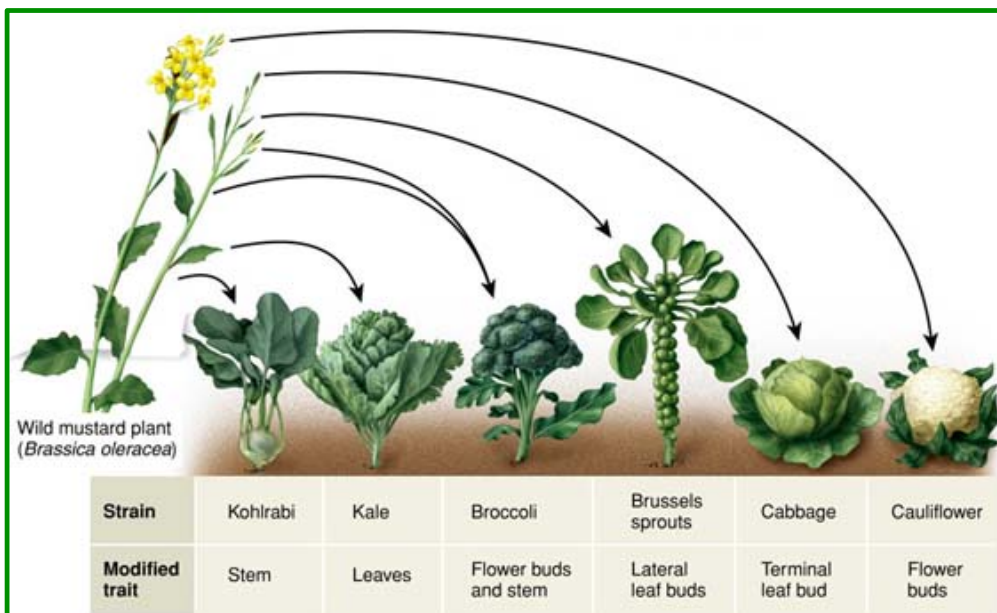
Gene Engineering Can Generate New Forms of Alleles of a Gene and, Therefore, Results in More Genetic Diversity



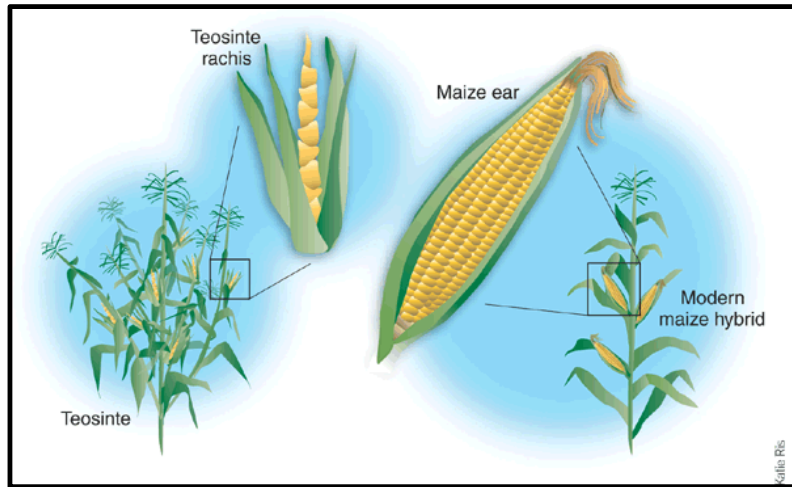
Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!



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



## *Engineering Corn From the Wild Grass Teosinte*



***Note: Architecture and Fruit (cob) Size***

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

## **Domesticated Animals Were Also “Engineered” By Breeding Wild Relatives**



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



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!*




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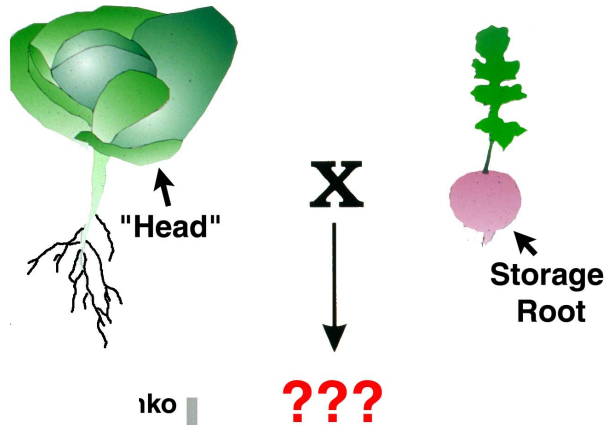


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## The Problem With Breeding the "Old Fashioned Way"

**Engineering A Novel Crop  
By "Wide" Breeding**

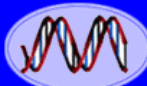
Cabbage (*Brassica*)      Radish (*Raphanus*)




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



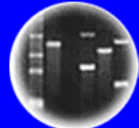





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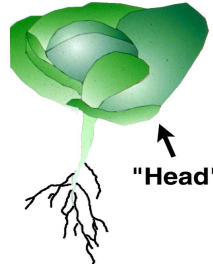


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## Engineering A Novel Crop By "Wide" Breeding


  

Cabbage (*Brassica*)



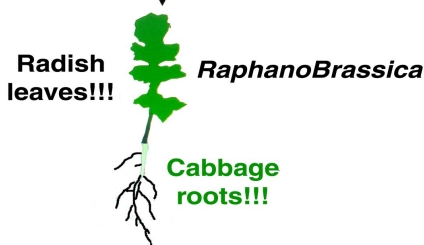
"Head"

Radish (*Raphanus*)




Storage  
Root

X



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

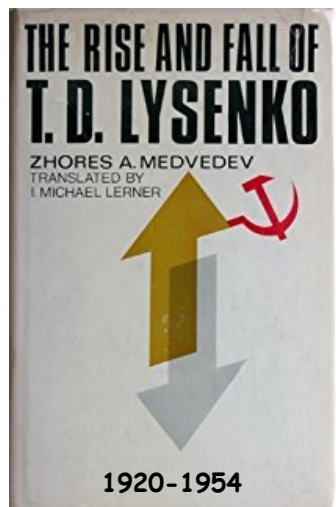
## How Ideology Destroys Science & Leads to Horrific Tragedy




**THE MURDER OF  
NIKOLAI VAVILOV**


THE STORY OF STALIN'S PERSECUTION OF ONE OF THE  
GREAT SCIENTISTS OF THE TWENTIETH CENTURY


**PETER PRINGLE**

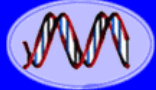


1920-1954





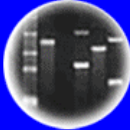




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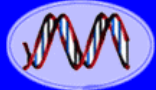


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## Karpechenko's Dream Come True!!!!



*Grafting Potato and Tomato Plants!*



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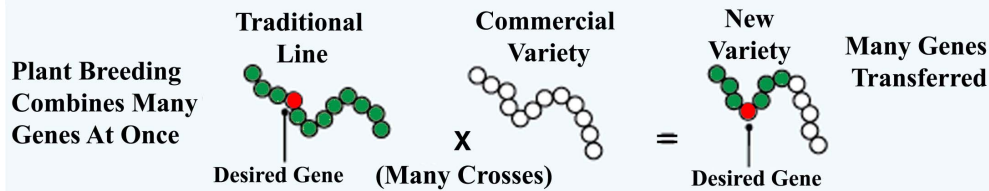
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**Genetic Engineering is a TECHNIQUE!**

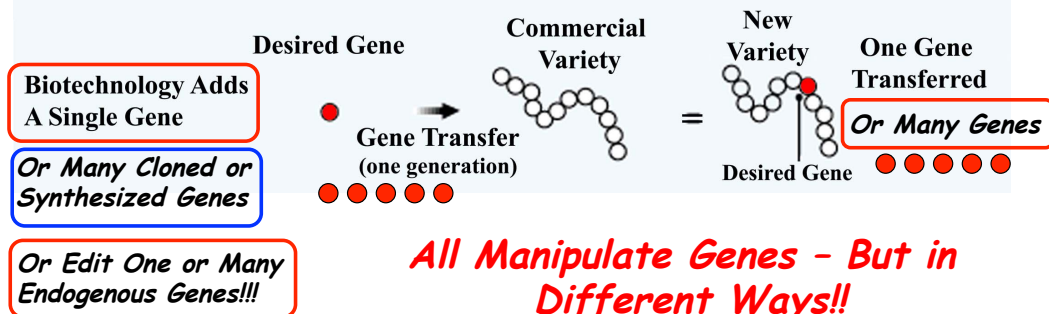
*How Do Classical Genetic Engineering  
Methods Differ From Those Using  
DNA and 21st Century Technologies?*

# Classical vs. DNA or Molecular Genetic Engineering Techniques

## TRADITIONAL PLANT BREEDING



## PLANT BIOTECHNOLOGY

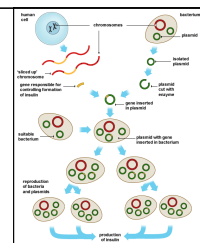
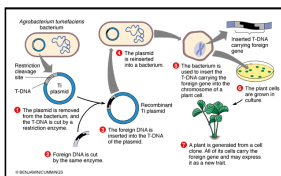


## What Are The Limitations of Classical Breeding/Genetic Engineering?

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?

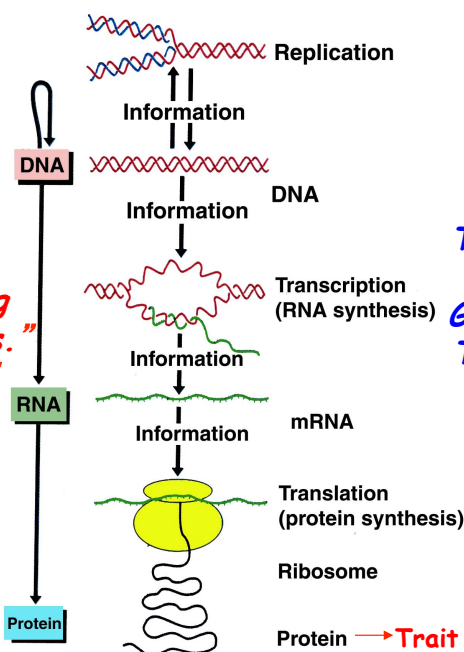
1. Any Gene From Any Organism Can Be Used In Any Organism - There Are No Breeding Barriers (e.g., genes of all sequenced genomes)
2. New Genes Can Be Engineered - Genes That Work Better and/or Produce New Proteins (i.e., create new genetic variability and/or alleles)
3. Existing Genes Can Be Engineered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



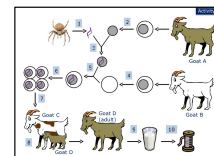
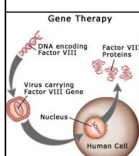
## THE FACTS ARE:

*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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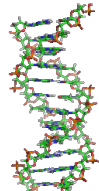
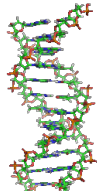


Cloning: Ethical Issues  
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## The "Ultimate" in Genetics Engineering is to Synthesize a Genome

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

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

