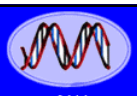
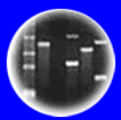

DNA
Genetic Code of Life


Entire Genetic Code of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

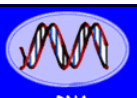
HC70A Spring 2021
Genetic Engineering in Medicine, Agriculture, and Law

Professor Bob Goldberg

Lecture 6

21st Century Genetic Engineering Applications

1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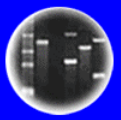
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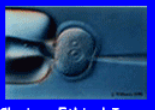
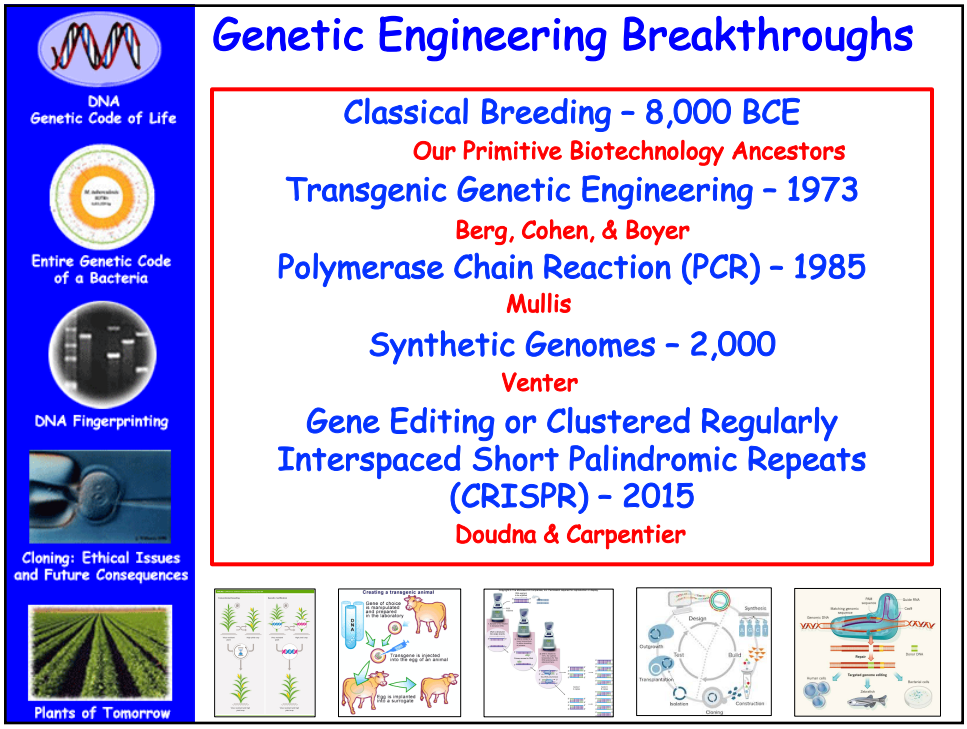

Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

Themes

1. What Are the Major Breakthoughs to Engineer Cells?
2. What is Marker Assisted Breeding and How Can It Speed Up Crop Improvement?
3. What Are l Applications of Transgenic Genetic Engineering?
4. How Can Genetic Engineering Be Used To Eliminate or Reduce Mosquito Populations?
5. What is the CRISPR-Cas Bacterial Immunity System?
6. What Are the Individual Components of the CRISPR-Cas Immunity System?
7. How Can CRISPER-Cas9 be Used For Gene Editing?
8. What is Gene Drive and How Can it Be Used To Fight Malaria?
9. What Are the Ethical and Regulatory Concerns of Using Gene Drive Systems?
10. What Are Other Applications of CRISPR-Cas9 Editing?

2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Genetic Engineering Breakthroughs
Classical Breeding – 8,000 BCE
Our Primitive Biotechnology Ancestors
Transgenic Genetic Engineering – 1973
Berg, Cohen, & Boyer
Polymerase Chain Reaction (PCR) – 1985
Mullis
Synthetic Genomes – 2,000
Venter
Gene Editing or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – 2015
Doudna & Carpentier

3


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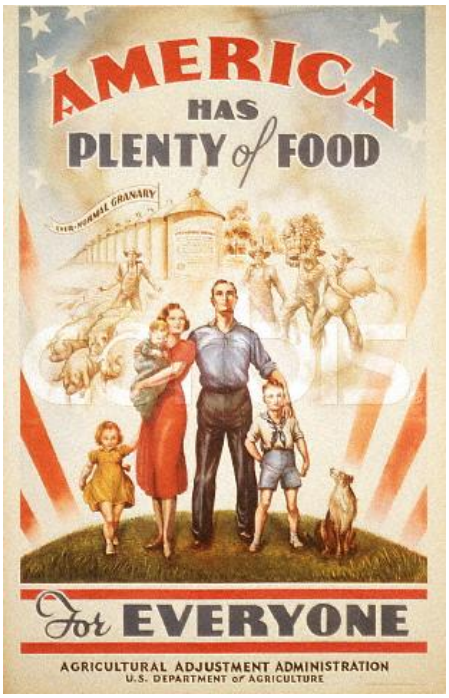
Plants of Tomorrow
21st Century "Classical" Breeding
1. Classical Breeding By Selective Mating (Thousands of Years)
2. Insertion of New Genes Into An Organism's Chromosomes (50 Years)
3. Editing Existing Genes Like A "Word Program" (1-2 Years)
Breeding or DNA Manipulation – They Are the SAME
&
Called Gene Manipulation
WHAT IS A GMO???

4



How Was This Accomplished Over the Past 100+ Years?

Advances in Classical Breeding Techniques!!

What About the Next 100 Years?

5

CROP YIELD INCREASES HAVE "ROCKETED UPWARDS" OVER THE LAST 100 YEARS AND CONTRIBUTED TO A LONGER AND "BETTER" LIFE

% Farm Workers	% Income on Food	Bushels/Acre		Life Span
55%	50% →	• 1920	30	← 48 Years
		• 1940	40	
		• 1960	60	
	22% →	• 1980	100	
1.5%	7% →	• 2020	172	← 80 Years

1920: 30 bushels/acre
1920: 1 farmer fed 10 people

2020: 172 bushels/acre
2020: 1 farmer feeds 200 people

Conclusion: Crop yields increased >500% over the past 100 years and lead to a similar reduction in food costs!!!!!

6

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

Tomato Genetic Diversity

Wheat Genetic Diversity

Mutations in a Gene That Change Its Chemical Sequence & Slightly Alters Its Function (e.g., fruit size, color)

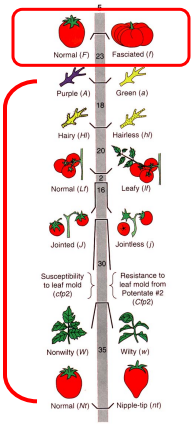
7

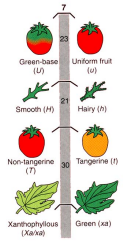
Alleles Reside at the Same Position on a Chromosome Because They Represent the SAME Gene

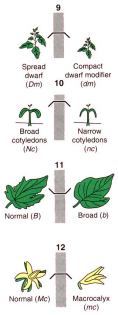
Alleles

Allele Phenotypes Specify Markers For Each Gene Location!

Different Genes

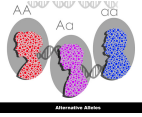




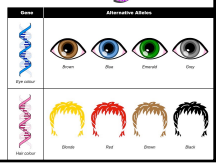


Gene Engineering Can Generate New Forms of Alleles of a Gene and, Therefore, Results in More Genetic Diversity

Single Nucleotide Polymorphisms or SNPs



mutations result in genetic diversity!!!

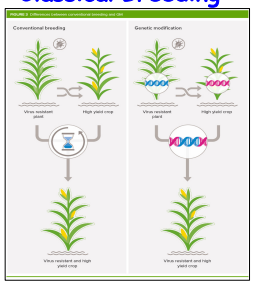


Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

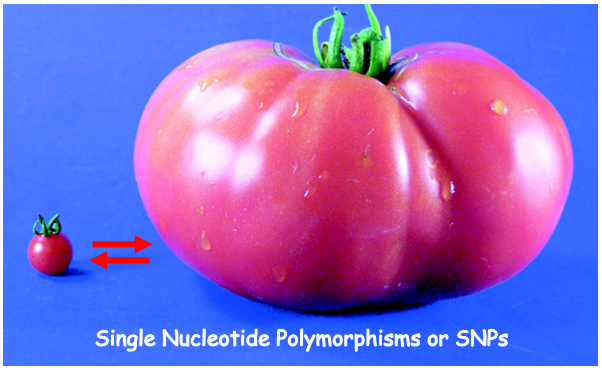
8

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

1. Classical Breeding



New Allele Combinations

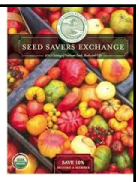


The Early Tomato "Bioengineers" Selected For Large Fruit Size Because it Provided More Food!

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

al·lele ə ˈlēl/ *Noun GENETICS plural noun: alleles*
one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.

9

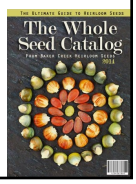


The Problem With Breeding the "Old Fashioned Way"

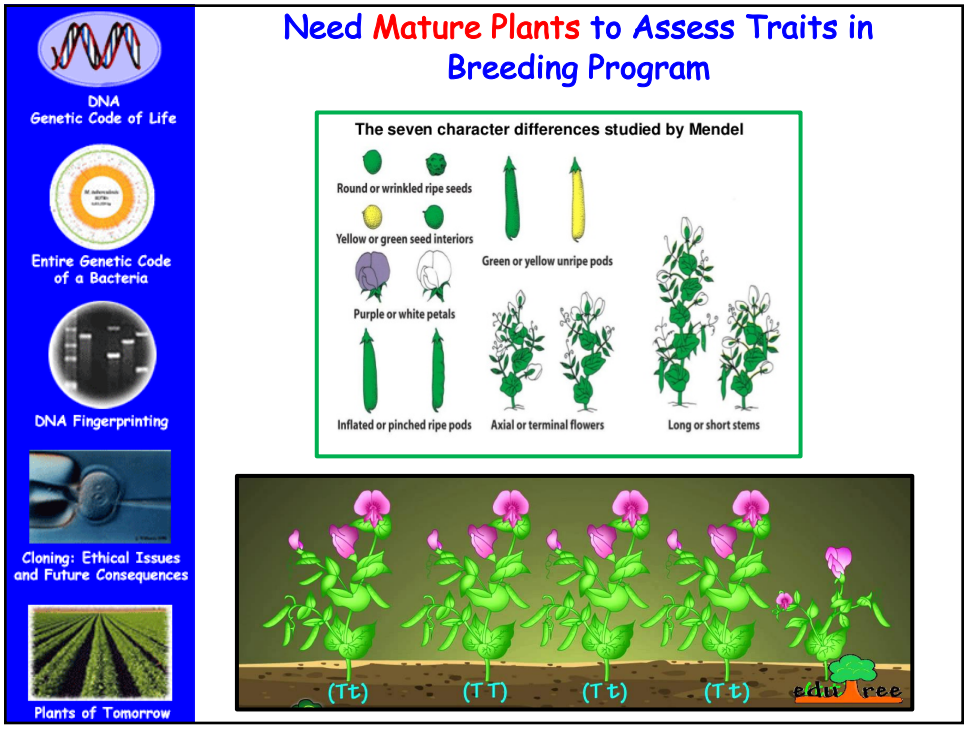
1. Cannot Predict Results!

2. Takes Many Generations – Slow!

3. Cannot Follow Traits Easily – e.g., Disease Resistance/Fruit Size!



10


DNA
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Entire Genetic Code of a Bacteria
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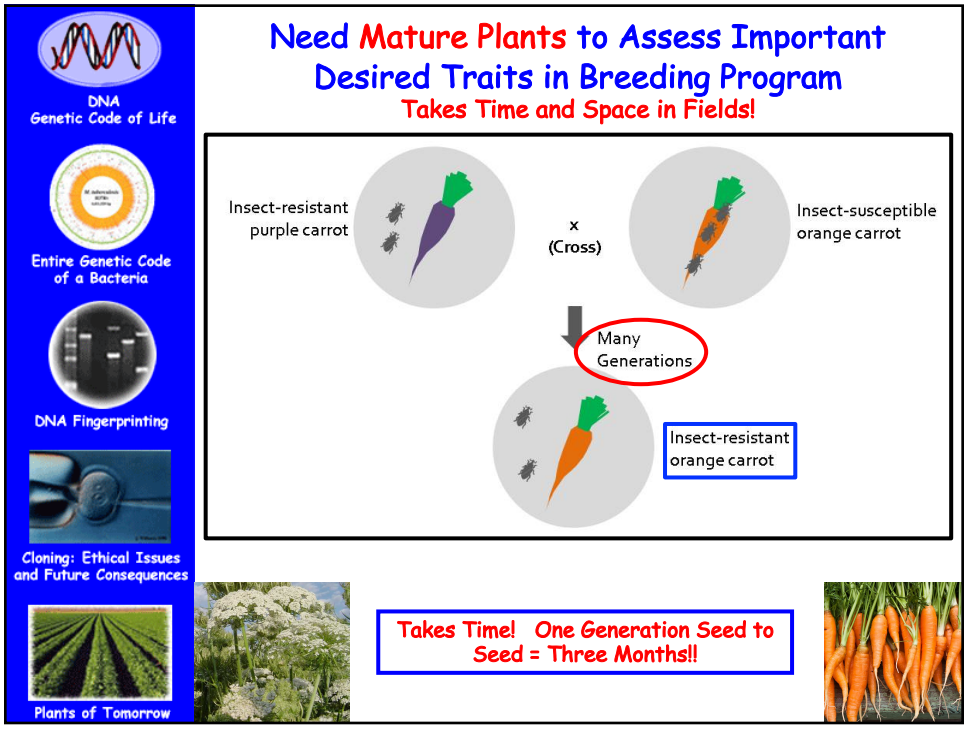
Plants of Tomorrow
Need Mature Plants to Assess Traits in Breeding Program
The seven character differences studied by Mendel
Round or wrinkled ripe seeds
Yellow or green seed interiors
Green or yellow unripe pods
Purple or white petals
Inflated or pinched ripe pods
Axial or terminal flowers
Long or short stems
(Tt)
(TT)
(Tt)
(Tt)
edu tree

11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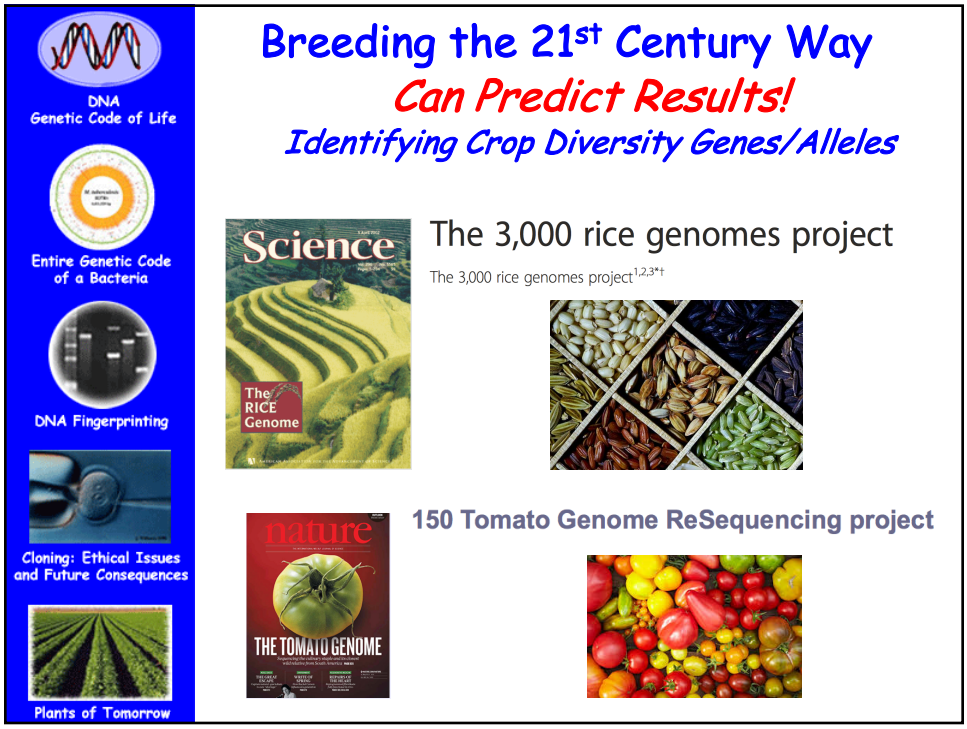
Need Mature Plants to Assess Important Desired Traits in Breeding Program
Takes Time and Space in Fields!
Insect-resistant purple carrot
x
(Cross)
Insect-susceptible orange carrot
Many Generations
Insect-resistant orange carrot
Takes Time! One Generation Seed to Seed = Three Months!!

12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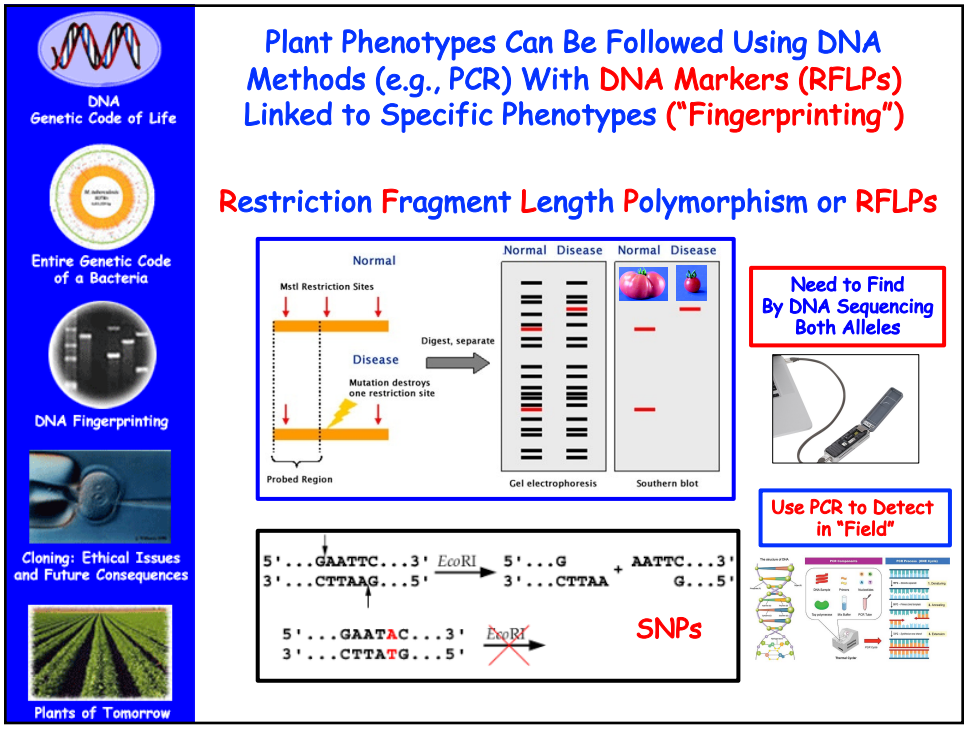
Breeding the 21st Century Way
Can Predict Results!
Identifying Crop Diversity Genes/Alleles
Science
The RICE Genome
The 3,000 rice genomes project
The 3,000 rice genomes project[1,2,3*†]
nature
THE TOMATO GENOME
150 Tomato Genome ReSequencing project

13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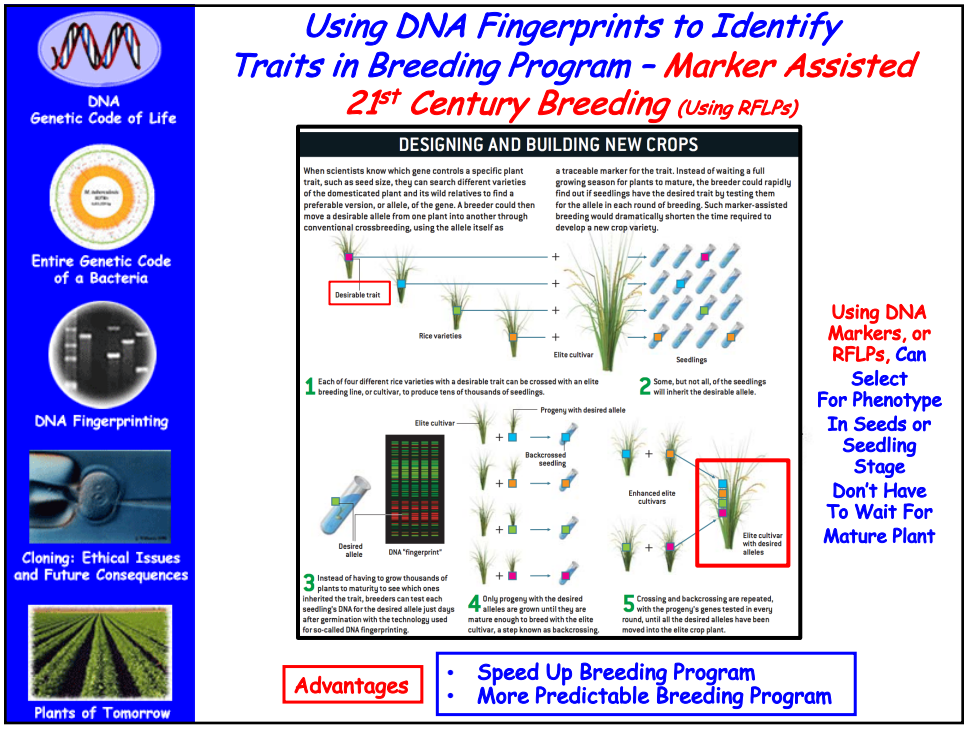
Plant Phenotypes Can Be Followed Using DNA Methods (e.g., PCR) With DNA Markers (RFLPs) Linked to Specific Phenotypes ("Fingerprinting")
Restriction Fragment Length Polymorphism or RFLPs
Normal
MstI Restriction Sites
Disease
Mutation destroys one restriction site
Probed Region
Digest, separate
Normal Disease
Normal Disease
Gel electrophoresis
Southern blot
Need to Find By DNA Sequencing Both Alleles
Use PCR to Detect in "Field"
5'...GAATTC...3'
3'...CTTAAG...5'
EcoRI
5'...G
3'...CTTAA
+
AATTC...3'
G...5'
5'...GAATAC...3'
3'...CTTATG...5'
EcoRI
SNPs

14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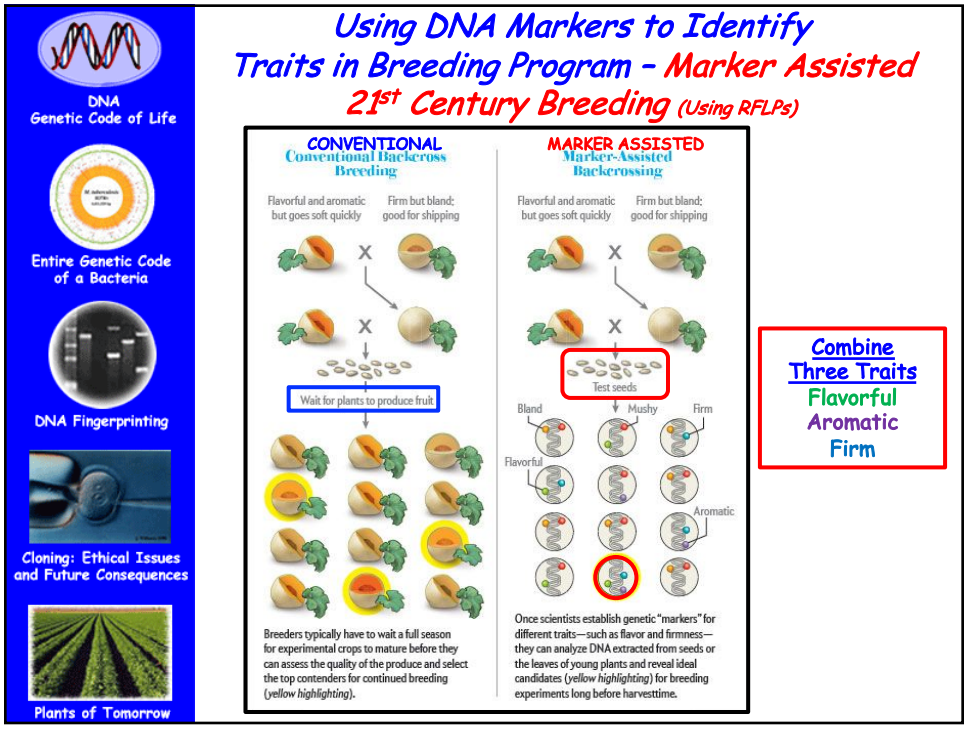
Using DNA Fingerprints to Identify Traits in Breeding Program – Marker Assisted 21st Century Breeding (Using RFLPs)
DESIGNING AND BUILDING NEW CROPS
When scientists know which gene controls a specific plant trait, such as seed size, they can search different varieties of the domesticated plant and its wild relatives to find a preferable version, or allele, of the gene. A breeder could then move a desirable allele from one plant into another through conventional crossbreeding, using the allele itself as a traceable marker for the trait. Instead of waiting a full growing season for plants to mature, the breeder could rapidly find out if seedlings have the desired trait by testing them for the allele in each round of breeding. Such marker-assisted breeding would dramatically shorten the time required to develop a new crop variety.
Desirable trait
Rice varieties
Elite cultivar
Seedlings
1 Each of four different rice varieties with a desirable trait can be crossed with an elite breeding line, or cultivar, to produce tens of thousands of seedlings.
2 Some, but not all, of the seedlings will inherit the desirable allele.
Progeny with desired allele
Elite cultivar
Backcrossed seedling
Desired allele
DNA "fingerprint"
Enhanced elite cultivars
Elite cultivar with desired alleles
3 Instead of having to grow thousands of plants to maturity to see which ones inherited the trait, breeders can test each seedling's DNA for the desired allele just days after germination with the technology used for so-called DNA fingerprinting.
4 Only progeny with the desired alleles are grown until they are mature enough to breed with the elite cultivar, a step known as backcrossing.
5 Crossing and backcrossing are repeated, with the progeny's genes tested in every round, until all the desired alleles have been moved into the elite crop plant.
Using DNA Markers, or RFLPs, Can Select For Phenotype In Seeds or Seedling Stage Don't Have To Wait For Mature Plant
Advantages
• Speed Up Breeding Program
• More Predictable Breeding Program

15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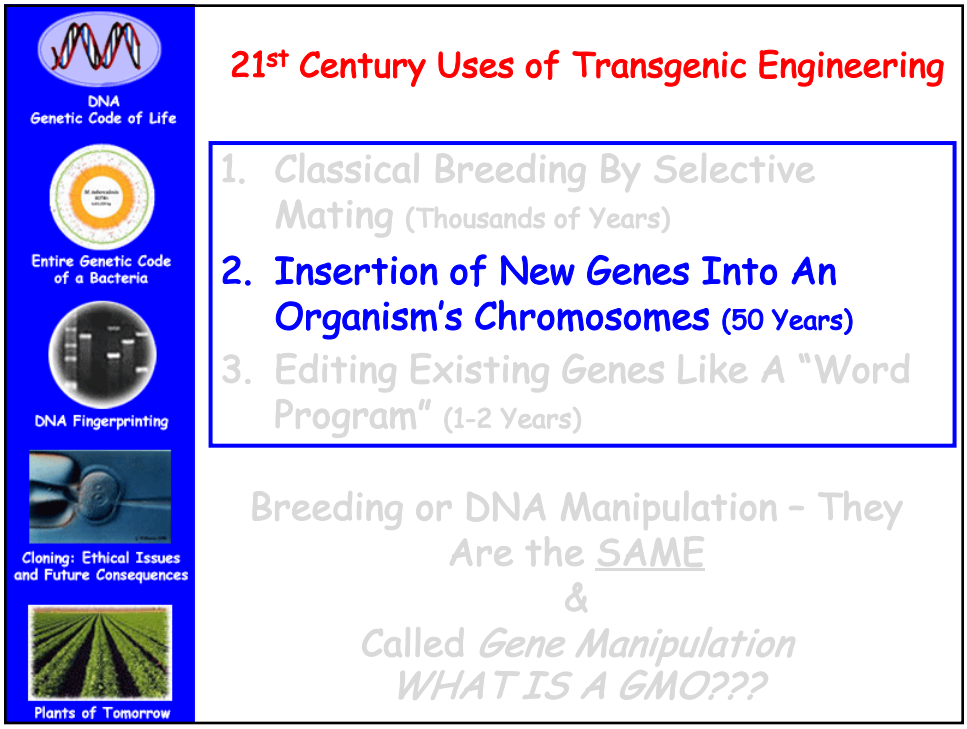
Using DNA Markers to Identify Traits in Breeding Program – Marker Assisted 21st Century Breeding (Using RFLPs)
CONVENTIONAL
Conventional Backcross Breeding
Flavorful and aromatic but goes soft quickly
Firm but bland; good for shipping
Wait for plants to produce fruit
Breeders typically have to wait a full season for experimental crops to mature before they can assess the quality of the produce and select the top contenders for continued breeding (yellow highlighting).
MARKER ASSISTED
Marker-Assisted Backcrossing
Flavorful and aromatic but goes soft quickly
Firm but bland; good for shipping
Test seeds
Bland
Mushy
Firm
Flavorful
Aromatic
Once scientists establish genetic "markers" for different traits—such as flavor and firmness—they can analyze DNA extracted from seeds or the leaves of young plants and reveal ideal candidates (yellow highlighting) for breeding experiments long before harvesttime.
Combine Three Traits
Flavorful
Aromatic
Firm

16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Breeding or DNA Manipulation – They Are the SAME
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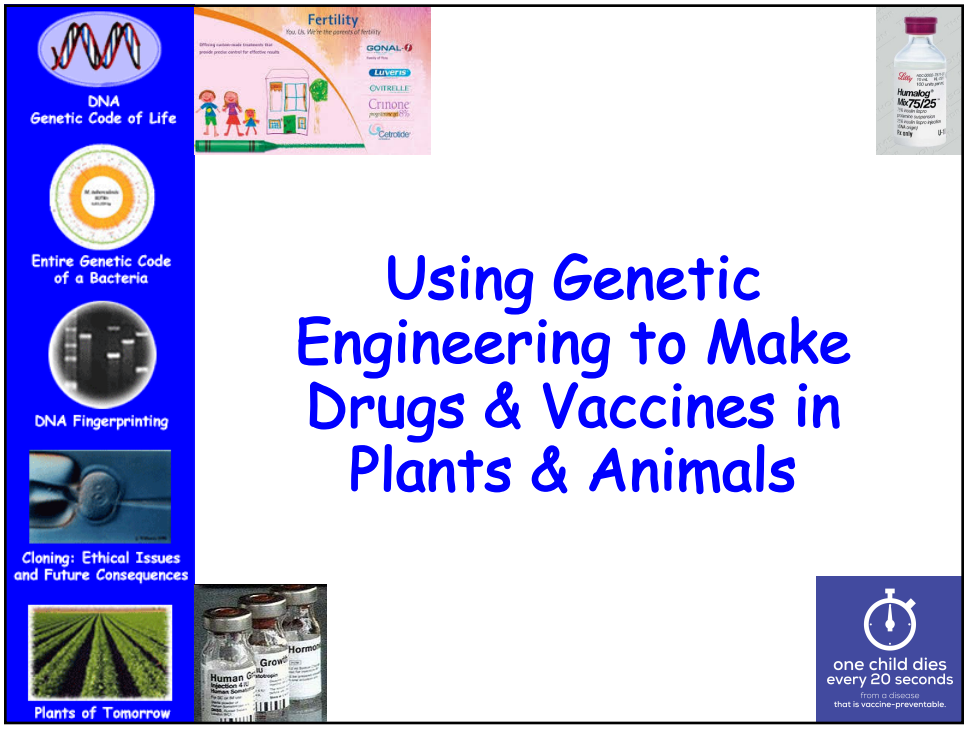
WHAT IS A GMO???

17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Fertility
GONAL-f
Luveris
OVITRELLE
Crinone
Cetrotide
Humalog
Using Genetic Engineering to Make Drugs & Vaccines in Plants & Animals
one child dies every 20 seconds
from a disease that is vaccine-preventable.

18

DNA
Genetic Code of Life

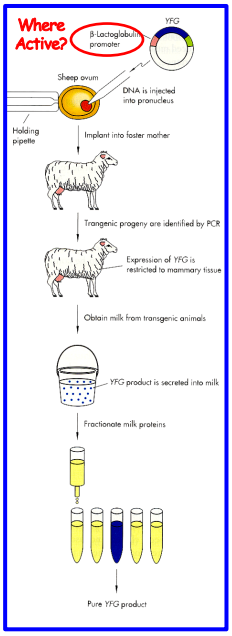
Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Animals Can Be Used as Factories to Produce Large Amounts of Human Proteins



Advantages of Molecular Pharming

1. Many human proteins need to be modified after translation to be active. Only eukaryotic cells can do this.
2. Bacteria need big fermenters + elaborate protein purification schemes- Farm animals can be used for this purpose w/o special processing/machinery.
3. Proteins stable, can be made in large amounts, and purified easily

19

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Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Genetically Engineered Drug-Producing Mammals Can Also Be Cloned

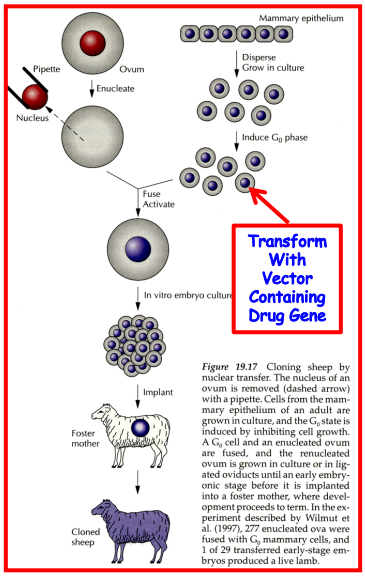


Figure 19.17 Cloning sheep by nuclear transfer. The nucleus of an ovum is removed (dashed arrow) with a pipette. Cells from the mammary epithelium of an adult are grown in culture, and the G_0 state is induced by inhibiting cell growth. A G_0 cell and an enucleated ovum are fused, and the renucleated ovum is grown in culture or in ligated oviducts until an early embryonic stage before it is implanted into a foster mother, where development proceeds to term. In the experiment described by Wilmut et al. (1997), 277 enucleated ova were fused with G_0 mammary cells, and 1 of 29 transferred early-stage embryos produced a live lamb.

Somatic Cells Can Also Be Genetically Engineered and Then Inserted Into Egg

20

February 7, 2009

F.D.A. Approves Drug From Gene-Altered Goats

Antithrombin-Treat Anti-Thrombin Deficiency A Dominant Human Genetic Disorder

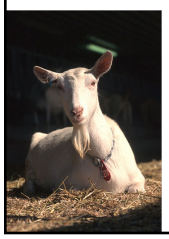
New Drug From Genetically Engineered Goat

FDA OKs ATryn, 1st Drug Made in Milk of a Genetically Engineered Animal

By Miranda Hitti
WebMD Health News

Feb. 6, 2009 -- The FDA today approved ATryn, the first drug made in genetically engineered animals.

Issues
Food Supply?
Containment?
Animal Health?
Effective Drug?



Bioengineering on the Farm

The Food and Drug Administration has approved the first drug produced in the milk of genetically engineered animals.

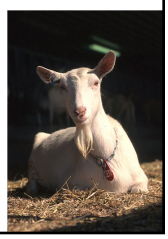
MODIFYING THE DNA
A human gene that produces the blood protein antithrombin is inserted into a short strand of goat DNA.

IMPLANTING THE DNA
The modified DNA is injected into the nucleus of a fertilized goat egg, which is then implanted into a female.

TESTING THE OFFSPRING
Kids born from the modified eggs are tested for the presence of antithrombin in their milk. Promising kids are bred normally to create a herd of modified goats.

EXTRACTING THE PROTEIN
Milk from the herd is filtered and purified. Annually, each goat can produce as much antithrombin as 90,000 human blood donations.

Sources: GTC Biotherapeutics



21

And Don't Forget Plants!

First plant-made biologic approved

Carrot cell bioreactors

The US Food and Drug Administration in May approved Elelyso (taliglucerase alfa), an enzyme produced in genetically engineered carrot cells, for treating type 1 Gaucher's disease. This is the first plant-made drug approved by the regulators, and for Israeli company Protalix BioTherapeutics of Carmiel, it is the first product made in their ProCellEx protein expression system to reach the market. The plant cell platform produces recombinant proteins with a glycan and amino acid structure similar to naturally produced human counterparts. Some 10,000 patients worldwide have Gaucher's, a rare genetic disorder in which individuals fail to produce the enzyme glucocerebrosidase.

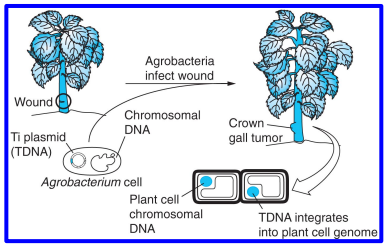
Drug-making plant blooms

Approval of a 'biologic' manufactured in plant cells may pave the way for similar products.

PLANTS IN THE PIPELINE
Manufacturers have begun or completed phase II clinical trials on a handful of biologics made in plants, and hope to follow Elelyso to market.

Drug	Condition	Company	Platform
Locteron (interferon-α)	Hepatitis C	Biolex Therapeutics	Duckweed
H5N1 vaccine	Influenza	Medicago	Tobacco
VEN100	Antibiotic-associated diarrhoea	Ventria Bioscience	Rice
CaroRx	Dental caries	Planet Biotechnology	Tobacco

Elelyso® Made in Engineered Carrot Cells To Treat Gaucher's Disease – A Lysosomal Storage Disease That Prevents Molecules From Being Degraded and Disposed of Properly in Cells – 100x Prevalence in Ashkenazi Jews. Gene on Chromosome 1, and Encodes a Glucocerebrosidase.
Advantages of Plants?



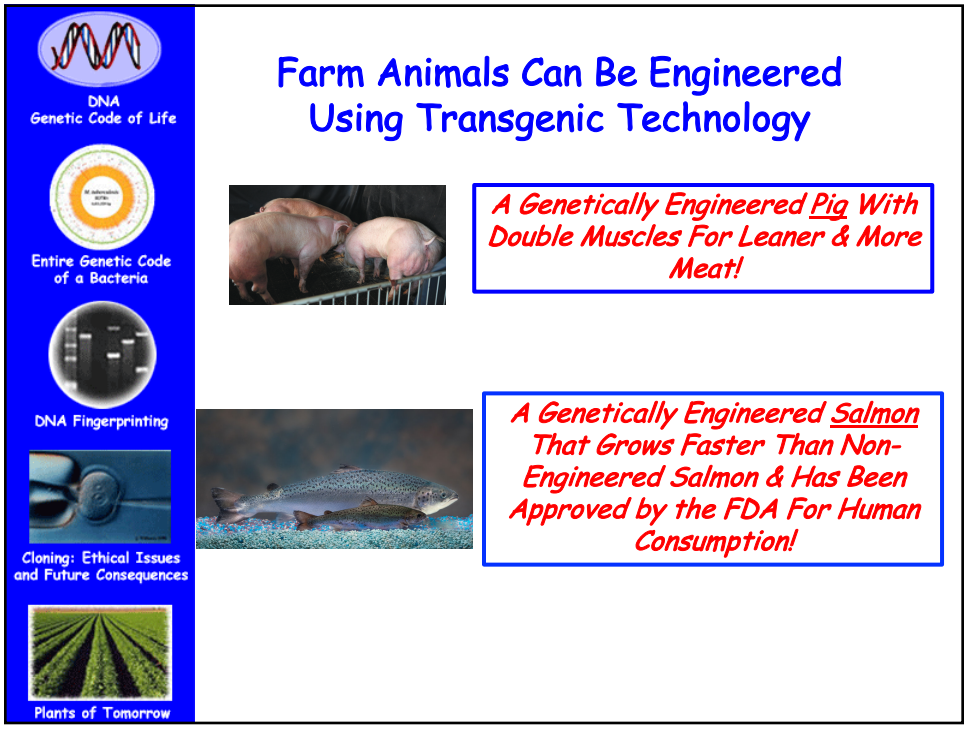
22

23

Crops Can Be Engineered Many Other Traits Using Transgenic Technology

Bacteria Insect Resistance Genes
Plant Virus "Immunity" Genes
Bacteria Herbicide Tolerance Genes
Bacteria and Plant Chimeric Male Fertility Genes

24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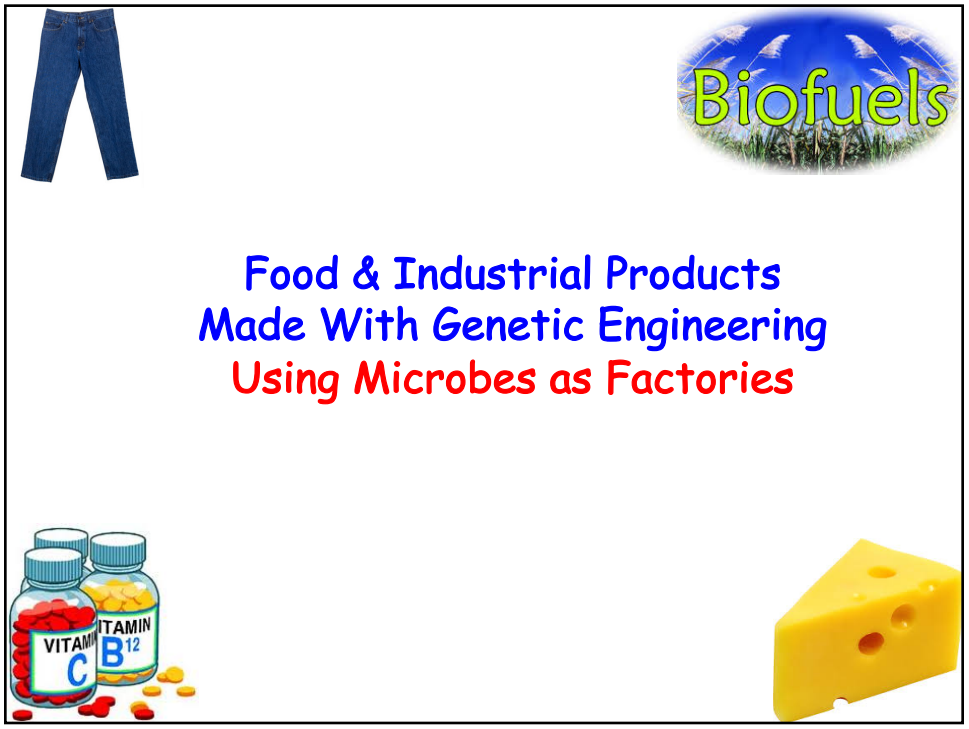
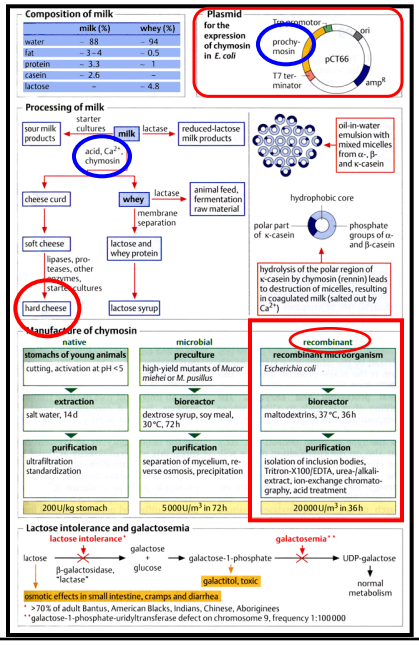
Farm Animals Can Be Engineered Using Transgenic Technology
A Genetically Engineered Pig With Double Muscles For Leaner & More Meat!
A Genetically Engineered Salmon That Grows Faster Than Non-Engineered Salmon & Has Been Approved by the FDA For Human Consumption!

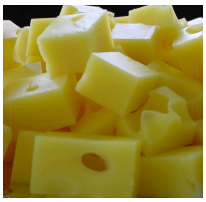
25


Biofuels
Food & Industrial Products
Made With Genetic Engineering
Using Microbes as Factories
VITAMIN C
B12

26

Recombinant Chymosin Is Used To Make Cheese

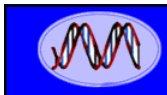




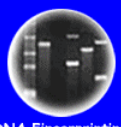
Chymosin (Rennin) Acts On Milk Proteins To Coagulate Milk → Cheese

Is Cheese A GMO?

27


DNA
Genetic Code of Life


Entire Genetic Code of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences

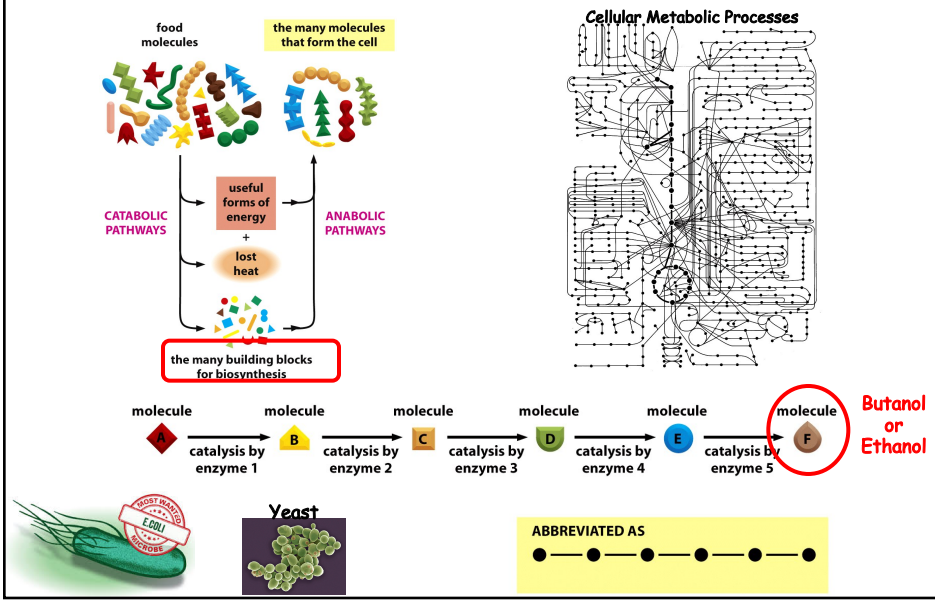

Plants of Tomorrow

Plant Based Foods Using Genetic Engineering

Genetically Engineered Yeast That Synthesizes a Soybean Protein Giving the Impossible Burger Its Red Color

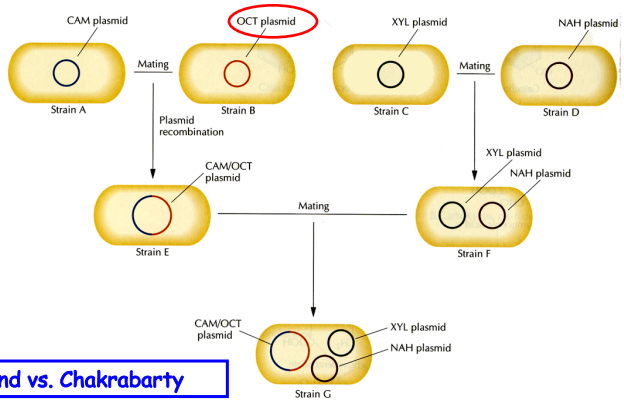
28

Metabolites Are Produced By Cellular Pathways That Use Specific Enzymes and Genes To Synthesize Specific Small Molecules



29

Bacteria Can Be Engineered To Degrade Several Different "Toxic" Compounds



Pseudomonas

A Landmark Decision- Diamond vs. Chakrabarty

Chakrabarty US Patent 4,259,444 1981
Genetically Engineered Microorganisms Are "Inventions"

Life Can Be Patented !

Figure 13.5 Schematic representation of the development of a bacterial strain that can degrade camphor, octane, xylene, and naphthalene. Strain A, which contains a CAM (camphor-degrading) plasmid, is mated with strain B, which carries an OCT (octane-degrading) plasmid. Following plasmid transfer and homologous recombination between the two plasmids, strain E carries a CAM and OCT biodegradative fusion plasmid. Strain C, which contains a XYL (xylene-degrading) plasmid, is mated with strain D, which contains a NAH (naphthalene-degrading) plasmid, to form strain F, which carries both of these plasmids. Finally, strains E and F are mated to yield strain G, which carries the CAM/OCT fusion plasmid, the XYL plasmid, and the NAH plasmid.

30

Engineering *E.coli* To Synthesize Indigo- The Major Blue Dye For Jeans & Other Clothes & Uses

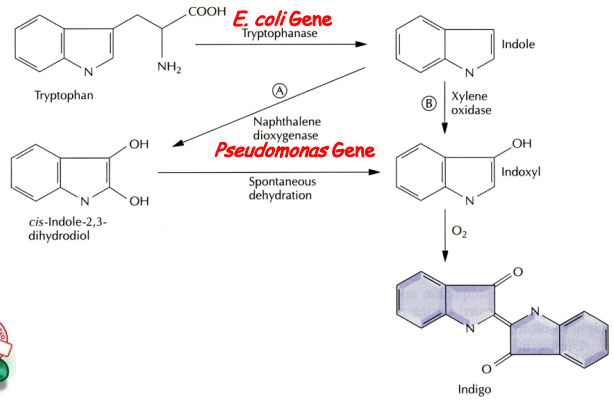


Figure 12.8 Indigo biosynthesis from tryptophan in genetically engineered *E. coli*. Tryptophanase is an *E. coli* enzyme. In pathway A, the naphthalene dioxygenase is derived from the NAH plasmid; in pathway B, the xylene oxidase is from the TOL plasmid. *E. coli* transformants that synthesize indigo contain either pathway A or B but not both pathways.

$200M/Year Industry
Indigo Previously Obtained From Plants!

31

Engineering *E.coli* Pathways To Make BioFuel

nature Vol 451|3 January 2008 doi:10.1038/nature06450

LETTERS

Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels

Shota Atsumi[1], Taizo Hanai[1] & James C. Liao[1,2]

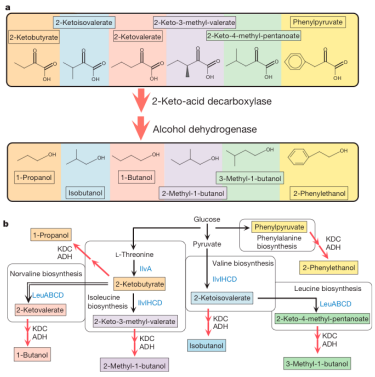


Figure 1 | Production of higher alcohols through the synthetic non-fermentative pathways. **a**, Various 2-keto acid precursors lead to corresponding alcohols through 2-ketoacid decarboxylase and alcohol dehydrogenase. **b**, The synthetic networks for the non-fermentative alcohol production in engineered *E. coli*. Red arrows represent the 2-keto acid decarboxylation and reduction pathway. Blue enzyme names represent amino acid biosynthesis pathways. The double lines represent a side pathway leading to norvaline and 1-butanol biosynthesis.

32

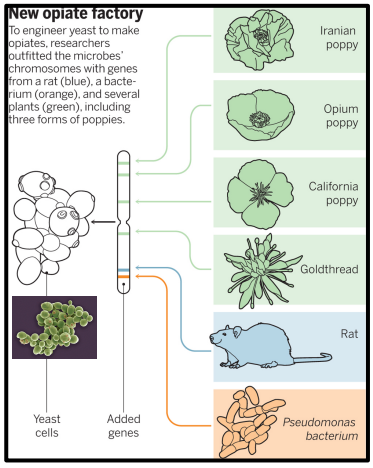
CARBON DIOXIDE-EATING BACTERIA OFFER HOPE FOR GREEN PRODUCTION

Lab workhorse *E. coli* engineered to make nutrients from greenhouse gas rather than from sugars.

Cell, November 27, 2019

33

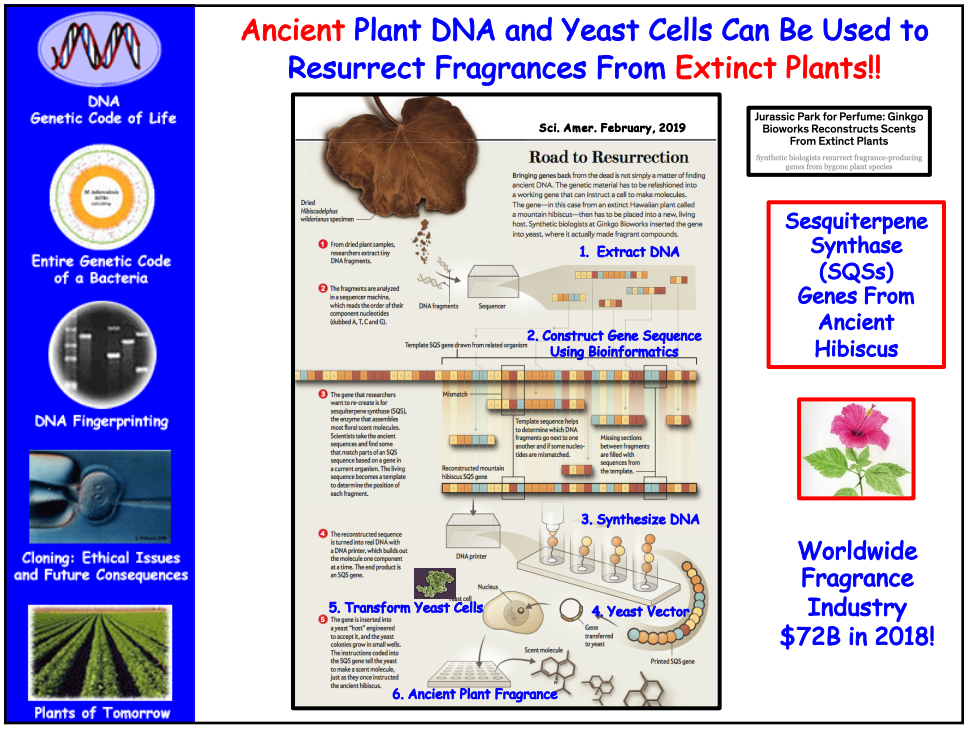
Complete Biosynthesis of Plant-Based Opioids in Yeast and *E. coli*



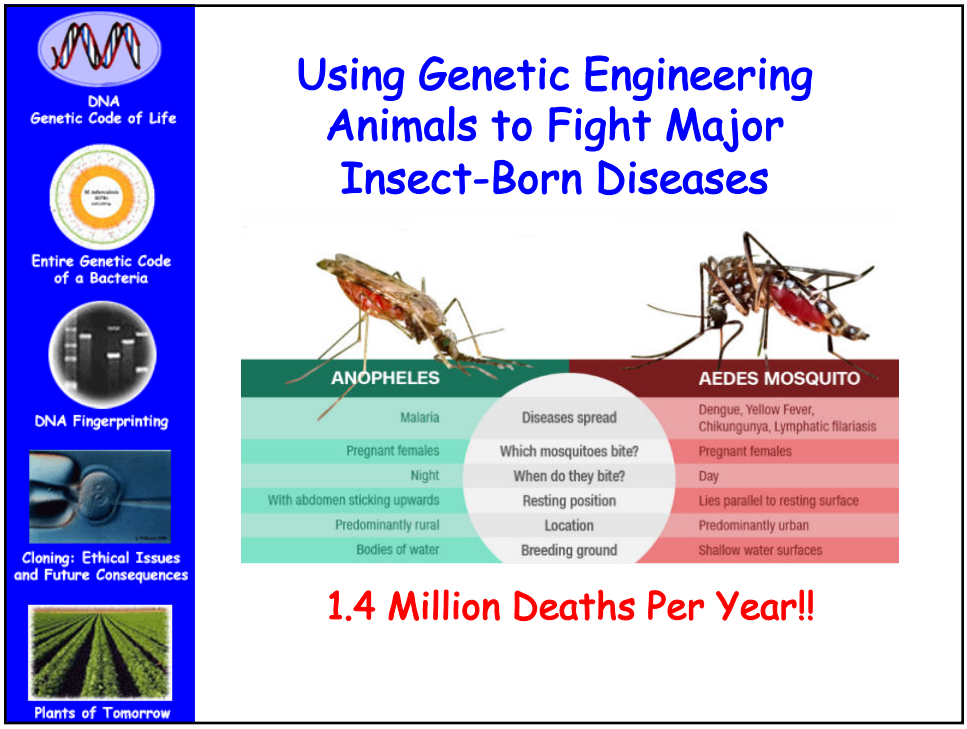
Six Genes From Three Different Organisms To Reconstruct the Poppy Opioid Pathway in Yeast!

Total biosynthesis of opiates by stepwise fermentation using engineered *Escherichia coli*

34


DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Ancient Plant DNA and Yeast Cells Can Be Used to Resurrect Fragrances From Extinct Plants!!
Sci. Amer. February, 2019
Road to Resurrection
1. Extract DNA
2. Construct Gene Sequence Using Bioinformatics
3. Synthesize DNA
4. Yeast Vector
5. Transform Yeast Cells
6. Ancient Plant Fragrance
Jurassic Park for Perfume: Ginkgo Bioworks Reconstructs Scents From Extinct Plants
Sesquiterpene Synthase (SQSs) Genes From Ancient Hibiscus
Worldwide Fragrance Industry $72B in 2018!

35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Using Genetic Engineering Animals to Fight Major Insect-Born Diseases
ANOPHELES
AEDES MOSQUITO
Malaria
Diseases spread
Dengue, Yellow Fever, Chikungunya, Lymphatic filariasis
Pregnant females
Which mosquitoes bite?
Pregnant females
Night
When do they bite?
Day
With abdomen sticking upwards
Resting position
Lies parallel to resting surface
Predominantly rural
Location
Predominantly urban
Bodies of water
Breeding ground
Shallow water surfaces
1.4 Million Deaths Per Year!!

36

DNA
Genetic Code of Life

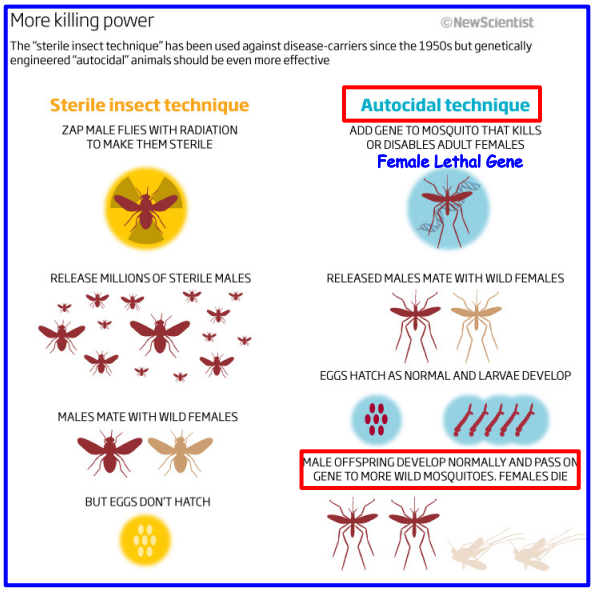
Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Using Genetic Engineering to Fight Mosquito-Transmitted Diseases



37

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FDA approves releasing GMO mosquitoes to fight Zika in Florida

The Florida Keys approve a trial release of genetically modified mosquitoes to combat Zika

Other tests have reduced mosquito populations by 90 percent

EPA **Guidance for Industry** FDA

Regulation of Mosquito-Related Products

1. **Examples of New Animal Drugs – Regulated by FDA**

   a. Products intended to reduce the virus/pathogen load within a mosquito, including reduction in virus/pathogen replication and spread within the mosquito and/or reduction in virus/pathogen transmissibility from mosquitoes to humans.

   b. Products intended to prevent mosquito-borne disease in humans or animals.

2. **Example of Pesticide Products – Regulated by EPA**

   Products intended to reduce the population of mosquitoes (for example, by killing them at some point in their life cycle, or by interfering with their reproduction or development).[3]

38


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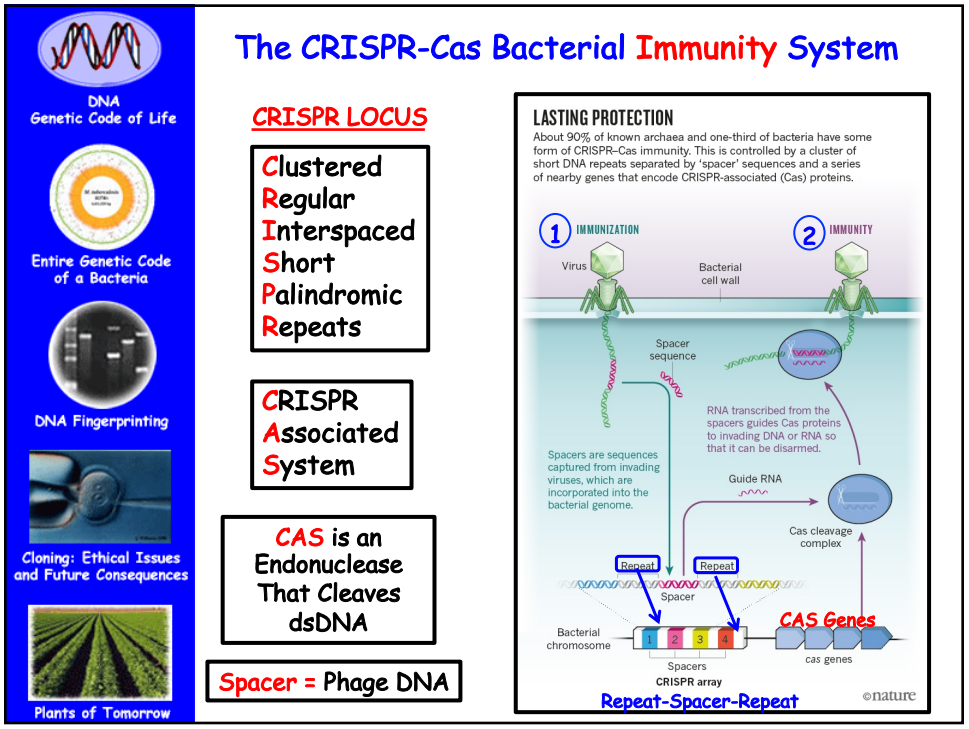
Genetic Engineering is a TECHNIQUE!
1. Classical Breeding By Selective Mating (Thousands of Years)
2. Insertion of New Genes Into An Organism's Chromosomes (50 Years)
3. Editing Existing Genes Like A "Word Program" (5 Years)
Breeding or DNA Manipulation – They Are the SAME
&
Called Gene Manipulation
WHAT IS A GMO???

39


DNA
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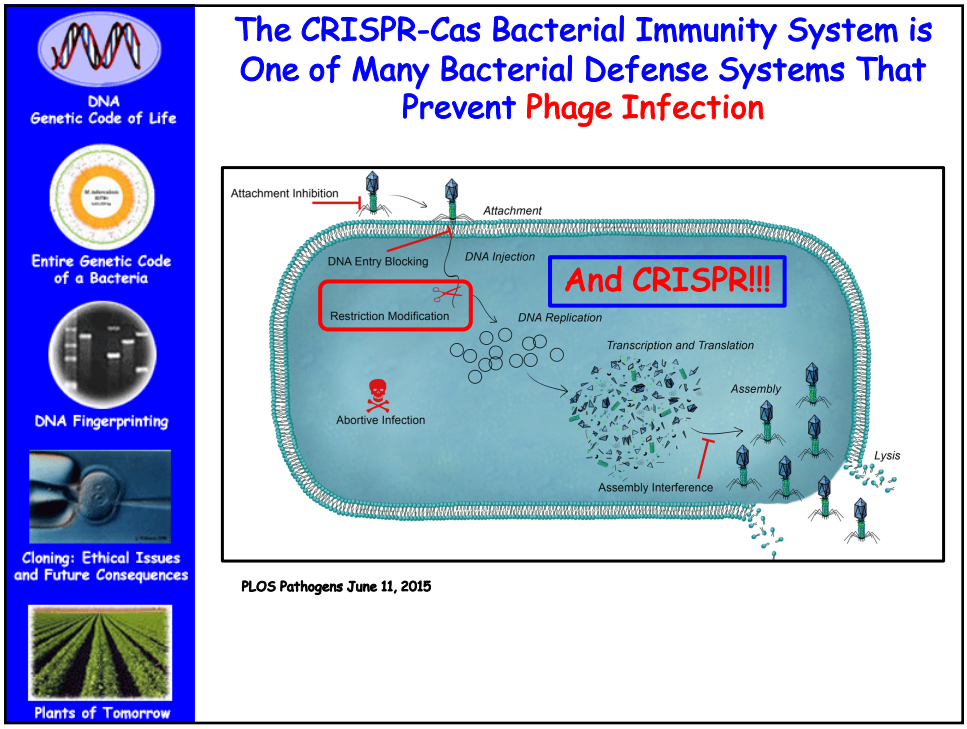
Plants of Tomorrow
The CRISPR-Cas Bacterial Immunity System
CRISPR LOCUS
Clustered
Regular
Interspaced
Short
Palindromic
Repeats
CRISPR
Associated
System
CAS is an Endonuclease That Cleaves dsDNA
Spacer = Phage DNA
LASTING PROTECTION
About 90% of known archaea and one-third of bacteria have some form of CRISPR–Cas immunity. This is controlled by a cluster of short DNA repeats separated by 'spacer' sequences and a series of nearby genes that encode CRISPR-associated (Cas) proteins.
1 IMMUNIZATION
2 IMMUNITY
Virus
Bacterial cell wall
Spacer sequence
RNA transcribed from the spacers guides Cas proteins to invading DNA or RNA so that it can be disarmed.
Spacers are sequences captured from invading viruses, which are incorporated into the bacterial genome.
Guide RNA
Cas cleavage complex
Repeat
Repeat
Spacer
Bacterial chromosome
CAS Genes
cas genes
Spacers
CRISPR array
Repeat-Spacer-Repeat
©nature

40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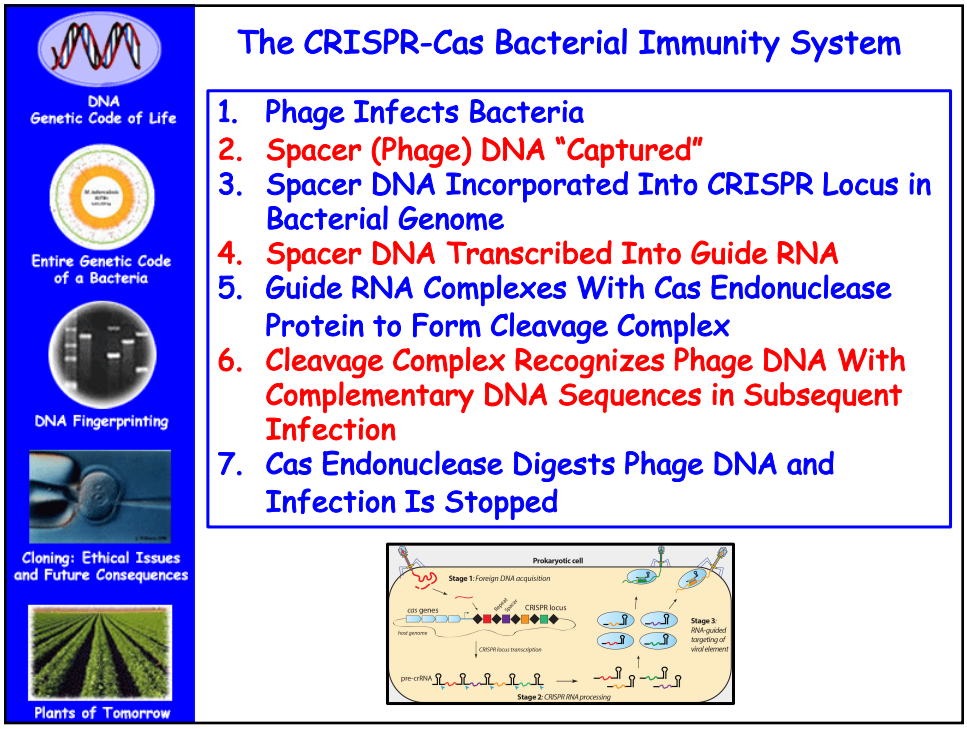
The CRISPR-Cas Bacterial Immunity System is One of Many Bacterial Defense Systems That Prevent Phage Infection
Attachment Inhibition
Attachment
DNA Entry Blocking
DNA Injection
And CRISPR!!!
Restriction Modification
DNA Replication
Transcription and Translation
Assembly
Abortive Infection
Lysis
Assembly Interference
PLOS Pathogens June 11, 2015

41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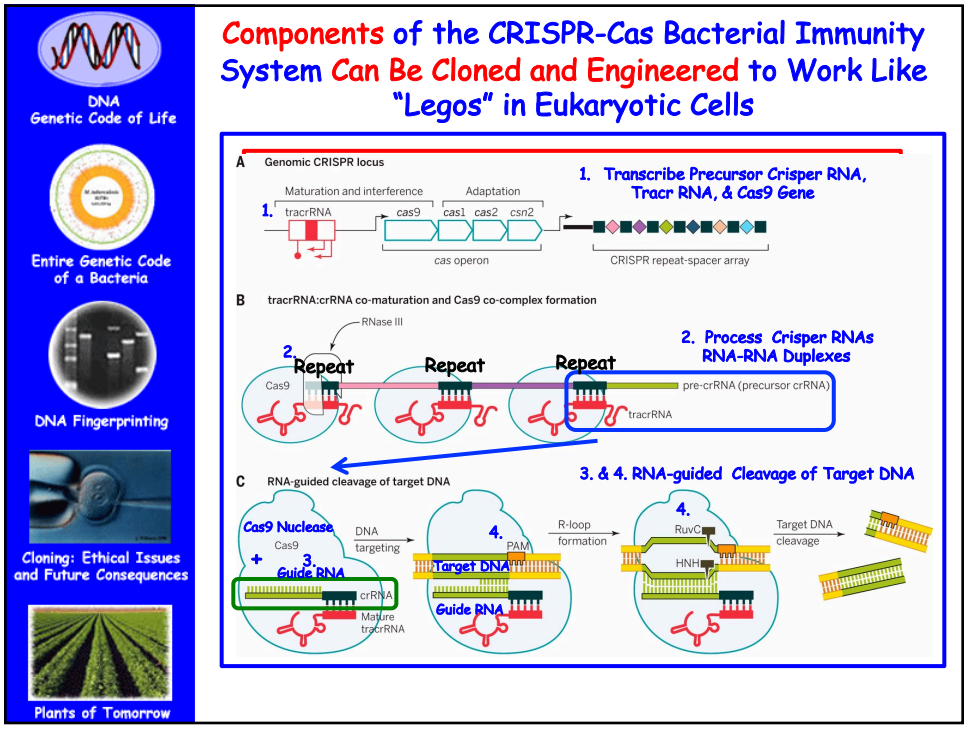
Plants of Tomorrow
The CRISPR-Cas Bacterial Immunity System
1. Phage Infects Bacteria
2. Spacer (Phage) DNA "Captured"
3. Spacer DNA Incorporated Into CRISPR Locus in Bacterial Genome
4. Spacer DNA Transcribed Into Guide RNA
5. Guide RNA Complexes With Cas Endonuclease Protein to Form Cleavage Complex
6. Cleavage Complex Recognizes Phage DNA With Complementary DNA Sequences in Subsequent Infection
7. Cas Endonuclease Digests Phage DNA and Infection Is Stopped
Prokaryotic cell
Stage 1: Foreign DNA acquisition
cas genes
CRISPR locus
Stage 3: RNA-guided targeting of viral element
pre-crRNA
Stage 2: CRISPR RNA processing

42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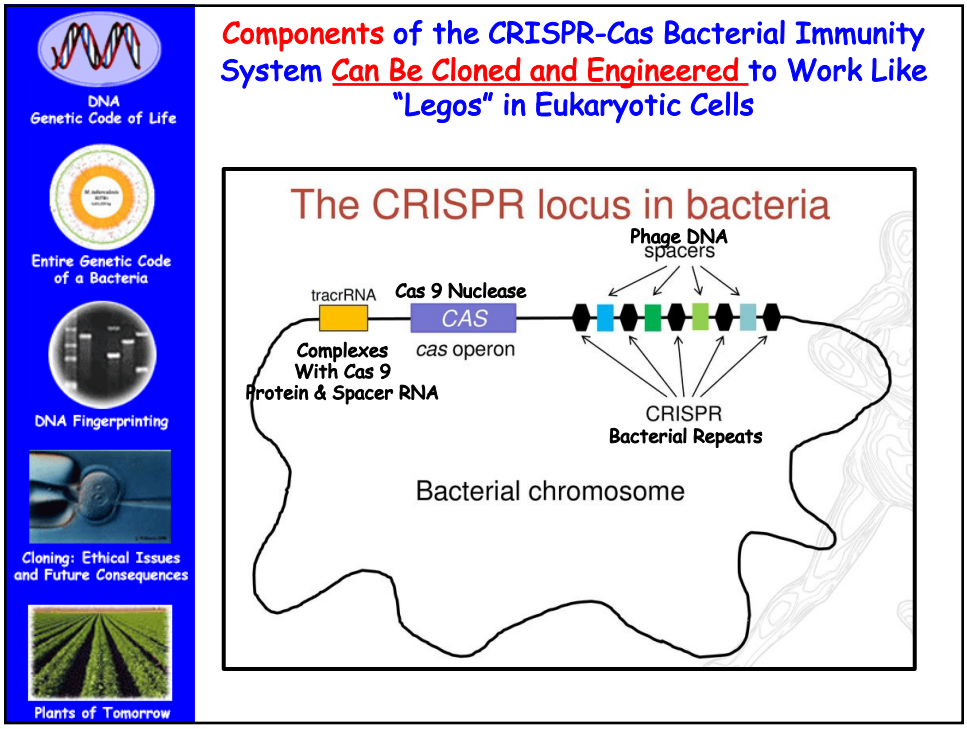
Components of the CRISPR-Cas Bacterial Immunity System Can Be Cloned and Engineered to Work Like "Legos" in Eukaryotic Cells
A Genomic CRISPR locus
1. Transcribe Precursor Crisper RNA, Tracr RNA, & Cas9 Gene
Maturation and interference
Adaptation
1.
tracrRNA
cas9
cas1 cas2 csn2
cas operon
CRISPR repeat-spacer array
B tracrRNA:crRNA co-maturation and Cas9 co-complex formation
RNase III
2.
Repeat
Repeat
Repeat
2. Process Crisper RNAs RNA-RNA Duplexes
Cas9
pre-crRNA (precursor crRNA)
tracrRNA
C RNA-guided cleavage of target DNA
3. & 4. RNA-guided Cleavage of Target DNA
Cas9 Nuclease
Cas9
+
3.
Guide RNA
crRNA
Mature tracrRNA
DNA targeting
4.
PAM
Target DNA
Guide RNA
R-loop formation
4.
RuvC
HNH
Target DNA cleavage

43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Plants of Tomorrow
Components of the CRISPR-Cas Bacterial Immunity System Can Be Cloned and Engineered to Work Like "Legos" in Eukaryotic Cells
The CRISPR locus in bacteria
Phage DNA spacers
tracrRNA
Cas 9 Nuclease
CAS
cas operon
Complexes With Cas 9 Protein & Spacer RNA
CRISPR Bacterial Repeats
Bacterial chromosome

44


DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
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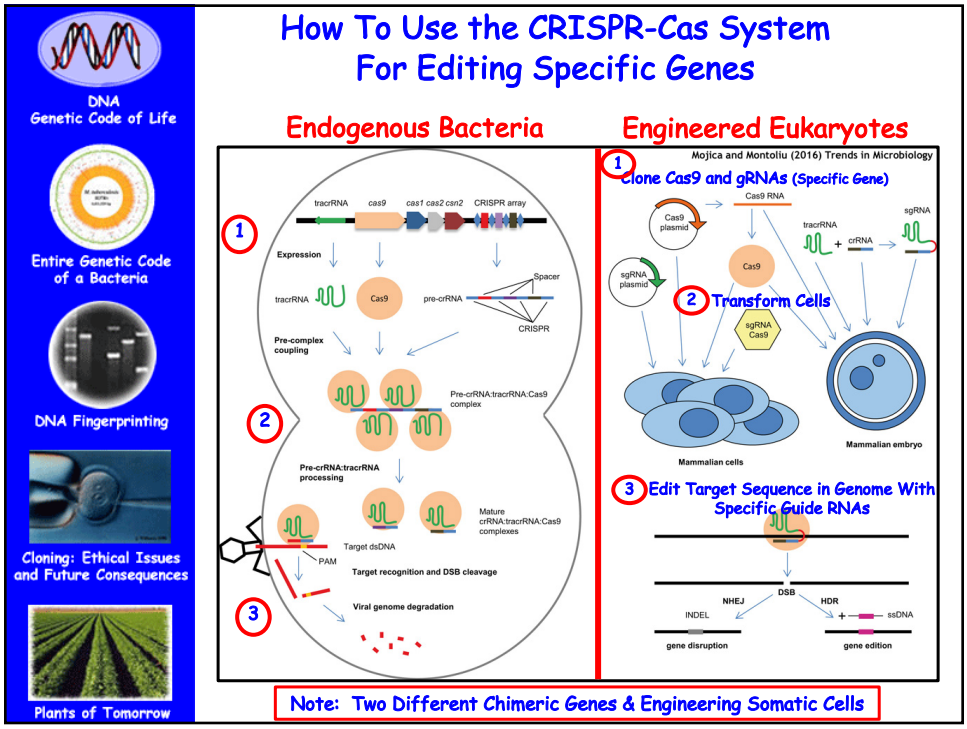
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang
CRISPR-Cas9 Editing (Molecular Typewriter)
PATENTED
Genomic DNA
Cas9
PAM
Target Sequence
crRNA
Guide RNA
tracrRNA
PATENTED
CRISPR Technology
Editing Human Cells

45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Plants of Tomorrow
How To Use the CRISPR-Cas System
For Editing Specific Genes
Endogenous Bacteria
Engineered Eukaryotes
tracrRNA
cas9
cas1 cas2 csn2
CRISPR array
Expression
Spacer
pre-crRNA
CRISPR
Pre-complex coupling
Pre-crRNA:tracrRNA:Cas9 complex
Pre-crRNA:tracrRNA processing
Mature crRNA:tracrRNA:Cas9 complexes
Target dsDNA
PAM
Target recognition and DSB cleavage
Viral genome degradation
Mojica and Montoliu (2016) Trends in Microbiology
Clone Cas9 and gRNAs (Specific Gene)
Cas9 RNA
Cas9 plasmid
sgRNA plasmid
tracrRNA
crRNA
sgRNA
Transform Cells
sgRNA Cas9
Mammalian cells
Mammalian embryo
Edit Target Sequence in Genome With Specific Guide RNAs
DSB
NHEJ
HDR
INDEL
ssDNA
gene disruption
gene edition
Note: Two Different Chimeric Genes & Engineering Somatic Cells

46


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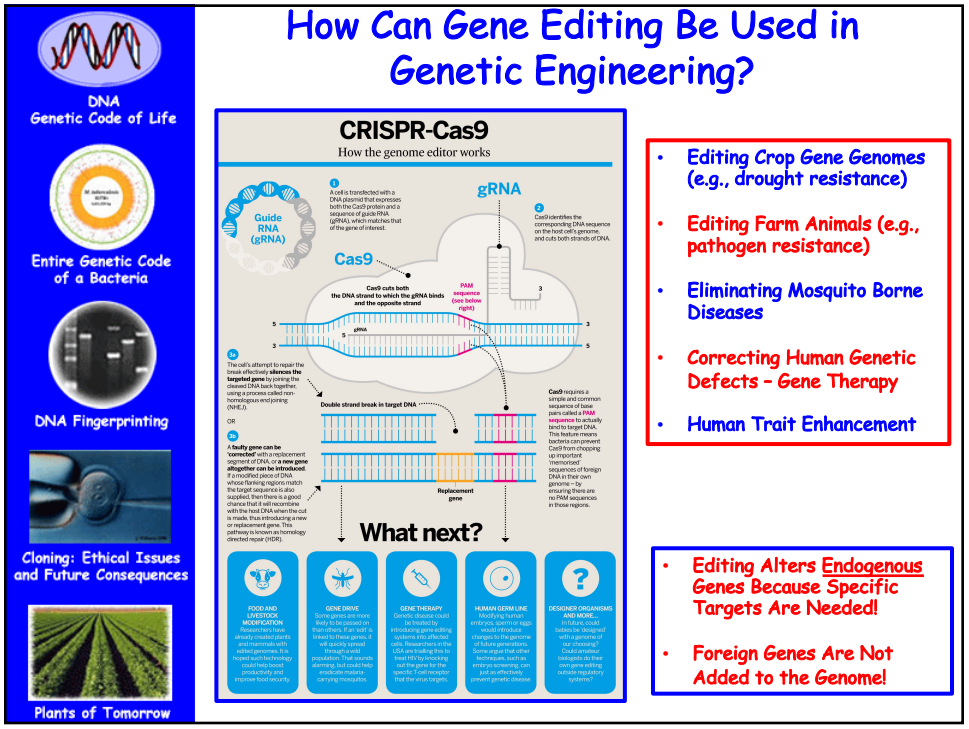
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Advantages of Gene Editing Over "Cohen-Boyer" Transgenic Genetic Engineering
• Simple Method to Edit, Correct, or Modify Any Endogenous Gene
• Multiple Genes Can Be Corrected at Once
• Dominant Alleles Can Be Mutated & Turned Off
Disadvantages of Gene Editing Over "Cohen-Boyer" Genetic Engineering
• Cannot Add Foreign Genes (e.g., GFP)
• Limited to Species-Specific Gene Corrections

47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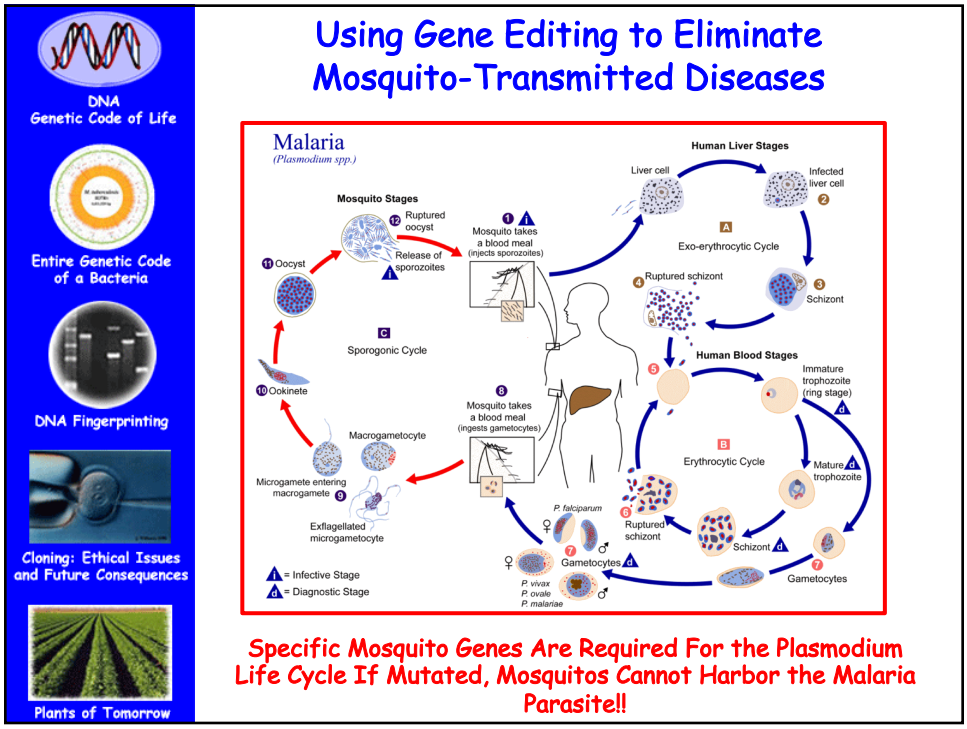
How Can Gene Editing Be Used in Genetic Engineering?
CRISPR-Cas9
How the genome editor works
Guide RNA (gRNA)
gRNA
Cas9
What next?
• Editing Crop Gene Genomes (e.g., drought resistance)
• Editing Farm Animals (e.g., pathogen resistance)
• Eliminating Mosquito Borne Diseases
• Correcting Human Genetic Defects – Gene Therapy
• Human Trait Enhancement
• Editing Alters Endogenous Genes Because Specific Targets Are Needed!
• Foreign Genes Are Not Added to the Genome!

48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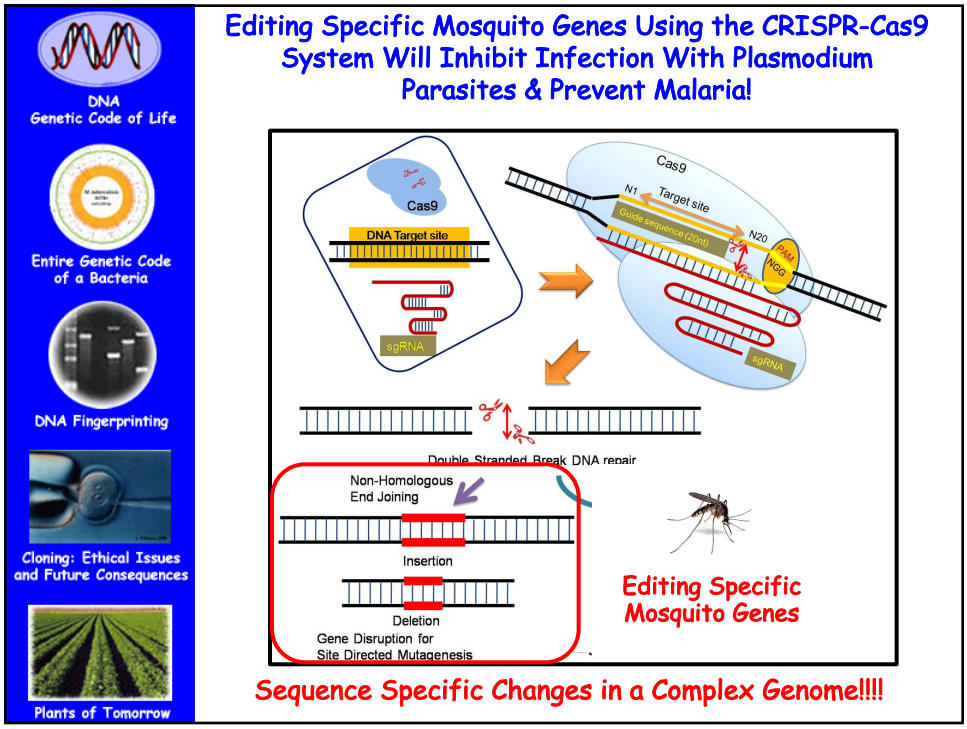
Plants of Tomorrow
Using Gene Editing to Eliminate Mosquito-Transmitted Diseases
Malaria
(Plasmodium spp.)
Human Liver Stages
Liver cell
Infected liver cell
Exo-erythrocytic Cycle
Ruptured schizont
Schizont
Human Blood Stages
Immature trophozoite (ring stage)
Mature trophozoite
Erythrocytic Cycle
Ruptured schizont
Schizont
Gametocytes
Gametocytes
P. falciparum
P. vivax
P. ovale
P. malariae
Mosquito takes a blood meal (injects sporozoites)
Mosquito Stages
Ruptured oocyst
Release of sporozoites
Oocyst
Sporogonic Cycle
Ookinete
Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
Exflagellated microgametocyte
= Infective Stage
= Diagnostic Stage
Specific Mosquito Genes Are Required For the Plasmodium Life Cycle If Mutated, Mosquitos Cannot Harbor the Malaria Parasite!!

49


DNA
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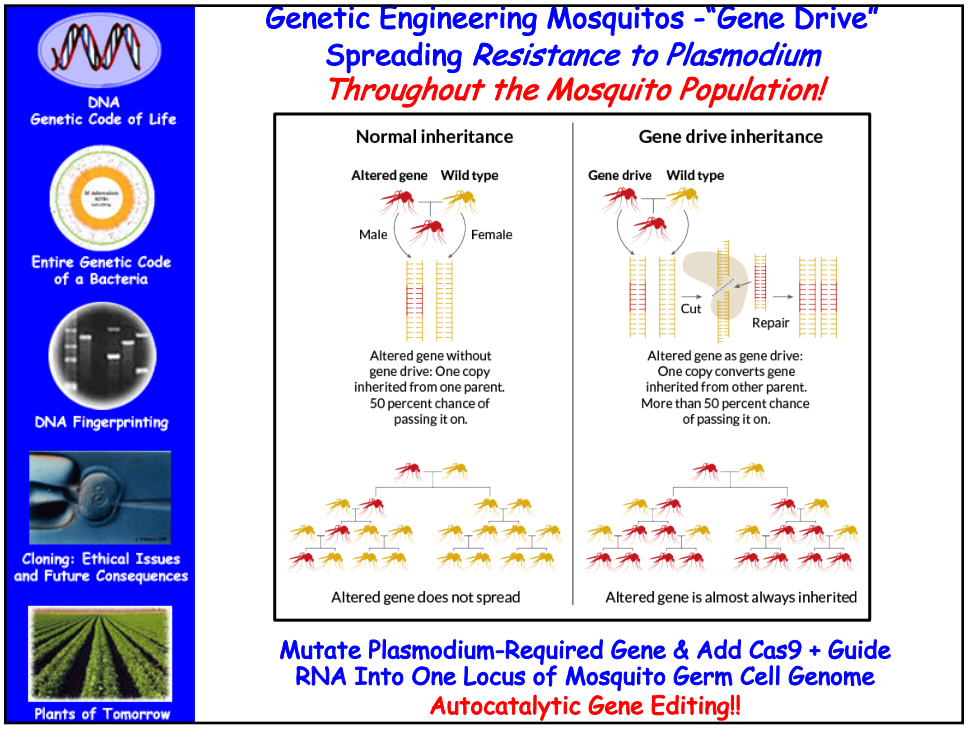
Plants of Tomorrow
Editing Specific Mosquito Genes Using the CRISPR-Cas9 System Will Inhibit Infection With Plasmodium Parasites & Prevent Malaria!
Cas9
DNA Target site
sgRNA
Cas9
N1
Target site
Guide sequence (20nt)
N20
PAM
NGG
sgRNA
Double Stranded Break DNA repair
Non-Homologous End Joining
Insertion
Deletion
Gene Disruption for Site Directed Mutagenesis
Editing Specific Mosquito Genes
Sequence Specific Changes in a Complex Genome!!!!

50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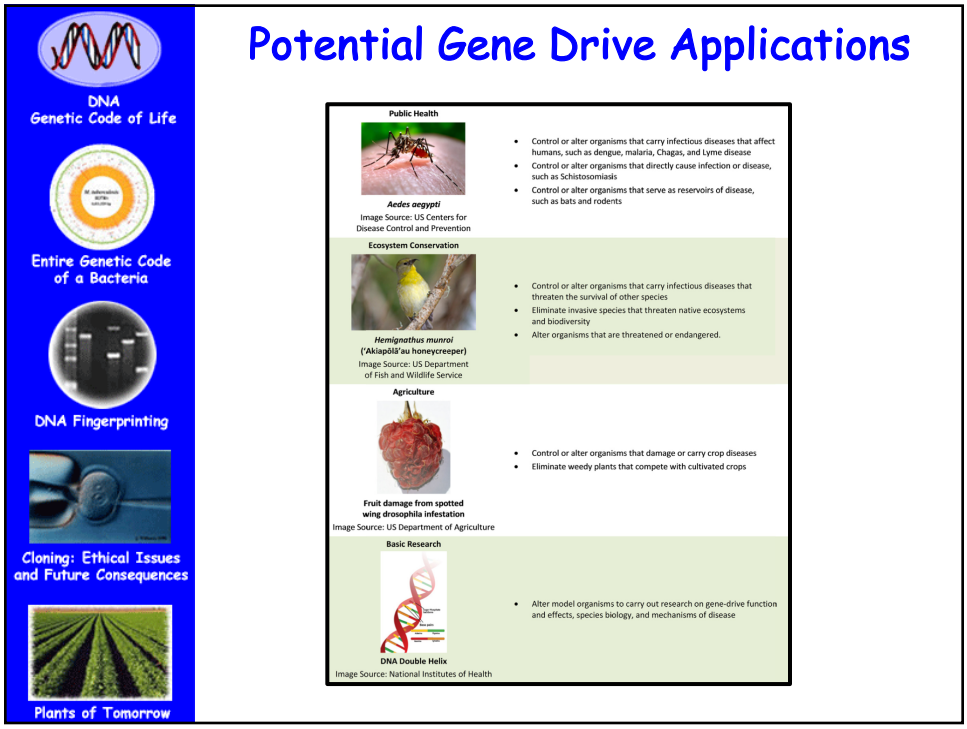
Plants of Tomorrow
Genetic Engineering Mosquitos -"Gene Drive"
Spreading Resistance to Plasmodium
Throughout the Mosquito Population!
Normal inheritance
Altered gene
Wild type
Male
Female
Altered gene without gene drive: One copy inherited from one parent. 50 percent chance of passing it on.
Altered gene does not spread
Gene drive inheritance
Gene drive
Wild type
Cut
Repair
Altered gene as gene drive: One copy converts gene inherited from other parent. More than 50 percent chance of passing it on.
Altered gene is almost always inherited
Mutate Plasmodium-Required Gene & Add Cas9 + Guide RNA Into One Locus of Mosquito Germ Cell Genome
Autocatalytic Gene Editing!!

51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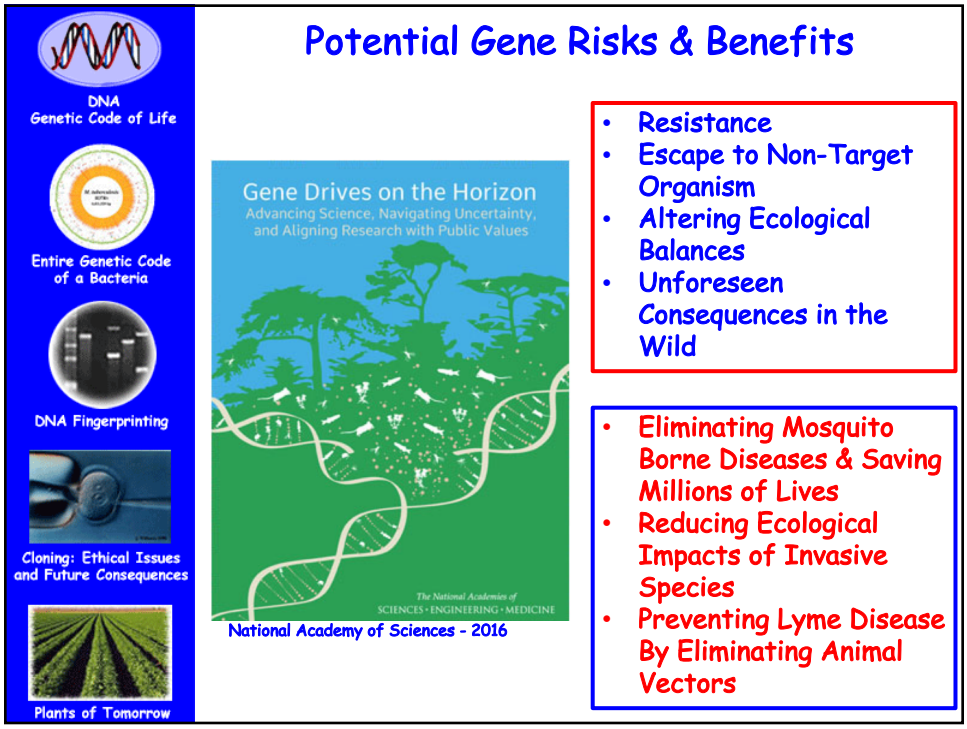
Potential Gene Drive Applications
Public Health
Aedes aegypti
Image Source: US Centers for Disease Control and Prevention
Control or alter organisms that carry infectious diseases that affect humans, such as dengue, malaria, Chagas, and Lyme disease
Control or alter organisms that directly cause infection or disease, such as Schistosomiasis
Control or alter organisms that serve as reservoirs of disease, such as bats and rodents
Ecosystem Conservation
Hemignathus munroi ('Akiapōlā'au honeycreeper)
Image Source: US Department of Fish and Wildlife Service
Control or alter organisms that carry infectious diseases that threaten the survival of other species
Eliminate invasive species that threaten native ecosystems and biodiversity
Alter organisms that are threatened or endangered.
Agriculture
Fruit damage from spotted wing drosophila infestation
Image Source: US Department of Agriculture
Control or alter organisms that damage or carry crop diseases
Eliminate weedy plants that compete with cultivated crops
Basic Research
DNA Double Helix
Image Source: National Institutes of Health
Alter model organisms to carry out research on gene-drive function and effects, species biology, and mechanisms of disease

52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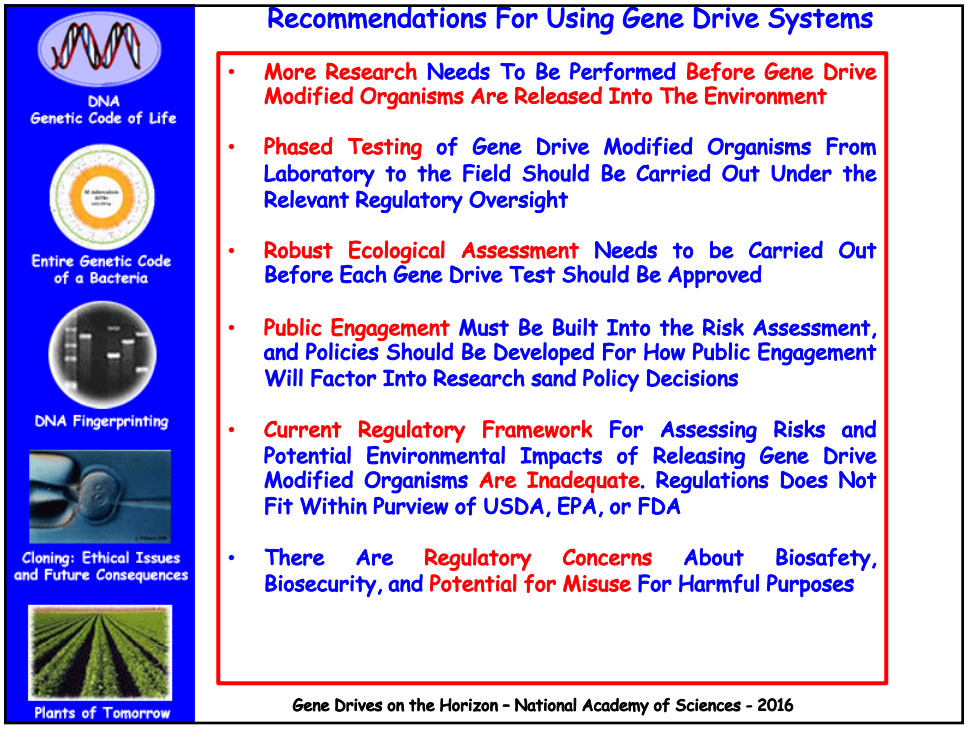
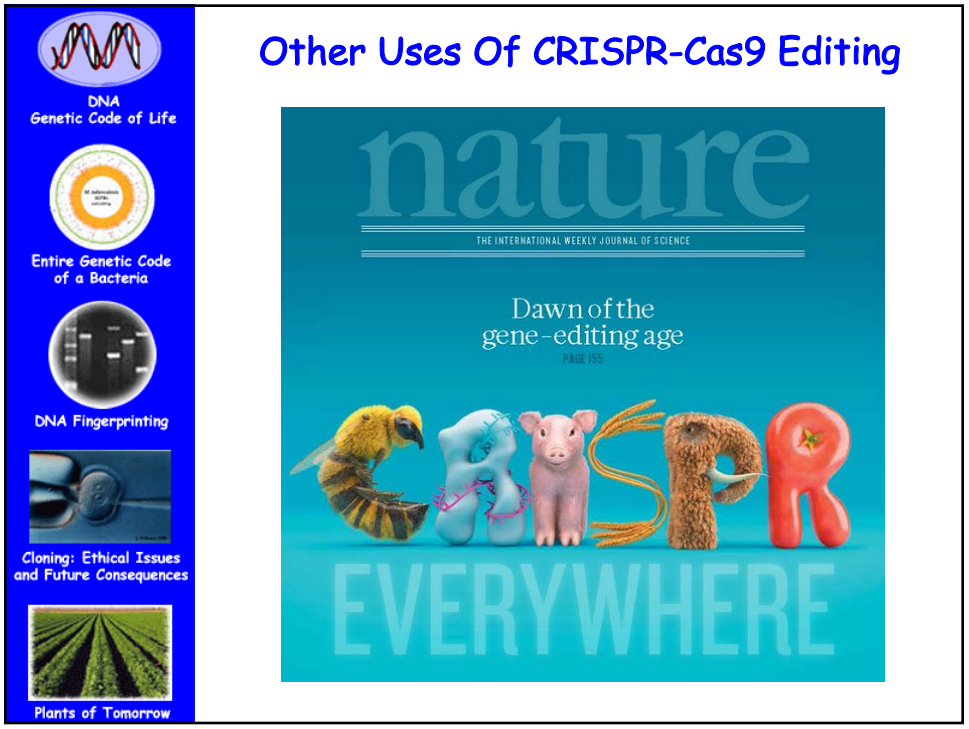
Plants of Tomorrow
Potential Gene Risks & Benefits
Gene Drives on the Horizon
Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values
The National Academies of SCIENCES • ENGINEERING • MEDICINE
National Academy of Sciences - 2016
• Resistance
• Escape to Non-Target Organism
• Altering Ecological Balances
• Unforeseen Consequences in the Wild
• Eliminating Mosquito Borne Diseases & Saving Millions of Lives
• Reducing Ecological Impacts of Invasive Species
• Preventing Lyme Disease By Eliminating Animal Vectors

53


DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Recommendations For Using Gene Drive Systems
• More Research Needs To Be Performed Before Gene Drive Modified Organisms Are Released Into The Environment
• Phased Testing of Gene Drive Modified Organisms From Laboratory to the Field Should Be Carried Out Under the Relevant Regulatory Oversight
• Robust Ecological Assessment Needs to be Carried Out Before Each Gene Drive Test Should Be Approved
• Public Engagement Must Be Built Into the Risk Assessment, and Policies Should Be Developed For How Public Engagement Will Factor Into Research sand Policy Decisions
• Current Regulatory Framework For Assessing Risks and Potential Environmental Impacts of Releasing Gene Drive Modified Organisms Are Inadequate. Regulations Does Not Fit Within Purview of USDA, EPA, or FDA
• There Are Regulatory Concerns About Biosafety, Biosecurity, and Potential for Misuse For Harmful Purposes
Gene Drives on the Horizon – National Academy of Sciences - 2016

54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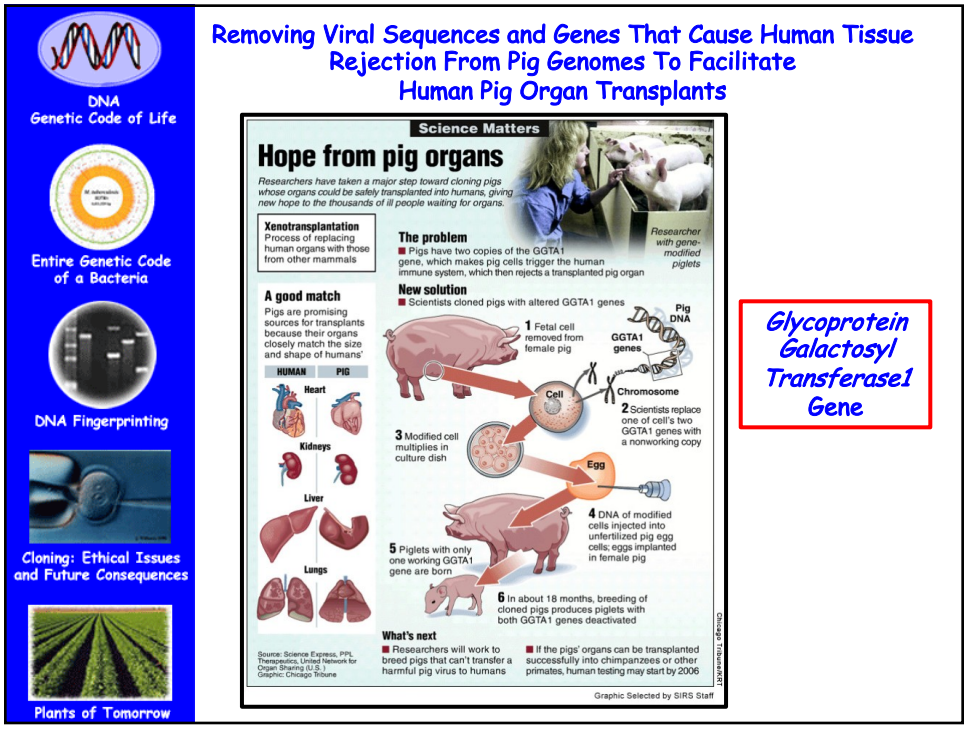
Other Uses Of CRISPR-Cas9 Editing
nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Dawn of the gene-editing age
PAGE 155
CRISPR
EVERYWHERE

55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Removing Viral Sequences and Genes That Cause Human Tissue Rejection From Pig Genomes To Facilitate Human Pig Organ Transplants
Science Matters
Hope from pig organs
Researchers have taken a major step toward cloning pigs whose organs could be safely transplanted into humans, giving new hope to the thousands of ill people waiting for organs.
Xenotransplantation
Process of replacing human organs with those from other mammals
The problem
Pigs have two copies of the GGTA1 gene, which makes pig cells trigger the human immune system, which then rejects a transplanted pig organ
Researcher with gene-modified piglets
A good match
Pigs are promising sources for transplants because their organs closely match the size and shape of humans'
HUMAN
PIG
Heart
Kidneys
Liver
Lungs
New solution
Scientists cloned pigs with altered GGTA1 genes
Pig DNA
GGTA1 genes
1 Fetal cell removed from female pig
Cell
Chromosome
2 Scientists replace one of cell's two GGTA1 genes with a nonworking copy
3 Modified cell multiplies in culture dish
Egg
4 DNA of modified cells injected into unfertilized pig egg cells; eggs implanted in female pig
5 Piglets with only one working GGTA1 gene are born
6 In about 18 months, breeding of cloned pigs produces piglets with both GGTA1 genes deactivated
What's next
Researchers will work to breed pigs that can't transfer a harmful pig virus to humans
If the pigs' organs can be transplanted successfully into chimpanzees or other primates, human testing may start by 2006
Source: Science Express, PPL Therapeutics, United Network for Organ Sharing (U.S.)
Graphic: Chicago Tribune
Graphic Selected by SIRS Staff
Glycoprotein Galactosyl Transferase1 Gene

56

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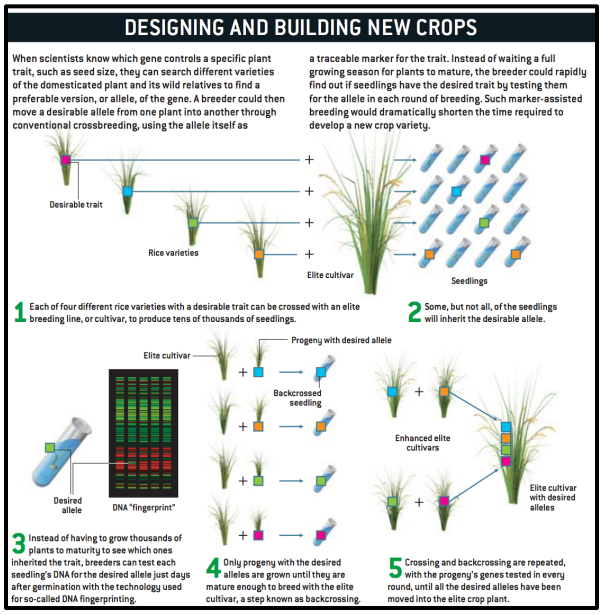
Using CRISPR-Cas9 Editing For Crop Improvement



57

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Using Gene Editing to Improve Crop Plants

Geneticists Have Used CRISPR Gene Editing to Create Crops That Grow More Food

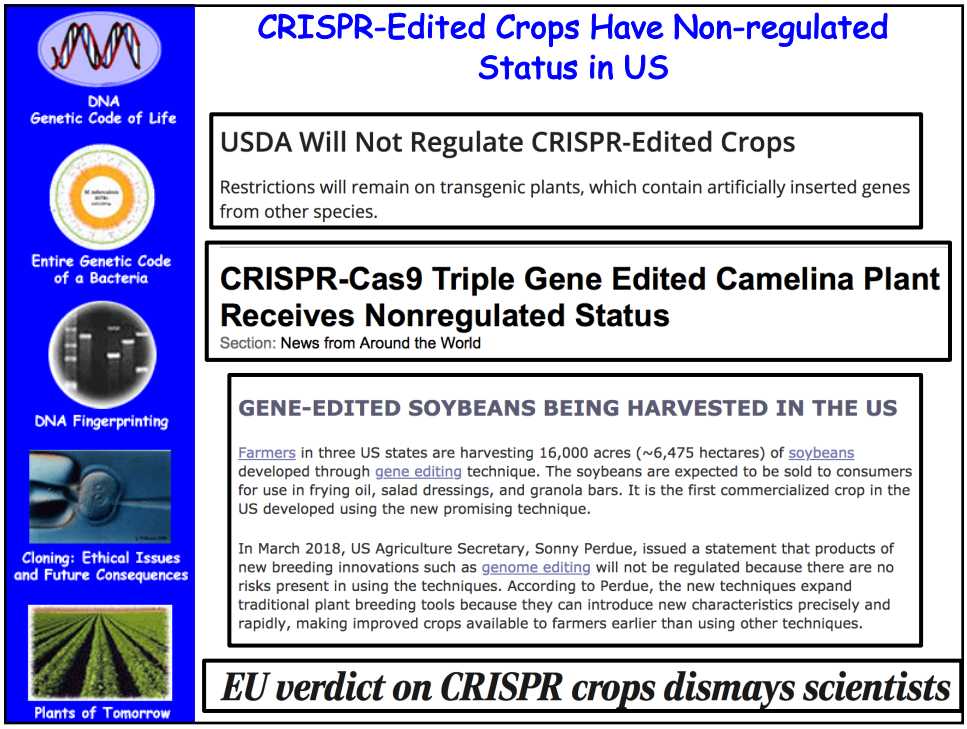
We're editing our way through global food shortage

SCIENTISTS USE CRISPR-CAS9 TECHNOLOGY TO IMPROVE DROUGHT AND SALT TOLERANCE IN RICE

GM Wheat Used to Make Bread with Less Gluten

Researchers Engineer Potyvirus Resistance Using CRISPR/Cas9

58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CRISPR-Edited Crops Have Non-regulated Status in US
USDA Will Not Regulate CRISPR-Edited Crops
Restrictions will remain on transgenic plants, which contain artificially inserted genes from other species.
CRISPR-Cas9 Triple Gene Edited Camelina Plant Receives Nonregulated Status
Section: News from Around the World
GENE-EDITED SOYBEANS BEING HARVESTED IN THE US
Farmers in three US states are harvesting 16,000 acres (~6,475 hectares) of soybeans developed through gene editing technique. The soybeans are expected to be sold to consumers for use in frying oil, salad dressings, and granola bars. It is the first commercialized crop in the US developed using the new promising technique.
In March 2018, US Agriculture Secretary, Sonny Perdue, issued a statement that products of new breeding innovations such as genome editing will not be regulated because there are no risks present in using the techniques. According to Perdue, the new techniques expand traditional plant breeding tools because they can introduce new characteristics precisely and rapidly, making improved crops available to farmers earlier than using other techniques.
EU verdict on CRISPR crops dismays scientists

59


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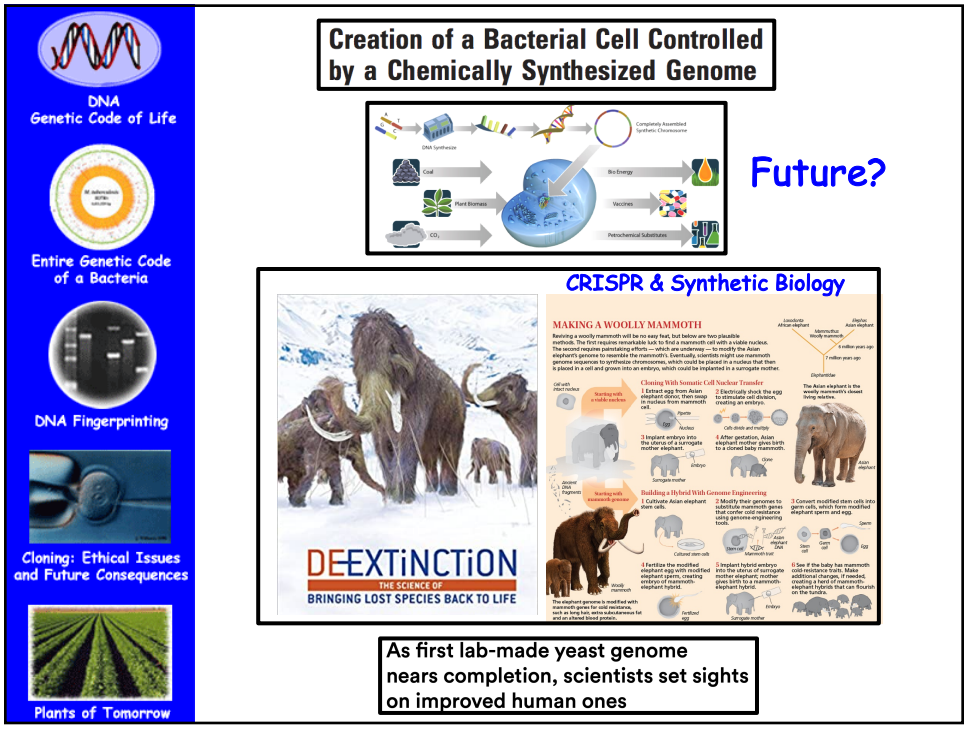
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Using CRISPR-Cas9 Editing For Correcting Human Genetic Disorders
Somatic Cell Gene Therapy
Ex Vivo
In Vivo
Germline Gene Therapy + Gene Enhancement
Editing humanity
The prospect of genetic enhancement

60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Future?
CRISPR & Synthetic Biology
DEEXTINCTION
THE SCIENCE OF
BRINGING LOST SPECIES BACK TO LIFE
MAKING A WOOLLY MAMMOTH
As first lab-made yeast genome nears completion, scientists set sights on improved human ones

61