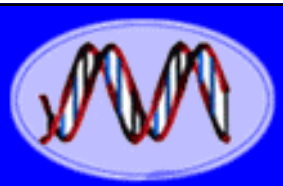
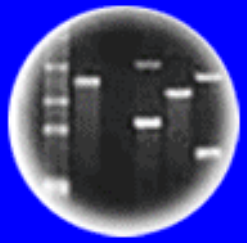


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

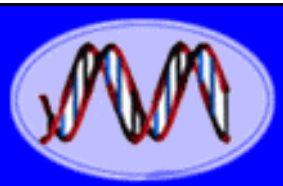
HC70A & PLSS059
Winter 2020
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & Channapatna Prakash

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

UCLA

TUSKEGEE UNIVERSITY



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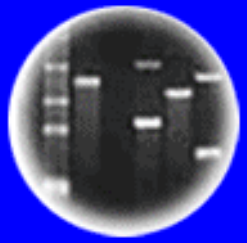


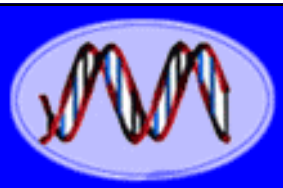
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

THEMES

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. 22nd Century Genetic Engineering - Synthetic Genomes



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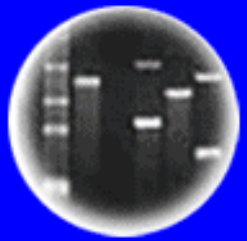

DNA Fingerprinting

Cloning: Ethical Issues
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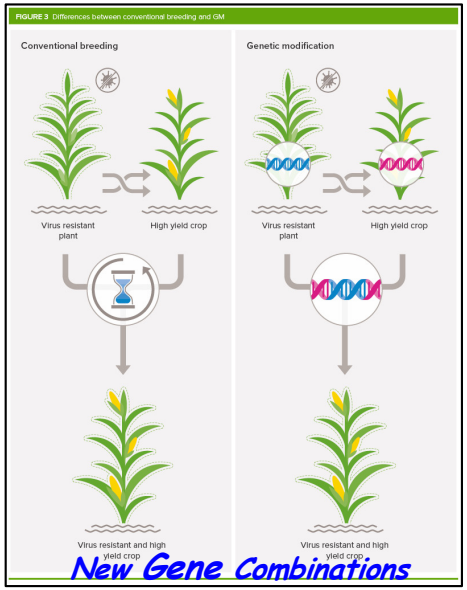
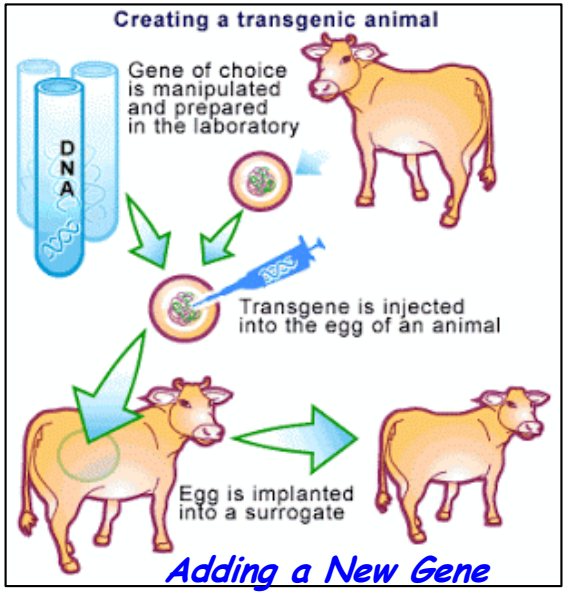
Three Genetic Engineering Techniques That Generate GMOs!!!

1. Classical Breeding



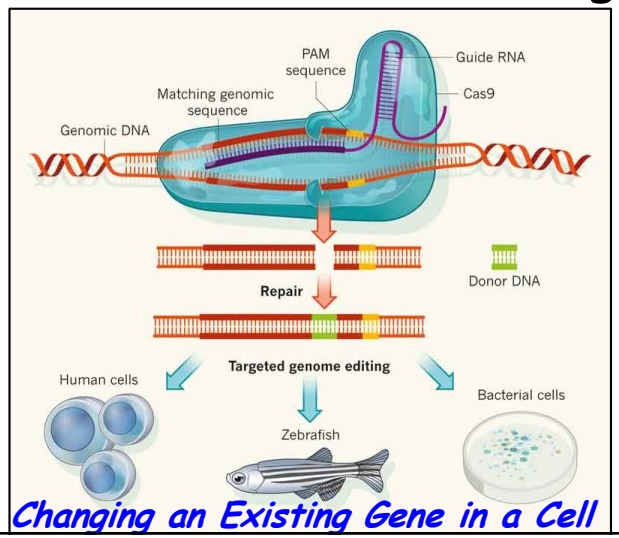
New Gene Combinations

2. Transgenic Organism



Adding a New Gene

3. CRISPR Gene Editing



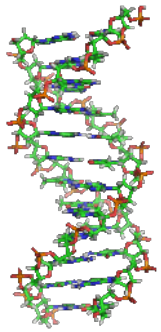
Changing an Existing Gene in a Cell

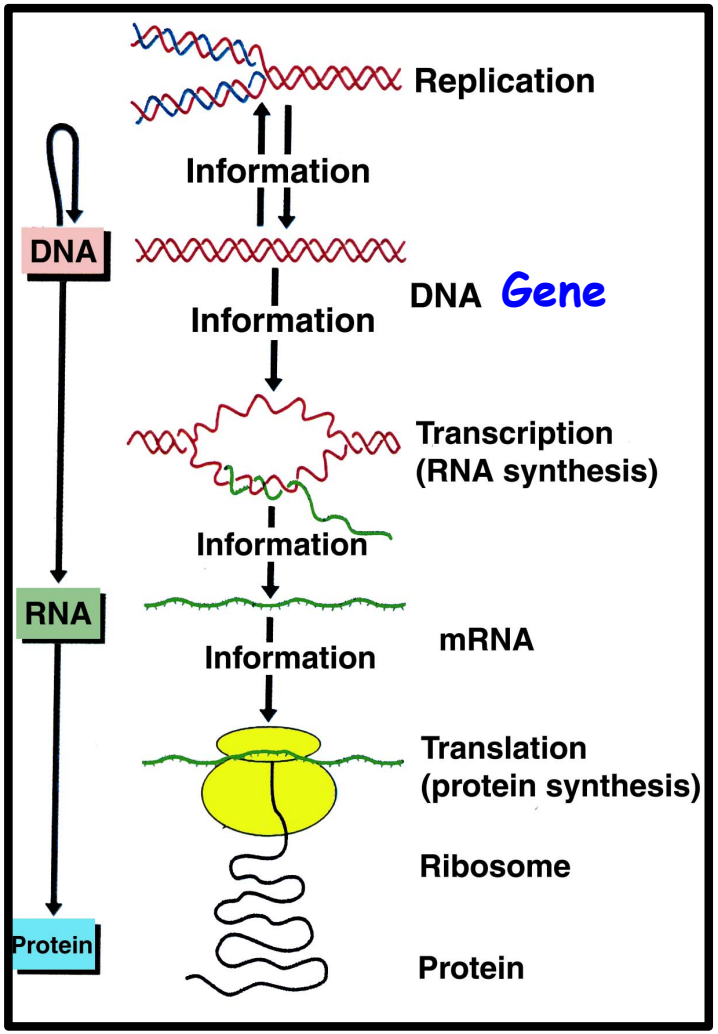
Goal

To
Direct the
Expression of
A New
"Desired"
Genetic Trait

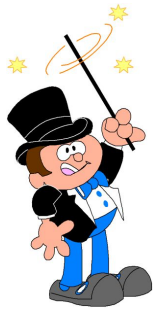
Genes & DNA Obey the Same Rules Using Either Classical or Modern DNA Engineering Approaches!! BOTH Produce GMOs!!!!!!!

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

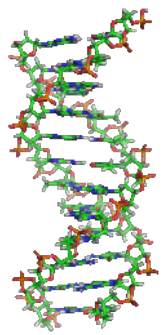




No Hocus Pocus!



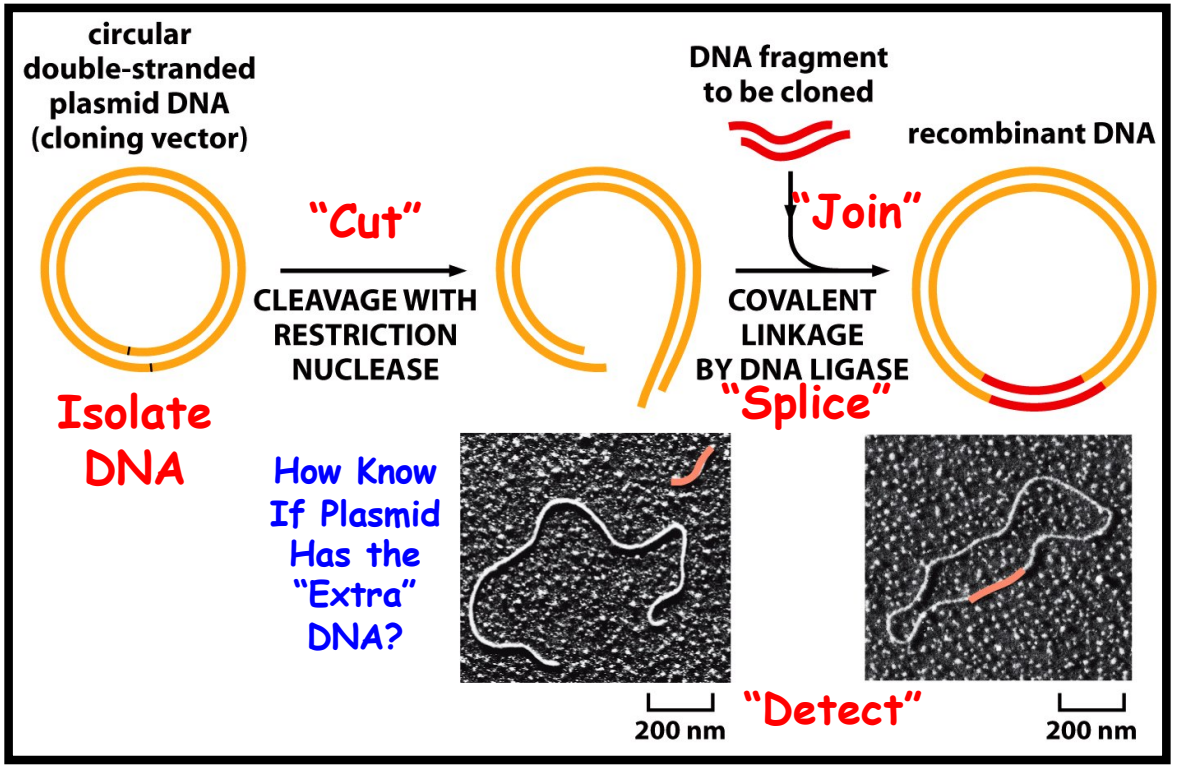
2. All Organisms Use The SAME Processes And "RULES" to Generate Traits!!

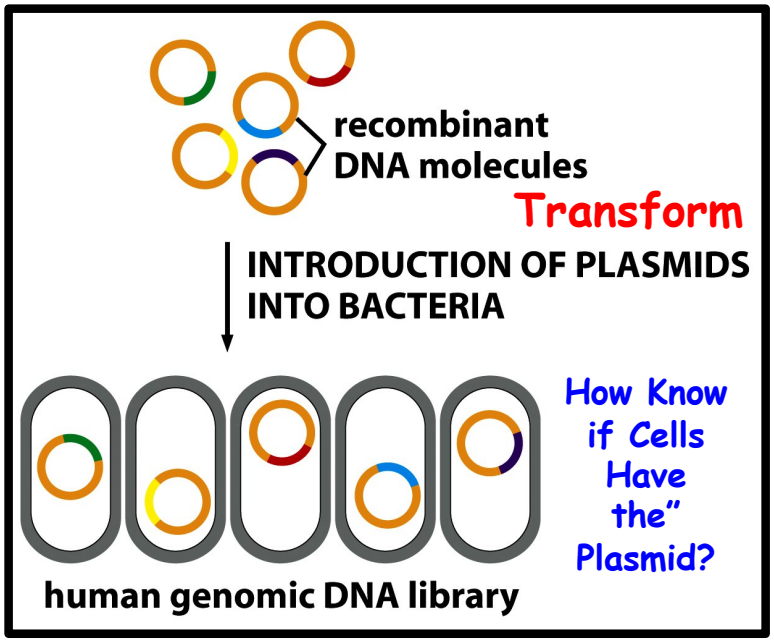


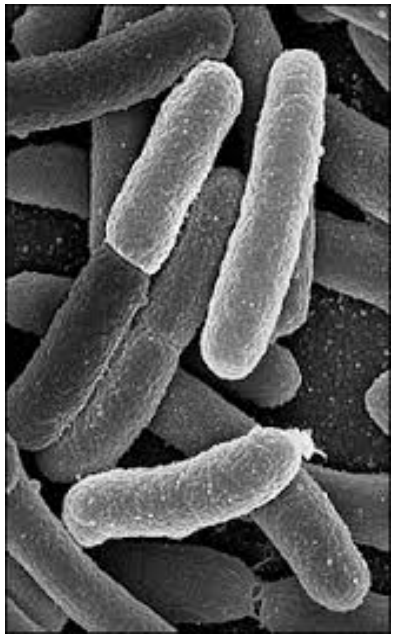
What "Tools" Are Needed For Genetic Engineering?

.

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*)



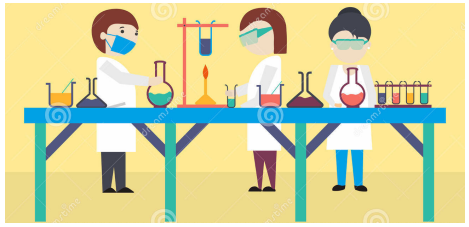




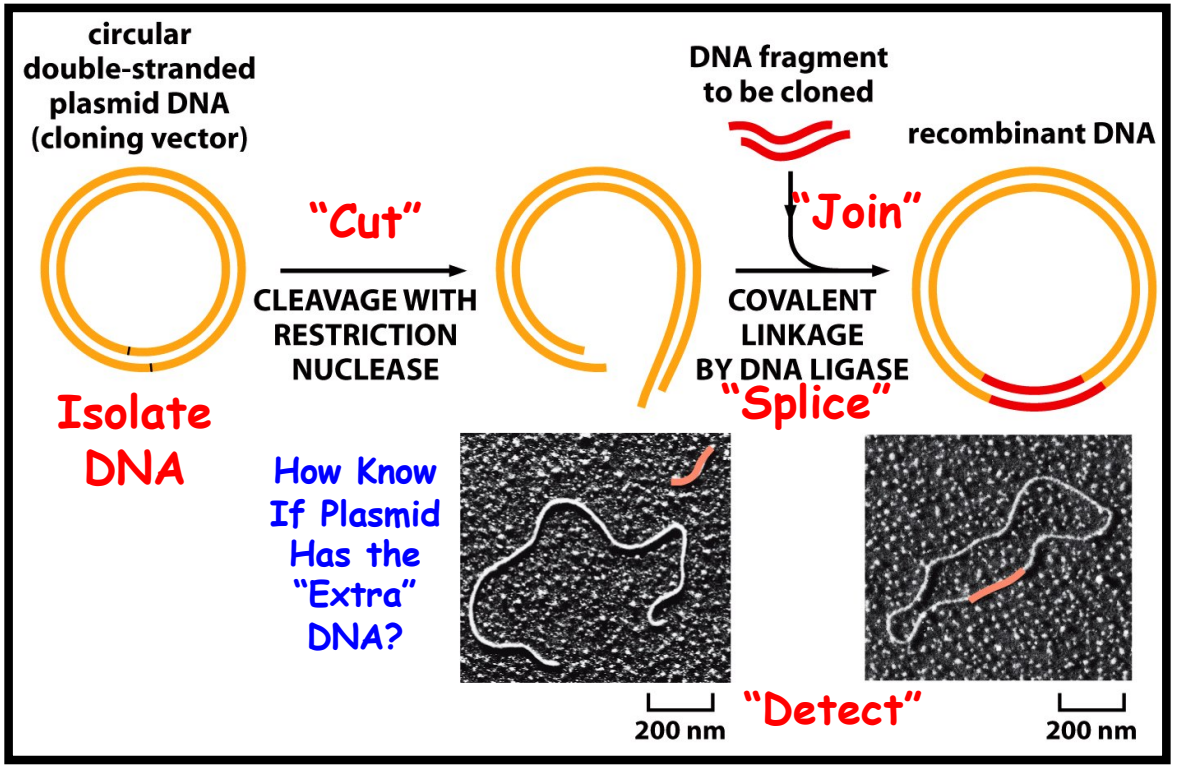
Lab Engineered DNA

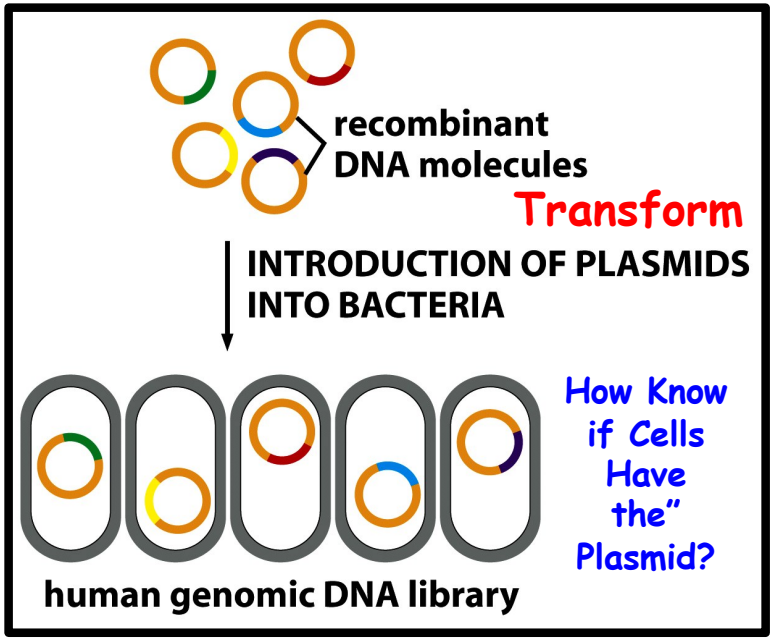
- Animals
- Plants
- Fungi & Other Bacteria

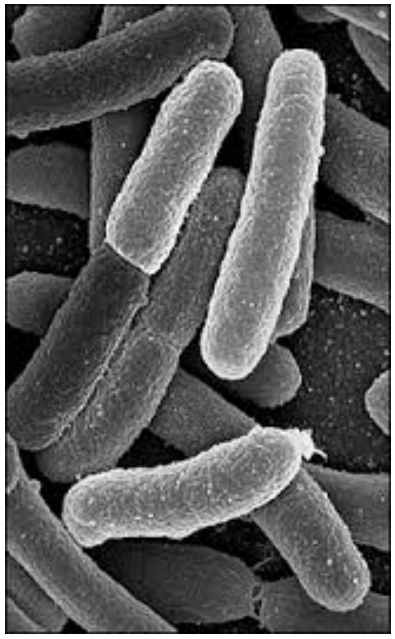
What Steps Are Required For Genetic Engineering?

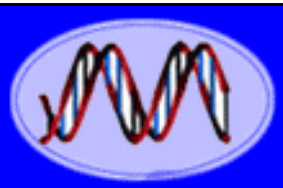


1. Isolate DNA From Cells
2. Isolate Vector (e.g., Plasmids)
3. Digest DNAs With the Same Restriction Enzyme
4. Anneal "Sticky" Ends of Two DNAs Together to Form Complementary Bases
5. Seal DNAs Together With DNA Ligase
6. Transform *E. coli* Cells With Recombinant DNA
7. Select For *E. coli* Cells With Recombinant Vectors
8. Demonstrate Directly That Recombinant Vector Contains Foreign DNA









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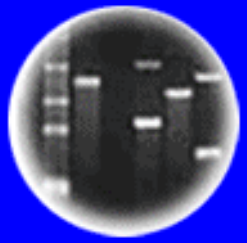


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Any Gene Can Be Isolated & Transferred to Any Organism Using Genetic Engineering!!

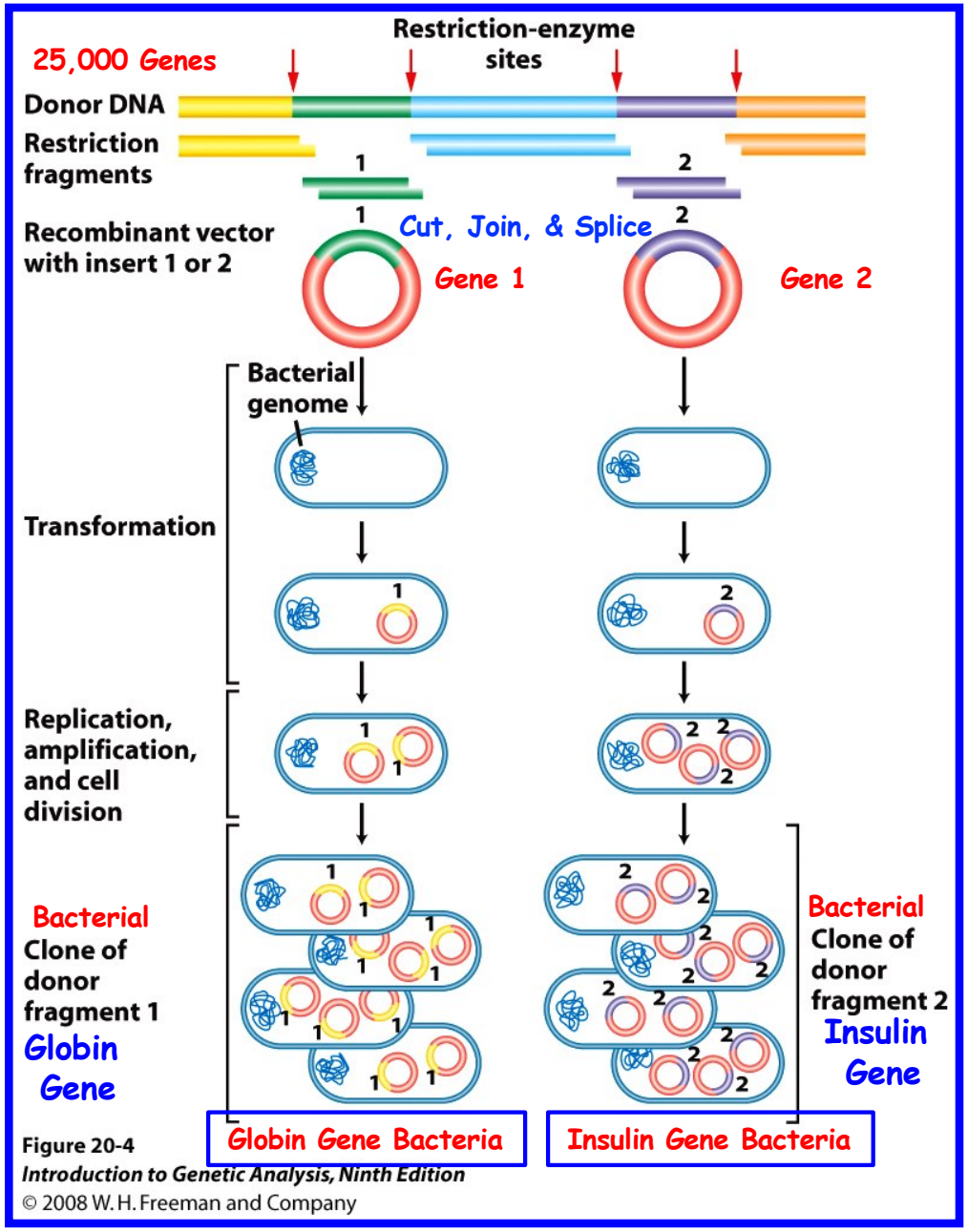
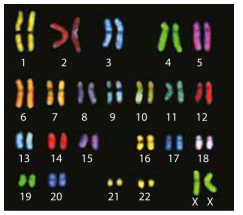
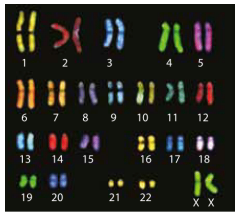


Figure 20-4
Introduction to Genetic Analysis, Ninth Edition

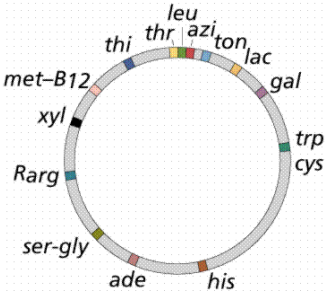


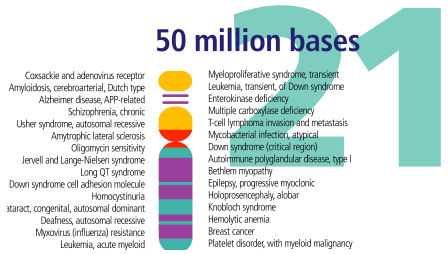
The First Step in Unraveling the Function of Every Gene in a Cell & Using the Information Obtained to Engineer New Cells

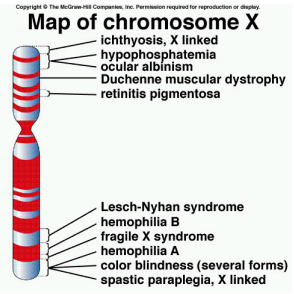


"Why" Clone Genes From An Organism's Genome?

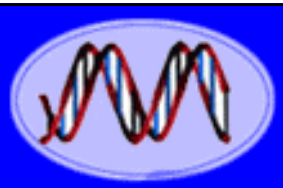
An Essential HC70A Concept!







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 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



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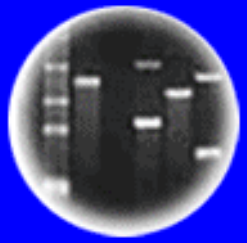

DNA Fingerprinting

Cloning: Ethical Issues
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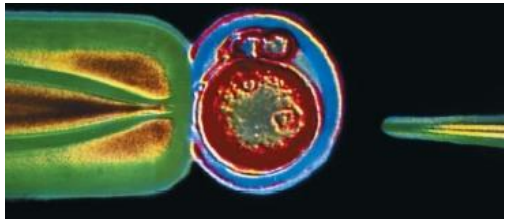
Plants of Tomorrow

Genetic Engineering Applications That Have Affected Society and Knowledge About Ourselves

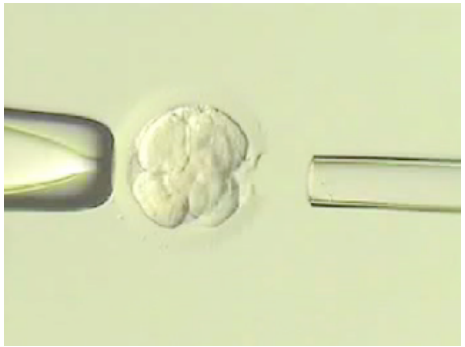
1. Recombinant DNA Drugs
2. Sequence of the Human Genome
3. DNA Home Testing Kits
4. Prenatal Genetic Diagnosis (PGD)
5. Ancient DNA
6. Human Ancestry and Origins
7. DNA Forensics
8. Synthetic Genomes



Determining the Genetic Identity of a Human Embryo Before Implantation!

Prenatal Genetic Diagnosis (PGD)

Fertility Clinics Scan for the Strongest Embryo



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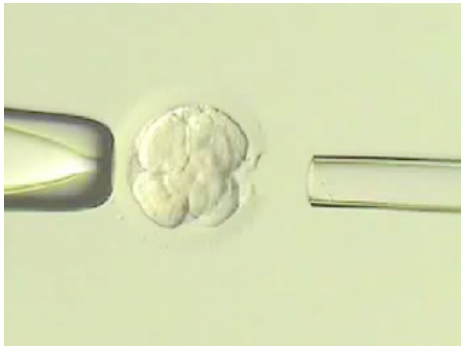
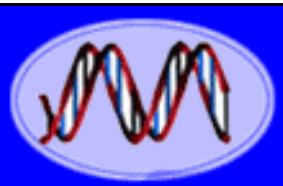


What Is the Significance of Genetic Engineering?

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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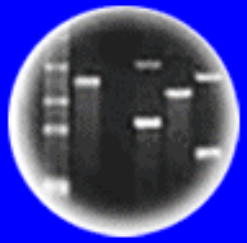


DNA Fingerprinting

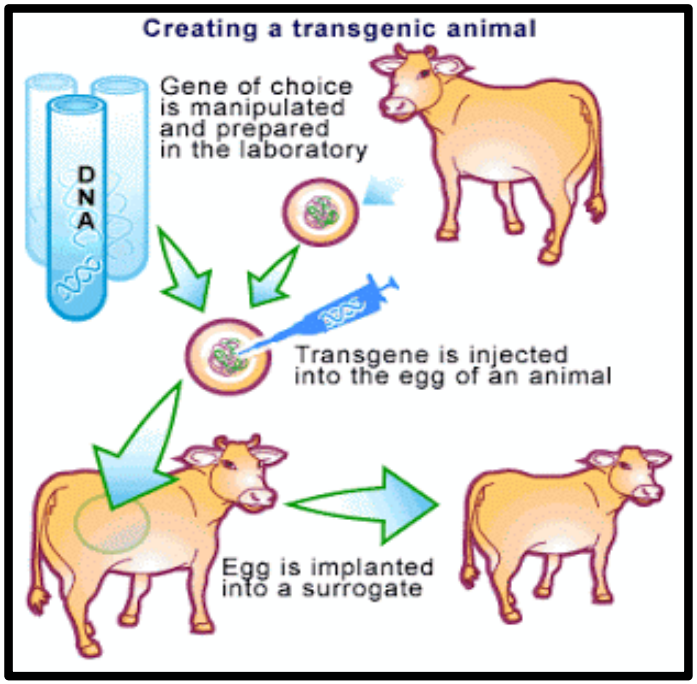
Cloning: Ethical Issues
and Future Consequences

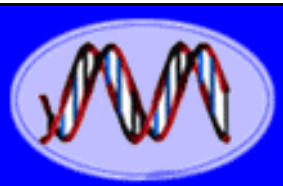
Plants of Tomorrow

What Can Be Done With Modern Genetic Engineering?

Some Examples

2. Transgenic Organisms





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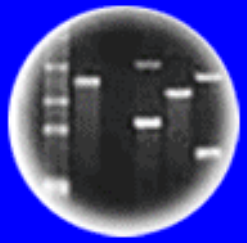

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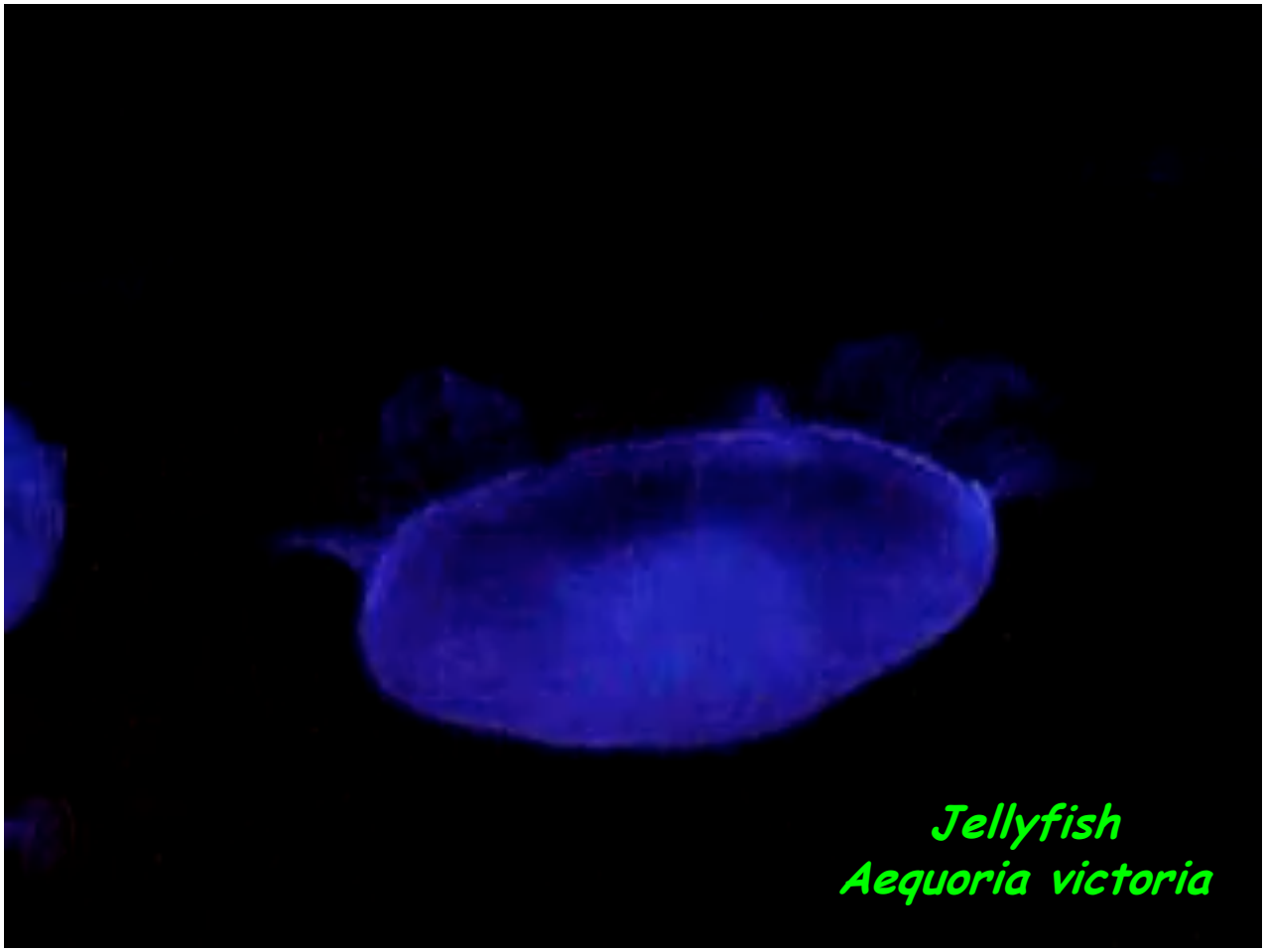
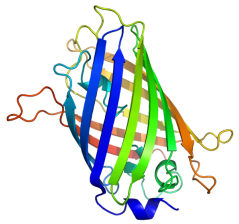
Using a Jellyfish Gene to Engineer *Glowing* Bacteria, Animals, and Plants!!!!



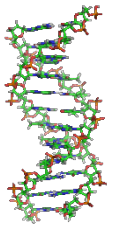
Green Fluorescence Protein (GFP)

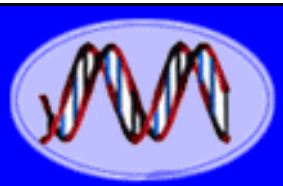
(238 amino acids)



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

Using Recombinant DNA to Isolate the Jellyfish GFP Gene





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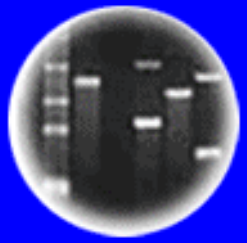

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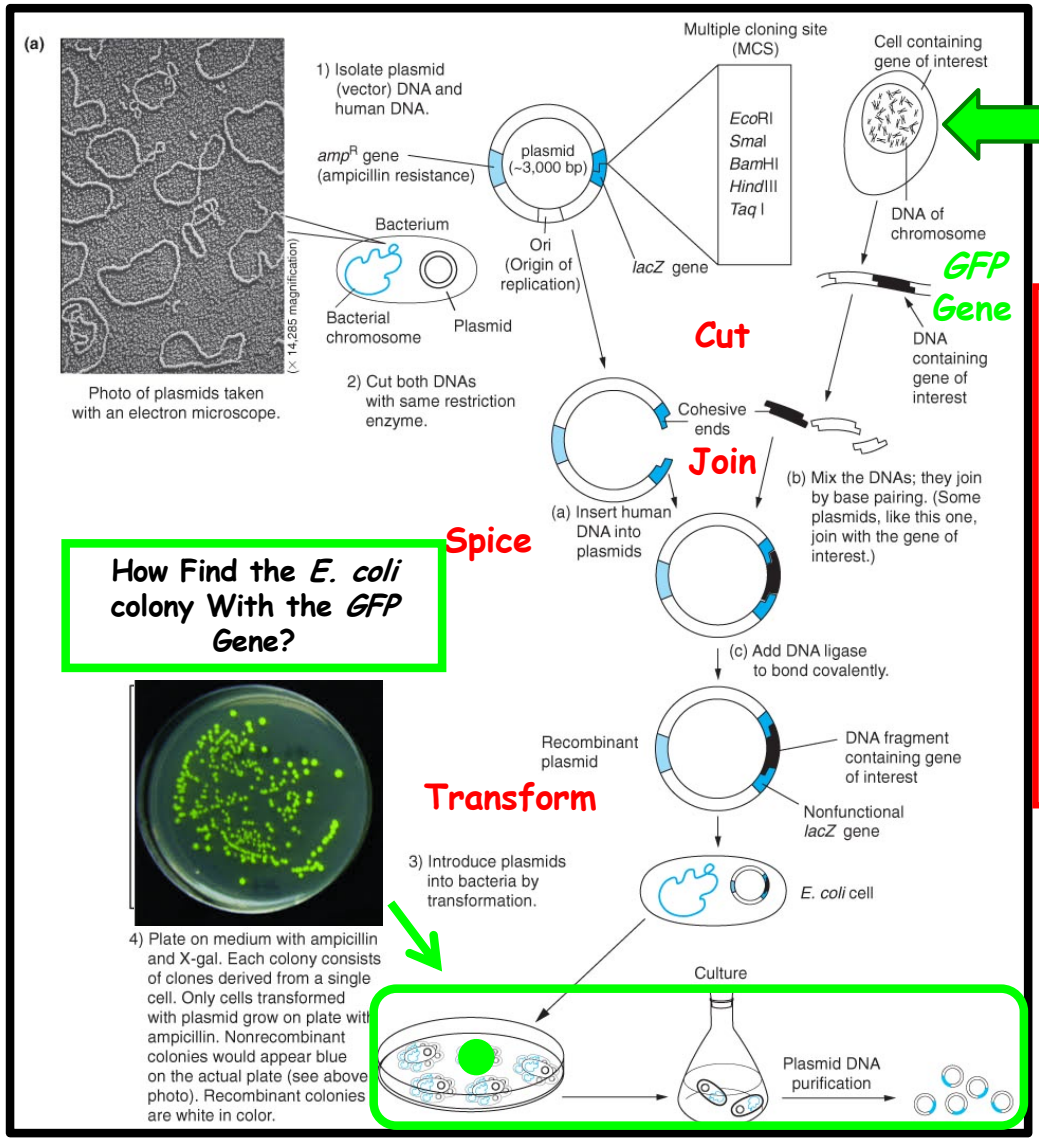
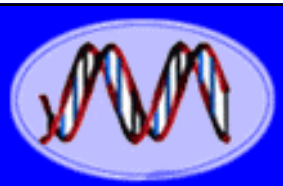


1. Clone Jelly Fish *GFP* Gene
2. Insert *GFP* Gene Into Plasmid Vector "Behind" a Specific "Switch"
4. Transform Into *E. coli* and Select Cells With Recombinant Plasmid
Determine if *GFP* Gene is Active

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



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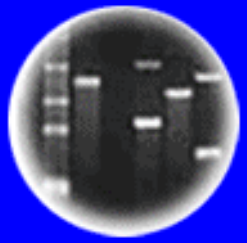

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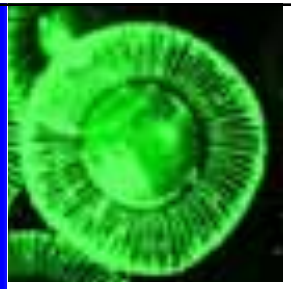
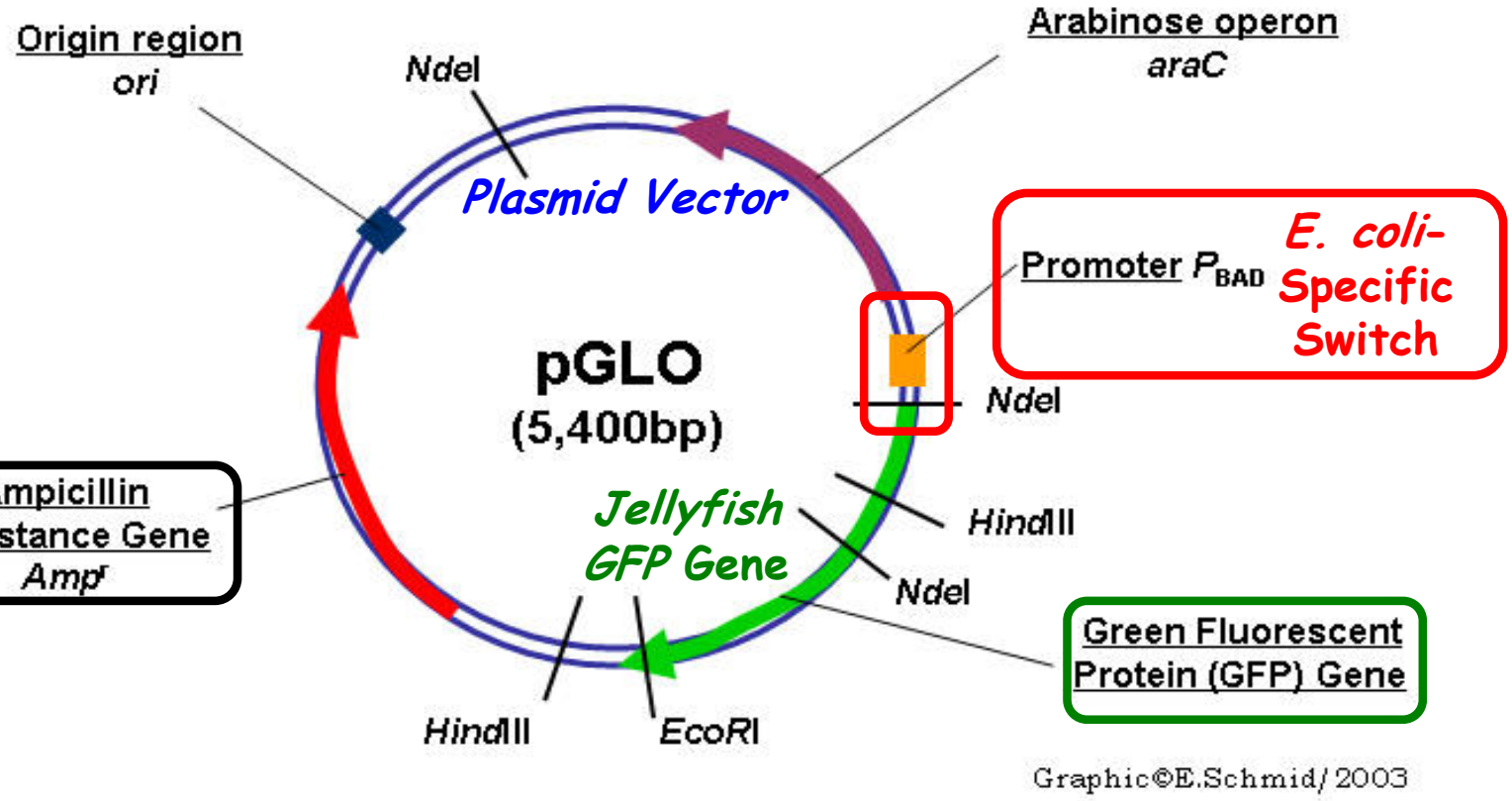
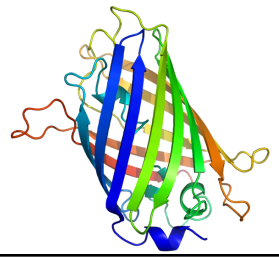


A Recombinant Plasmid Containing the GFP Gene

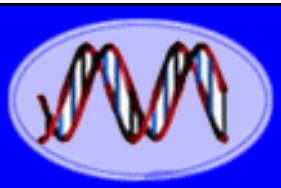
How Make it Active in Living Cells?



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



Engineering the Jellyfish GFP Gene to Be Active in Different Organisms



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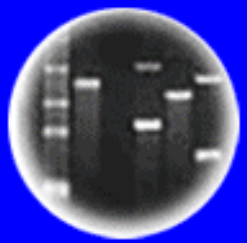
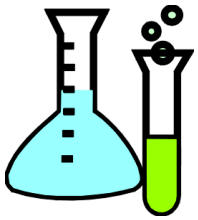


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

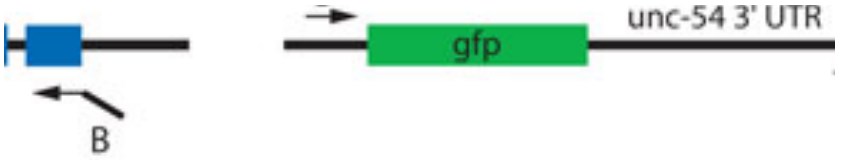
Plants of Tomorrow



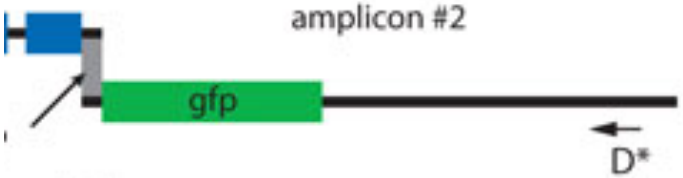
Isolate From a Gene "Cut"

Species-Specific "On Switch"

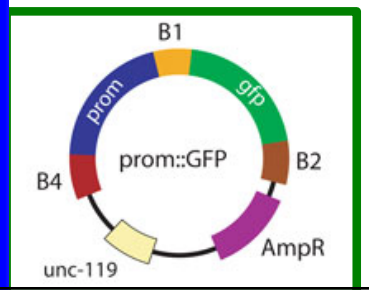
Jellyfish GFP Gene



Anneal & Splice

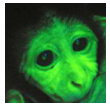


Engineered Chimeric GFP Gene

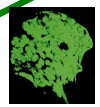


amplicon #3

Transform

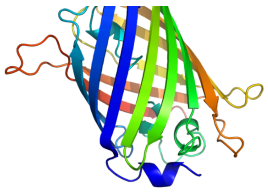


Animals



Plants

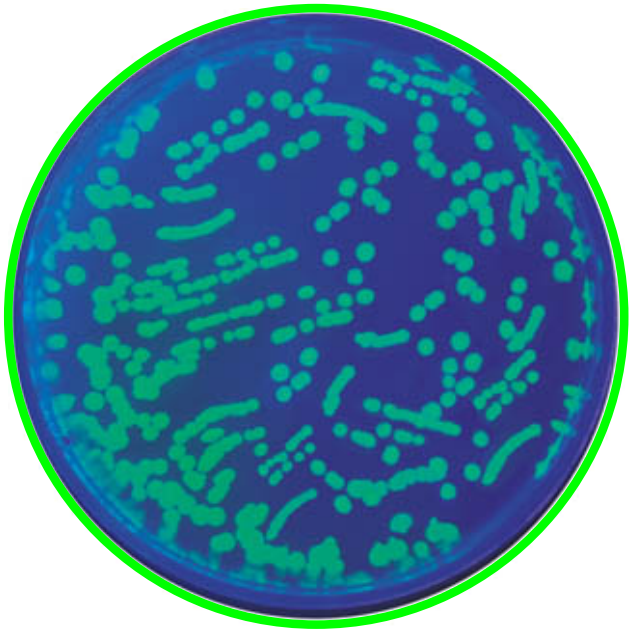
Bacteria

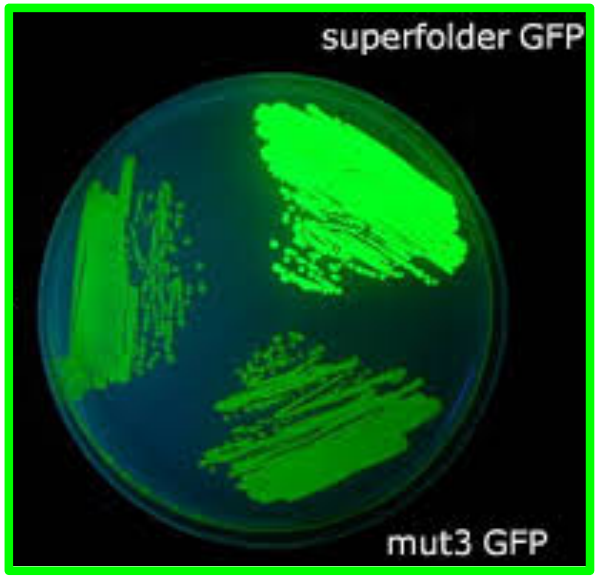


Essential HC70A Concept
Yo! It's in the Sequences!

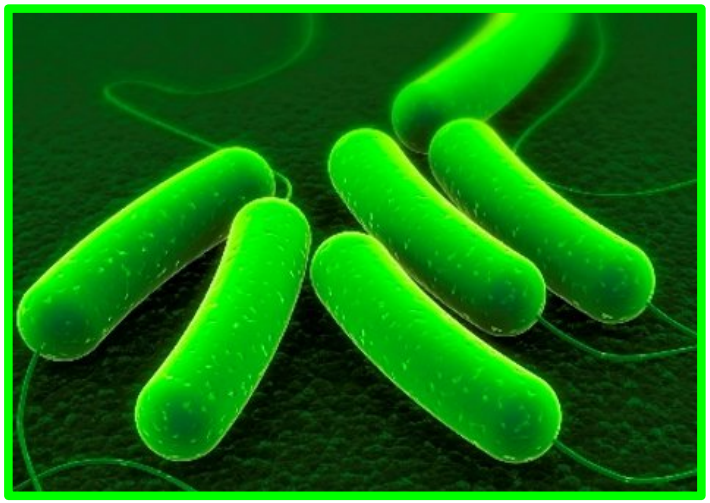
GloColi – Engineering *E. coli* With the Jellyfish GFP Gene!

What Are the Conceptual Implications of This Experiment?

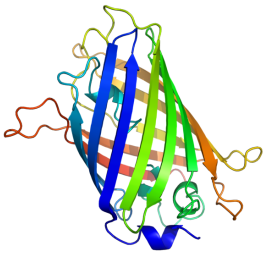




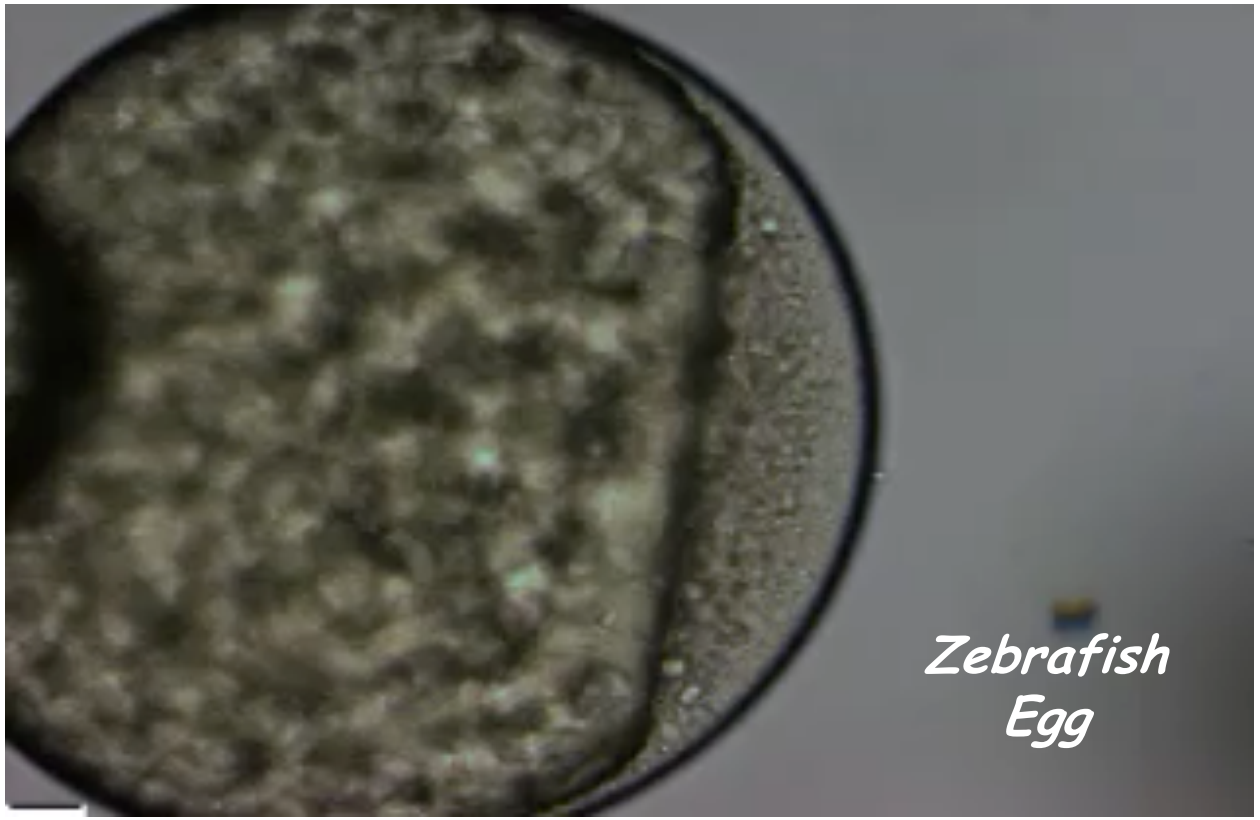
E. Coli Switch + Jellyfish GFP Gene



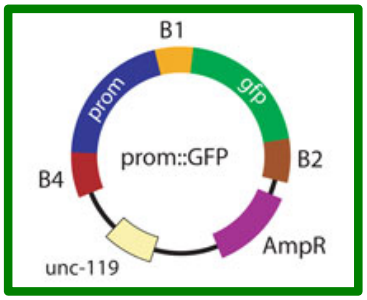
E. Coli Synthesizes GFP Protein!



Engineering a "GloFish"



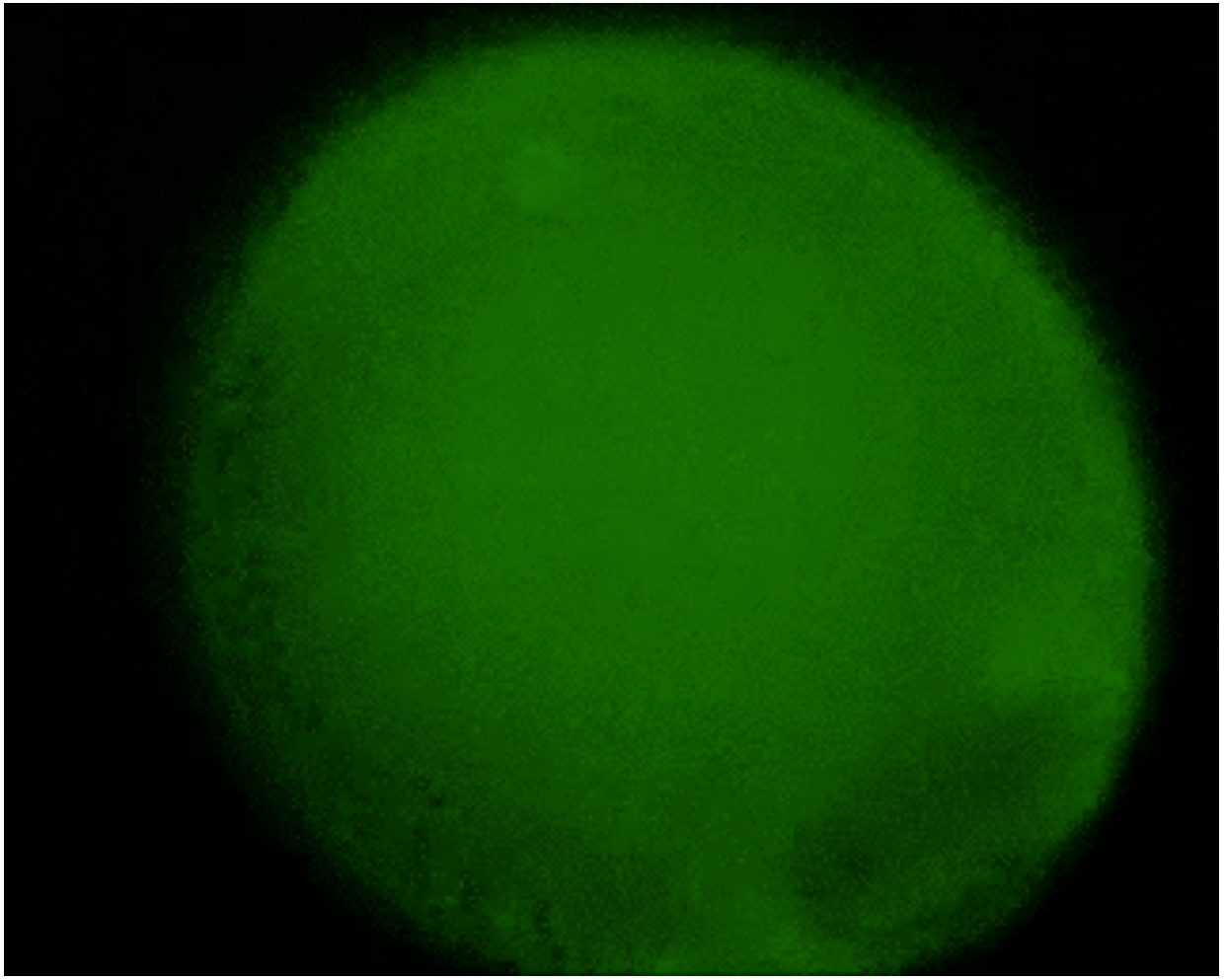
Zebrafish
Danio rerio



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

What Switch Used?

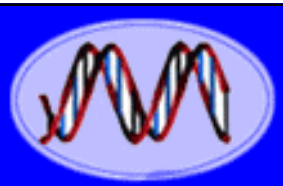
A "GloFish" Embryo!!



Zebrafish - Danio rerio

Genetically Engineered "GloFish!!"

Note Different Fluorescing Colors – Due to
Different Engineered Jellyfish Genes



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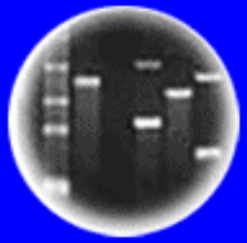


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Can GloFish Can Be Purchased In California?

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

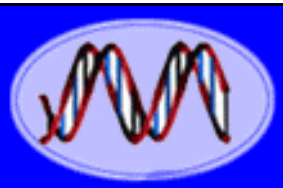
Regulation Makes it illegal to spawn, cultivate, or incubate any transgenic fish in the state controlled waters of the Pacific Ocean.

- **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!!

petco



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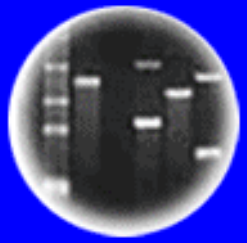

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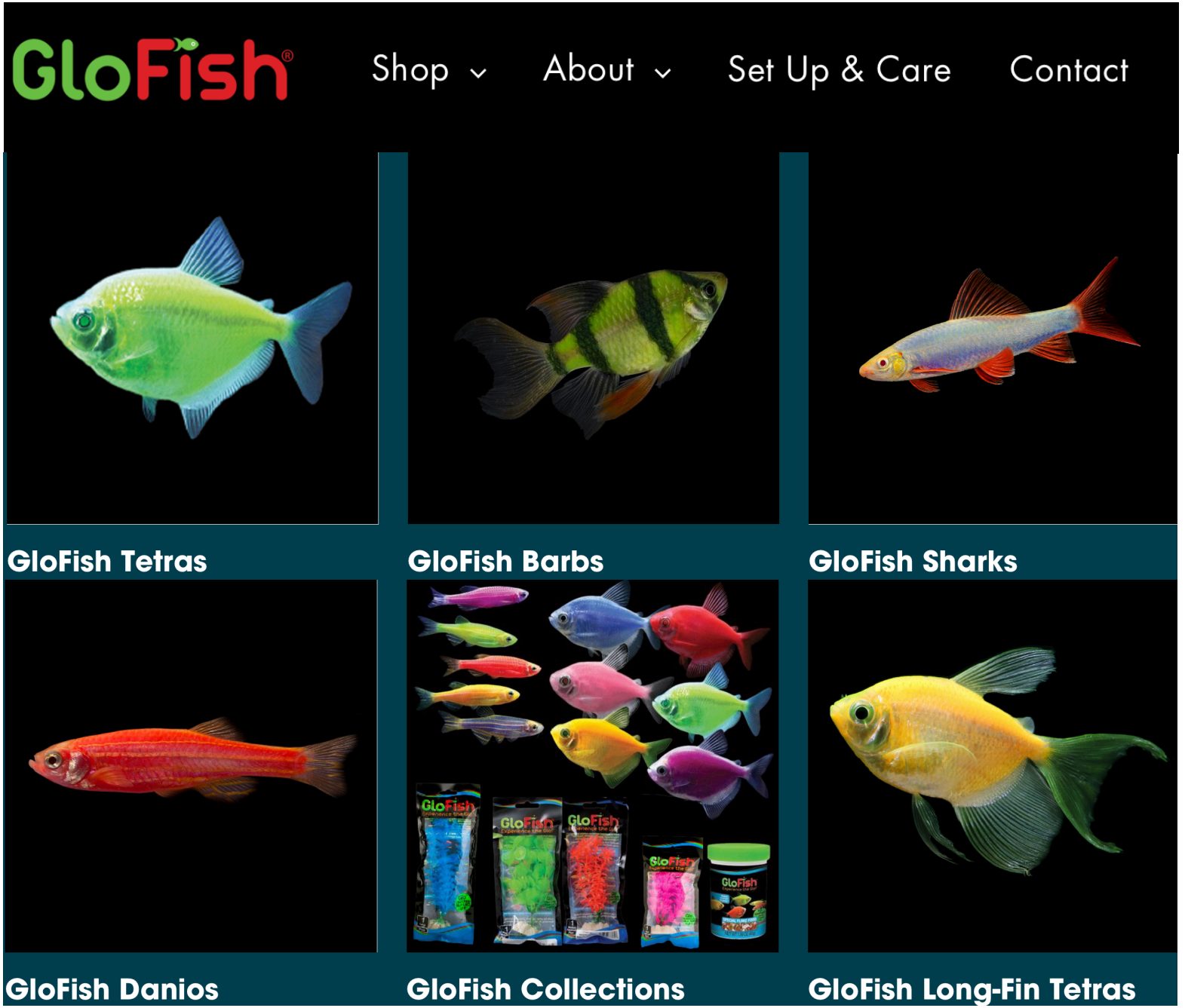
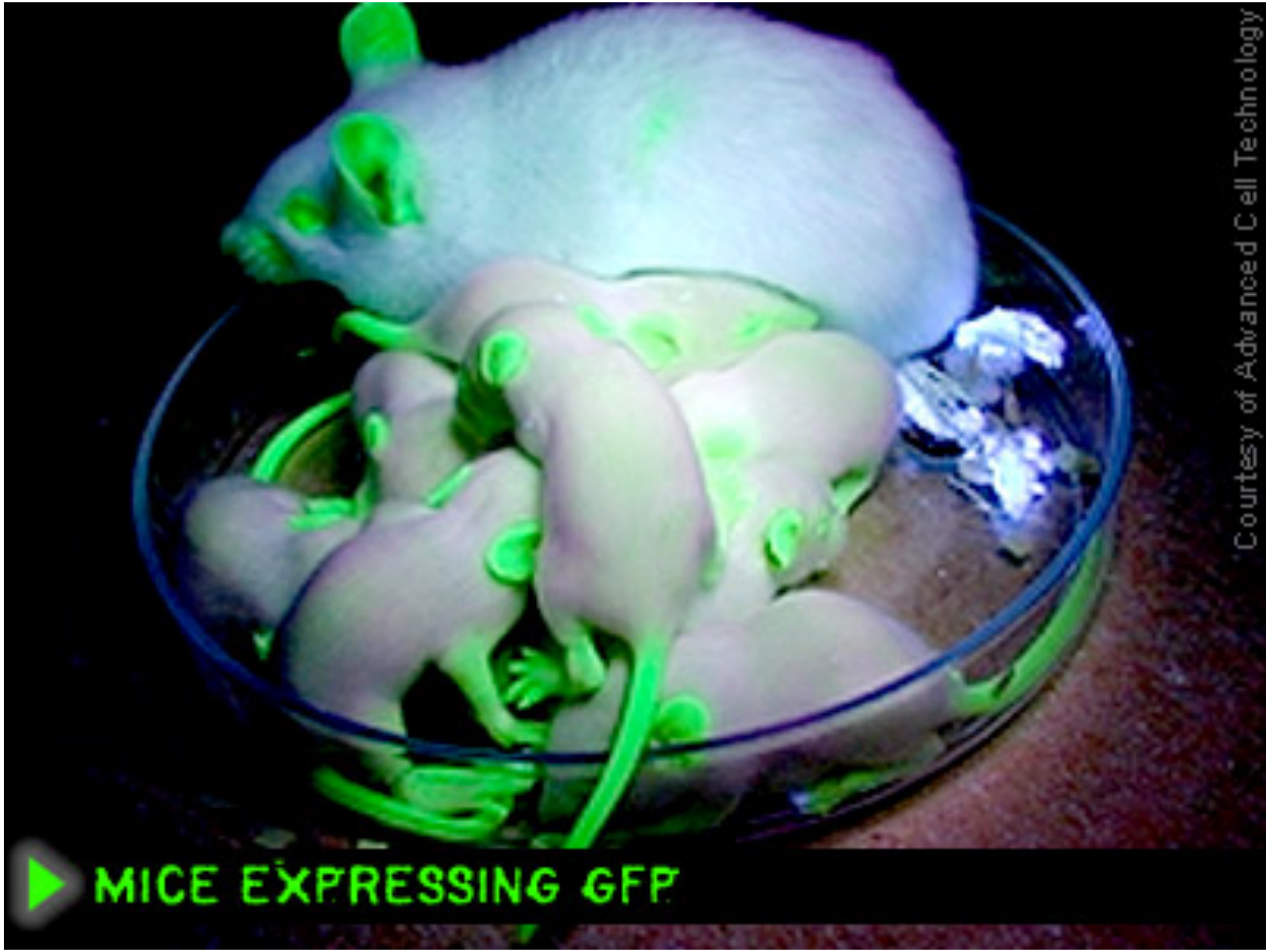


Austin company behind glow-in-the-dark fish in pet stores sells IP for $50 million

How About a Glo Fly!

What About "Glo Mice!!!"



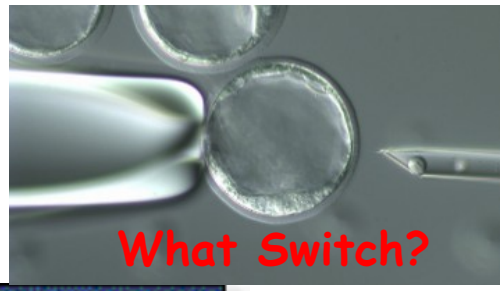
And Glo Monkeys, Cats and Pigs as Well!!

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

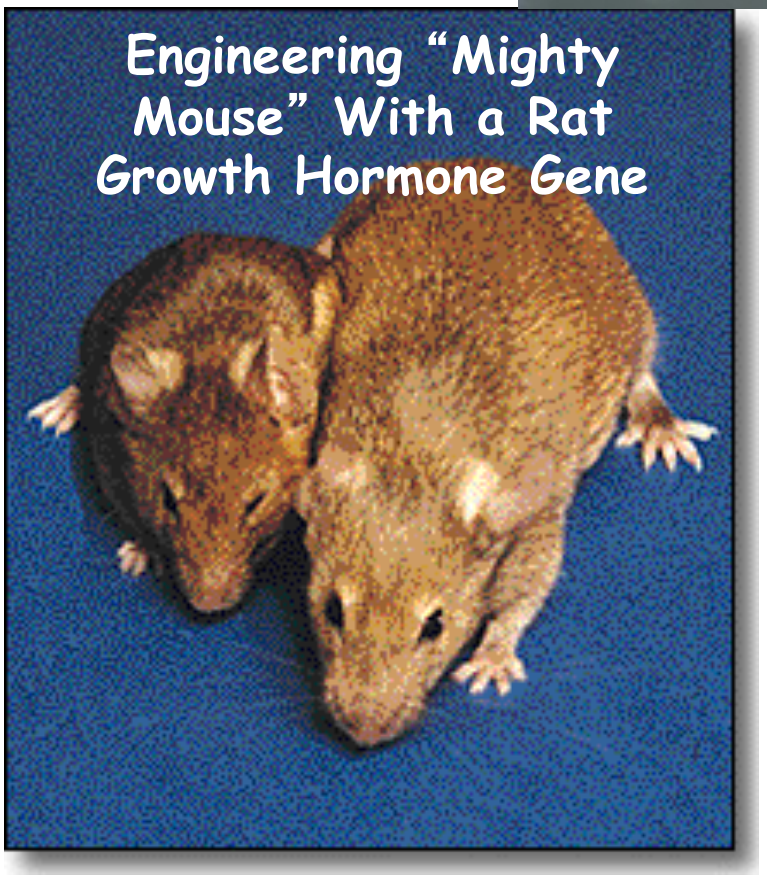
What are the Biological Implications of These Experiments?

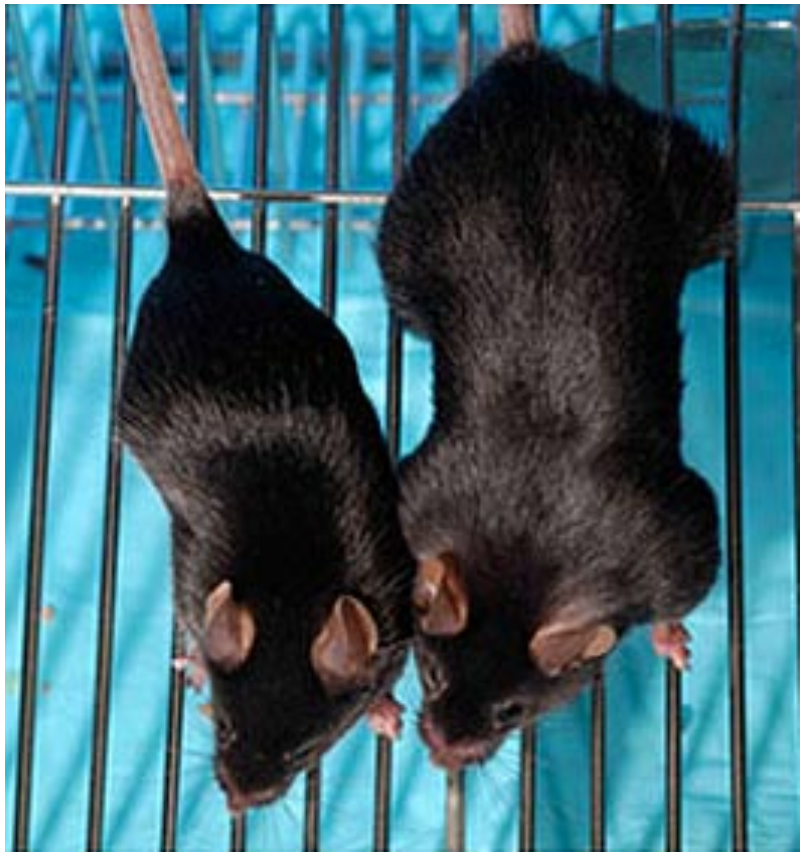
Dramatic growth of mice that develop from eggs microinjected with metallothionein–growth hormone fusion genes

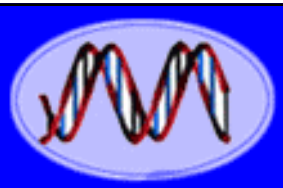
Richard D. Palmiter*, Ralph L. Brinster†, Robert E. Hammer†, Myrna E. Trumbauer†, Michael G. Rosenfeld‡, Neal C. Birnberg§ & Ronald M. Evans§



Nature, December, 1982
38 Years Ago!!!!!!!!







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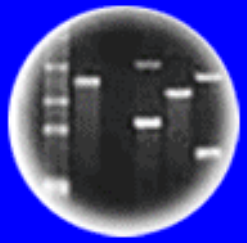

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Plants of Tomorrow

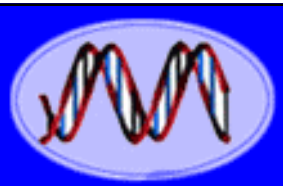
Genetic Engineering Faster Growing Salmon For More Productive Aquafarms!

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

FDA Approves Application for AquaBounty Salmon Facility in Indiana

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

FDA U.S. FOOD & DRUG ADMINISTRATION



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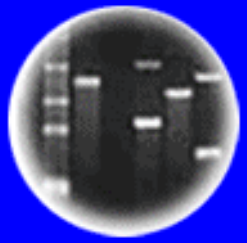

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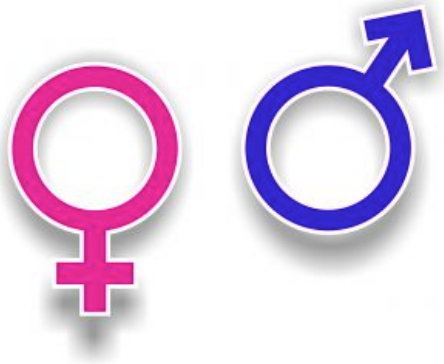
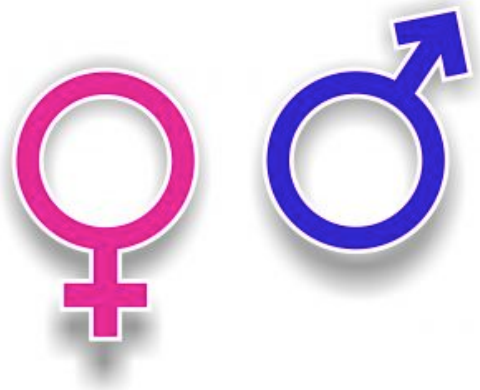
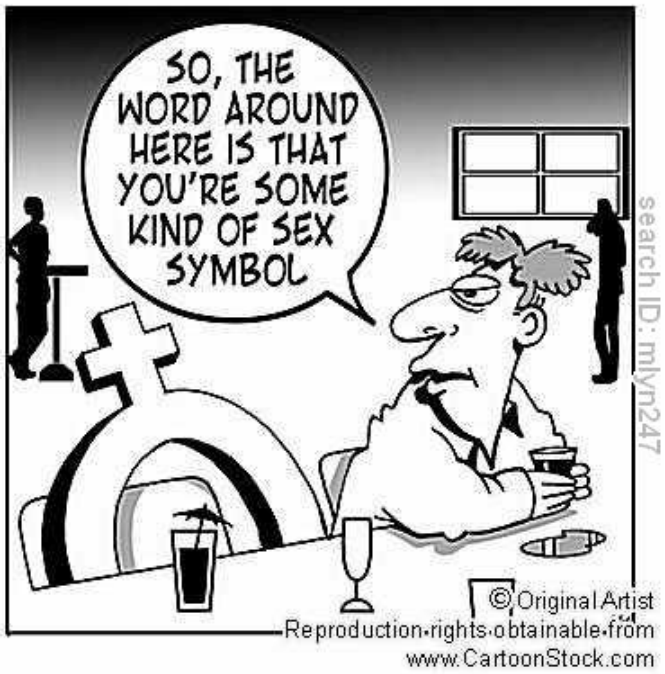
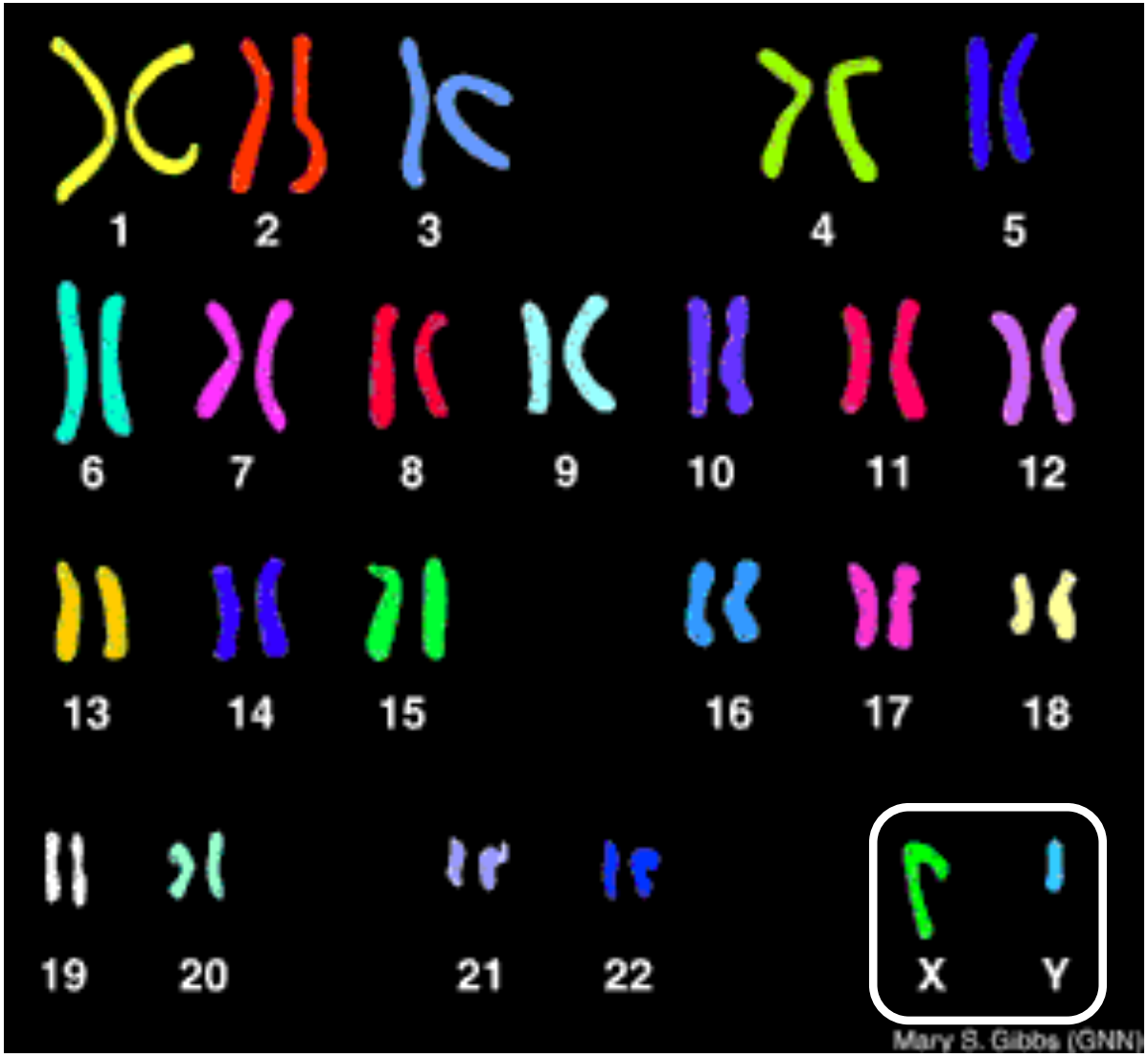


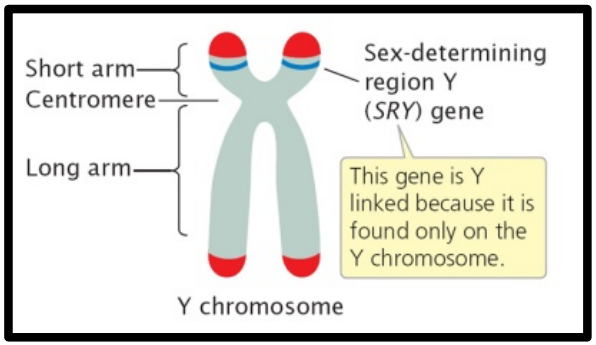


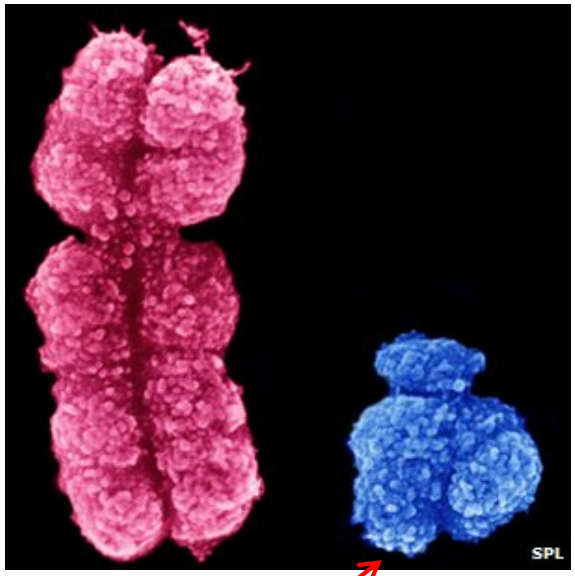
How About Engineering The Sex Of An Organism?



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





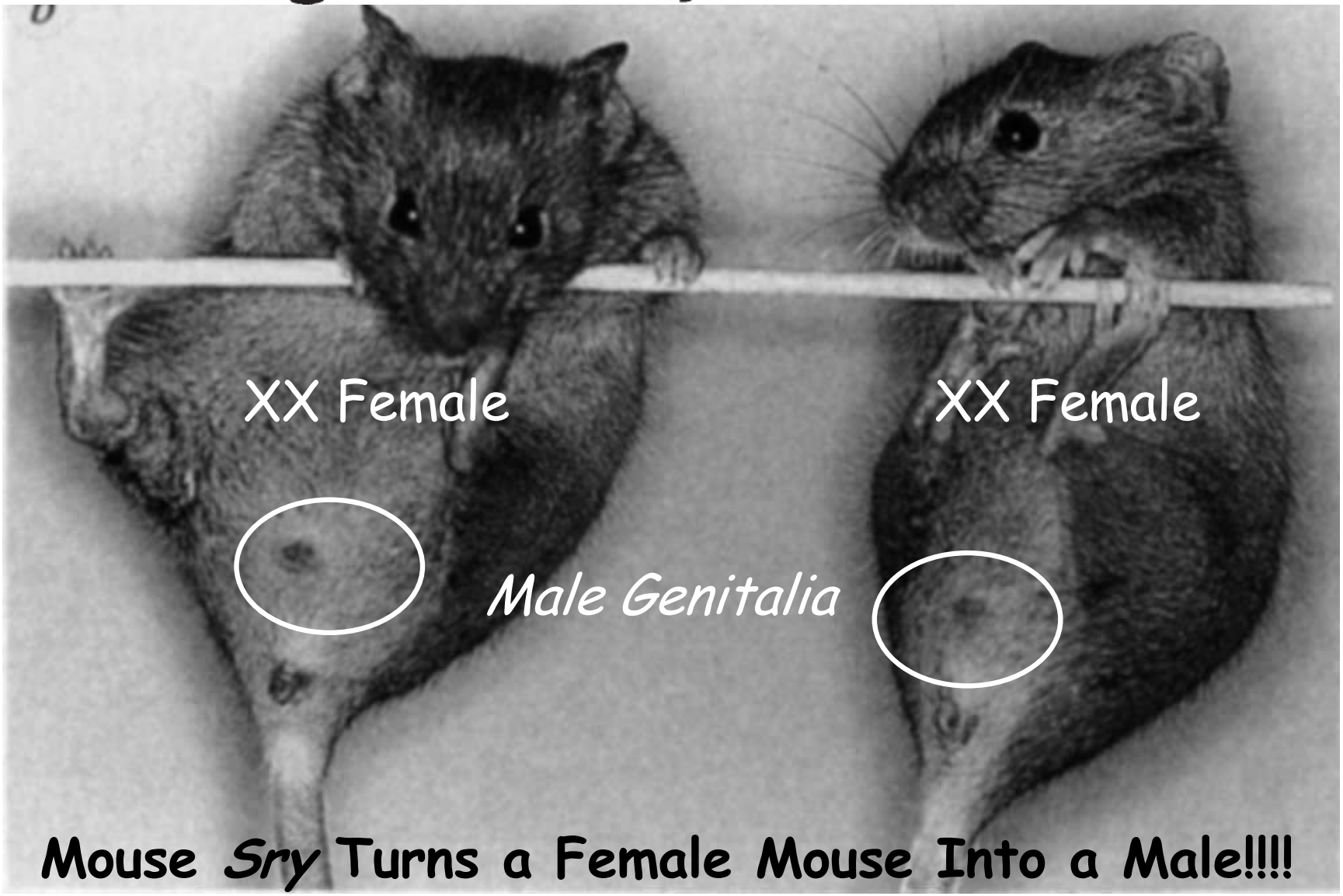


Male SRY Gene
(Sex Determining Region Y)
Regulates Other Genes
Turns on Switches

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

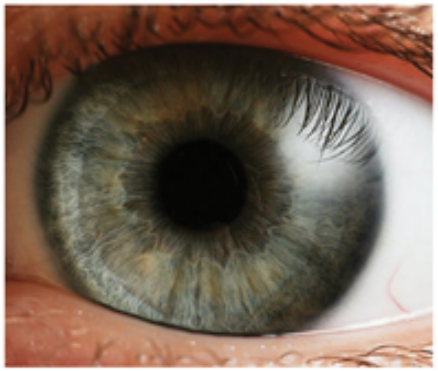
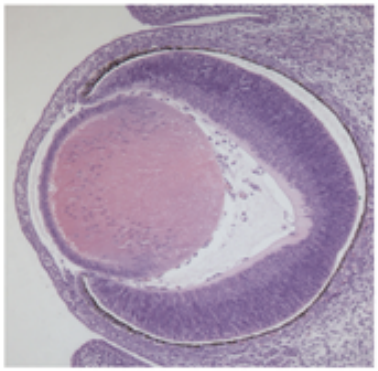
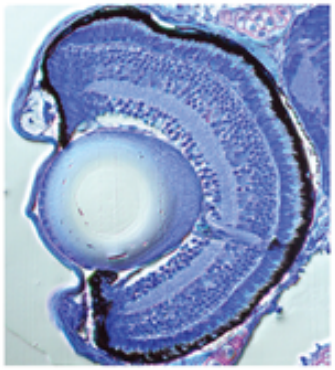
Male development of chromosomally female mice transgenic for *Sry*

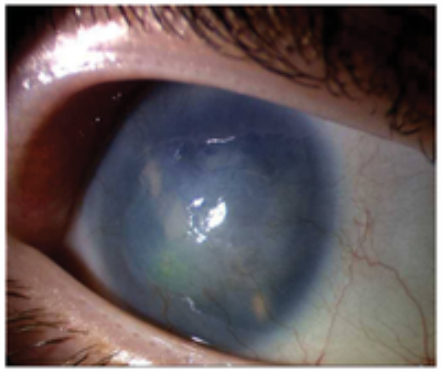
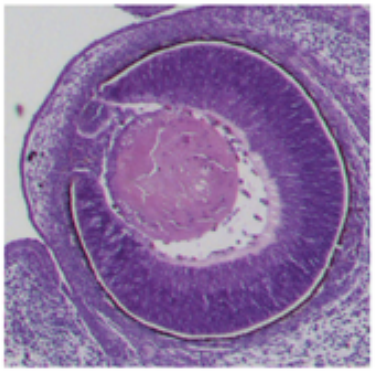
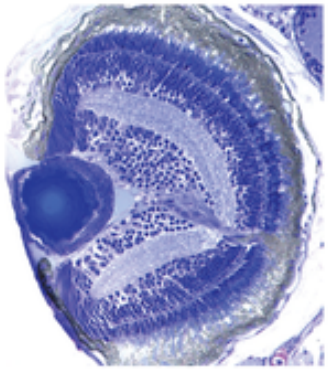
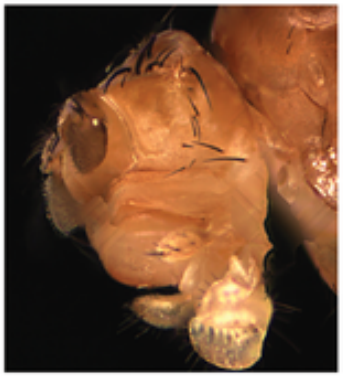
Nature, May 9, 1991



Functional Proof That Sry (TDF) Controls Male Sex 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	*Drosophila*
WT				
mut				
	***PAX6*$^{+/-}$**	***Pax6*$^{-/-}$**	***pax6b*$^{-/-}$**	***ey*$^{-/-}$**
EQs	cornea opaque iris absent 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*

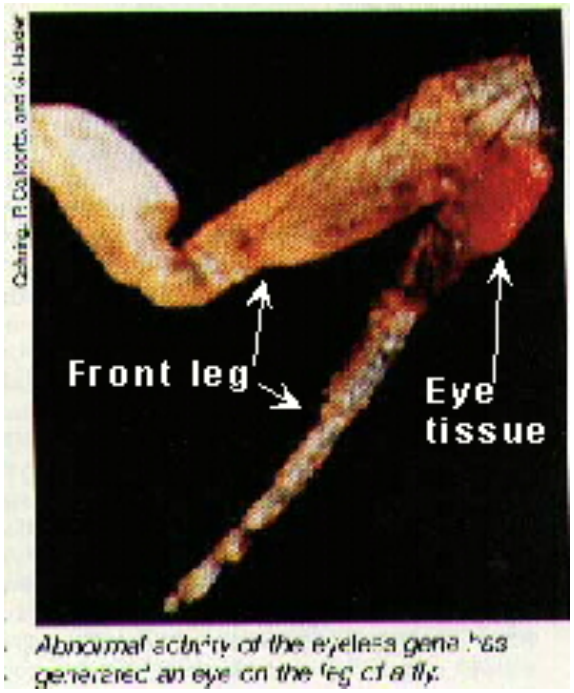
Science 267, 1788, 1995

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

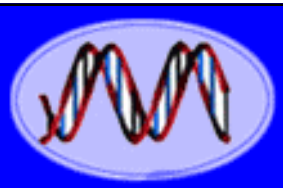
Different Fly Regions

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.



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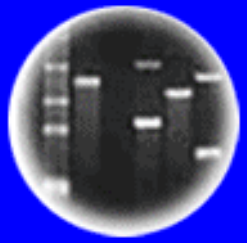

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What About Inserting Bacterial Genes Into Plants To Produce a Result With Significant Agricultural Application?

GARDEN | GUIDE

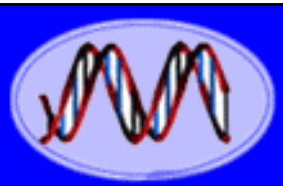
SUNSET

WHAT TO DO IN YOUR GARDEN IN SEPTEMBER

Southern California Checklist

DEBRA LAMBERT

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



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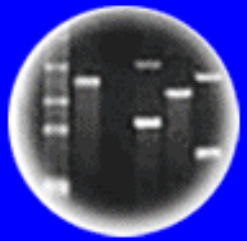

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How to Use Bt Pesticide as an Organic Pest Control

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

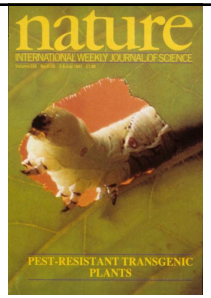
OMRI Listed

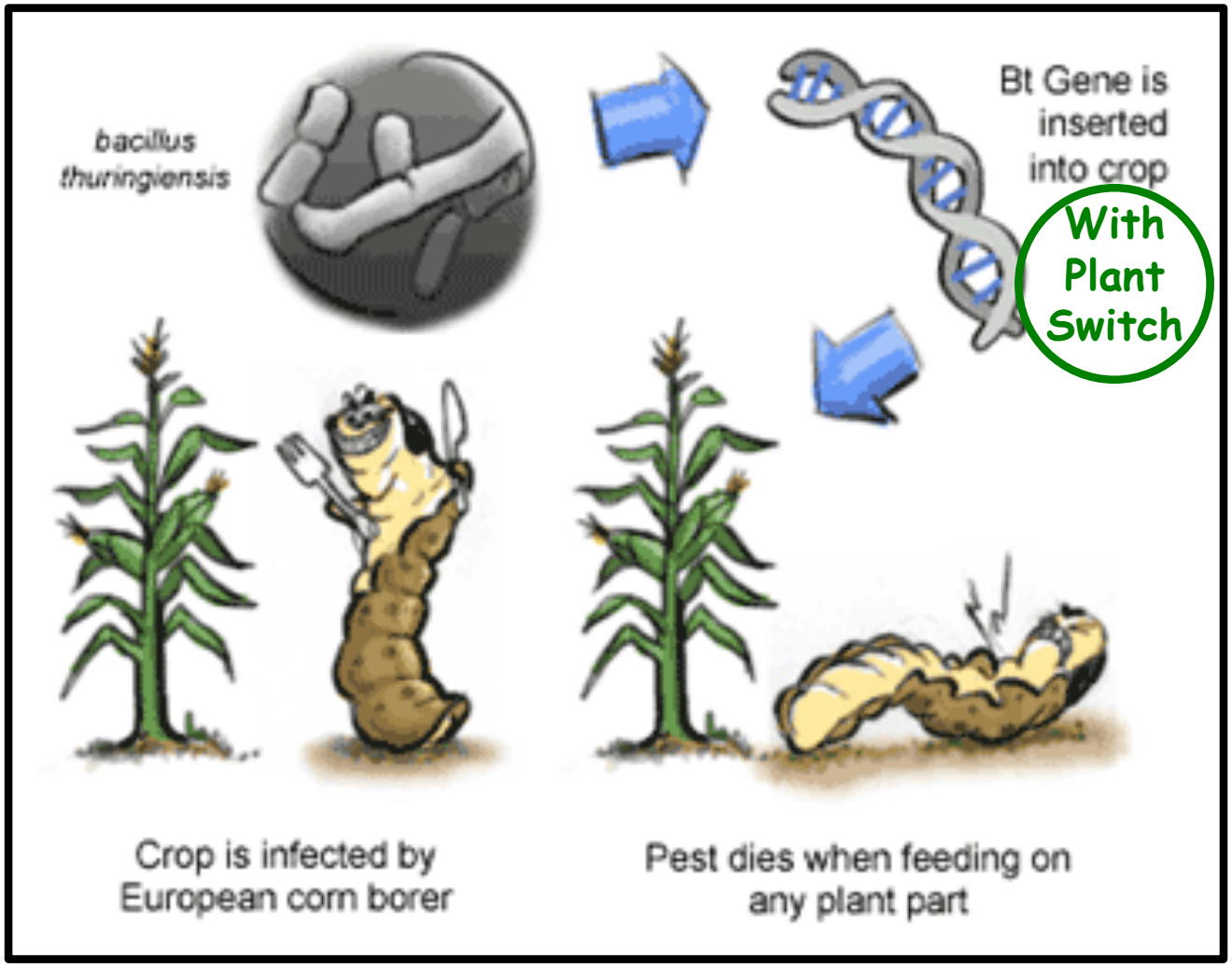
Monterey

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%

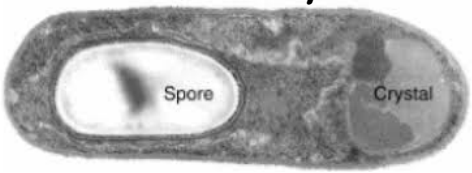
*The percent active ingredient does not indicate product performance and potency measurements are not federally standardized.

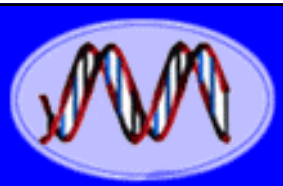
Crops Can Be Engineered With Bt For Insect Resistance





Bt Toxin in Spores





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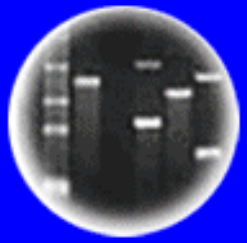
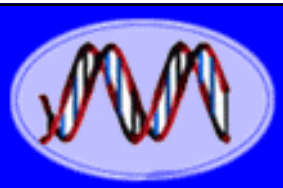


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Genetic Engineering a Plant to Resist Worms!
Implications For Agriculture



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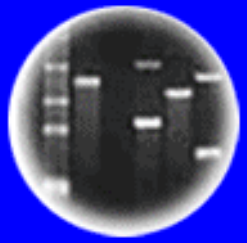

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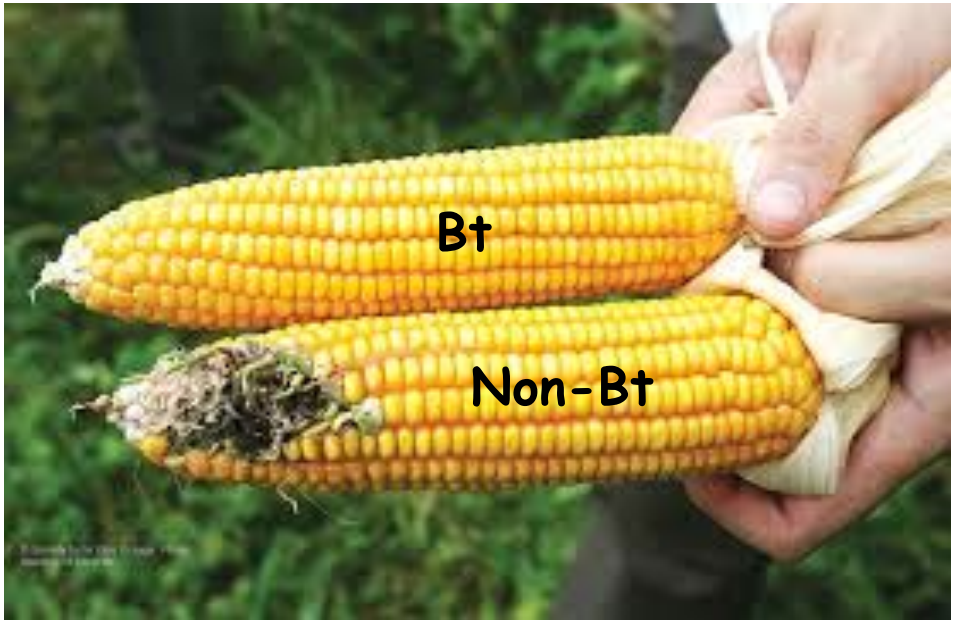
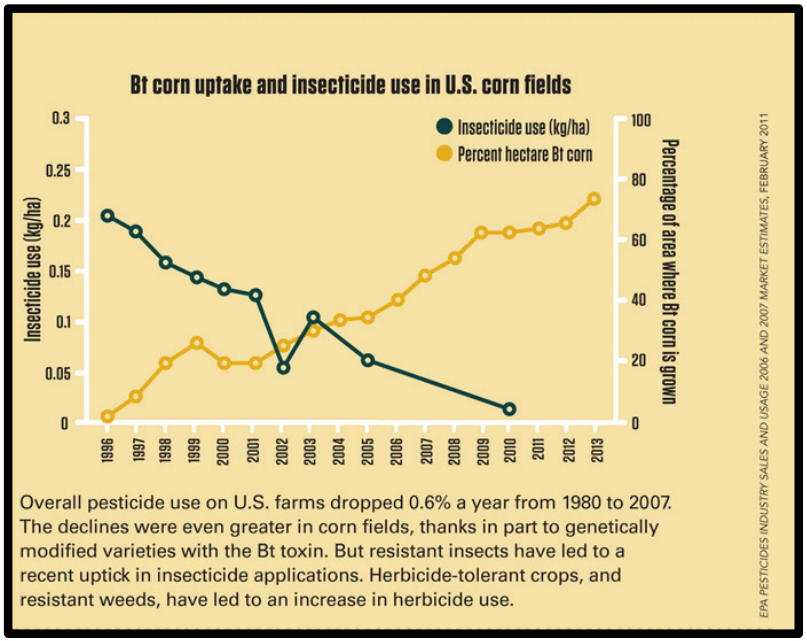
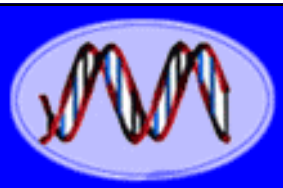
Adoption of Bt Corn By US Farmers Has Reduced the Use of Pesticides!!!!





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.



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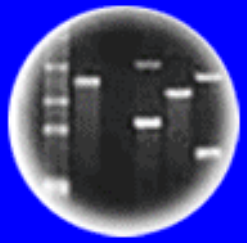

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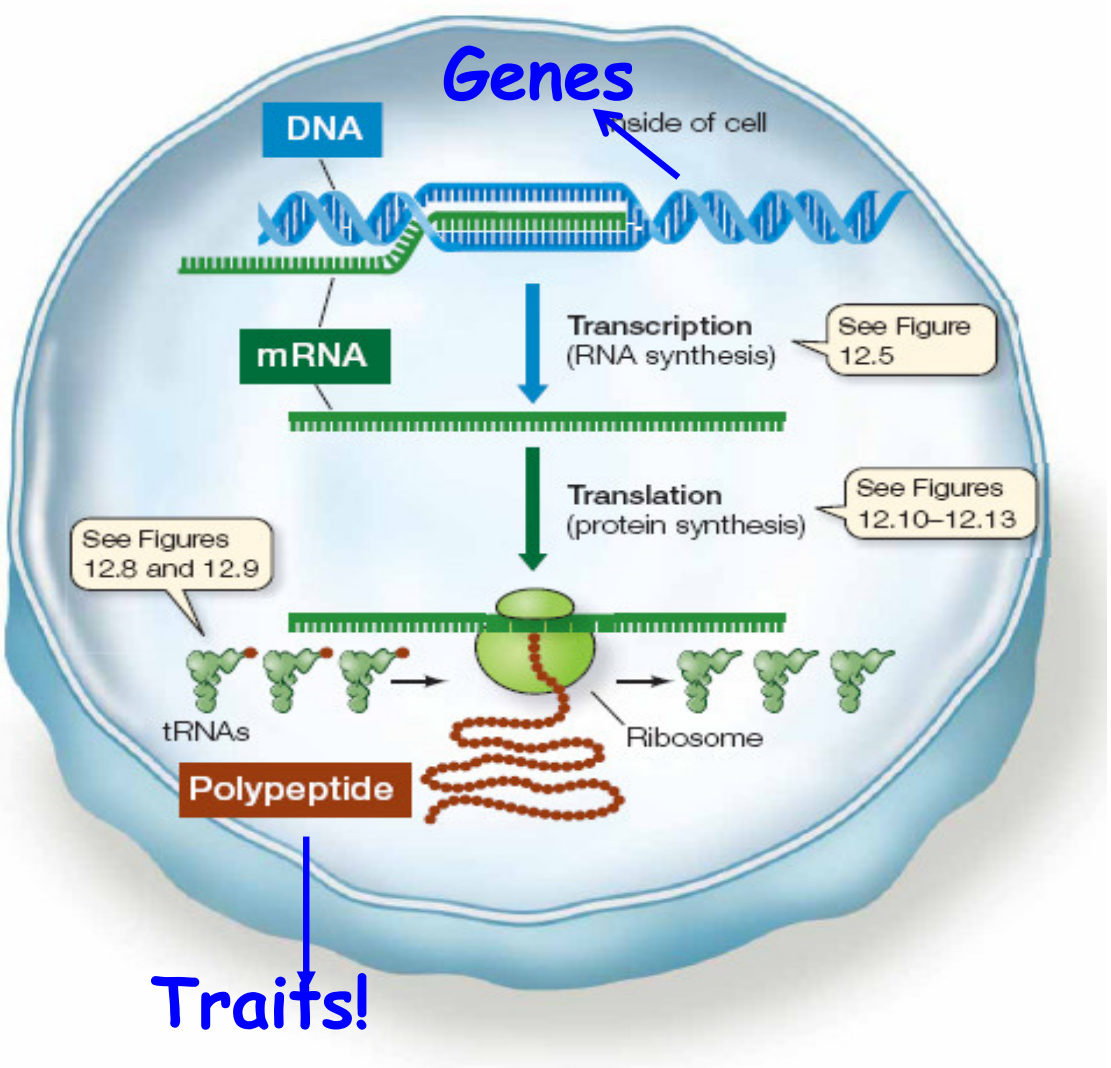
What Can We Infer FROM These Genetic Engineering Experiments About How Genes "Work" and Genetic Processes in All Living Organisms?

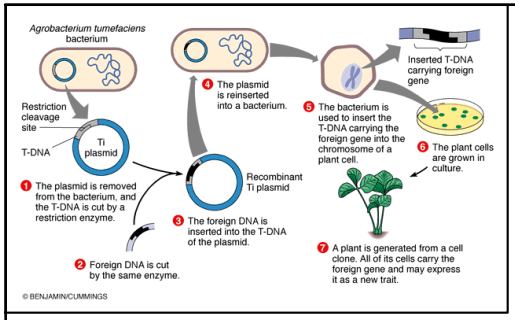


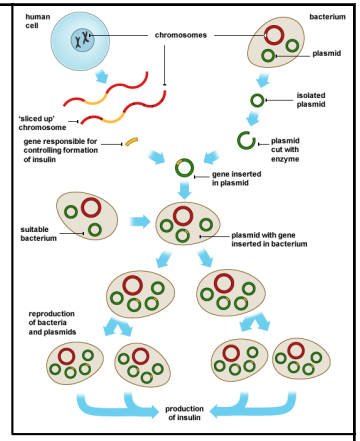
Observations and Inferences From Genetic Engineering Experiments

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

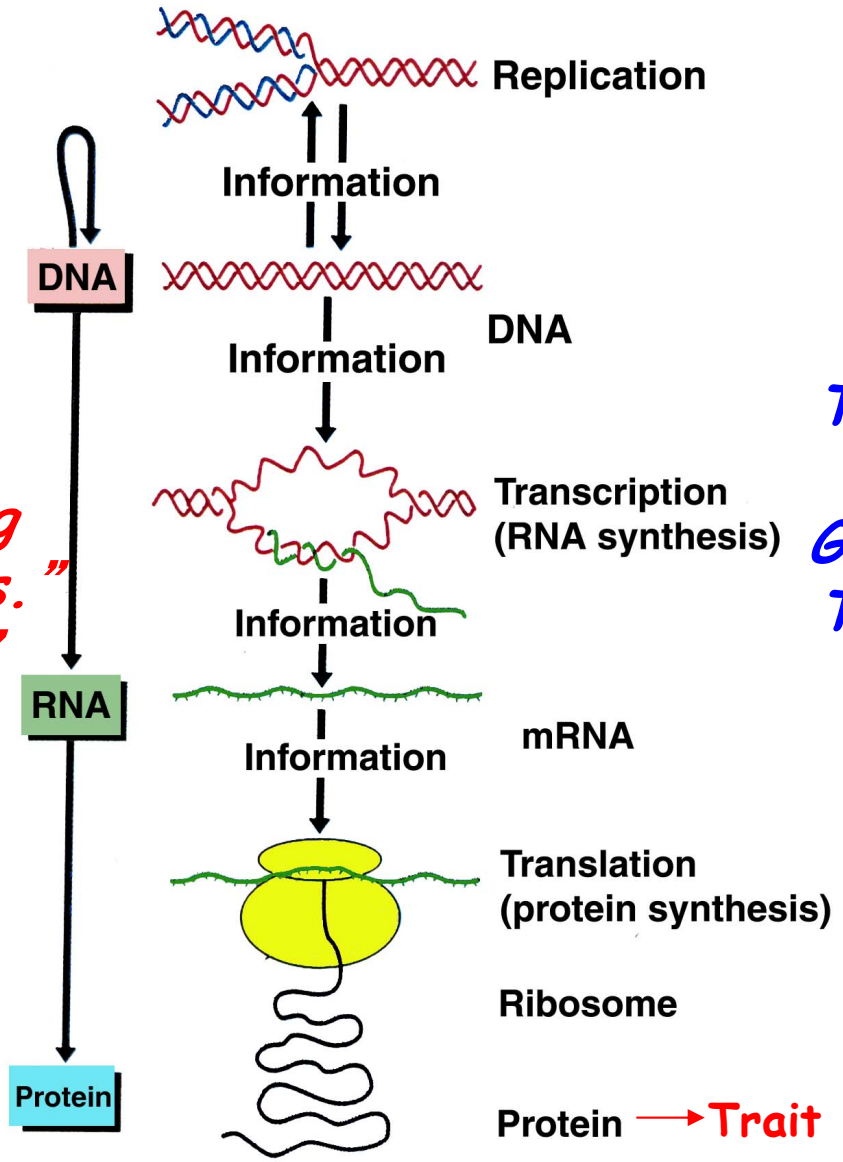
2. *Basic Genetic Processes Are Universal (Replication & DNA to RNA to Protein)*
   E. G. - The Bt Gene Directs the Production of Bt Protein in Crops.

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 Glo Fish & Bt Genes Are Inherited Generation After Generation.





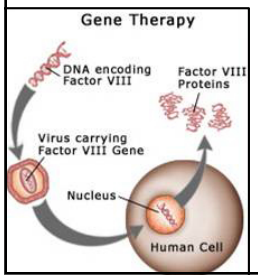
THE FACTS ARE:

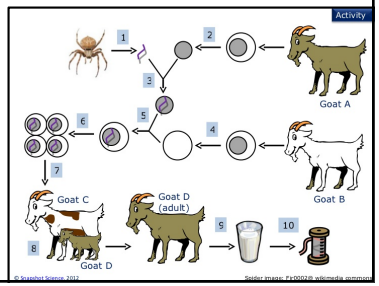


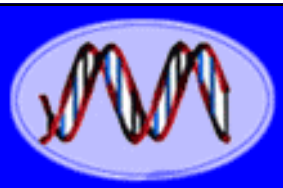
Can Intervene in This Process in Cells

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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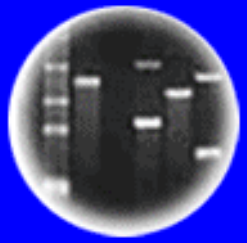

DNA Fingerprinting

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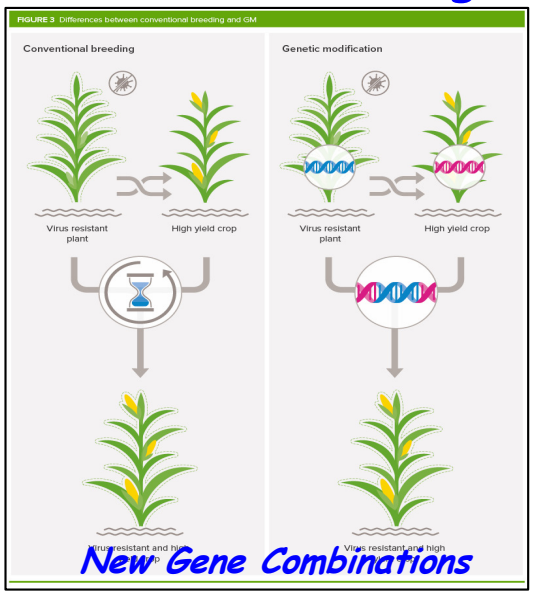
Plants of Tomorrow

There is Nothing New About Genetic Engineering!

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

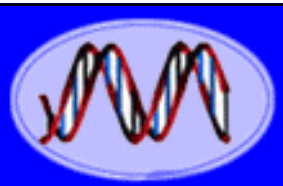
1. Classical Breeding



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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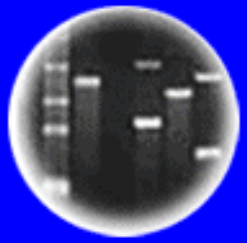

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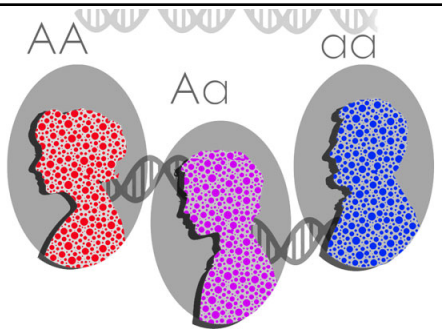
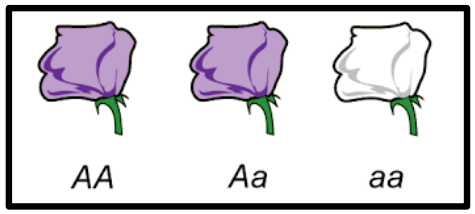
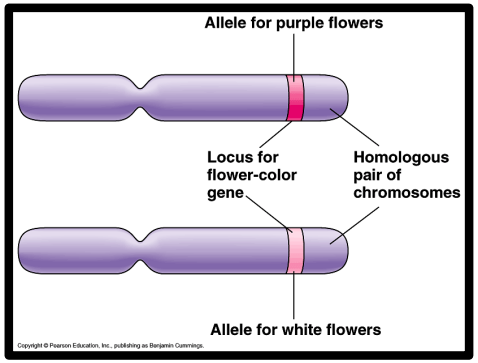


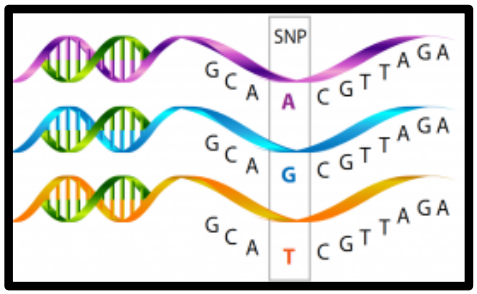
Populations of All Organisms Contain Genetic Variability

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

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





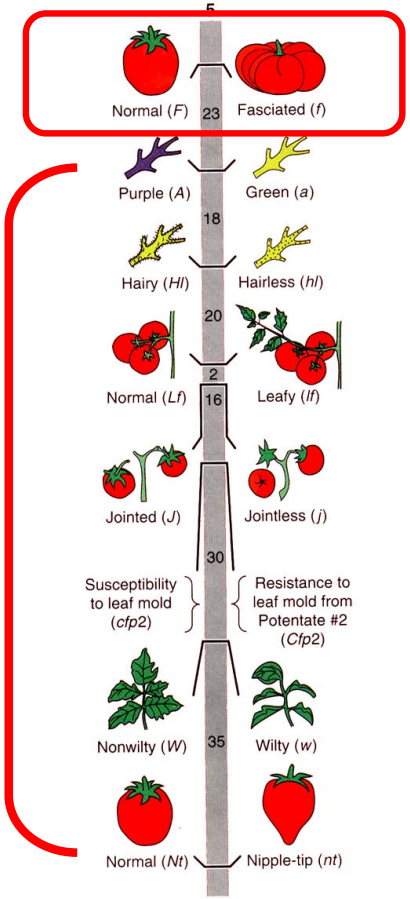


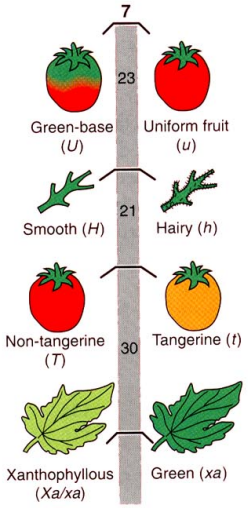
Mutations in a Gene That Change Its DNA Sequence & Slightly Alters Its Function (e.g., fruit size, color) and Produce Allelic Forms & Genetic Variability

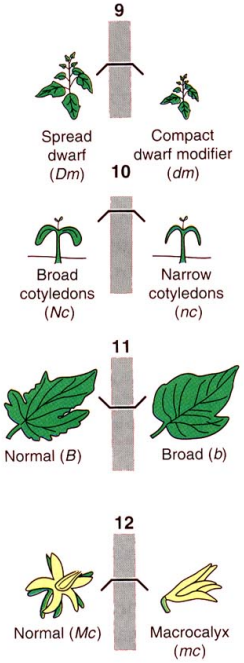
Alleles Reside at the Same Position on a Chromosome Because They Represent the SAME Gene

Alleles

Allele Phenotypes Specify Markers For Each Gene Location!



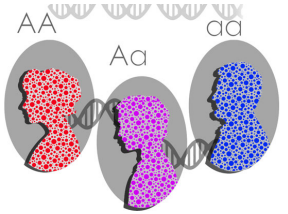


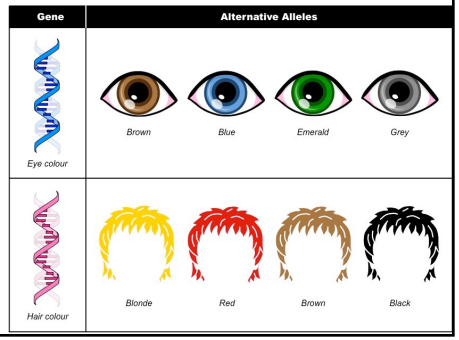


Different Genes

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

mutations result in genetic diversity!!!

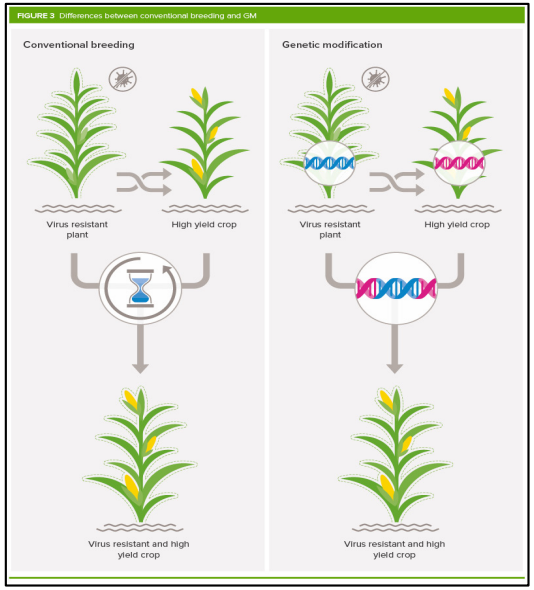




Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

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

1. Classical Breeding



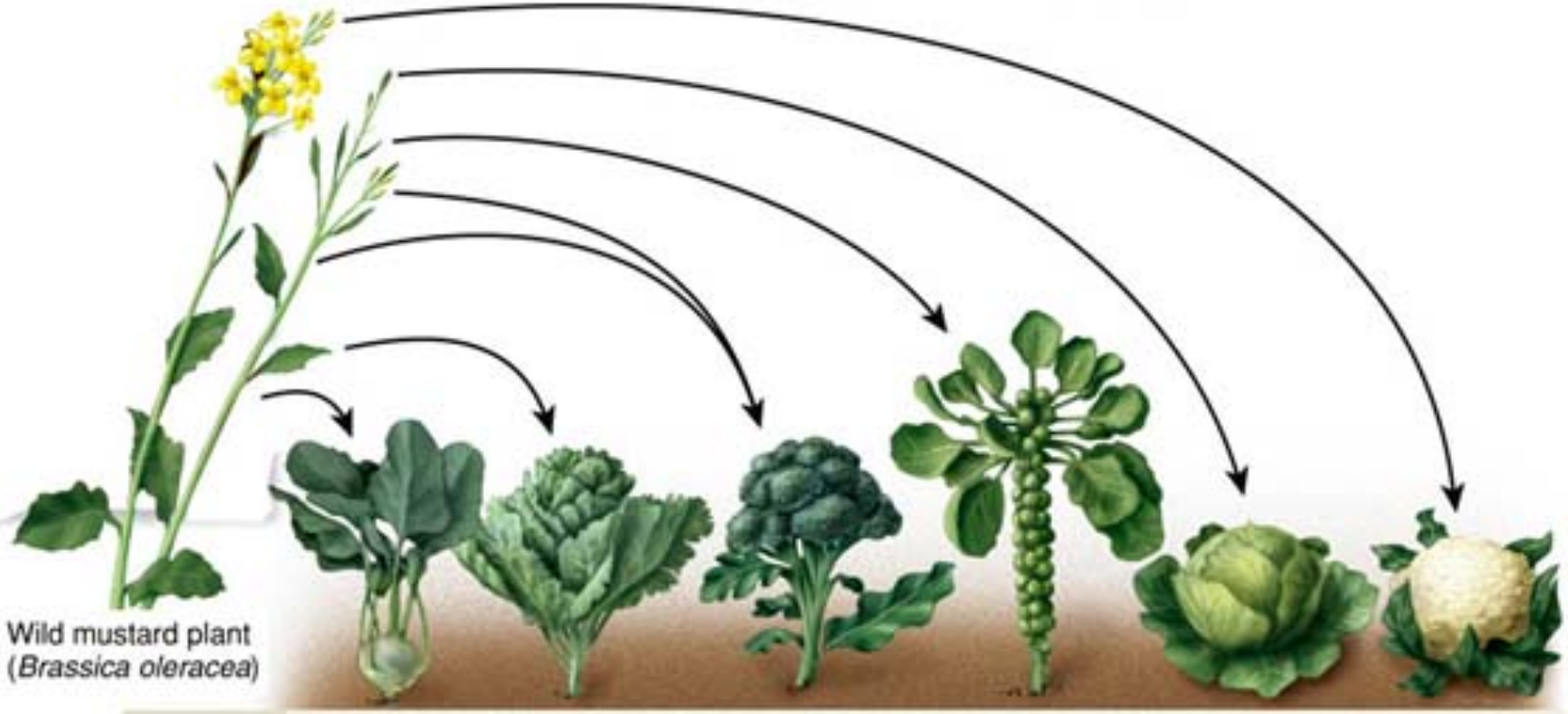
New Allele Combinations

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'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!!!!!!

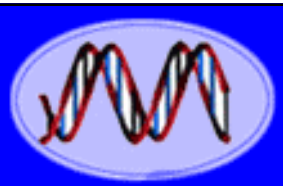


Strain	Kohlrabi	Kale	Broccoli	Brussels sprouts	Cabbage	Cauliflower
Modified trait	Stem	Leaves	Flower buds and stem	Lateral leaf buds	Terminal leaf bud	Flower buds

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

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



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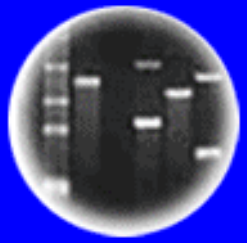

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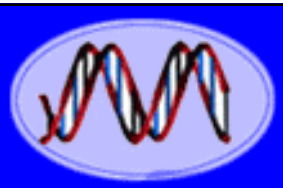
The Problem With Breeding the "Old Fashioned Way"

Cannot Predict Results!



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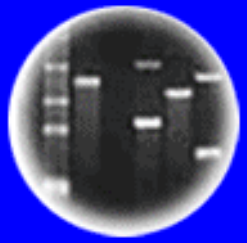

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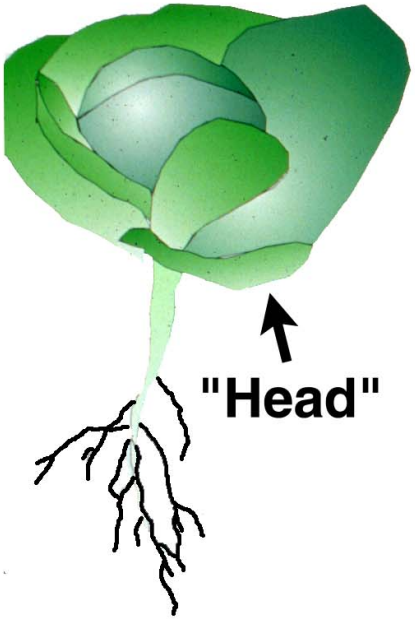
The Problem With Breeding the "Old Fashioned Way"

Engineering A Novel Crop By "Wide" Breeding

Cabbage (*Brassica*)



"Head"

X

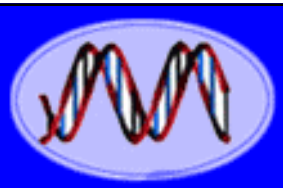
Radish (*Raphanus*)

Storage
Root

???

ıko

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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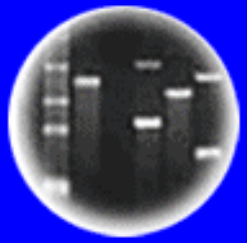

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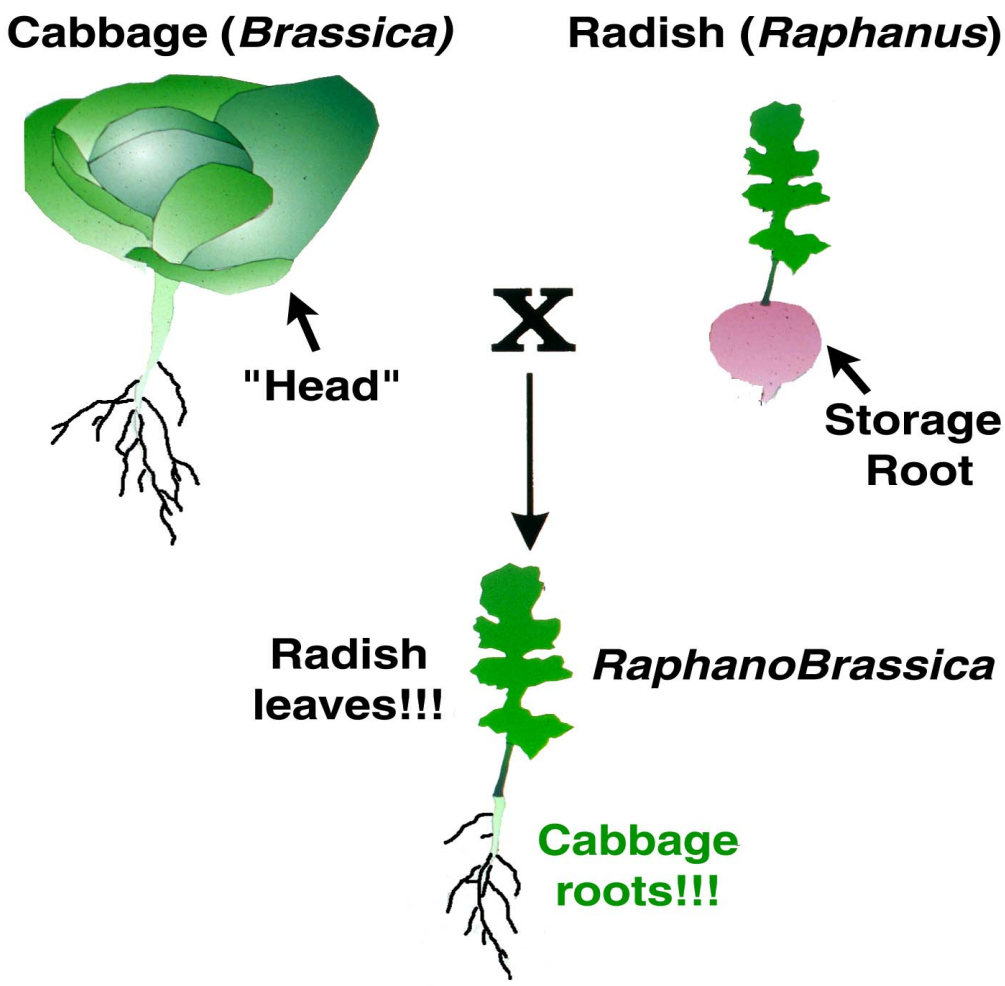
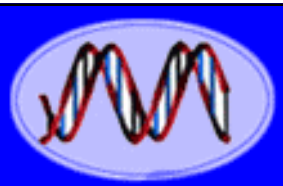


Results Show the Unpredictability of Classical Breeding Approaches!!
Compare With the Modern Genetic Engineering Examples Shown Previously



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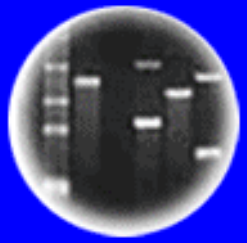

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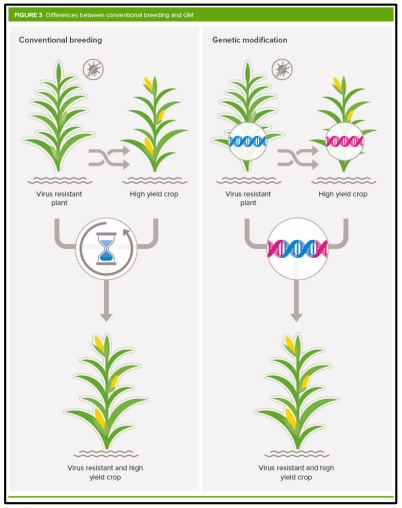
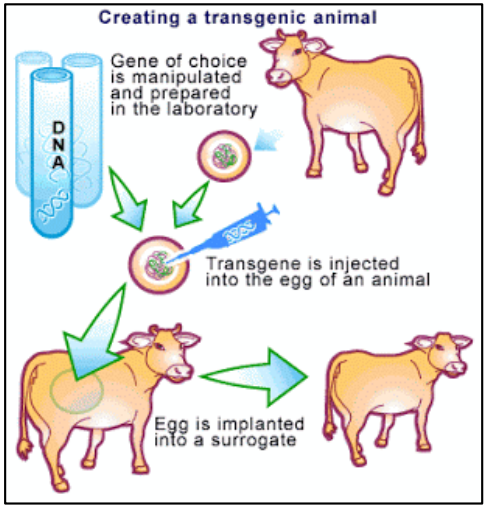
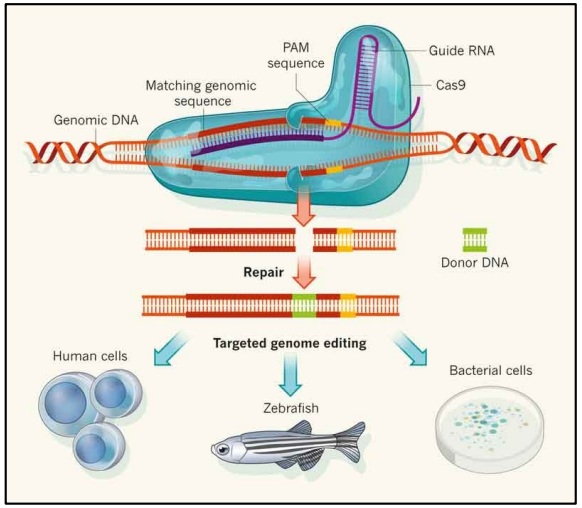
Genetic Engineering is a TECHNIQUE!

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



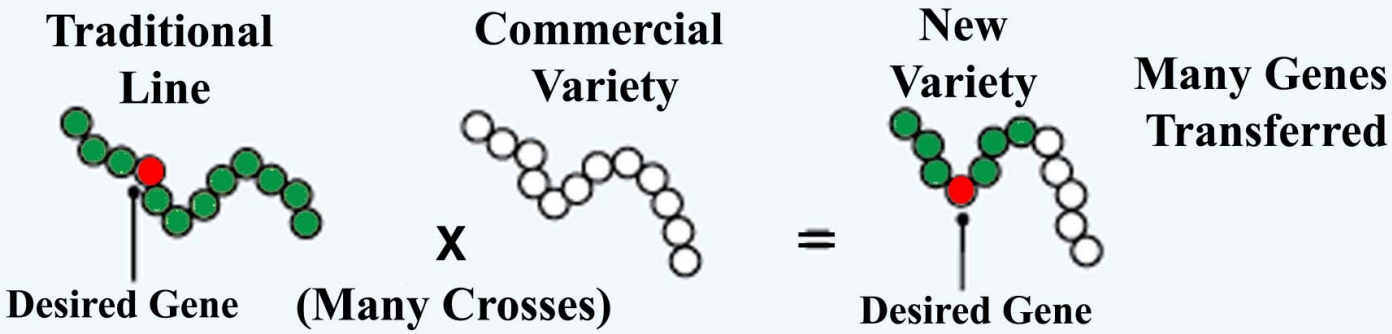




Classical vs. DNA or Molecular Genetic Engineering Techniques

TRADITIONAL PLANT BREEDING

Plant Breeding Combines Many Genes At Once

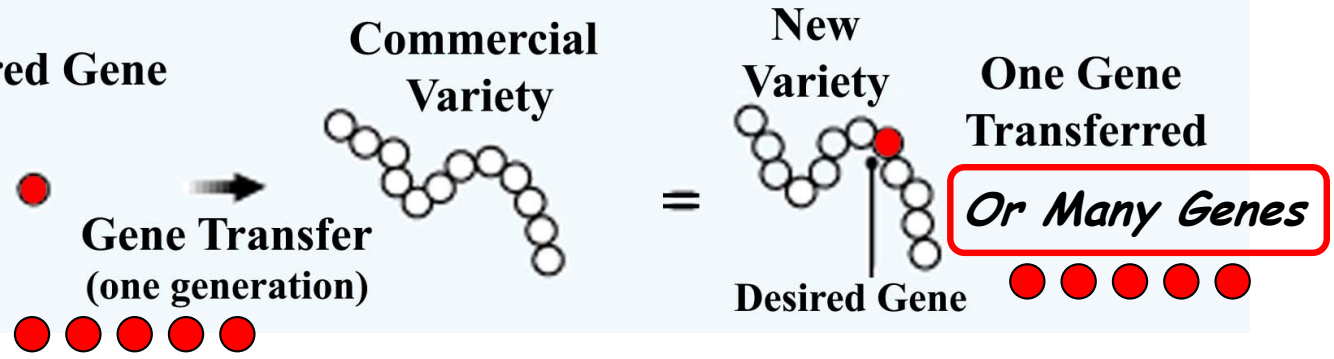


PLANT BIOTECHNOLOGY

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!!

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)

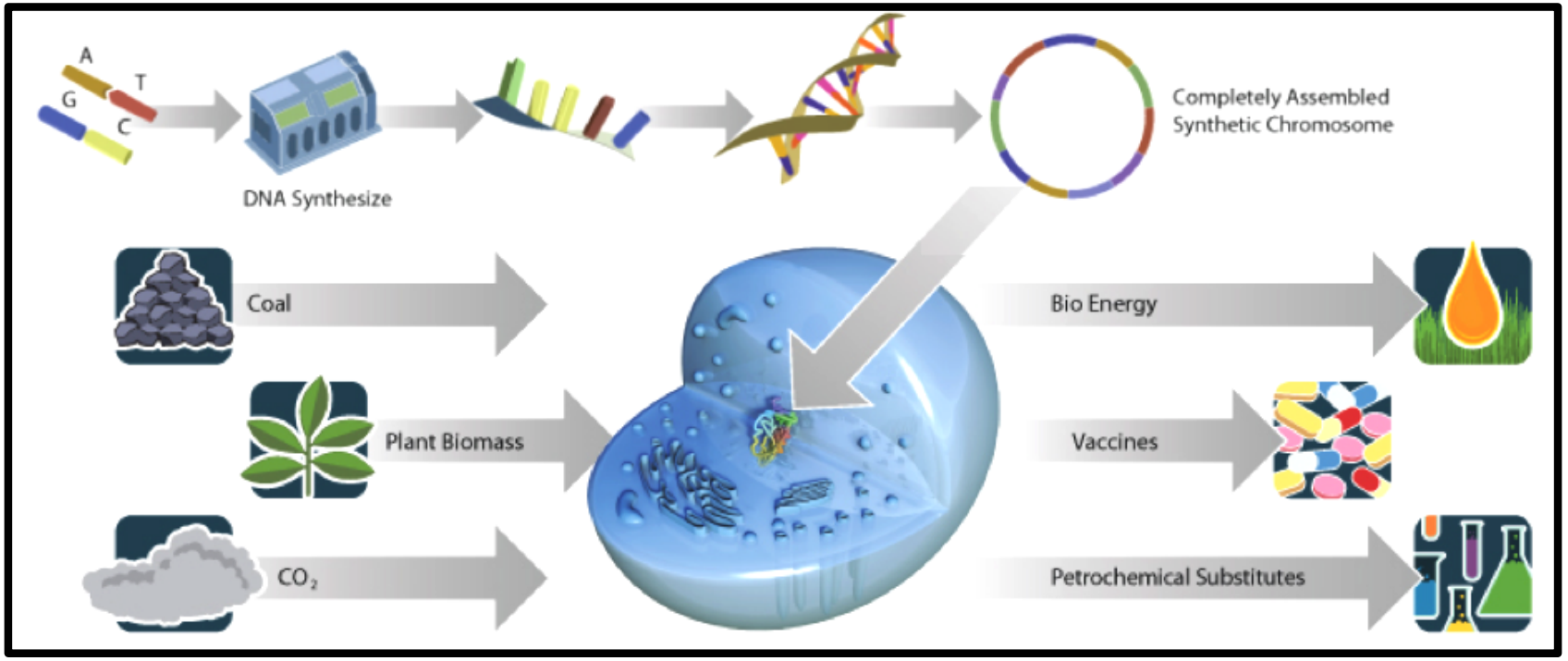
4. Speed - Can Engineer a New Organism in a Generation

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

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

There Are No Genetic Limits!

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome



Think of the Possibilities……..

As first lab-made yeast genome nears completion, scientists set sights on improved human ones