

DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

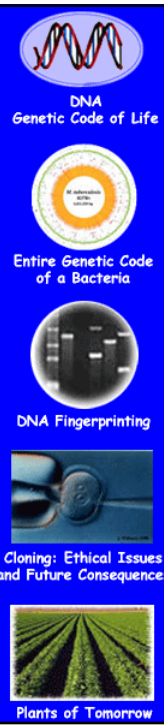
Plants of Tomorrow

HC70A
Spring 2021
**Genetic Engineering in Medicine,
 Agriculture, and Law**

Professor Bob Goldberg

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

1



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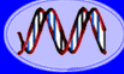
Cloning: Ethical Issues
and Future Consequences

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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?

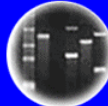
2




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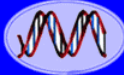


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Last Week's Lecture...a Reminder



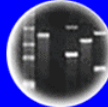
3




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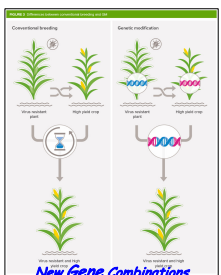
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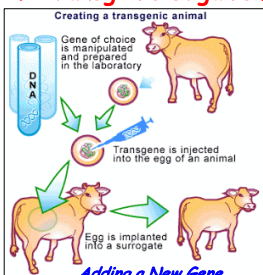
Three Genetic Engineering Techniques That Generate **GMOs!!!**

1. Classical Breeding



New Gene Combinations

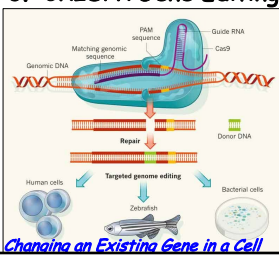
2. Transgenic Organism



Adding a New Gene

3. CRISPR Gene Editing

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

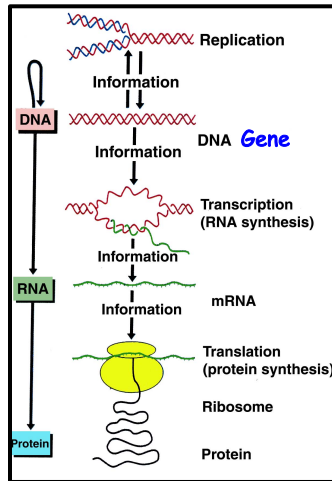
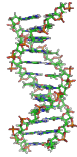


Changing an Existing Gene in a Cell

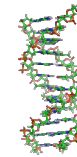
4

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

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

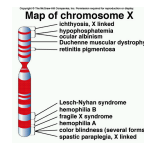
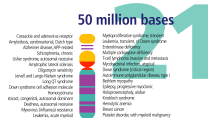
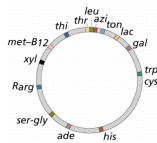


2. All Organisms Use The SAME Processes And "RULES" to Generate Traits!!



5

"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**

6

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!!



7

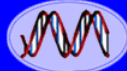
The Scientific Method



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



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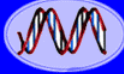


Cloning: Ethical Issues
and Future Consequences




Plants of Tomorrow

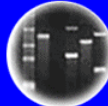
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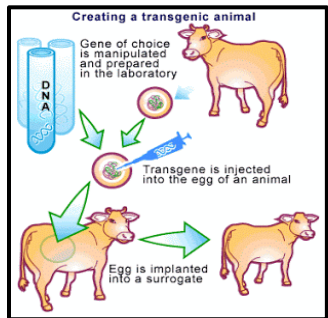
What Can Be Done With Modern Genetic Engineering?

Some Examples

Transgenic Organisms

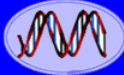
Adding a New Gene To an Organism's Chromosomes (Genome)

Method Two




Creating a transgenic animal
Gene of choice is manipulated and prepared in the laboratory
Transgene is injected into the egg of an animal
Egg is implanted into a surrogate

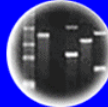
9




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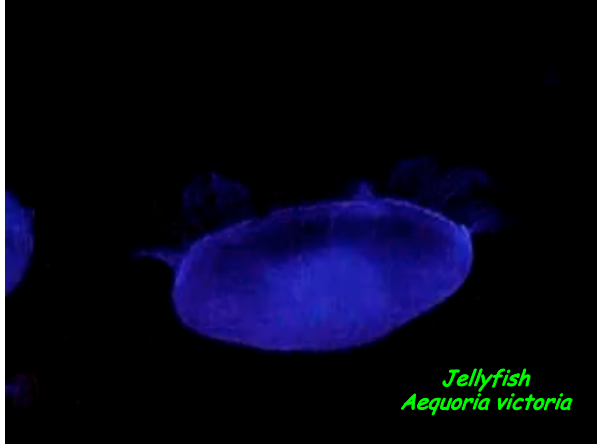


Cloning: Ethical Issues
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Using a Jellyfish Gene to Engineer *Glowing* Bacteria, Animals, and Plants!!!!




Jellyfish
Aequoria victoria

Green Fluorescence Protein (GFP)
(238 amino acids)



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



10

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* and Select Cells With Recombinant Plasmid
- Determine if GFP Gene is Active

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

11

A Typical Gene

ON OFF Switch

Regulatory region

Protein Coding Region

Termination region

5' end 3' end

Intergenic DNA Distal elements Promoter Termination region Intergenic DNA

↓ Transcription

Can Engineer Genes Like Lego Pieces

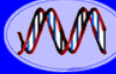
Specific DNA Sequences Perform Specific Functions


Essential HC70A Concept

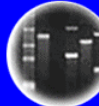
Need a **Species-Specific Switch** to Allow a Gene To Function in a Specific Organism


Bacterial Switch


12

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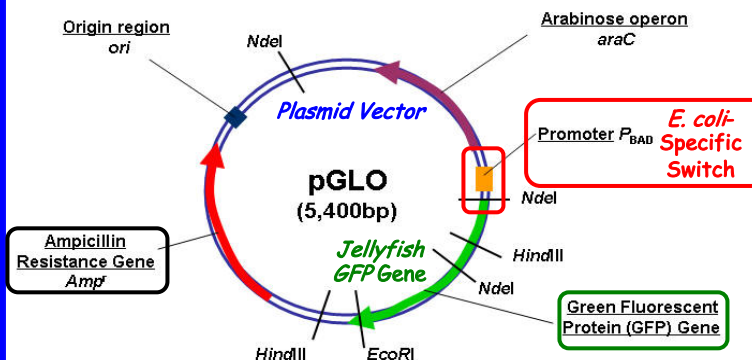
 DNA Fingerprinting

 Cloning: Ethical Issues and Future Consequences

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A Recombinant Plasmid Containing the GFP Gene

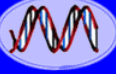
How Make it Active in Living Cells?




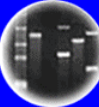
Graphic © E. Schmid / 2003


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


13

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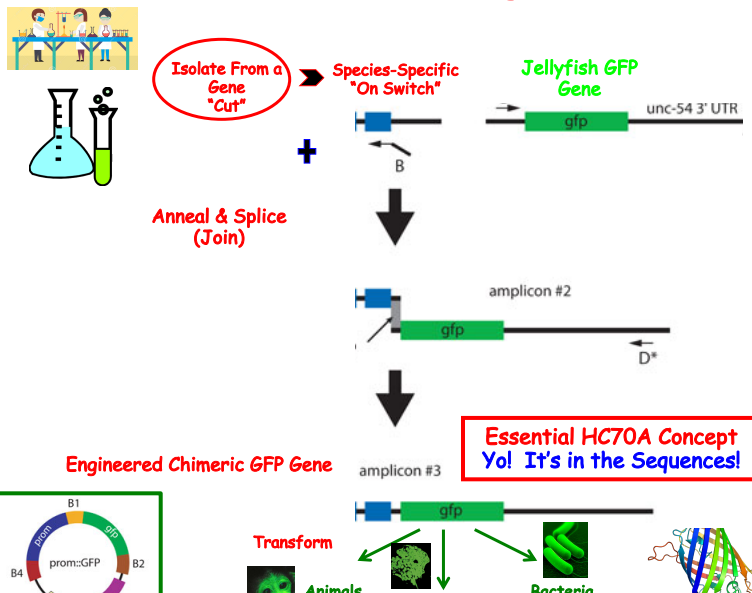
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


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Engineering the Jellyfish GFP Gene to Be Active in Different Organisms



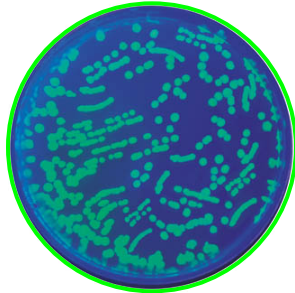
Essential HC70A Concept Yo! It's in the Sequences!

Transform  Animals  Plants  Bacteria

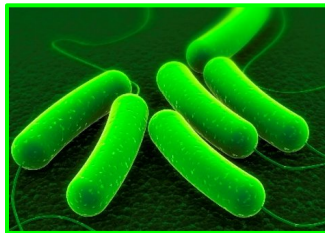
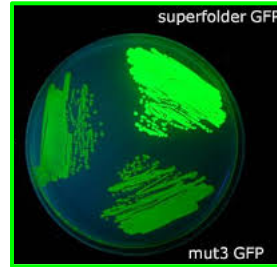
14

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

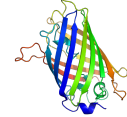
What Are the Conceptual Implications of This Experiment?



E. Coli Switch + Jellyfish GFP Gene

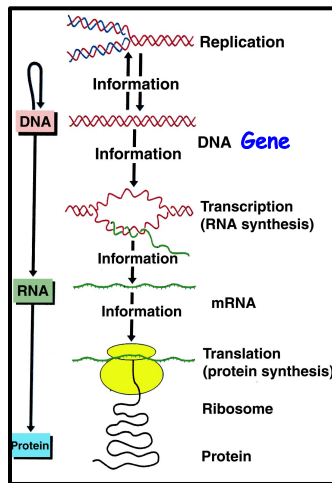
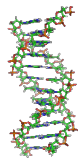


E. Coli Synthesizes GFP Protein!

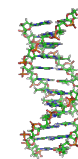


Genes & DNA Obey the Same Rules Using *Either* Classical or Modern DNA Engineering Approaches!! ***BOTH Produce GMOs!!!!!!***

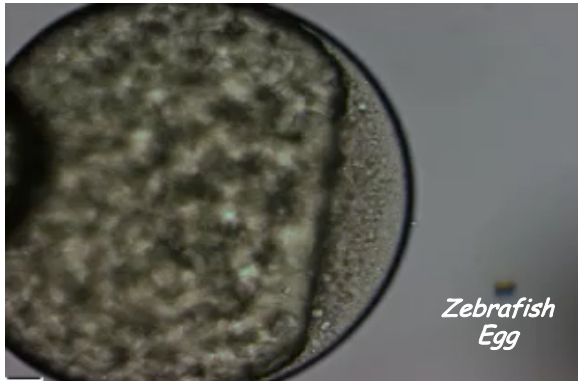
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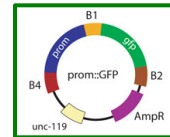
2. All Organisms Use The SAME Processes And "RULES" to Generate Traits!!



Engineering a "GloFish"



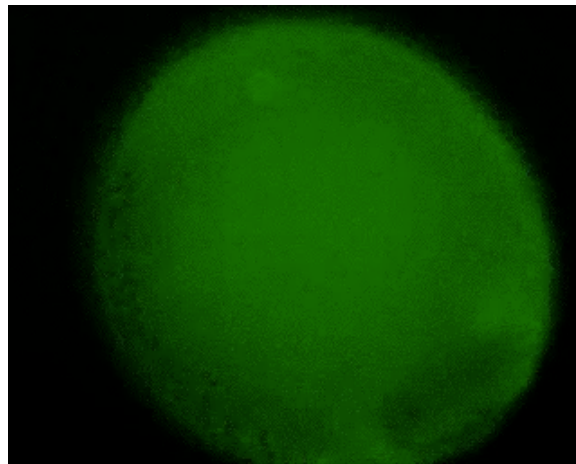
Zebrafish
Danio rerio



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

17

A "GloFish" Embryo!!



Zebrafish - *Danio rerio*

18

Genetically Engineered "GloFish!!"



Note Different Fluorescing Colors - Due to Different Engineered Jellyfish Genes

19



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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!!



20

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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

21

How About a Glo Fly!

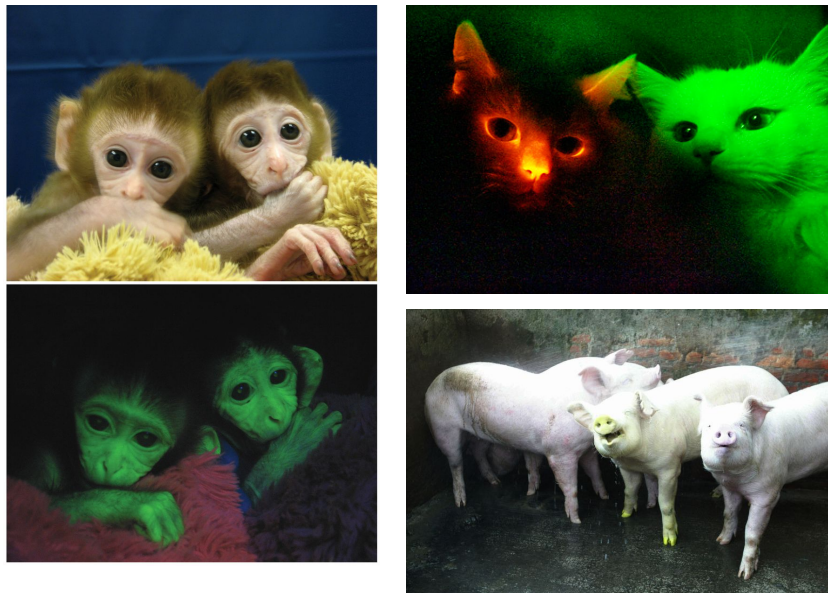
22

What About "Glo Mice!!!"



23

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



24

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



What are the Biological Implications of These Experiments?

25

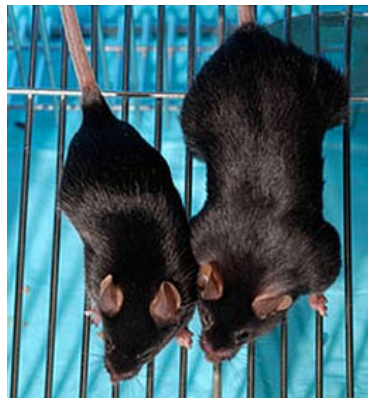
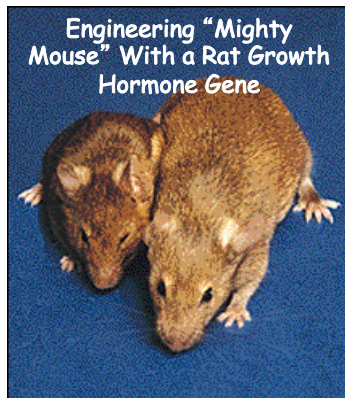
Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes

Richard D. Palmiter¹, Ralph L. Brinster², Robert E. Hammer¹, Myrna E. Trumbauer¹, Michael G. Rosenfeld¹, Neal C. Birnberg³ & Ronald M. Evans³

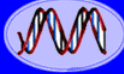


What Switch?


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



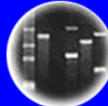
26




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Genetic Engineering Faster Growing Salmon For More Productive Aquafarms!

HOW THEY COMPARE




GM salmon Length: 24ins Weight: 6.6lb	Farm salmon Length: 13ins Weight: 2.8lb
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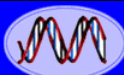
*Both fish are 18 months

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

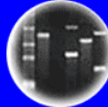





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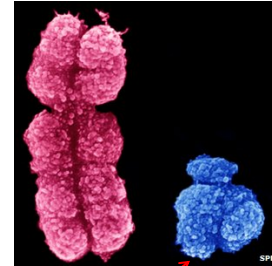
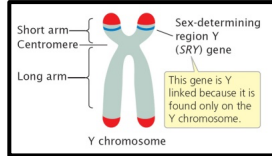
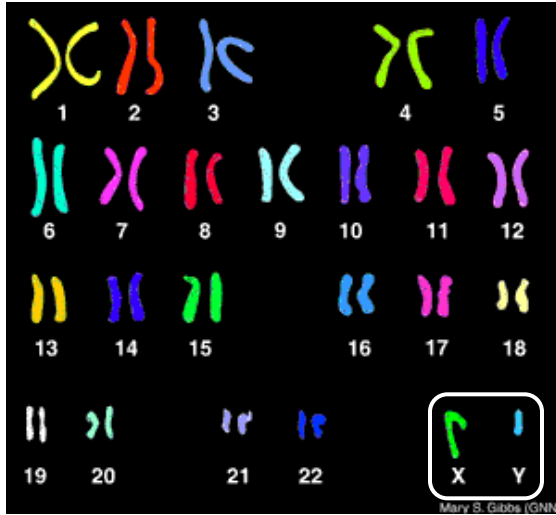





How About Engineering The Sex Of An Organism?



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www.CartoonStock.com

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

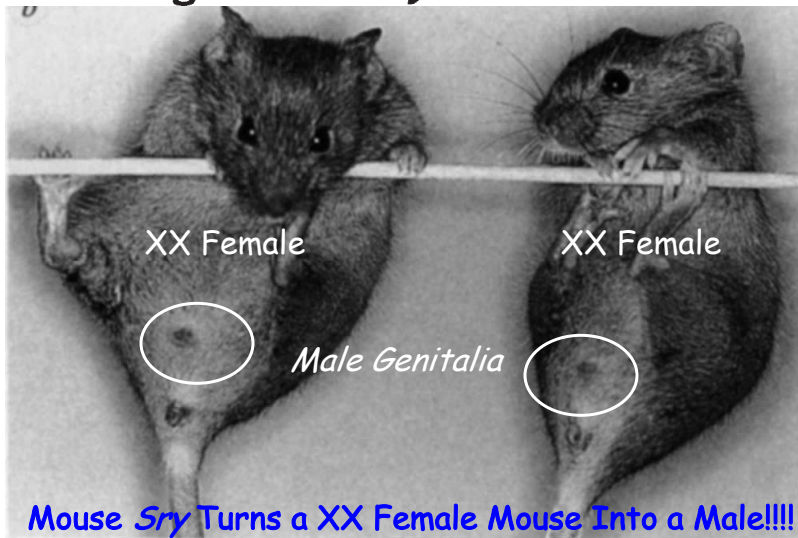


Male SRY Gene
(Sex Determining Region Y)
 Regulates Other Genes
 Turns on Switches

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

29

Male development of chromosomally female mice transgenic for Sry Nature, May 9, 1991



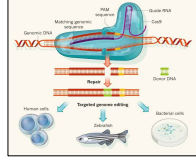
Mouse Sry Turns a XX Female Mouse Into a Male!!!!

Functional Proof That Sry (TDF) Controls Male Sex Development

30

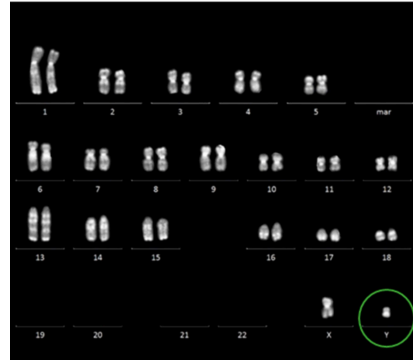
Engineering Male XY Pigs Into Female Pigs

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



31

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

	Human	Mouse	Zebrafish	<i>Drosophila</i>
Normal Gene WT				
Mutant Gene 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 absent anterior chamber absent	eye decreased size lens decreased size retina malformed	eye absent

32

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

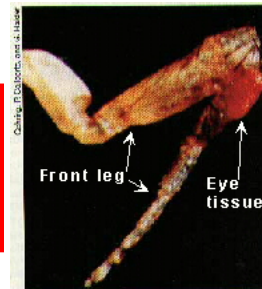


Different Fly Regions

Fly Leg Switch + Mouse Eye Gene

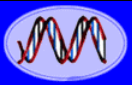


What Does This Experiment Tell Us About Mouse & Fly Eye Genes?




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


33




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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



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. ♦

34



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Plants of Tomorrow

How to Use Bt Pesticide as an Organic Pest Control

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

By Barbara Pleasant
April 24, 2013



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

Photo Courtesy Safe Brand



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


Other Ingredients: 100.00%

Total: 100.00%

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

35

Crops Can Be Engineered With Bt For Insect Resistance





Crop is infected by European corn borer

Pest dies when feeding on any plant part



Bt Toxin in Spores

Hornworm

36



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

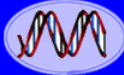


Plants of Tomorrow


Genetic Engineering a Plant to Resist Worms! Implications For Agriculture



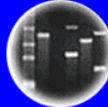
37




DNA
Genetic Code of Life




Entire Genetic Code
of a Bacteria



DNA Fingerprinting

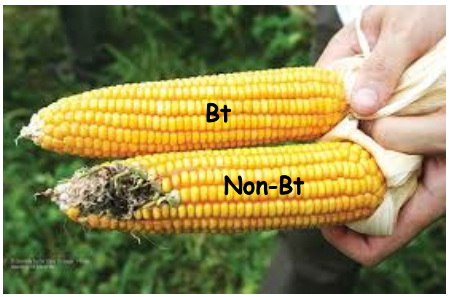


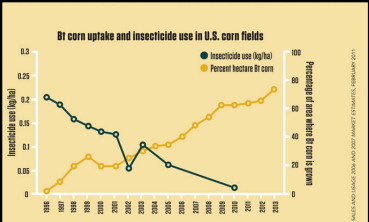
Cloning: Ethical Issues
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Adoption of Bt Corn By US Farmers Has Reduced the Use of Pesticides!!!!





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.

38

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

Traits!

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Genetic Code of Life

Entire Genetic Code
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DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

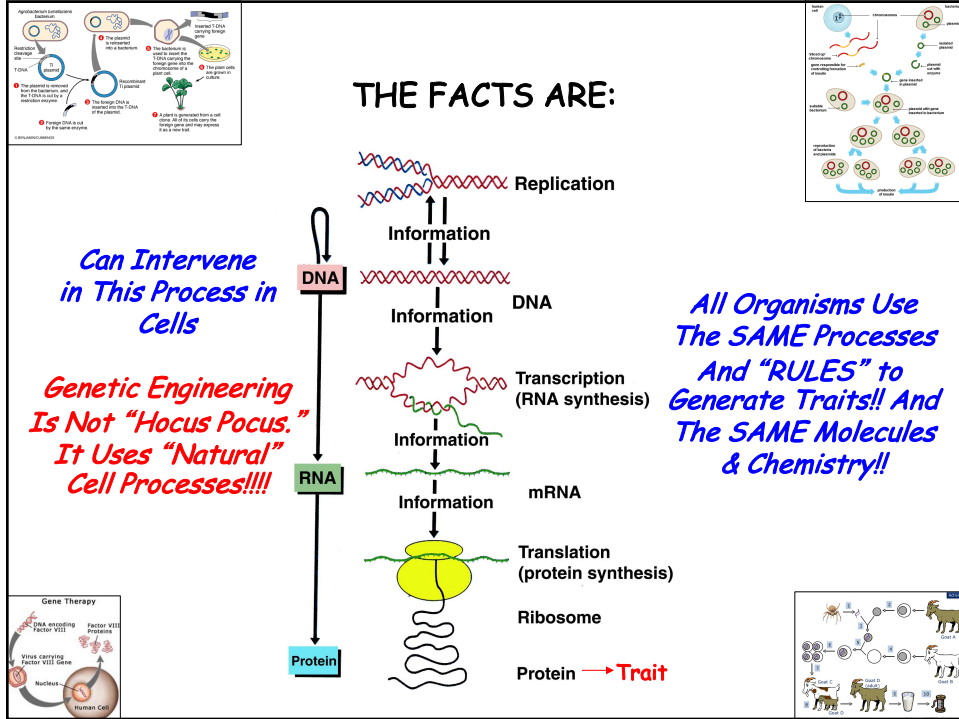
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39

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.

40



41

DNA Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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There is Nothing New About Genetic Engineering!

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

1. Classical Breeding

New Gene Combinations

42

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!

43



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Genetic Code of Life



Entire Genetic Code of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues and Future Consequences



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Populations of All Organisms Contain Genetic Variability



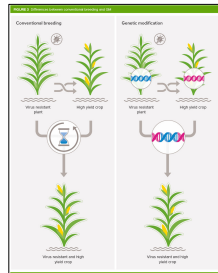




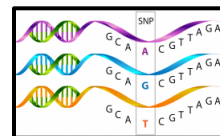
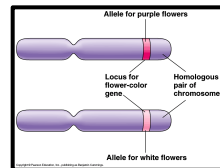
44

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!



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

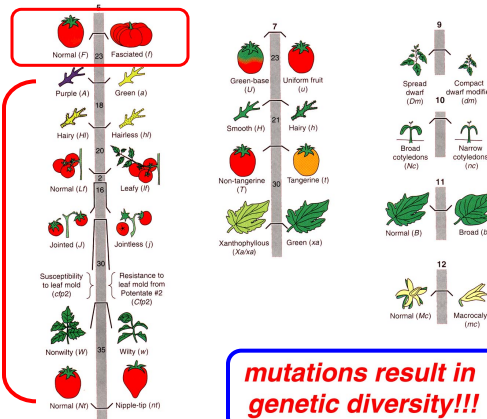
45

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!!!

Human Eye Colors

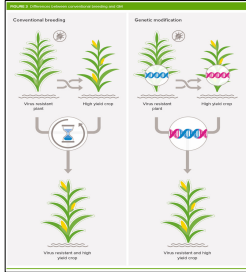


Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

46

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

1. Classical Breeding



New Allele Combinations



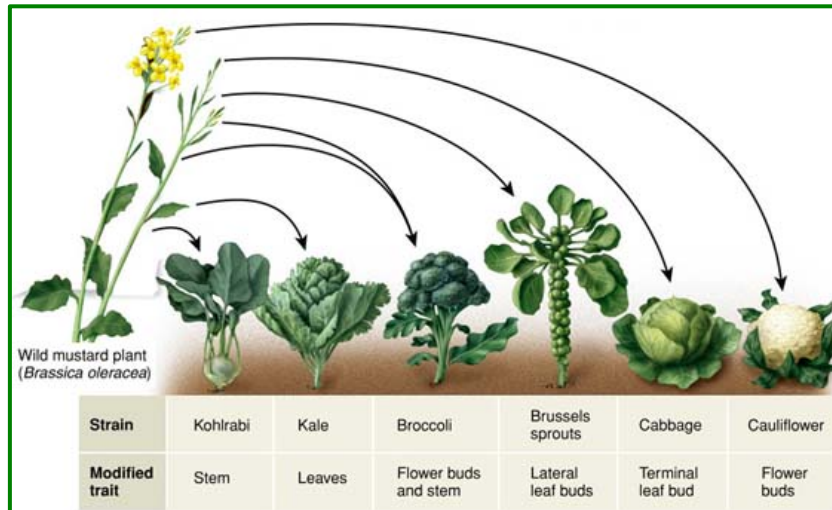
*The Early Tomato "Bioengineers" Selected For Large
Fruit Size Because it Provided More Food!*

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

allele α /ə/ 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.

47

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



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

48

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

49

DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

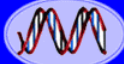
Cloning: Ethical Issues
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The Problem With Breeding the “Old Fashioned Way”

Cannot Predict Results!

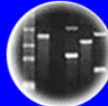
50




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Cloning: Ethical Issues
and Future Consequences

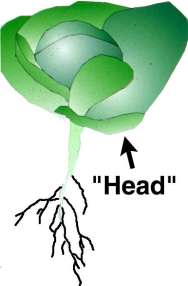


Plants of Tomorrow

The Problem With Breeding the "Old Fashioned Way"

Engineering A Novel Crop By "Wide" Breeding


Cabbage (*Brassica*)



"Head"

X

Radish (*Raphanus*)




Storage
Root


↓

???

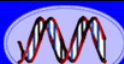
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Karpechenko, G.D., 1928. *Polyplloid hybrids of Raphanus sativus L. X Brassica oleracea L.* Zeitschrift für induktive Abstammungs- und Vererbungslehre 48, 1-85.



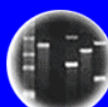
51




DNA
Genetic Code of Life




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DNA Fingerprinting



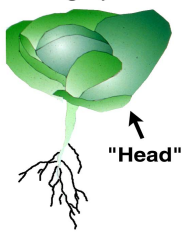
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Engineering A Novel Crop By "Wide" Breeding


Cabbage (*Brassica*)



"Head"

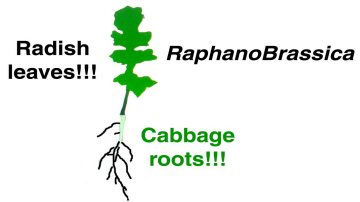
X

Radish (*Raphanus*)



Storage
Root

↓



Radish
leaves!!!

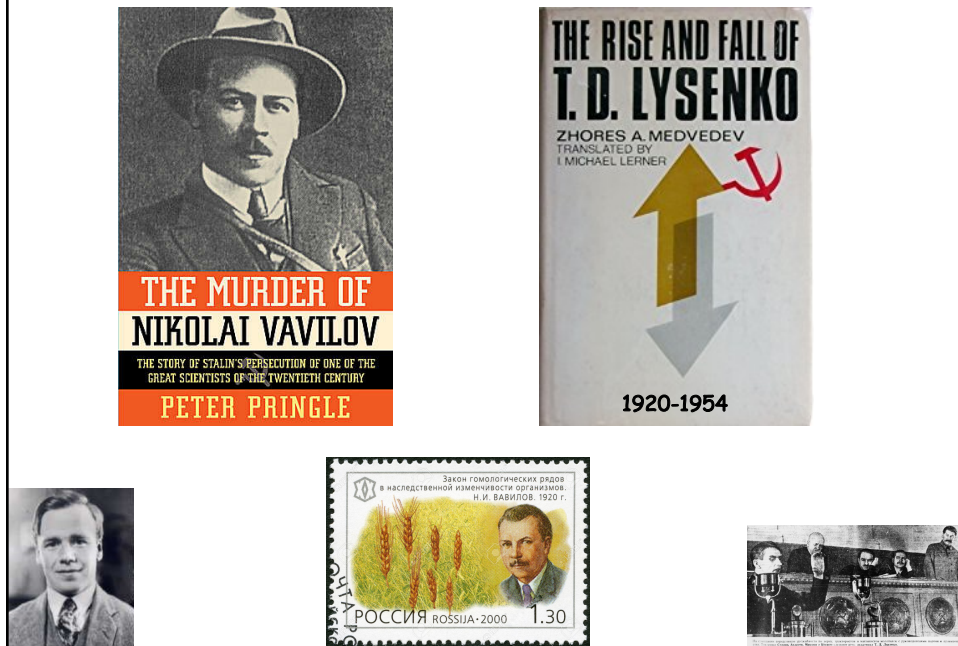
RaphanoBrassica

Cabbage
roots!!!

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

52

How Ideology Destroys Science & Leads to Horrific Tragedy



53

Karpechenko's Dream Come True!!!!

DNA Genetic Code of Life

Entire Genetic Code of a Bacteria

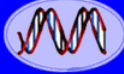
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences


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Grafting Potato and Tomato Plants!

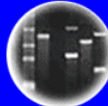
54




DNA
Genetic Code of Life




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DNA Fingerprinting



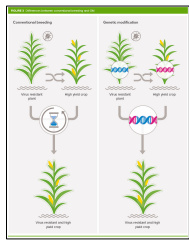
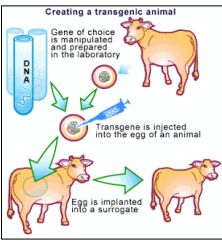
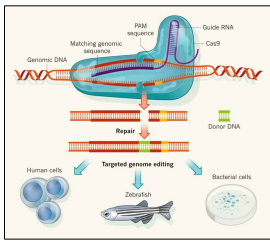
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Genetic Engineering is a TECHNIQUE!

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






55

Classical vs. DNA or Molecular Genetic Engineering Techniques


TRADITIONAL PLANT BREEDING

Traditional Line




Desired Gene

Commercial Variety



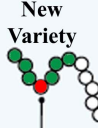
X

Commercial Variety



=

New Variety



Desired Gene


Many Genes Transferred

Plant Breeding Combines Many Genes At Once


PLANT BIOTECHNOLOGY

Biotechnology Adds A Single Gene

Desired Gene

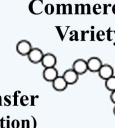


Gene Transfer (one generation)




→

Commercial Variety



=

New Variety



Desired Gene

One Gene Transferred
Or Many Genes

Or Many Cloned or Synthesized Genes

Or Edit One or Many Endogenous Genes!!!

All Manipulate Genes - But in Different Ways!!

56

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)

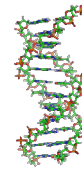
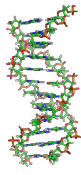
57

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!

58



We Live in The Age of DNA & Genetic Engineering!

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

