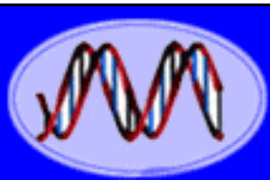
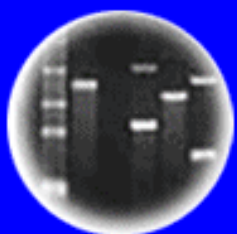


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A & SAS70A Winter 2019
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, John Harada, & Channapatna Prakash

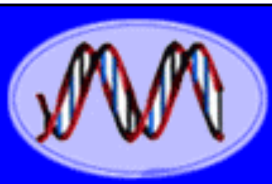
Lecture 6

Twenty-First Century Genetic Engineering Applications

UCLA

TUSKEGEE UNIVERSITY

UC DAVIS
UNIVERSITY OF CALIFORNIA



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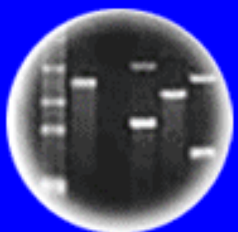

DNA Fingerprinting

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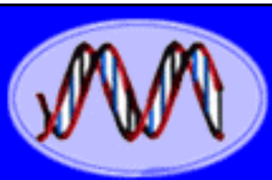
Themes

1. What is a GMO?
2. What Are the Three Procedures to Engineer Cells?
3. How Do Classical Breeding, Foreign Gene Insertion, and Editing Differ?
4. What is Marker Assisted Breeding and How Can It Speed Up Crop Improvement?
5. What Are Industrial Applications of Genetic Engineering?
6. How Can Genetic Engineering Be Used To Eliminate or Reduce Mosquito Populations?
7. What is the CRISPR-Cas Bacterial Immunity System?
8. What Are the Individual Components of the CRISPR-Cas Immunity System?
9. How Can CRISPER-Cas9 be Used For Gene Editing?
10. What is Gene Drive and How Can it Be Used To Fight Malaria?
11. What Are the Ethical and Regulatory Concerns of Using Gene Drive Systems?
12. What Are Other Applications of CRISPR-Cas9 Editing?
13. What Are the Ethical Concerns For Editing the Human Genome in Somatic and Germ Cells



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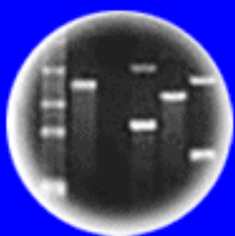

DNA Fingerprinting

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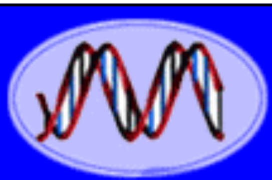
Breeding or DNA Manipulation – They Are the SAME

&

Called *Gene Manipulation*

WHAT IS A GMO???



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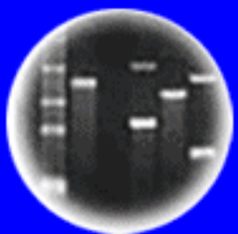

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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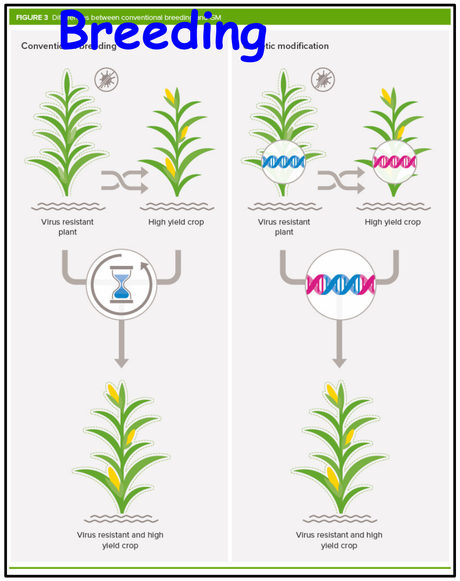
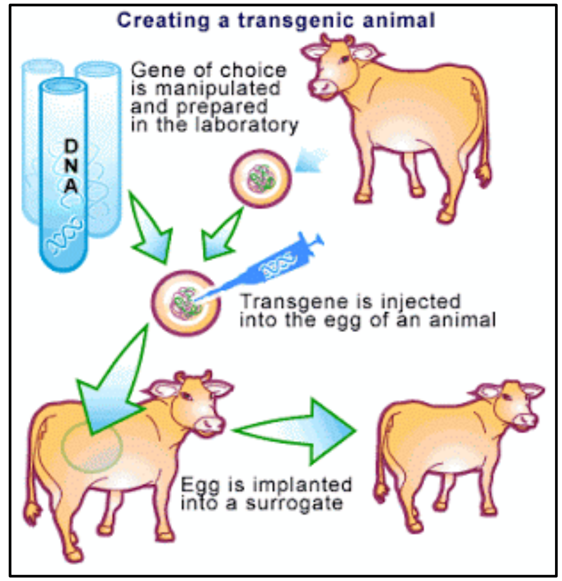
Three Genetic Engineering Techniques That Generate GMOs!!!

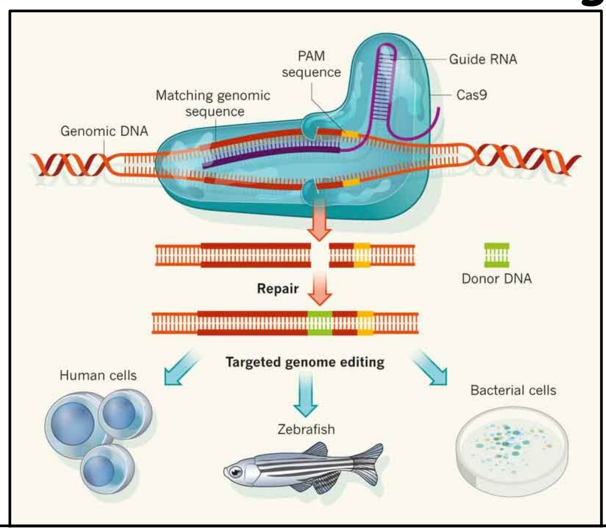
1. Classical Breeding

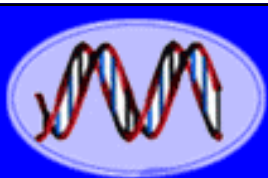


2. Transgenic Organism



3. CRISPR Gene Editing





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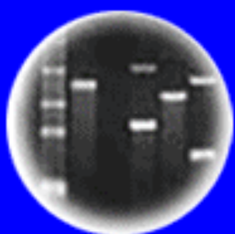

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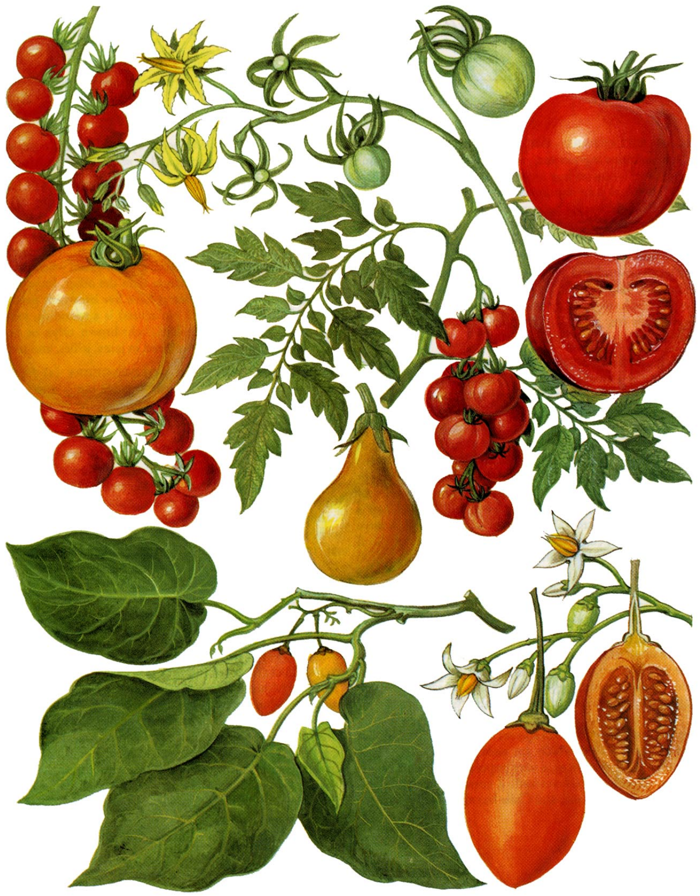
Breeding or DNA Manipulation - They Are the SAME
&
Called *Gene Manipulation*
WHAT IS A GMO???

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

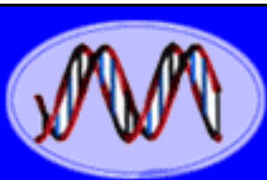
Tomato Genetic Diversity



Wheat Genetic Diversity

Nikolai Vavilov
1887-1943

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



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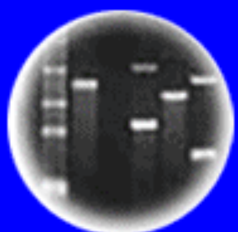

DNA Fingerprinting

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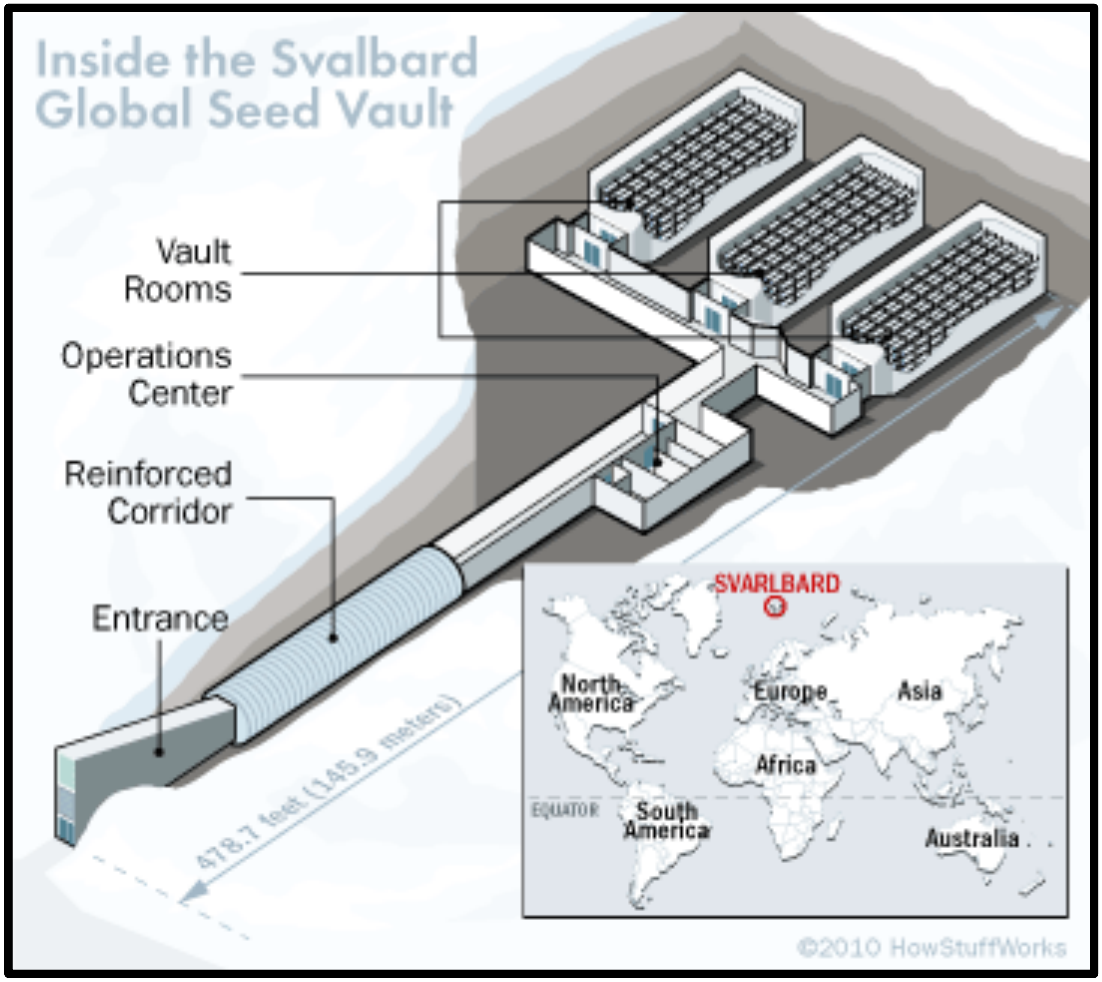
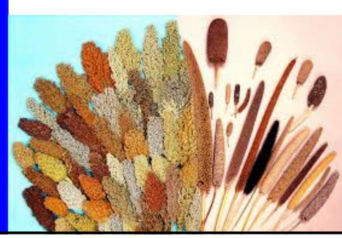
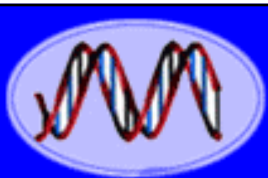
A Noah's Ark For Seeds & Crop Diversity







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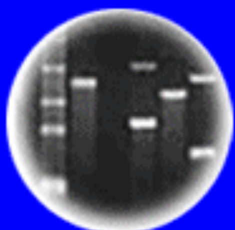

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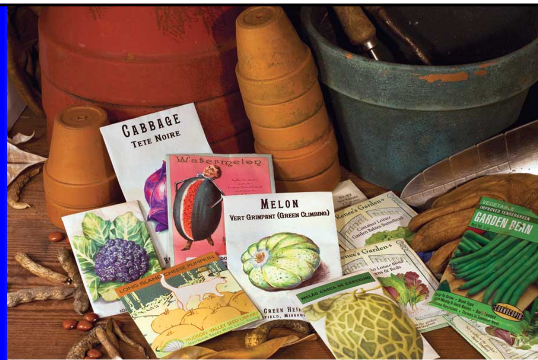
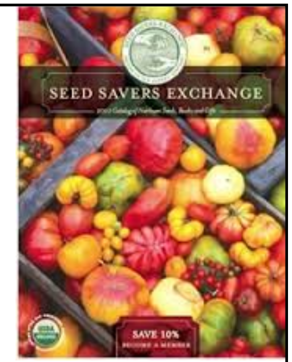


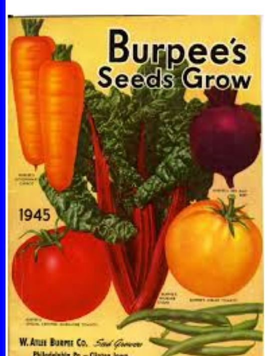


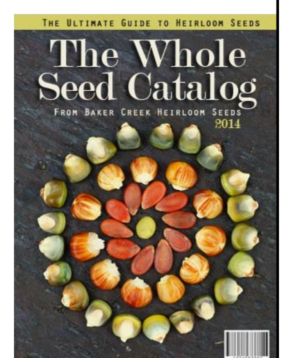
The Problem With Breeding the "Old Fashioned Way"

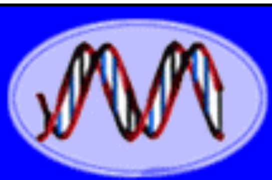
Cannot Predict Results!

Takes Many Generations – Slow!

Cannot Follow Traits Easily – e.g., Disease Resistance!







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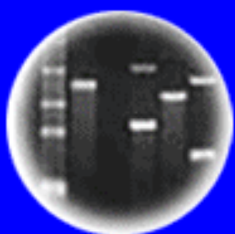

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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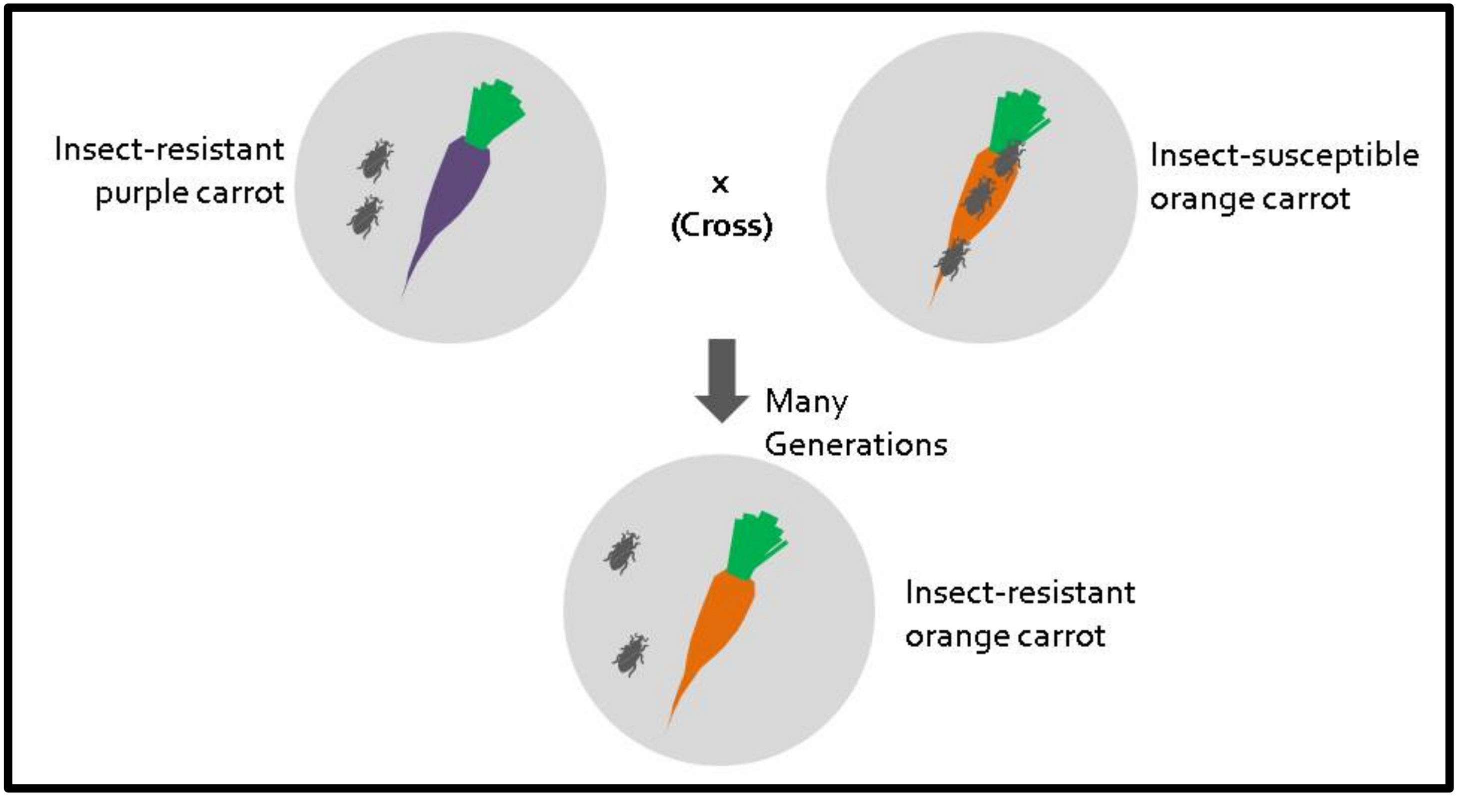
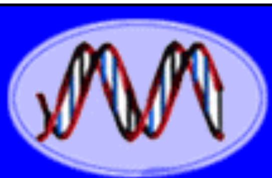
Need Mature Plants to Assess Important Phenotypes in Breeding Program





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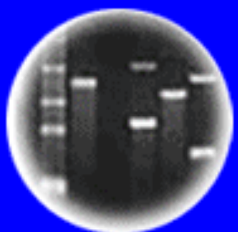

DNA Fingerprinting

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Breeding the 21st Century Way

Can Predict Results!

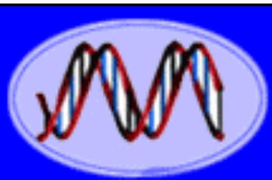
Identifying Crop Diversity Genes/Alleles

The 3,000 rice genomes project

The 3,000 rice genomes project[1,2,3*†]

150 Tomato Genome ReSequencing project

Using DNA Fingerprints to Identify Traits in Breeding Program – Marker Assisted 21st Century Breeding (Using RFLPs)



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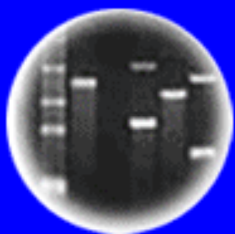

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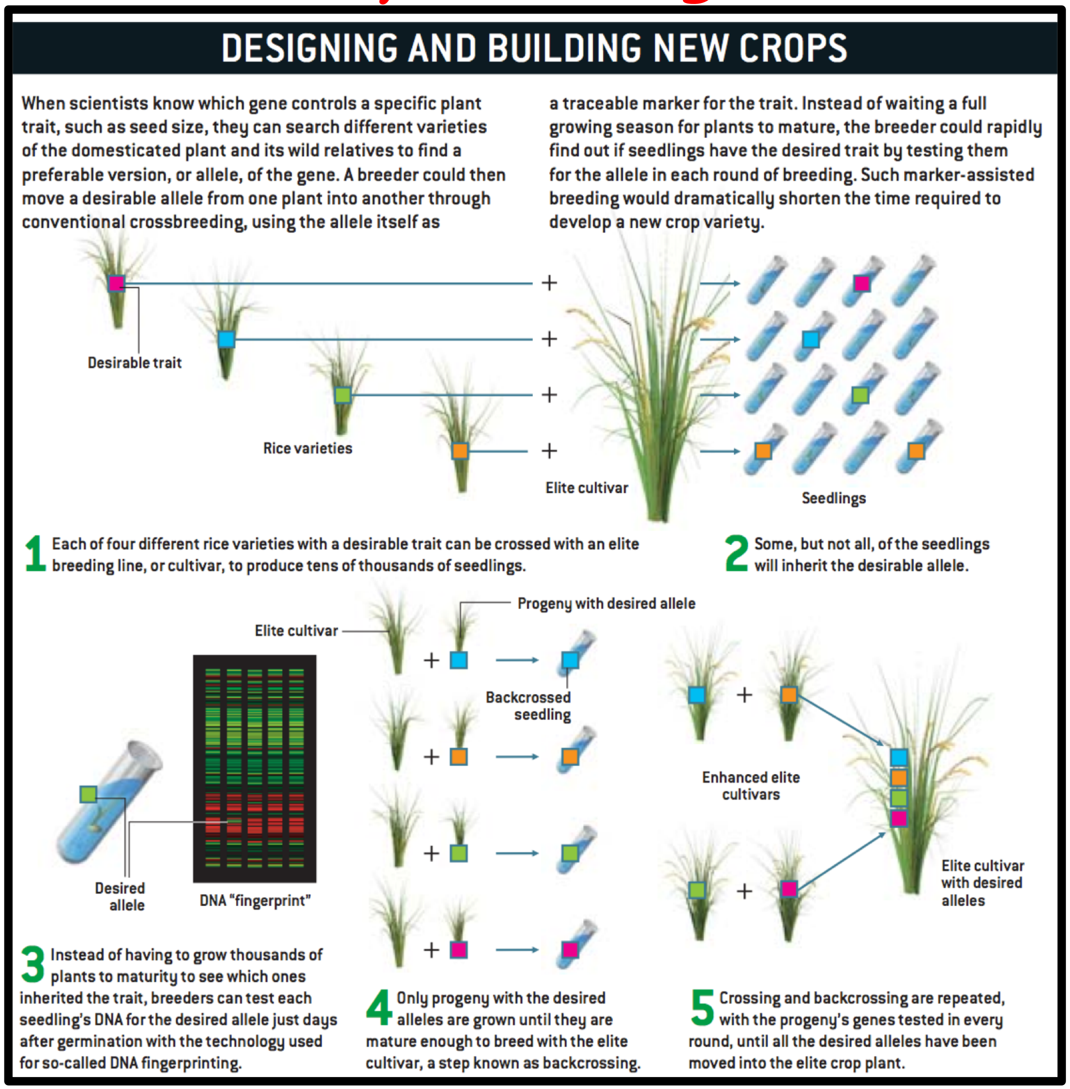
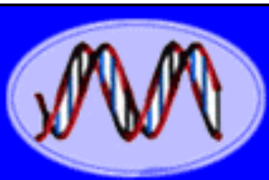
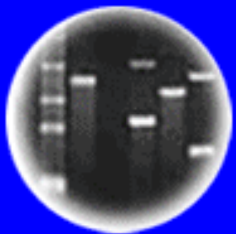


Advantages

- Speed Up Breeding Program
- More Predictable Breeding Program





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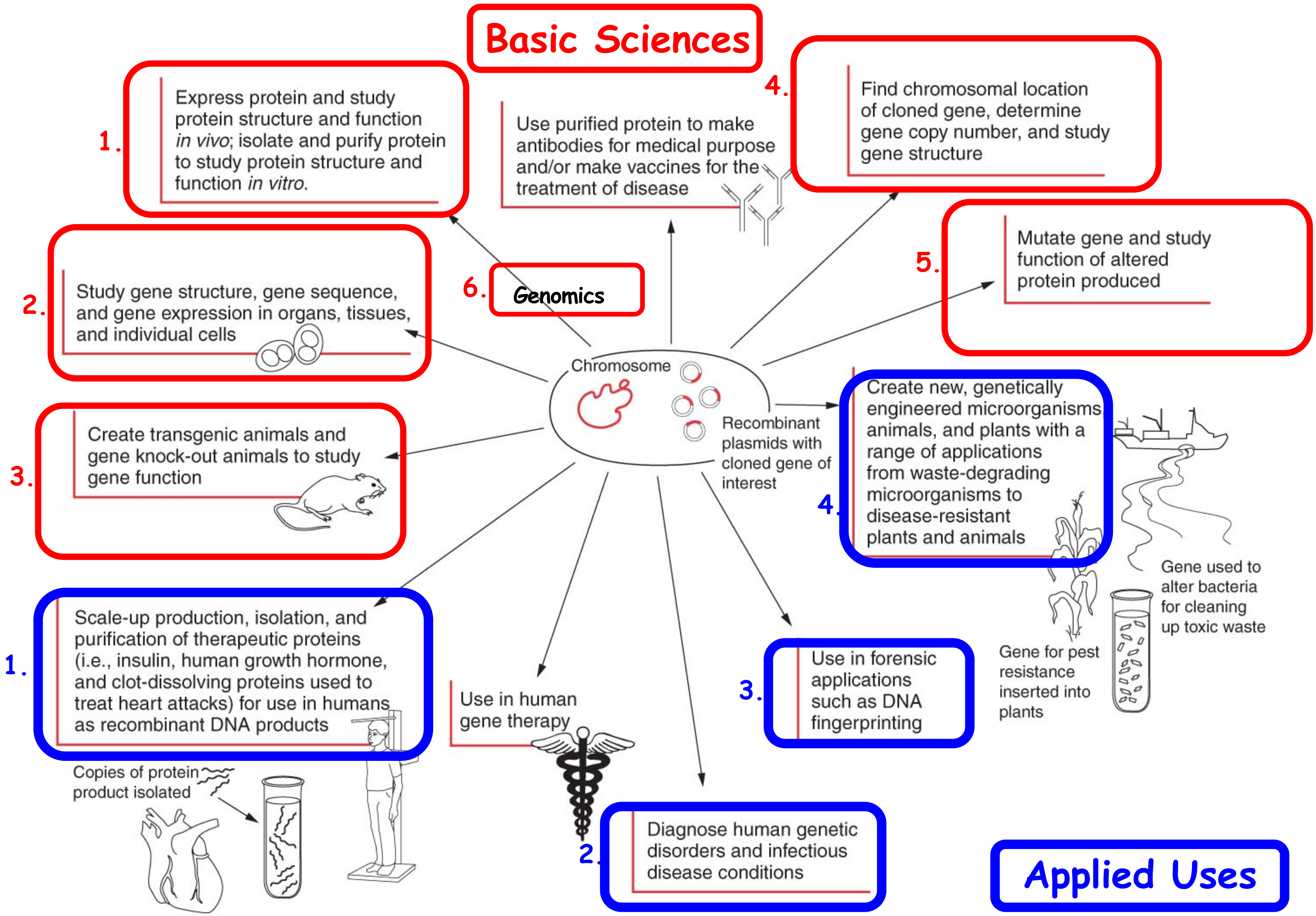
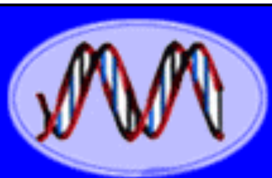
WHAT IS A GMO???

There Are Numerous Applications of "Cohen-Boyer" Genetic Engineering – Many Have Been Discussed in Class





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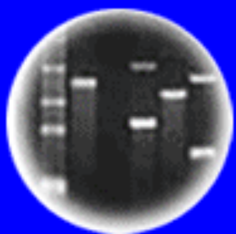

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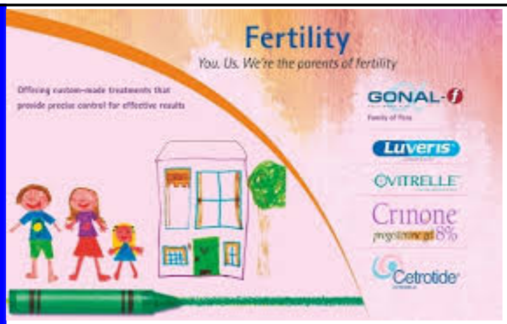
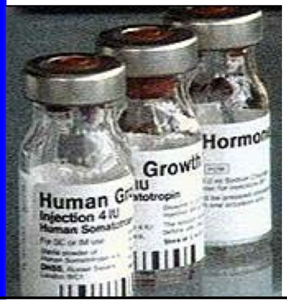
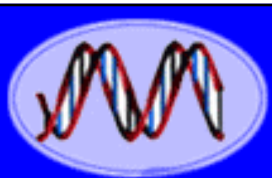


Using Genetic Engineering to Make Drugs & Vaccines

A $1.1 Trillion Dollar Market (2017)!!





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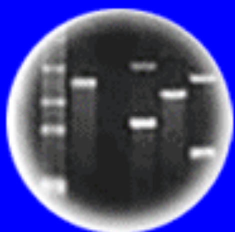

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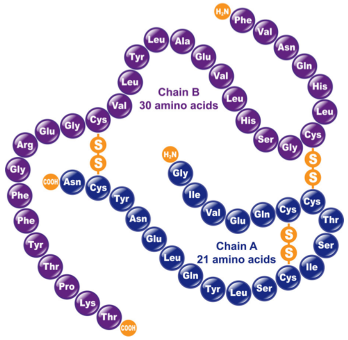
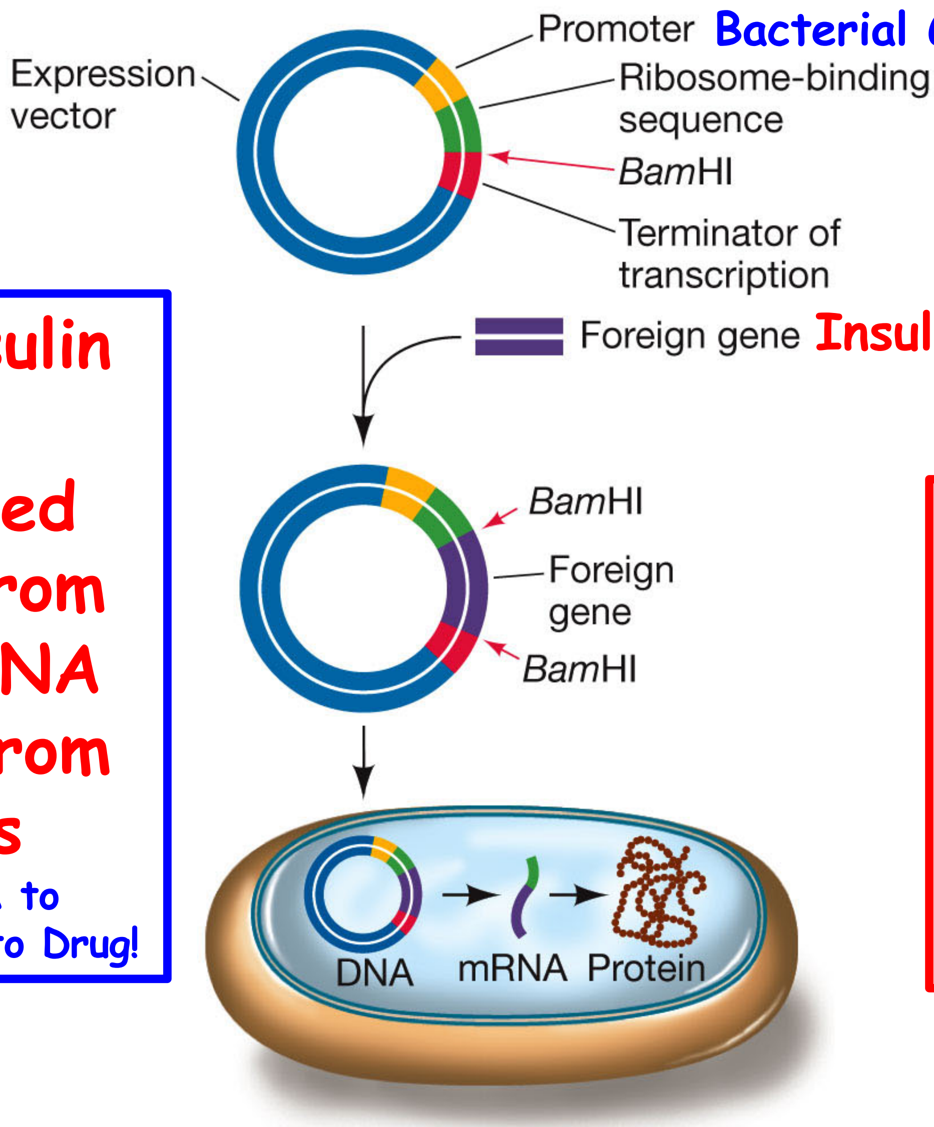
One of the Most Important Applications of Genetic Engineering Technology Has Been To Manufacture Drugs & Vaccines to Treat Human and Animal Diseases

Created a Multibillion Dollar Biotechnology Industry, Was Responsible For the Acceptance of Recombinant DNA Technology in the 1970s,& Lead to Pioneering Decisions in Patent Law

Engineering a Bacterial Cell to Make a Human Protein (e.g., Insulin)





Bacterial Gene Promoter/Switch

Insulin cDNA

Recall: Insulin cDNA Synthesized Directly From Insulin mRNA Isolated From Pancreas

mRNA to cDNA to Engineered E. coli to Drug!

What Needs To Be "Done" to the Human cDNA to Have it Expressed in Bacterial Cells?

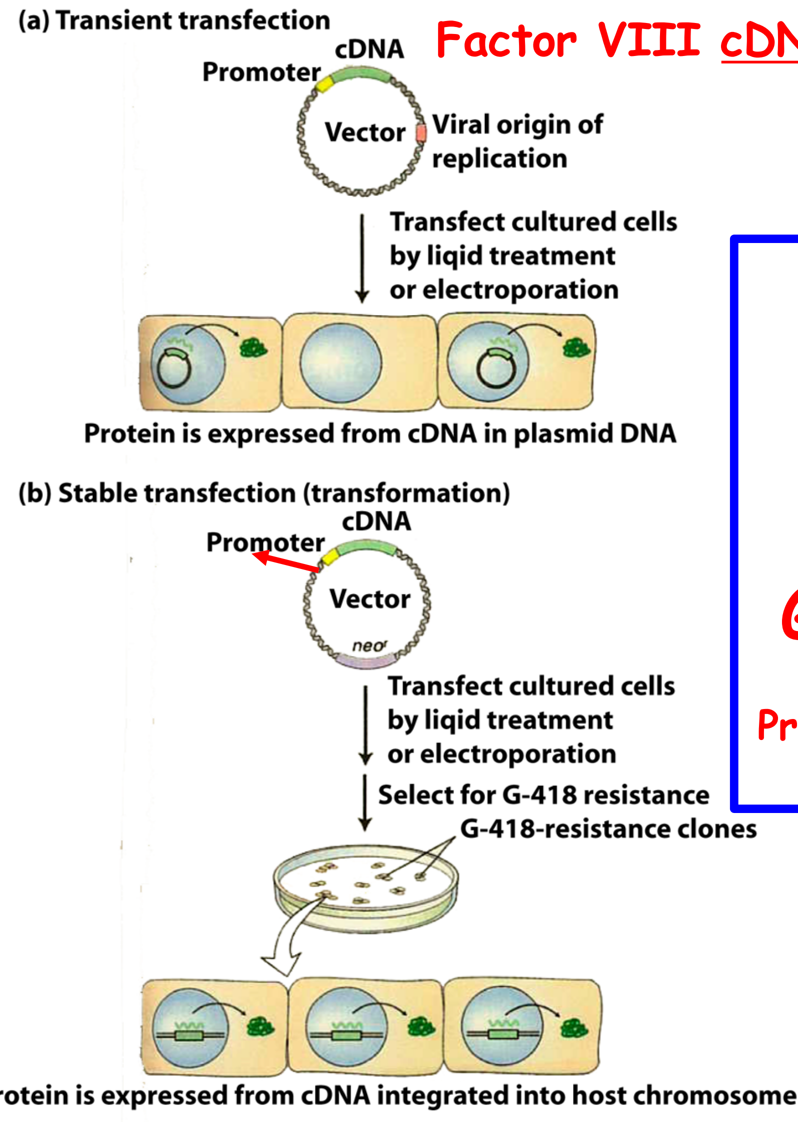
LIFE 8e, Figure 16.16

Engineering an Animal Cell to Make a Human Protein (e.g., Factor VIII)

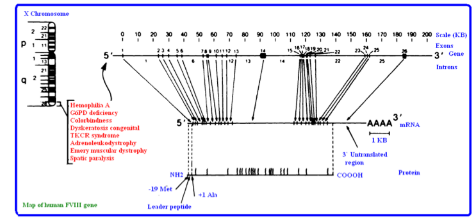
What is the Reason For Using Mammalian Cells?

[Chinese Hamster Cells (CHO)]

Recall:
Extraordinary Measures,
Pompe's Disease
&
α-Glucosidase Enzyme

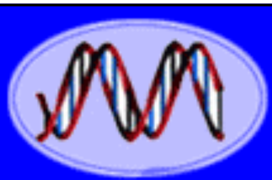


Factor VIII cDNA



Recall: Factor VIII Gene Isolated First Using Genetic Code &
Protein Sequence
Protein to Gene to mRNA to cDNA to Drug

Purify Factor VIII Protein!



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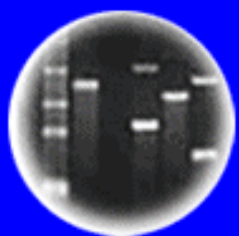

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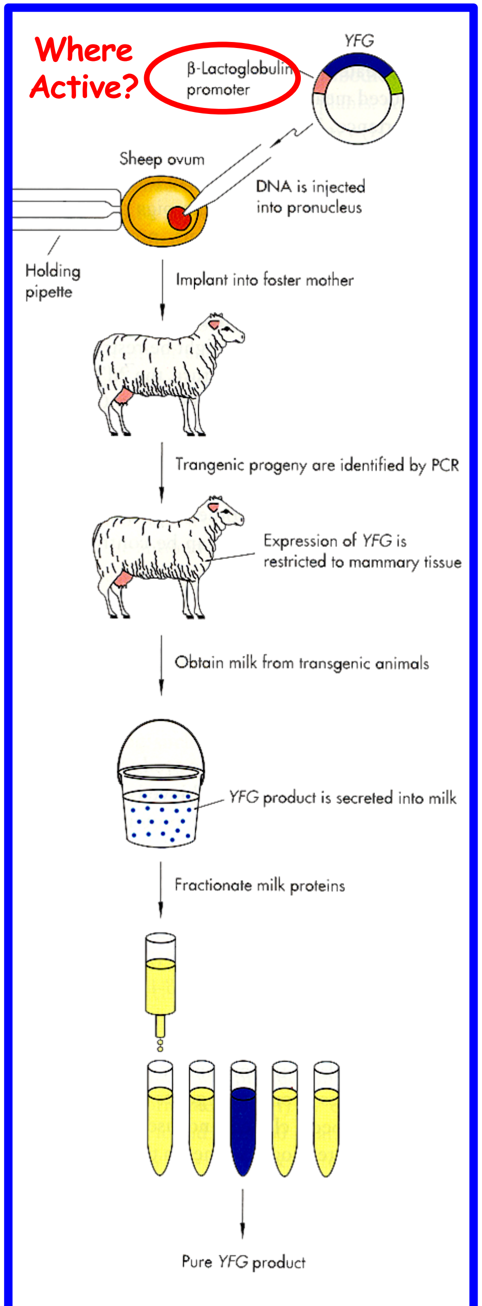
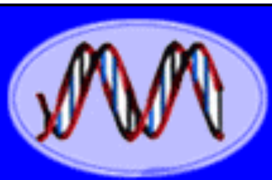
Animals Can Also be Used as Factories to Produce Large Amounts of Human Proteins



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

Genetically Engineered Drug-Producing Mammals Can Also Be Cloned



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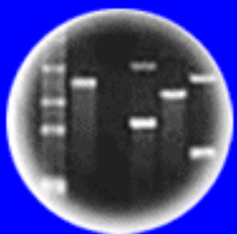

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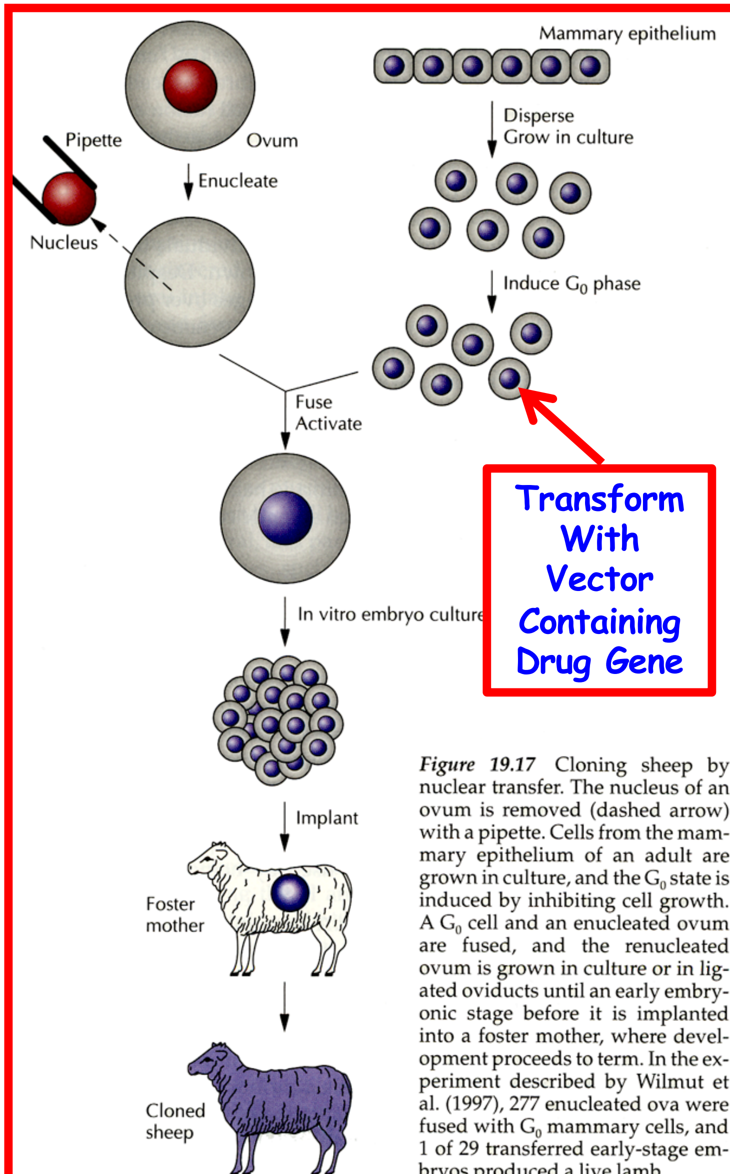


Figure 19.17 Cloning sheep by nuclear transfer. The nucleus of an ovum is removed (dashed arrow) with a pipette. Cells from the mammary epithelium of an adult are grown in culture, and the G_0 state is induced by inhibiting cell growth. A G_0 cell and an enucleated ovum are fused, and the renucleated 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

February 7, 2009

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

Antithrombin

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.

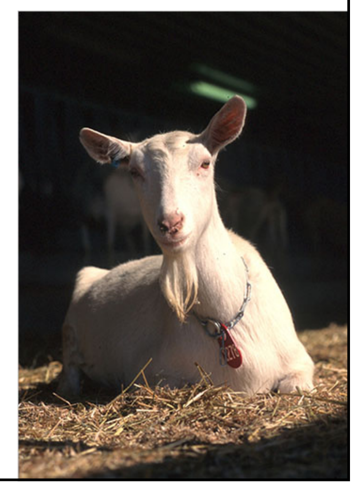
MODIFYING THE DNA
A human gene that produces the blood protein antithrombin is inserted into a short strand of goat DNA.

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



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.

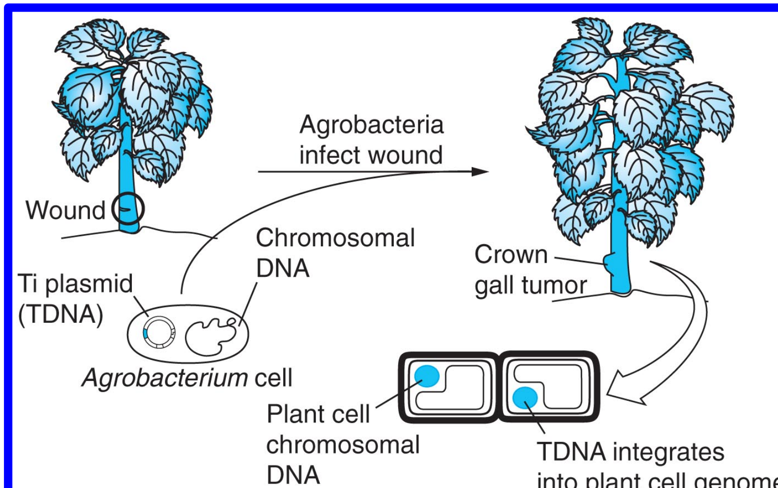
PLANTS IN THE PIPELINE

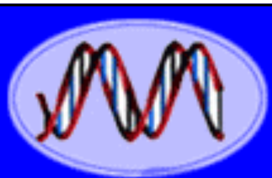
Manufacturers have begun or completed phase II clinical trials on a handful of biologics made in plants, and hope to follow Elelyso to market.

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

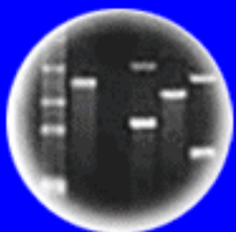
Elelyso® Made in Engineered Carrot Cells To Treat Gaucher's Disease – A Lysosomal Storage Disease That Prevents Molecules From Being Degraded and Disposed of Properly in Cells – 100x Prevalence in Ashkenazi Jews. Gene on Chromosome 1, and Encodes a Glucocerebrosidase.

Advantages of Plants?




DNA
Genetic Code of Life


Entire Genetic Code
of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues
and Future Consequences


Plants of Tomorrow

one child dies
every 20 seconds
from a disease
that is vaccine-preventable.

Fight the Flu
It starts with you

Using Genetic Engineering to Make Vaccines

Fight the Flu
It starts with you

one child dies
every 20 seconds
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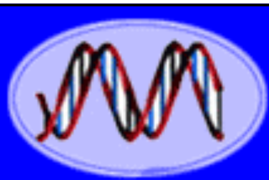

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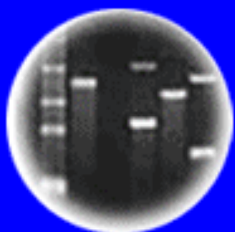


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

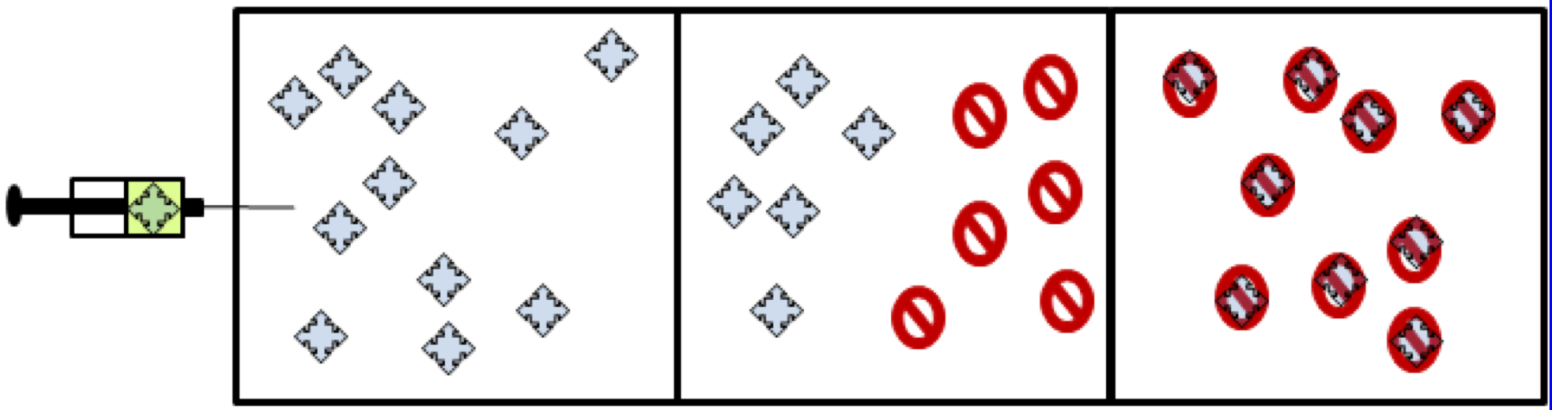
Vaccines Work With Body Immune System

(Adaptive Immunity)

HOW A VACCINE WORKS

Creating Immunity

1 2 3

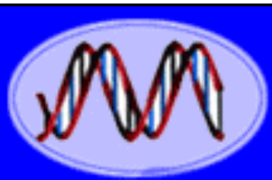


1. A weakened form of a disease antigen – that may be dead or living – is injected into the body.

2. The body reacts to the antigen by creating antibodies to attack it.

3. If the certain antigen ever enters the body again, the body's immune system antibodies will be able to fight against it.

Using Genetic Engineering To Make Vaccines



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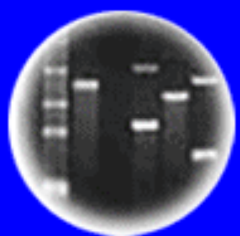


DNA Fingerprinting

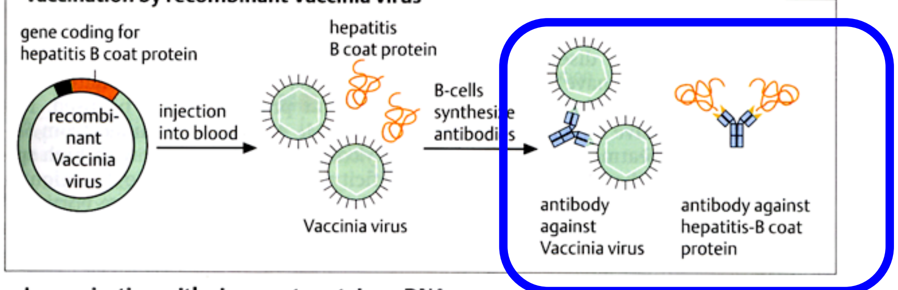
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

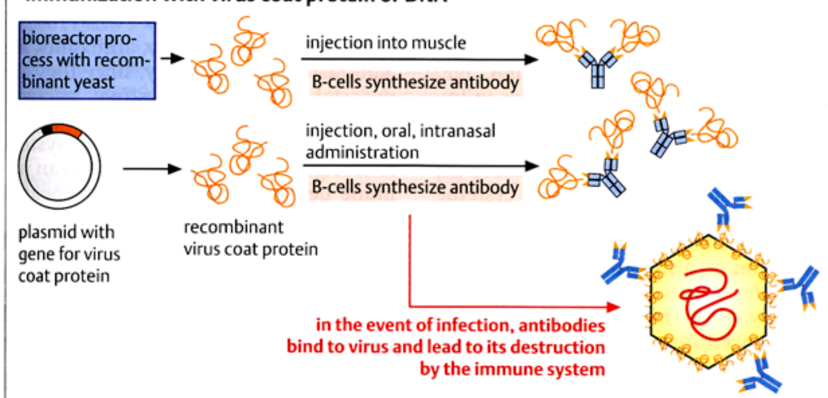
Recombinant vaccines (selection)

		antigen	status
viruses	hepatitis B	surface antigens	registered
	Herpes simplex type 2	surface antigens	clinical studies
	rabies vaccine	surface antigens	not registered
	yellow fever virus	surface antigens	preclinical studies
	AIDS virus	surface antigens	clinical studies
bacteria	*Streptococcus pneumoniae*	polysaccharide conjugate	registered
	Clostridium tetani	tetanus toxin	not registered
	Mycobacterium tuberculosis	surface antigens	clinical studies
parasites	*Plasmodium falciparum*	(malaria)	clinical studies
	Trypanosoma sp.	(sleeping sickness)	clinical studies
	Schistosoma mansoni	(bilharziosis)	clinical studies

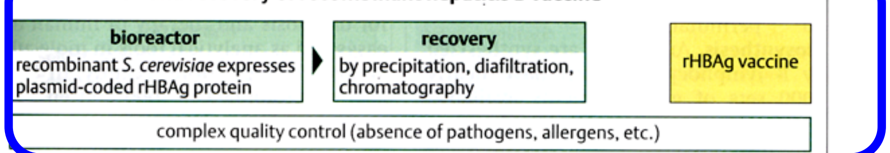
Vaccination by recombinant Vaccinia virus



Immunization with virus coat protein or DNA



Fermentation and recovery of recombinant hepatitis B vaccine



Clone Pathogenic Antigen Gene in *E. Coli* or Other Host (e.g., Yeast, Virus) And Synthesize Large Amounts of Antigen

Synthetic Biology Can Be Used to Rapidly Synthesize Vaccines

VACCINES

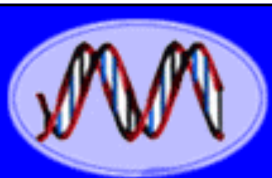
Synthetic Generation of Influenza Vaccine Viruses for Rapid Response to Pandemics

Synthetic Biologists Engineer A Custom Flu Vaccine In A Week

A synthetic biology method proves its chops.

Synthetic Biology Could Speed Flu Vaccine Production

Advanced genetic engineering is already changing vaccine development and could make inroads into other branches of medicine.



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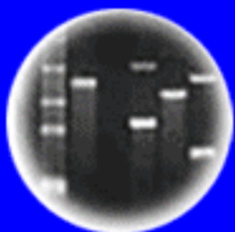

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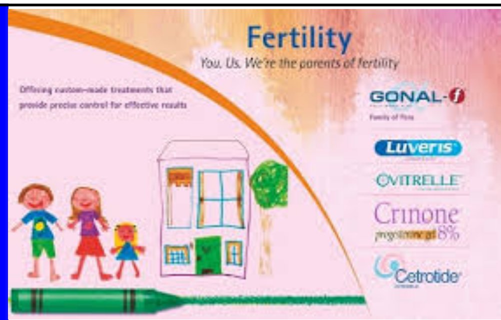


Vaccines Work!!!

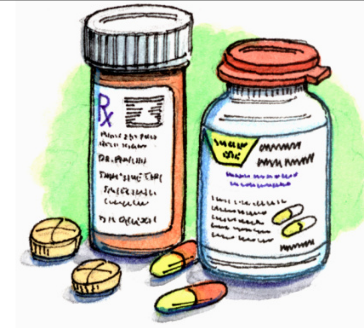


TABLE 12.1 Annual cases in Canada from various diseases before and after the introduction of vaccines against the causative agents of the diseases

Disease	Annual no. of cases before vaccine was introduced	No. of cases in 2002
Polio	20,000	0
Diphtheria	9,000	0
Rubella	69,000	16
Mumps	52,000	197
Haemophilus influenzae type b infection	2,000	48
Whooping cough	25,000	2,557
Measles	300,000	7

Measles outbreaks make 2018 a near-record year for U.S.

22 Cases –All Unvaccinated!

Measles has been declared a public health emergency in one Portland-area county.

California Vaccination Requirements

GUIDE TO IMMUNIZATIONS REQUIRED FOR SCHOOL ENTRY

Grades K-12

CDPH

INSTRUCTIONS

Use this guide as a quick reference to help you determine whether children seeking admission to your school meet California's school immunization requirements. For the actual laws, see Health and Safety Code, Division 105, Part 2, Chapter 1, Sections 120325-120380; California Code of Regulations, Title 17, Division 1, Chapter 4, Subchapter 8, Sections 6000-6075. If you have any questions, call the Immunization Coordinator at your local health department.

IMMUNIZATION REQUIREMENTS

To enter into public and private elementary and secondary schools (grades kindergarten through 12, including transitional kindergarten), children under age 18 years must have immunizations.

VACCINE	REQUIRED DOSES
Polio	**4 doses at any age, but...** 3 doses meet requirement for ages 4–6 years if at least one was given on or after the 4th birthday[1]; 3 doses meet requirement for ages 7–17 years if at least one was given on or after the 2nd birthday.[1]
Diphtheria, Tetanus, and Pertussis	***Age 6 years and under:*** DTP, DTaP or any combination of DTP or DTaP with DT (diphtheria and tetanus) **5 doses at any age, but...** 4 doses meet requirements for ages 4–6 years if at least one was on or after the 4th birthday.[1]
	Age 7 years and older: Tdap, Td, or DTP, DTaP or any combination of these **4 doses at any age, but...**3 doses meet requirement for ages 7–17 years if at least one was on or after the 2nd birthday.[1] If last dose was given before the 2nd birthday, one more (Tdap) dose is required.
Measles, Mumps, Rubella (MMR)	***Age 4-6 years* (kindergarten and above): 2 doses**[2] both on or after 1st birthday.[1]
	***7th grade:* 2 doses**[2] both on or after 1st birthday.[1]
	***Age 7-17 years and not entering or advancing into 7th grade:* 1 dose** on or after 1st birthday.[1]
Hepatitis B[3]	***Age 4-6 years* (kindergarten and above): 3 doses.**
Varicella	**1 dose**[4, 6]
Tdap Booster (Tetanus, reduced diphtheria, and pertussis)	***7th grade:*** **1 dose** on or after 7th birthday. [5, 7]

STATE NEWS

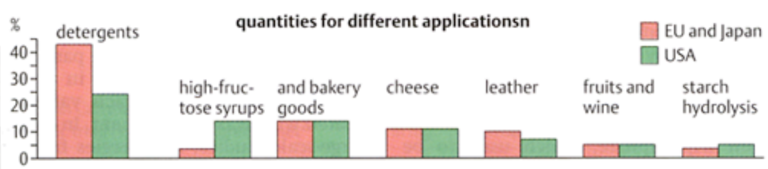
California Passes a 'No Exemption' Vaccination Policy for School Children. California Governor Jerry Brown signed S.B. 277 into law. The law will ban the use of personal or religious beliefs as grounds for exemption from vaccination, mandating that all children must be vaccinated by the beginning of school. California joins two other states, Mississippi and West Virginia, which do not have any exemptions for vaccination – though students in all three states may still opt out if a doctor says they should not get vaccinated for a medical reason. The law's passage comes following a deadly outbreak of measles in Disneyland.

Industrial & Food Products Made With Genetic Engineering

Bacteria & Other Microbes Are the Source Of Many Different Products

Enzymes as additives in industry

application	enzyme type	organisms (examples)	market size (% of total)	economic advantage
detergents	proteases, cellulases, lipases	*Bacillus licheniformis Aspergillus nidulans Trichoderma reesei*	40	1
starch hydrolysis	α-amylase	*Bacillus amyloliquefaciens*	5	3, 4
glucose iso-merization	glucose isomerase	*Streptomyces venezuelae*	7	1, 3
beer brewing	amylase	*Bacillus subtilis*	3	3, 4
fruit processing, wine	cellulases, hemicellulases, pectinases	*Aspergillus niger*	5	3, 4, 5, 6
flour, bakery goods	α-amylase, proteases	*Aspergillus oryzae*	8	1, 3
cheese manufacture, aroma	proteases, chymosin, lipases	animal rennin, *Rhizomucor miehei, Saccharomyces cerevisiae*	12	2
silage and animal feed	phytases	*Aspergillus niger*	8	3
paper and textiles	α-amylase, lipase	*Bacillus, Humicola*	2	4
leather treatment	proteases	*Aspergillus oryzae*	10	1, 7

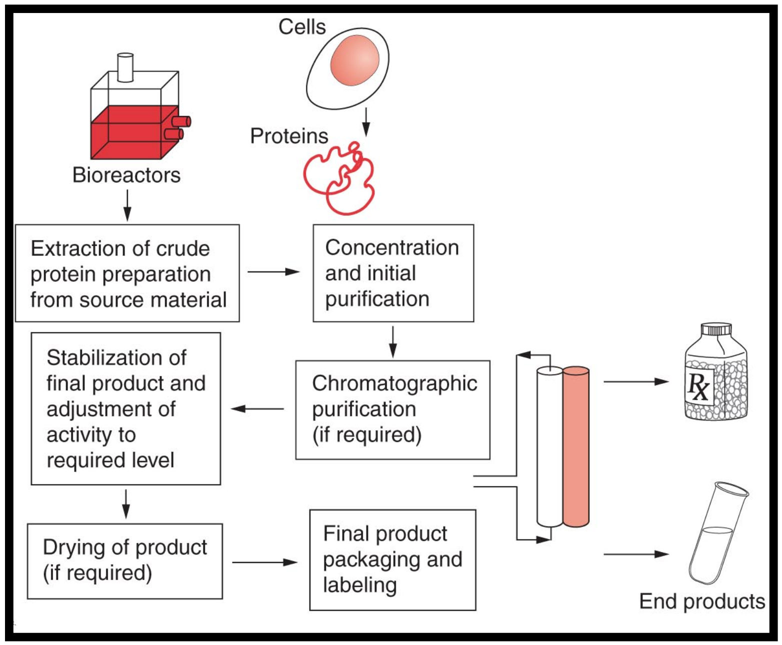


process/application	enzyme cost per unit quantity (US $)
starch liquefaction	ca. $ 2 per t starch
glucose from starch	$ 3.5 per t starch
isomerization of glucose	$ 6 per t starch
HFS in USA	$ 6–7 per t starch
ethanol	$ 1 per t starch
beer	$ 0.1 per 100 L
bakery goods USA	$ 0.1 per 100 kg flour
bakery goods EU	$ 0.1–0.5 per 100 kg flour
fruit juice	$ 0.1–0.5 per 100 L juice
wine	$ 0.1–0.5 per 100 L wine
stabilization of fruit lemonade by glucose oxidase	$ 0.3–0.8 per 1000 L
cheese manufacture	$ 0.05 per 100 L milk
detergents	$ 0.05 per kg detergent
leather tanning	$ 1.2–3 per t skin

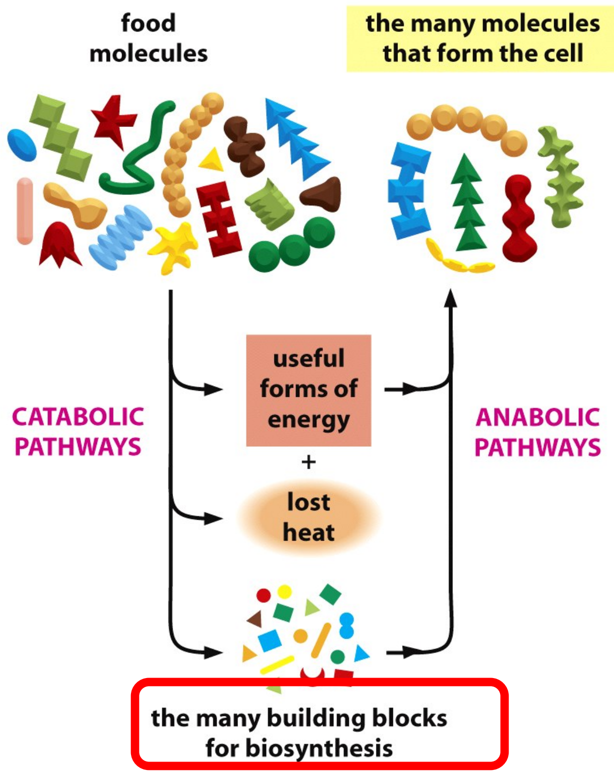
important goals in application technology

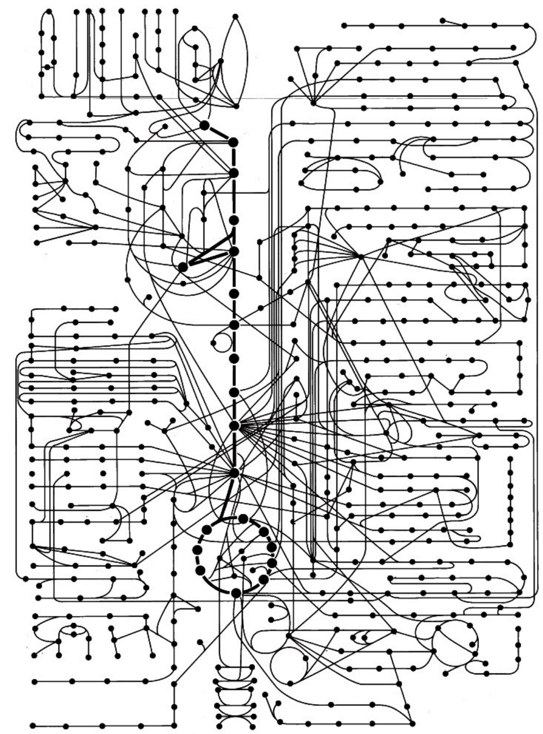
1 higher product quality
2 improved taste
3 better yields
4 reduced process costs
5 better filtration
6 better conservation
7 improved working conditions, reduced environmental load

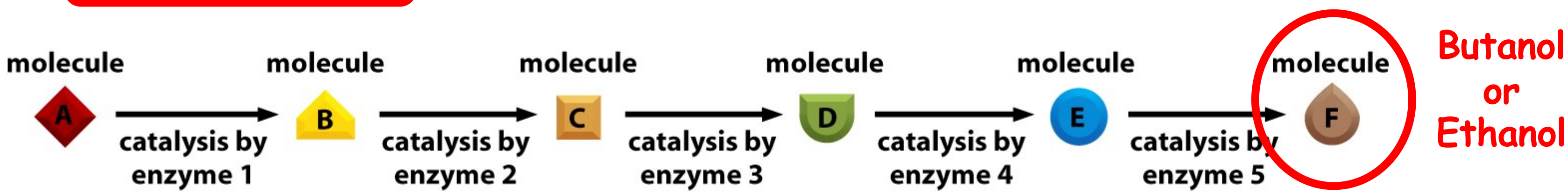
Specific Proteins and/or Metabolic Pathways Can Be Improved and/or Manipulated By Recombinant DNA!

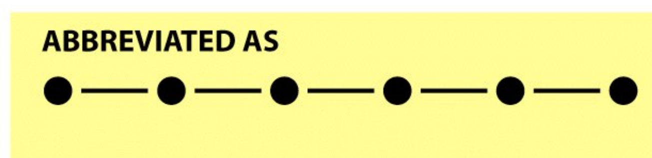


Metabolites Are Produced By Cellular Pathways That Use Specific Enzymes and Genes To Synthesize Specific Small Molecules









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]

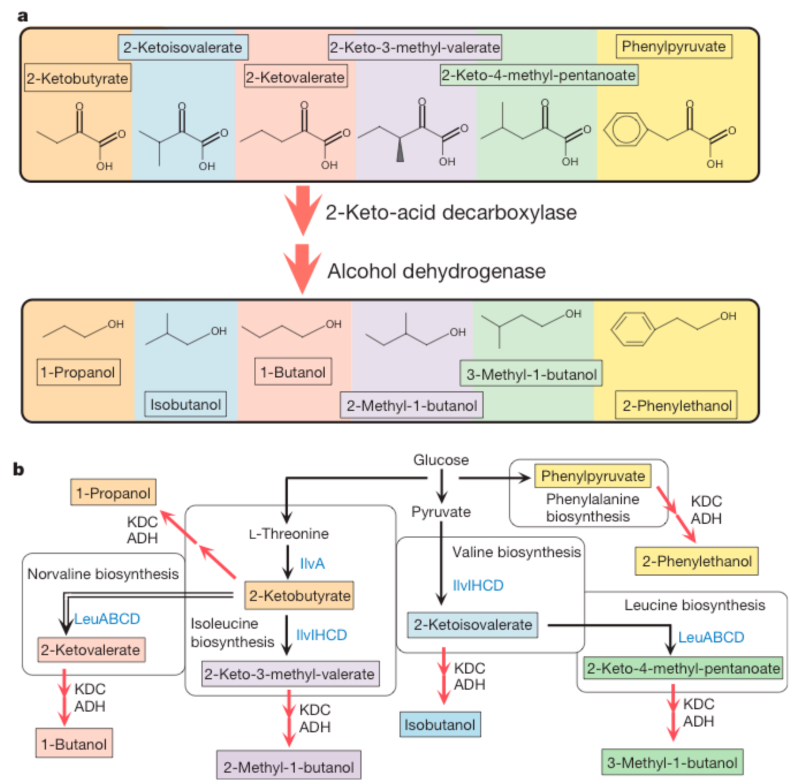


Figure 1 | Production of higher alcohols through the synthetic non-fermentative pathways. a, Various 2-keto acid precursors lead to corresponding alcohols through 2-ketoacid decarboxylase and alcohol dehydrogenase. **b,** The synthetic networks for the non-fermentative alcohol production in engineered *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.

Bacteria Can Be Engineered To Degrade Biomass Waste-Containing Cellulose (e.g., paper)

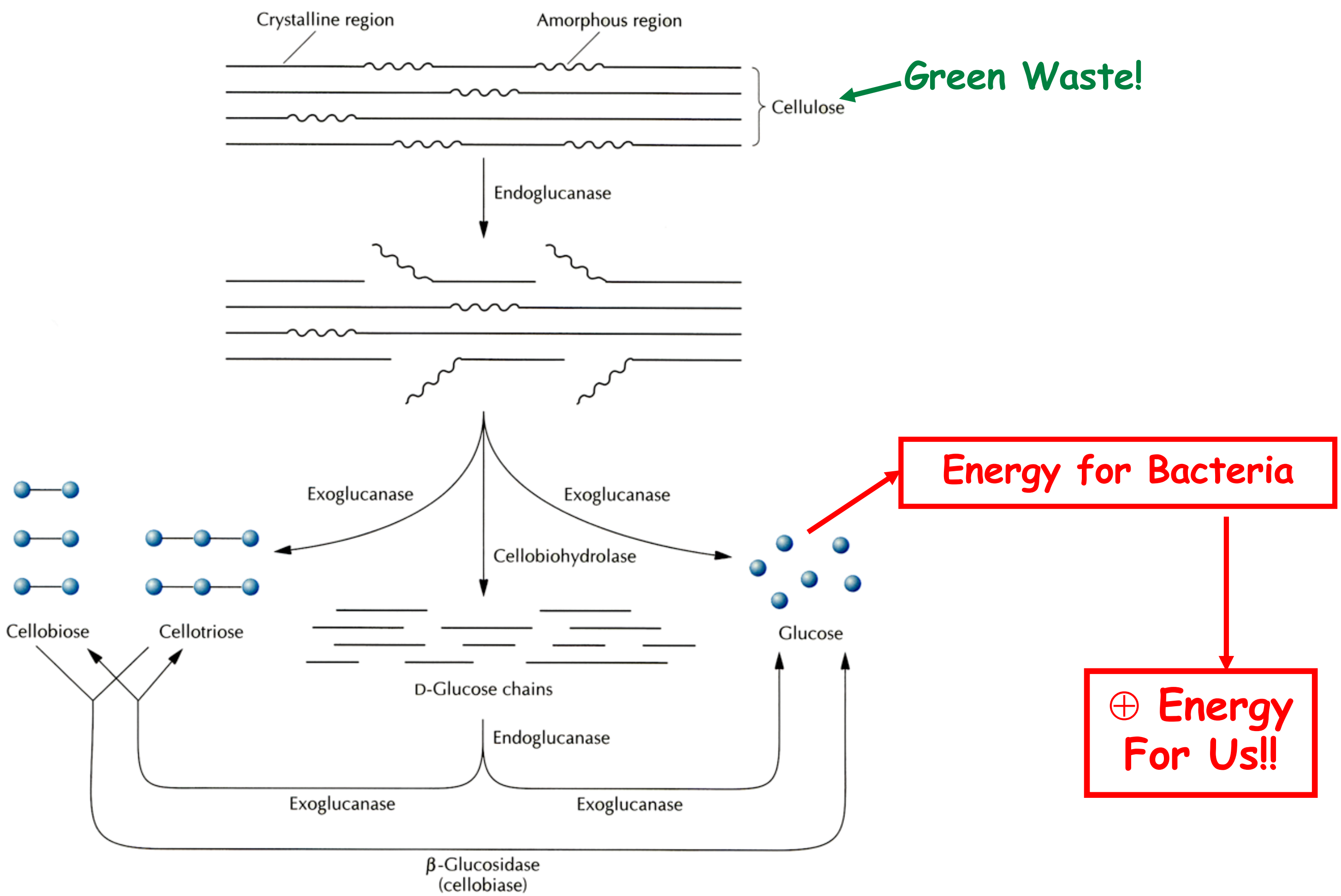


Figure 13.27 Enzymatic biodegradation of cellulose. Cellulose hydrolysis begins with the cleavage of β-1,4-linkages within the accessible amorphous regions of the cellulose chains by endoglucanase(s). This reaction is followed by the removal of oligosaccharides from the reducing ends of the partially cleaved cellulose chains by exoglucanase(s) and cellobiohydrolase(s). The degradation of cellulose is completed when the cellobiose and cellotriose are converted to glucose by β-glucosidase.

Agriculture, Timber Processing, Human Activities: e.g., Plants Left Over From Harvests, Animal Manure With Grasses, Municipal Water Paper, Cotton Leftovers, Hay, Etc.

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

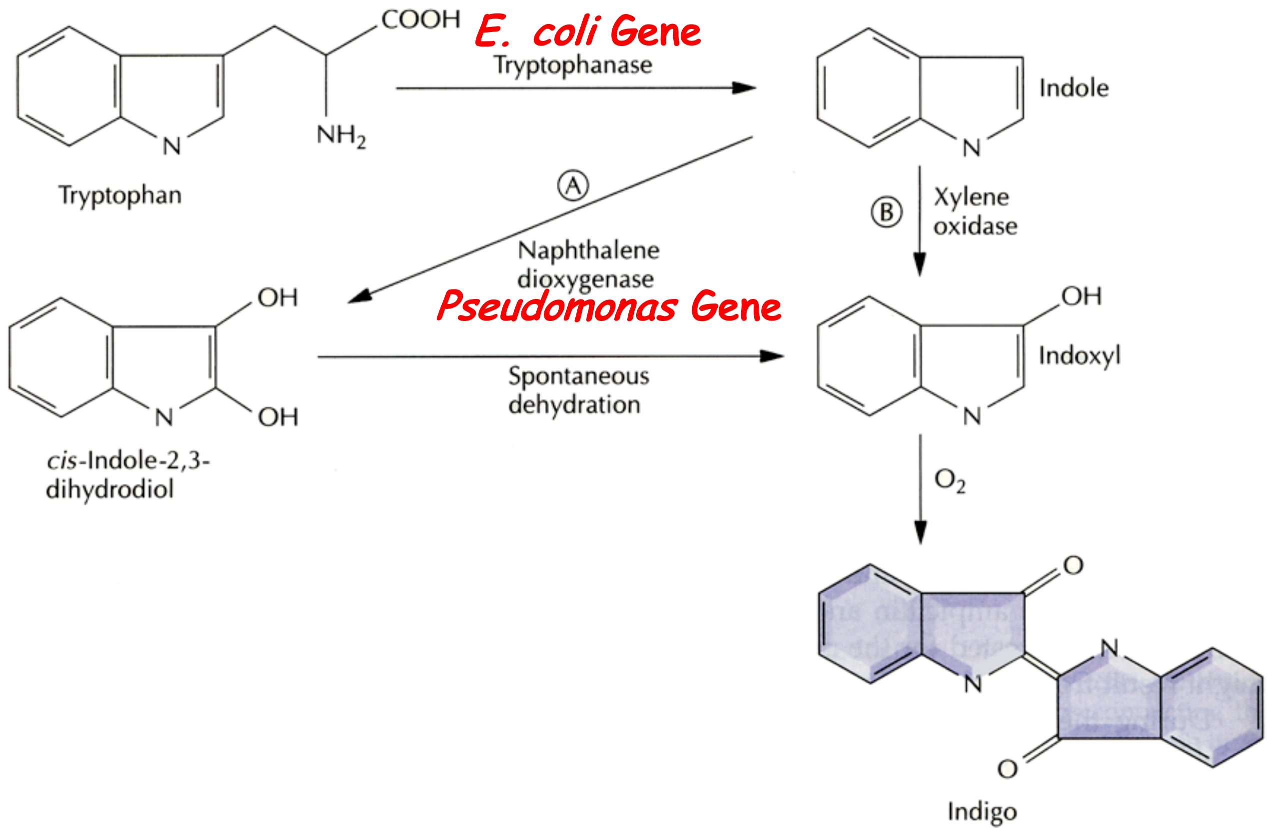


Figure 12.8 Indigo biosynthesis from tryptophan in genetically engineered *E. coli*. Tryptophanase is an *E. coli* enzyme. In pathway A, the naphthalene dioxygenase is derived from the NAH plasmid; in pathway B, the xylene oxidase is from the TOL plasmid. *E. coli* transformants that synthesize indigo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

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

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

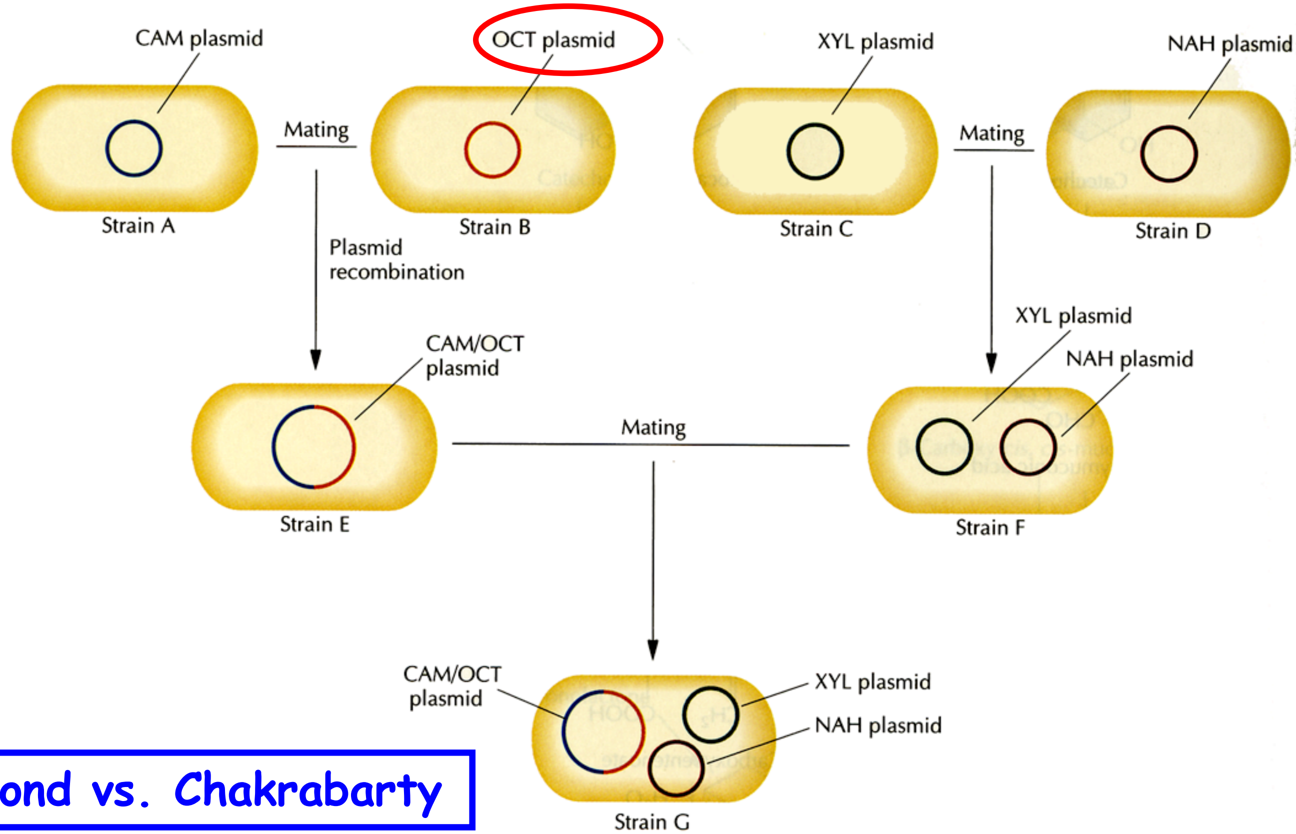


Figure 13.5 Schematic representation of the development of a bacterial strain that can degrade camphor, octane, xylene, and naphthalene. Strain A, which contains a CAM (camphor-degrading) plasmid, is mated with strain B, which carries an OCT (octane-degrading) plasmid. Following plasmid transfer and 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.

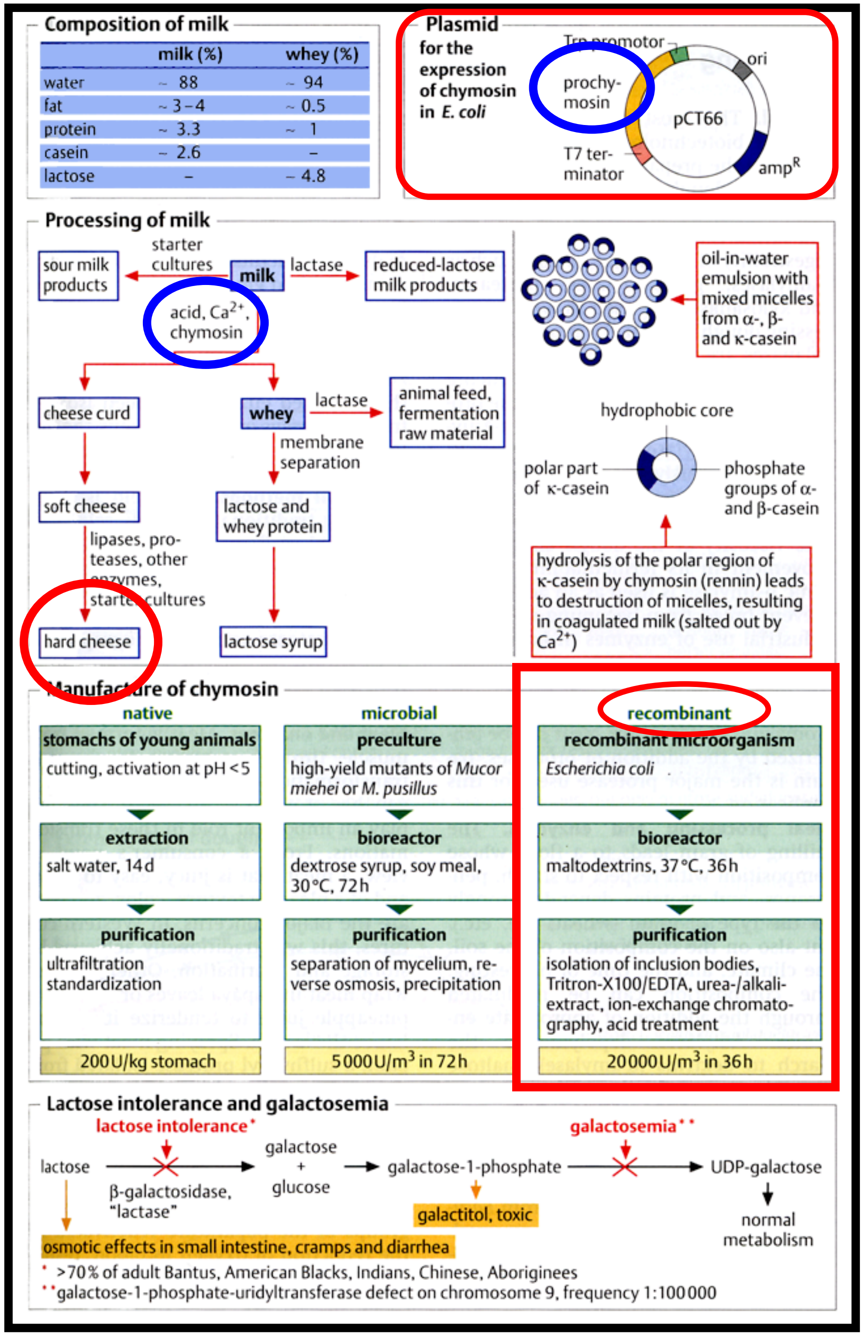
Pseudomonas

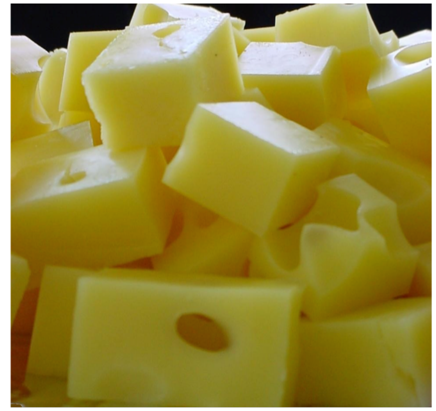
A Landmark Decision- Diamond vs. Chakrabarty

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

Life Can Be Patented !

Recombinant Chymosin Is Used To Make Cheese





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

Is Cheese A GMO?

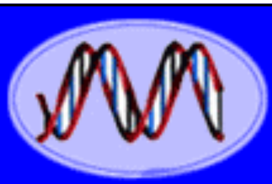
Chymosin In Cheese Making

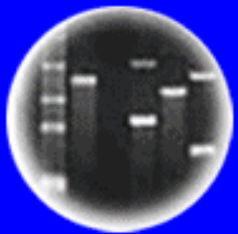
1. ~80-90% of Cheeses Are Made With Recombinant Chymosin (a Protease)
2. Approved For Use In Cheese Making By FDA - 1992
3. Not Different From Non-Recombinant Chymosin- ∴ GRAS- Generally Regarded As Safe & No Labeling Needed — Because Not An Additive & Not Different From Non-Recombinant Chymosin!!

Is Cheese Made Using Recombinant Chymosin a GMO?

Industry Adds Claim That Recombinant Chymosin is "Kosher" & "Vegetarian"

Why No Fuss?





Genetic Engineering Can Be Used To Make Better/More Effective Antibiotics

Occurrence

taxonomic group	relative number (%)
Actinomycetes	50
other bacteria	10
fungi	20
lichens	1
algae	2
plants	15
animals	2

~25 000 compounds from nature

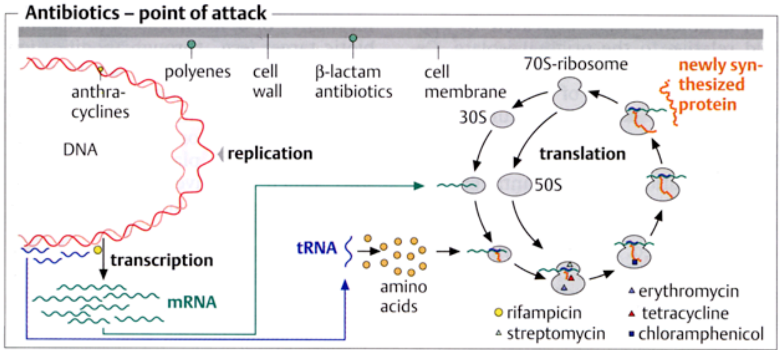
Systemic antibiotics (2001)

type	value (billion US $)
cephalosporins	6.7
penicillins	4.6
chinolones (synthetic)	4.6
macrolides	4.3
tetracyclines	0.7
aminoglycosides	0.6
peptide antibiotics, glycopeptides	0.5
other	2.2
total	24,2

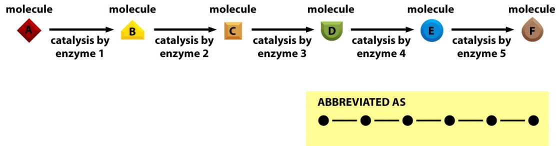
Classification by chemical structure

1	carbohydrate antibiotics	aminoglycosides	streptomycin (medicine), kasugamycin (rice fungicide)
2	macrocyclic lactones	macrolides polyene antibiotics ansamycines	erythromycin (medicine) pimaricin (cheese production) rifamycin (against tuberculosis)
3	chinones and related antibiotics	tetracyclines anthracyclines	tetracycline, chlorotetrycycline (medicine, feed antibiotic) doxorubicin (cancer therapy)
4	amino acid and peptide antibiotics	amino acid derivatives β-lactam antibiotics peptide antibiotics chromopeptides glycopeptides	cyclosporin (organ transplantation) phosphinothricin (plant protection) penicillins, cephalosporins (medicine) bacitracin (medicine), virginiamycin (feed antibiotic) actinomycin (cancer therapy), bleomycin (cancer therapy), vancomycin (medicine), avoparcine (cattle feed antibiotic)
5	N-heterocyclic compounds	nucleoside antibiotics	polyoxins, blasticidin S (fungicides for plant protection)
6	O-heterocyclic compounds	polyether antibiotics	monensin (chicken feed)
7	alicyclic compounds	cycloalkane derivatives	cycloheximide (leaf fungicide)
8	aromatic antibiotics	benzene derivatives	chloramphenicol (medicine) griseofulvin (fungicide)

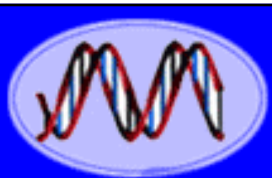
Antibiotics – point of attack



By Modifying Pathways Leading to Antibiotics In Bacterial Cells. But Need To Know Genes/Proteins in Pathway
&
By Finding Their Targets In Pathogens As Well



Ancient Plant DNA and Yeast Cells Can Be Used to Resurrect Fragrances From Extinct Plants!!



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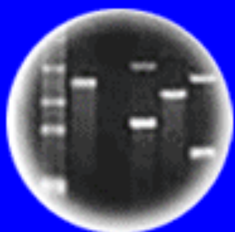

DNA Fingerprinting

Cloning: Ethical Issues
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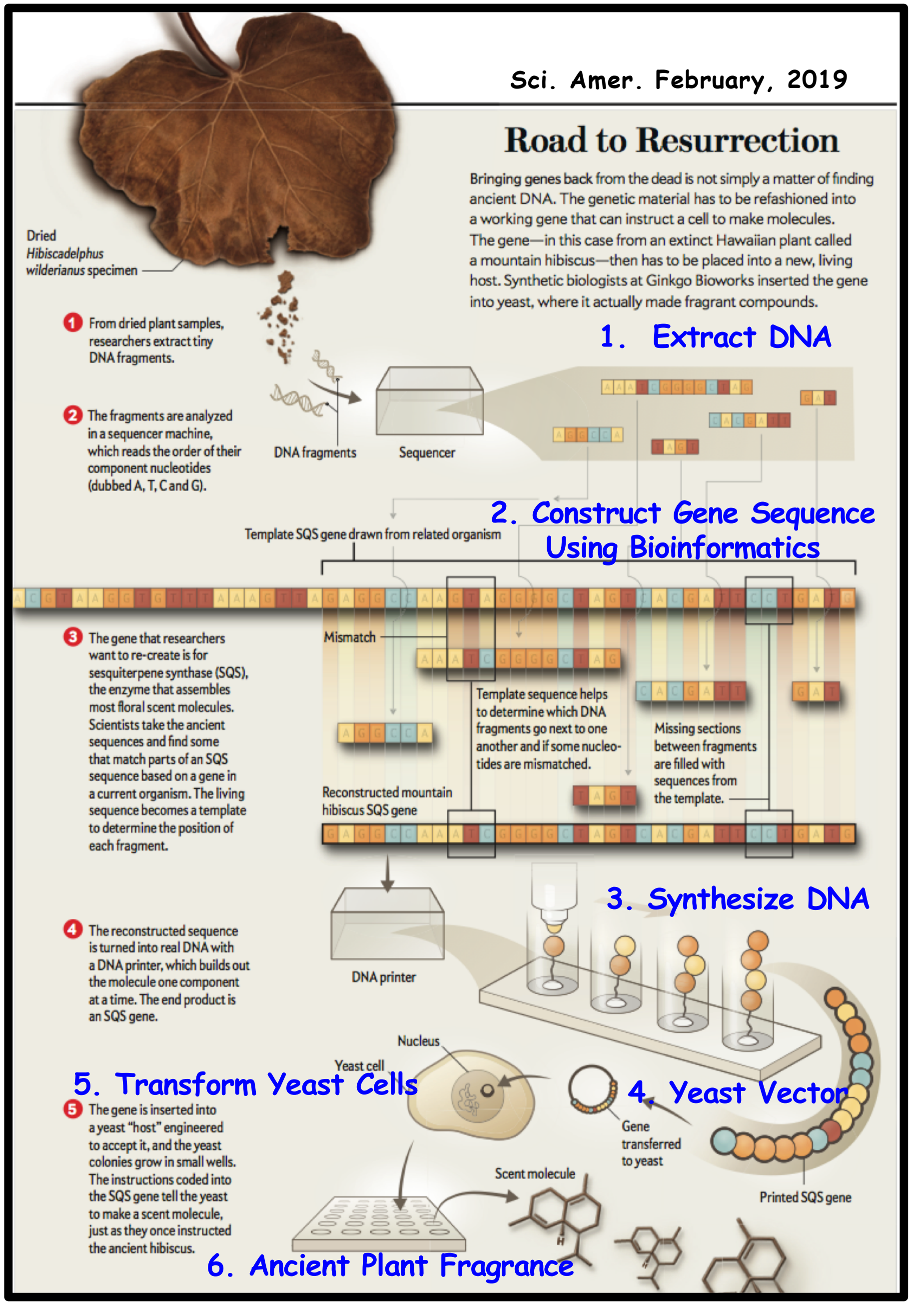
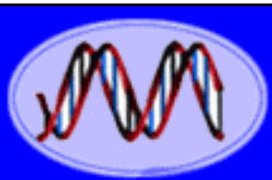
Plants of Tomorrow



Sesquiterpene Synthase (SQSs) Genes From Ancient Hibiscus

Worldwide Fragrance Industry $72B in 2018!



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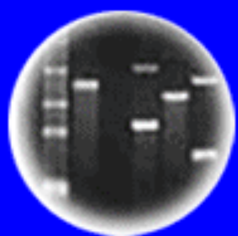

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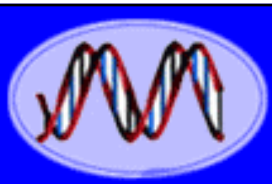
Plants of Tomorrow

Using Genetic Engineering Animals to Fight Major Insect-Born Diseases

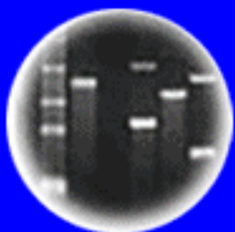
ANOPHELES		AEDES MOSQUITO
Malaria	Diseases spread	Dengue, Yellow Fever, Chikungunya, Lymphatic filariasis
Pregnant females	Which mosquitoes bite?	Pregnant females
Night	When do they bite?	Day
With abdomen sticking upwards	Resting position	Lies parallel to resting surface
Predominantly rural	Location	Predominantly urban
Bodies of water	Breeding ground	Shallow water surfaces

WHO: Zika virus 'spreading explosively,' level of alarm 'extremely high'



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

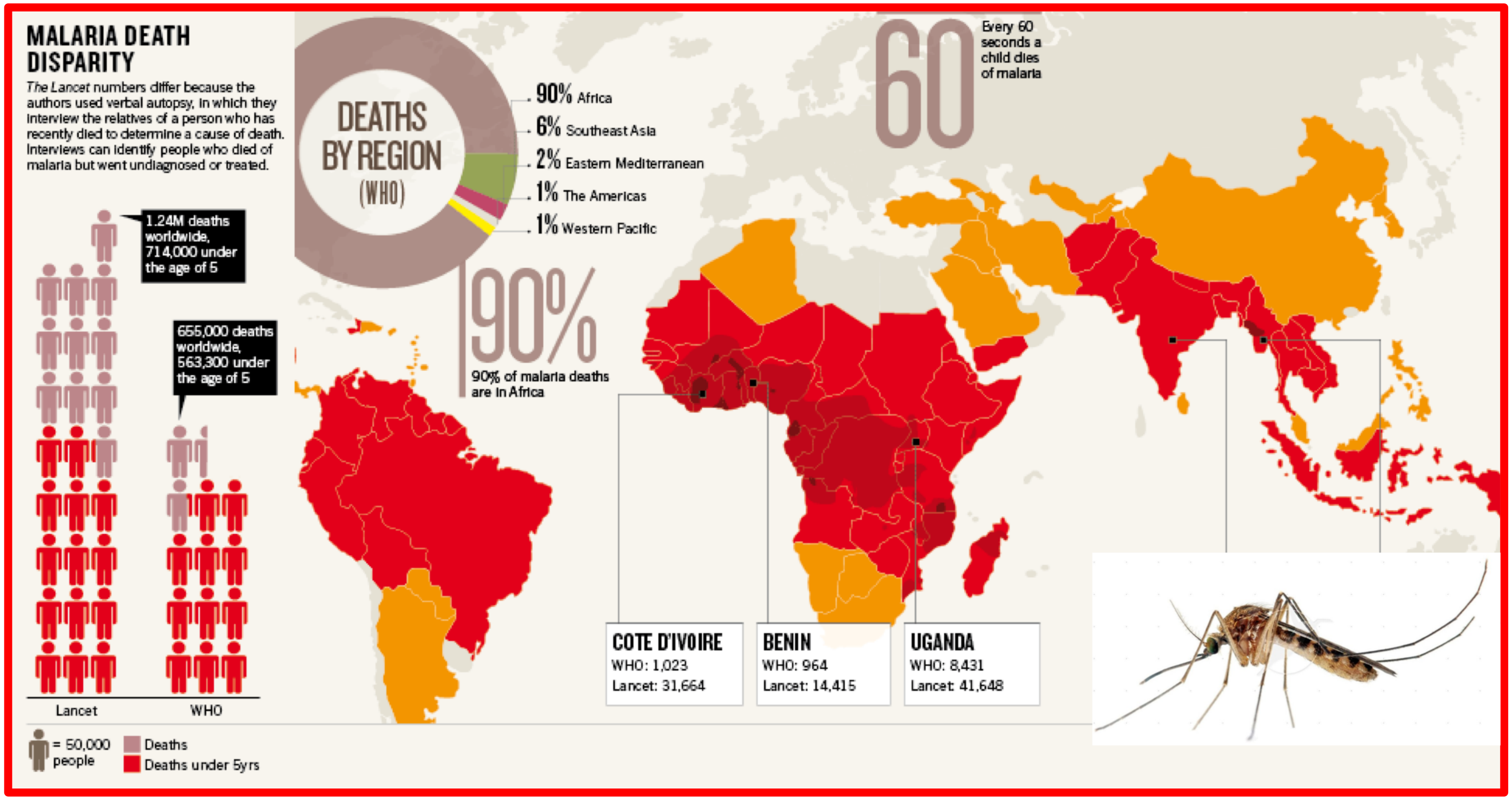


DNA Fingerprinting

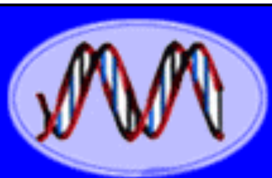
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Using Genetic Engineering to Fight Malaria



1.4 Million Deaths Per Year



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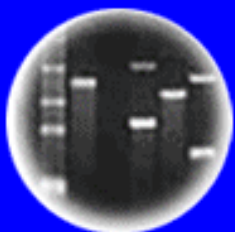

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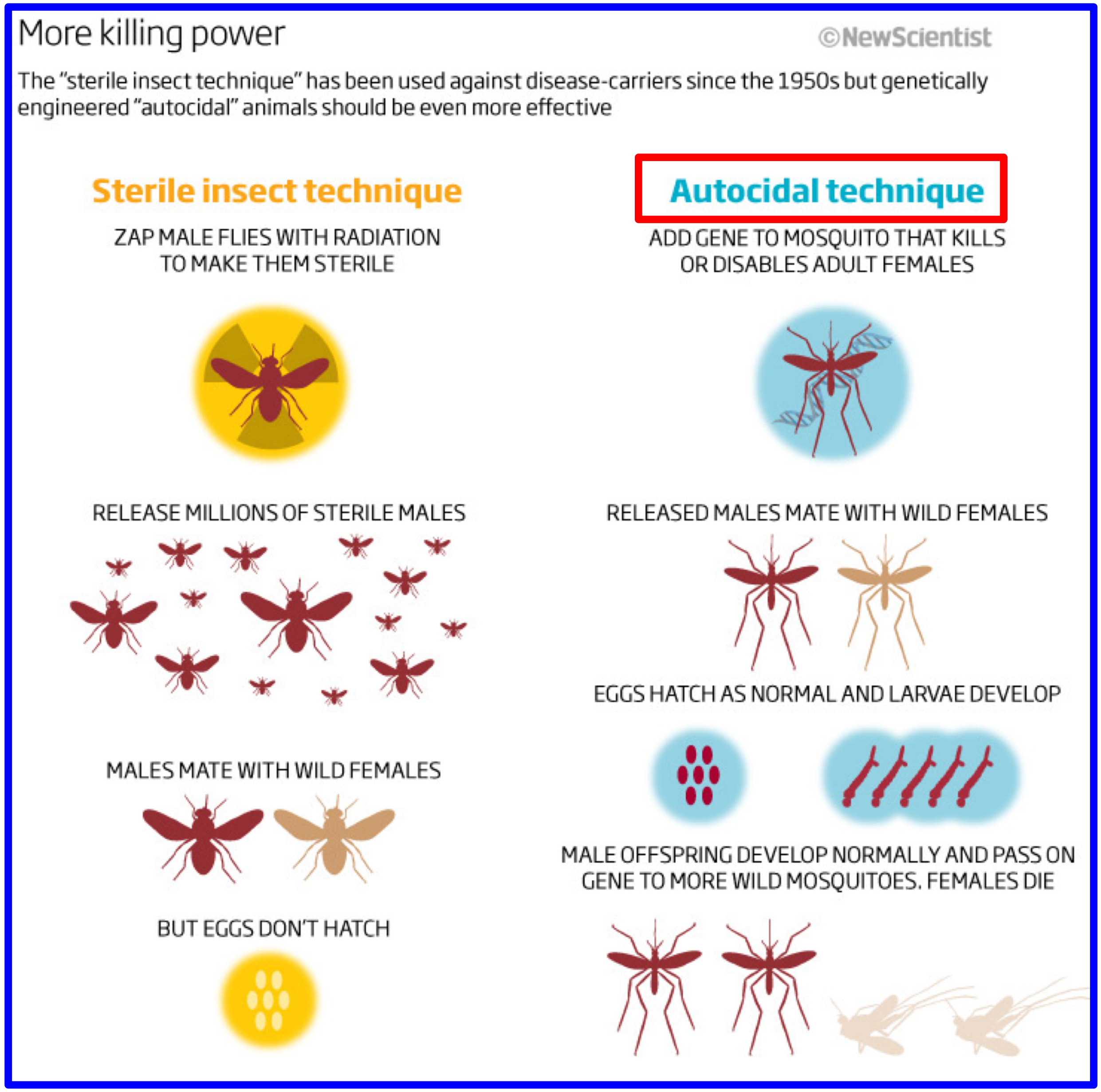
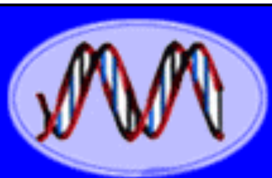
Using Genetic Engineering to Fight Mosquito-Transmitted Diseases





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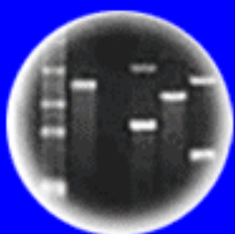

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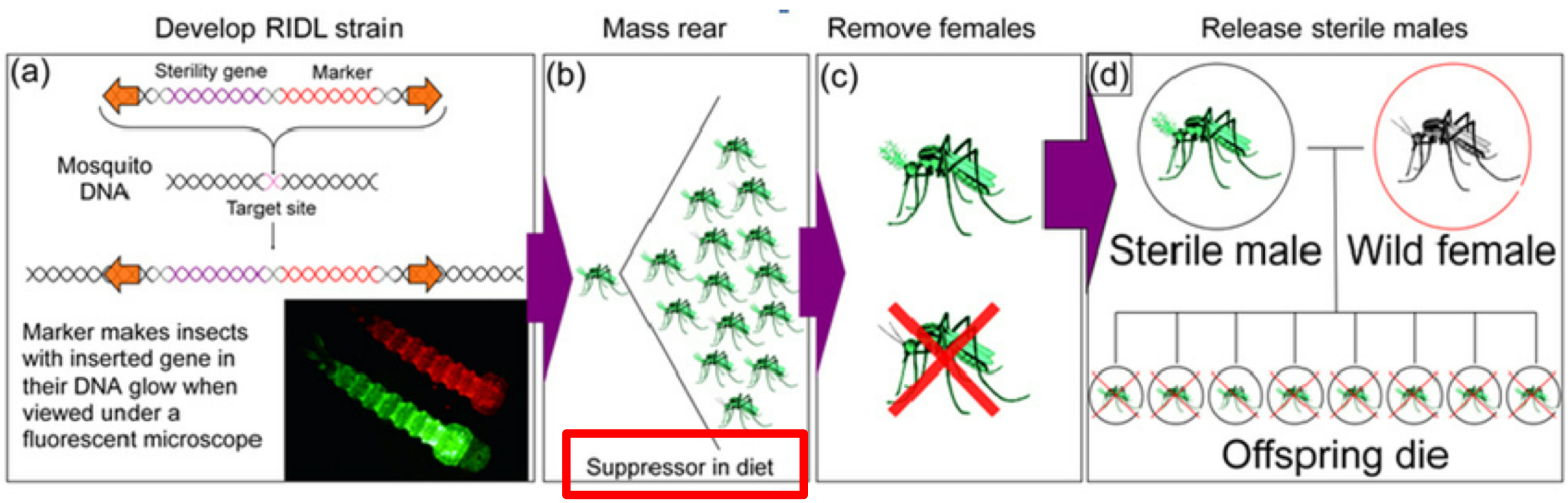
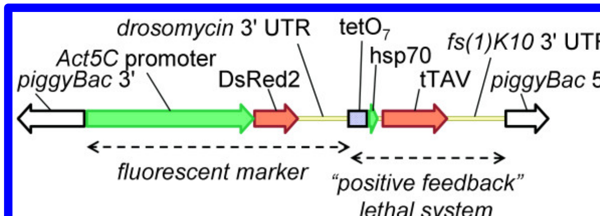
Using Genetic Engineering to Fight Other Mosquito-Transmitted Diseases

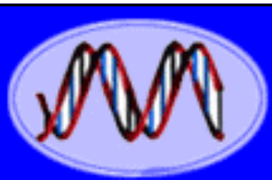
Release of Insects Carrying a Dominant Lethal Allele



Releases of the genetically engineered Oxitec mosquito, commonly known as 'Friendly *Aedes aegypti*', reduced the dengue mosquito population in an area of Juazeiro, Brazil by 95%, well below the modelled threshold for epidemic disease transmission.



OXITEC



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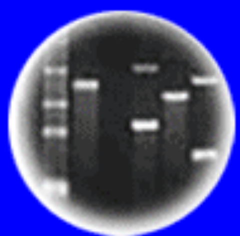

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FDA approves releasing GMO mosquitoes to fight Zika in Florida

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

Other tests have reduced mosquito populations by 90 percent

Guidance for Industry

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).[5]

Genetic Engineering is a TECHNIQUE!

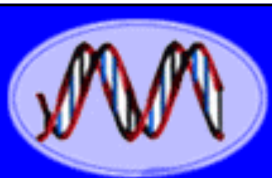
1. Classical Breeding By Selective Mating (Thousands of Years)
2. Insertion of New Genes Into An Organism's Chromosomes (50 Years)
3. **Editing Existing Genes Like A "Word Program" (1-2 Years)**

Breeding or DNA Manipulation - They Are the SAME

&

Called *Gene Manipulation*

WHAT IS A GMO???



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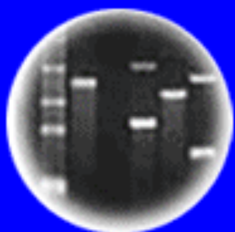

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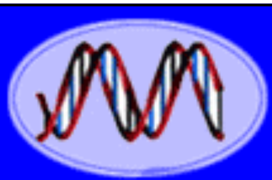

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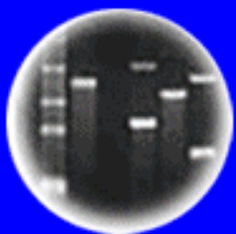

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New Weapon to Fight Zika: The Mosquito

How mosquitoes with 'self-destruct' genes could save us from Zika virus

A Call to Fight Malaria One Mosquito at a Time by Altering DNA

Engineering Mosquitoes' Genes to Resist Malaria

Gene-Engineered Mosquitoes Can't Spread Malaria: Researchers

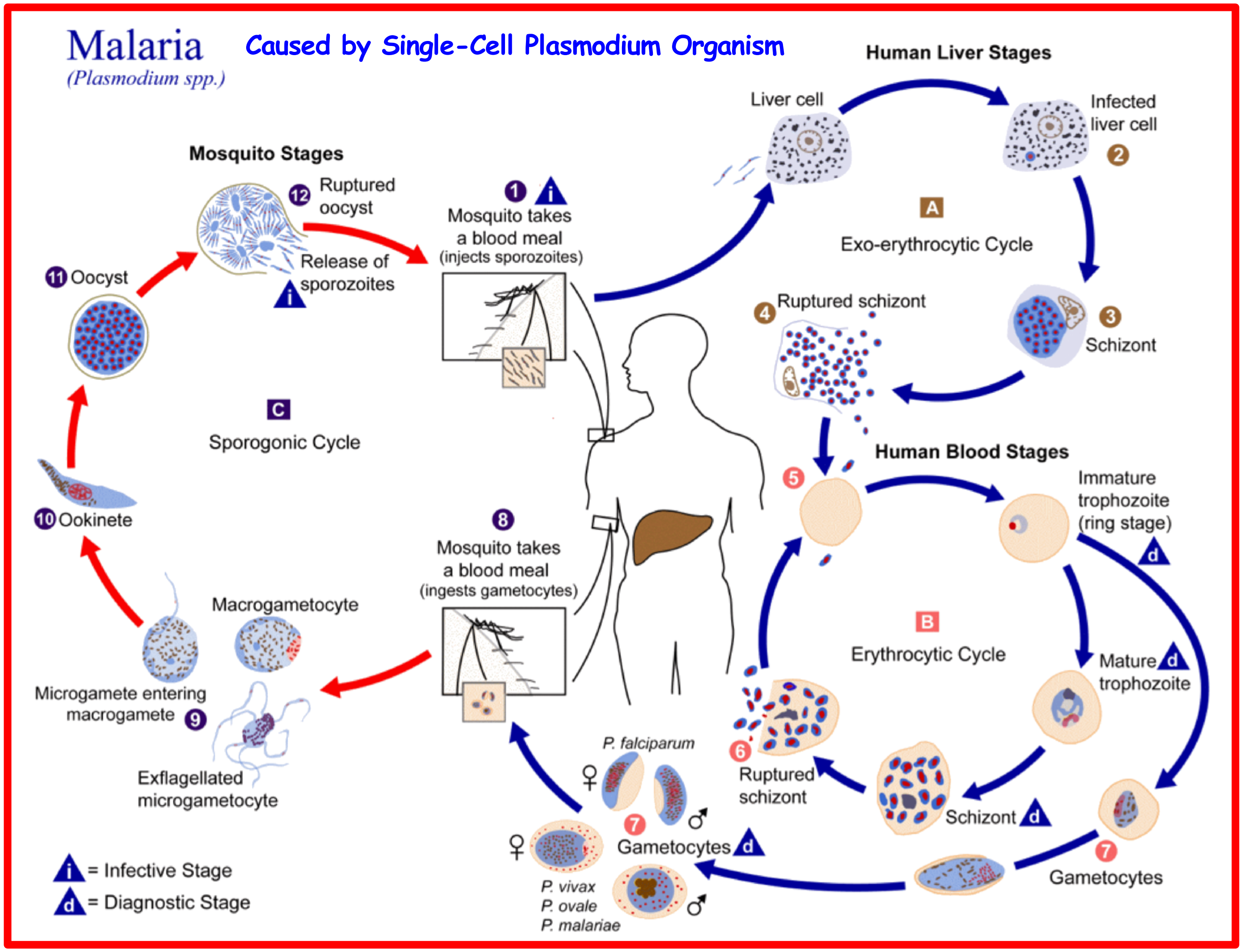
by MAGGIE FOX

Researchers in California say they have genetically engineered mosquitoes that cannot be infected with the malaria parasite — and they've done it in a way that virtually guarantees the trait will spread quickly in a population.

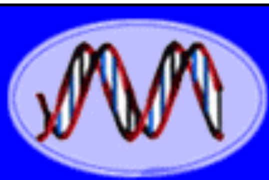
Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*

PNAS, November, 2015

Mosquito Genes Required For Harboring Disease Parasites Are Targets For Genetic Engineering & Disease Control



Mutate Genes & Prevent Pathogen From Residing in Mosquito



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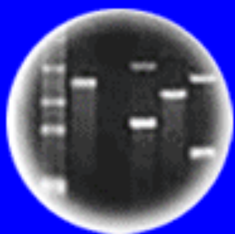

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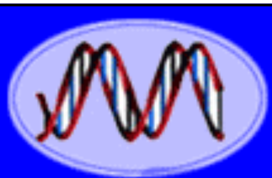

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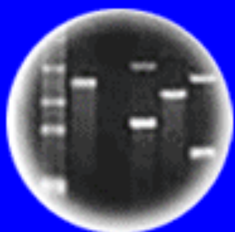

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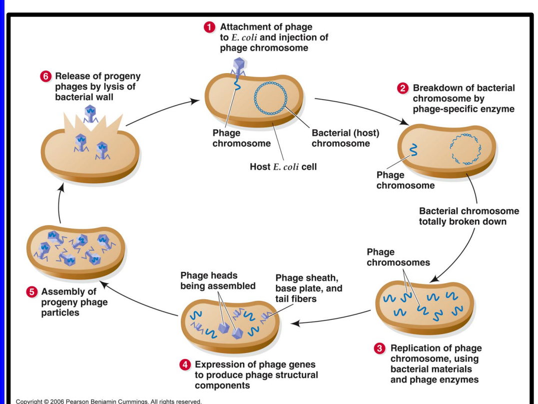
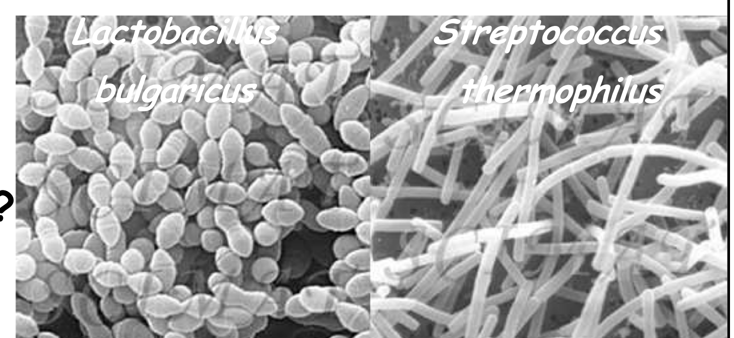
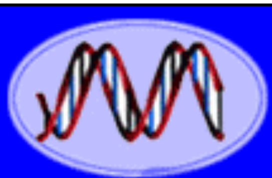
What Does Yogurt Making Have To Do With Discovering CRISPR-Cas9?

Lactobacillus bulgaricus and Streptococcus thermophilus



What Happens If Viruses Infect Bacterial Cultures?





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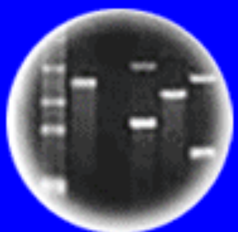

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The CRISPR-Cas Bacterial Immunity System

CRISPR & Cas Discovered
In Yogurt Bacteria Resistant
To Viral Infections!

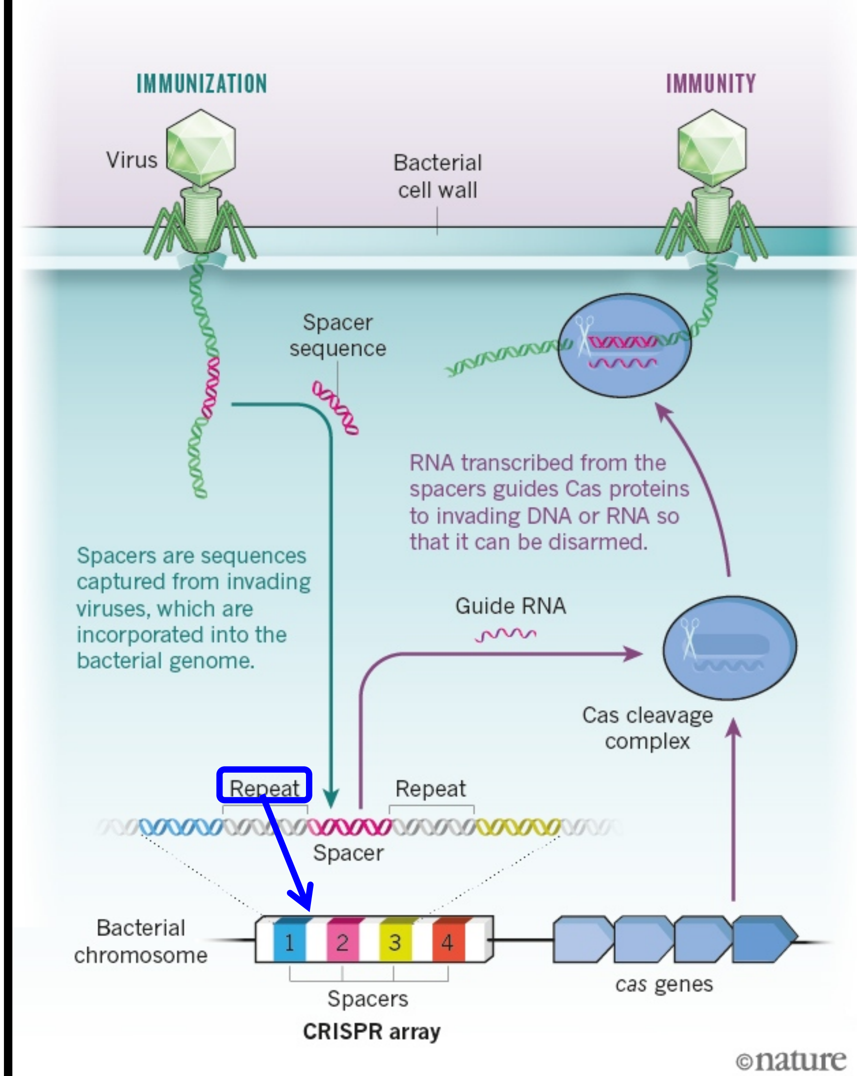
Clustered
Regular
Interspaced
Short
Palindromic
Repeats

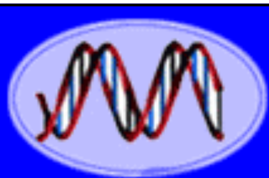
CRISPR
Associated
System

Cas is an
Endonuclease
That Cleaves
dsDNA

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.





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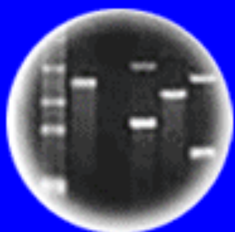

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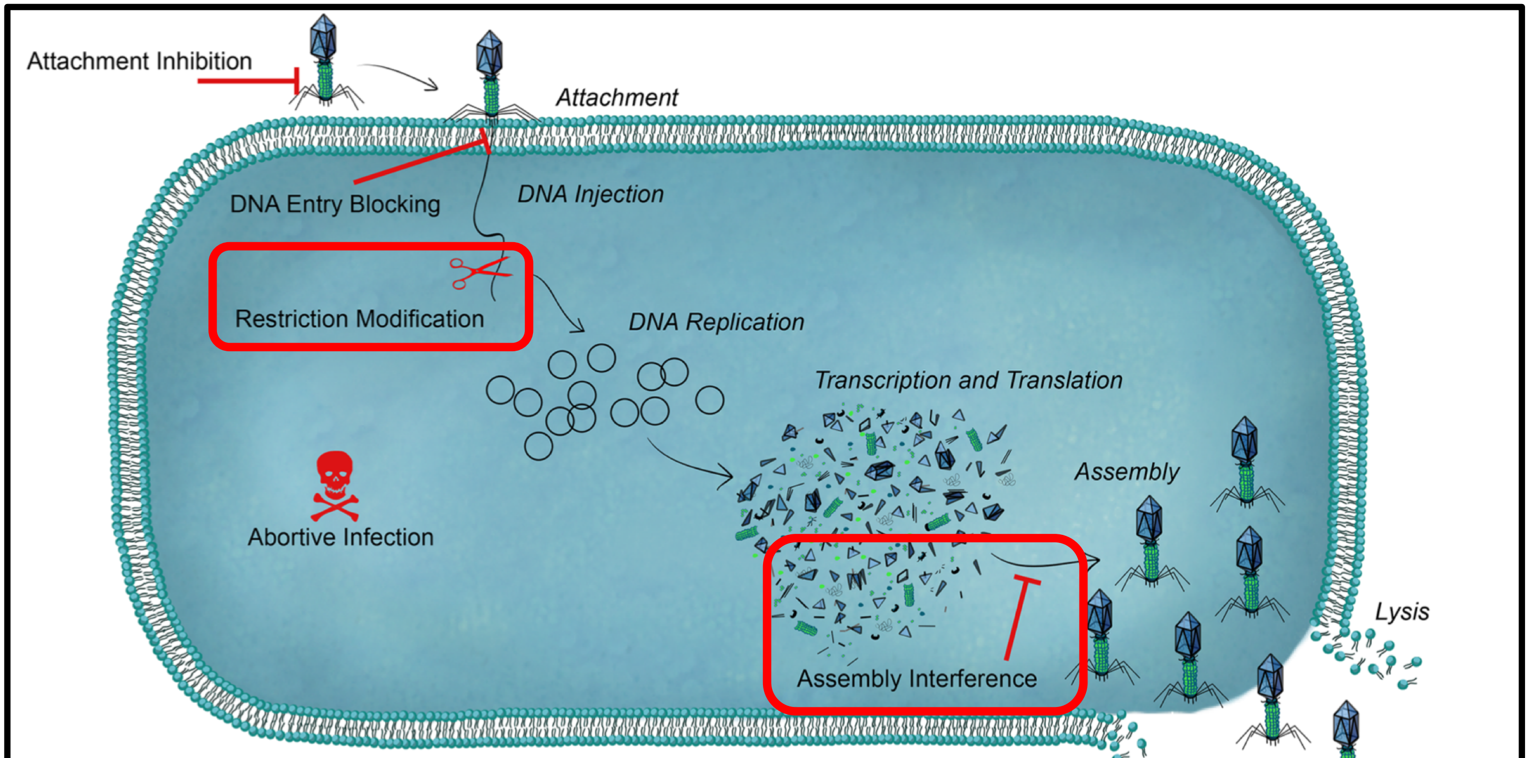
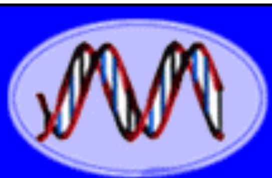
The CRISPR-Cas Bacterial Immunity System is One of Many Bacterial Defense Systems That Prevent Phage Infection



PLOS Pathogens June 11, 2015



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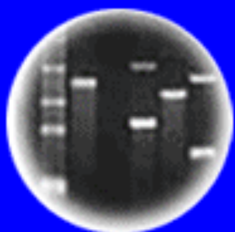

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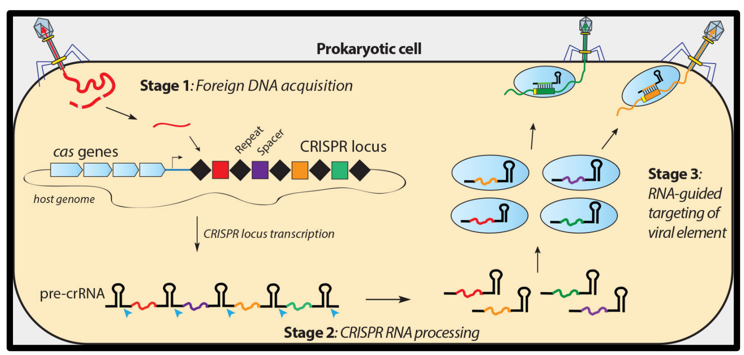
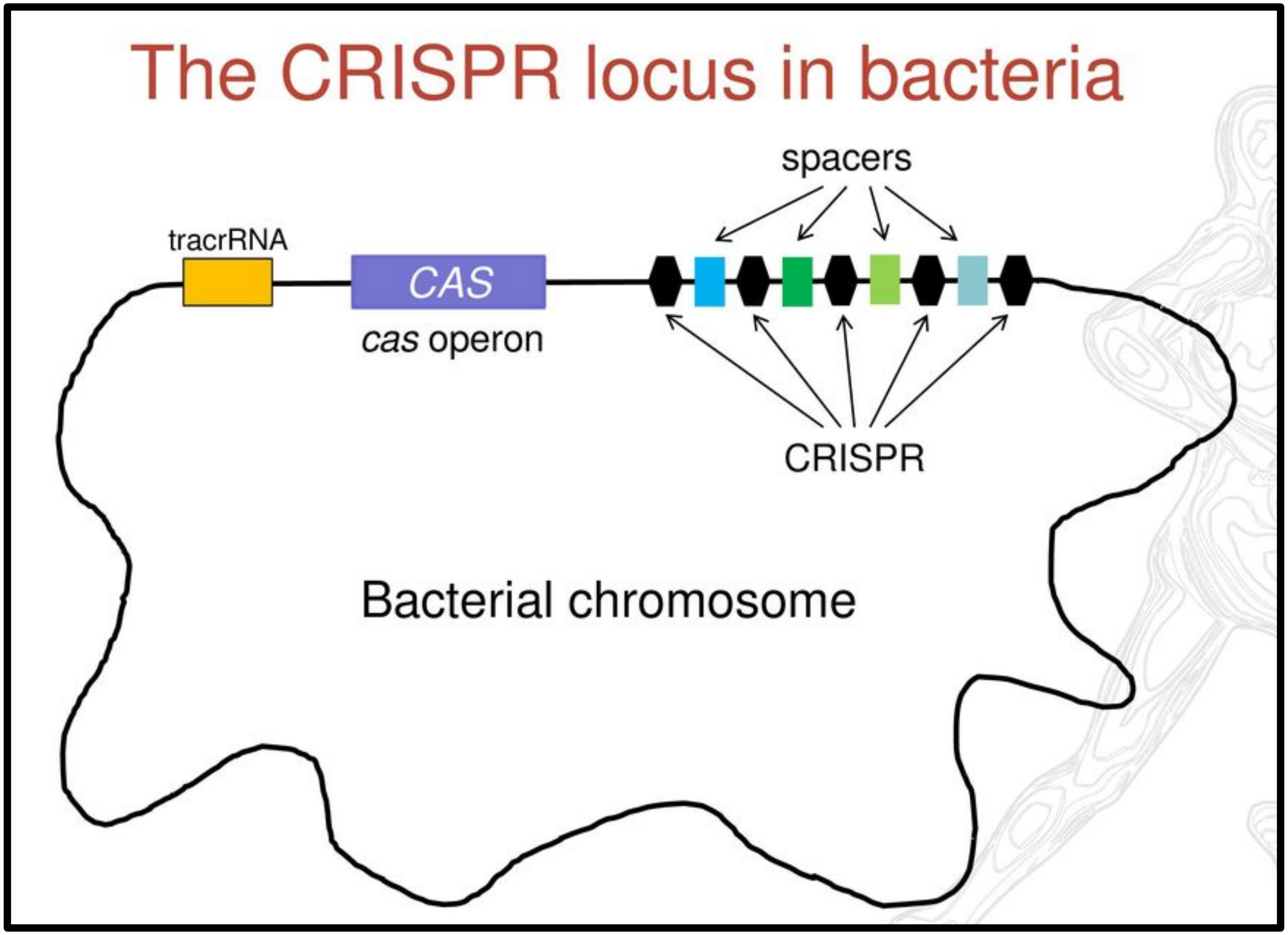
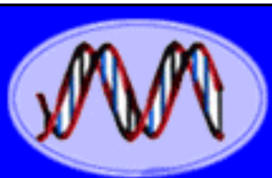
The CRISPR-Cas Bacterial Immunity System

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



Components of the CRISPR-Cas Bacterial Immunity System Can Be Cloned and Engineered to Work Like "Legos" in Eukaryotic Cells





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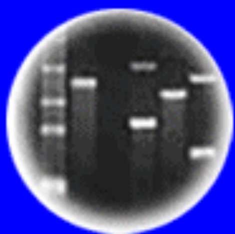

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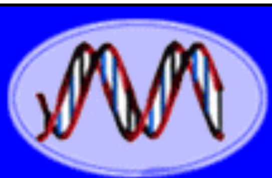

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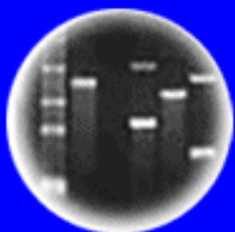

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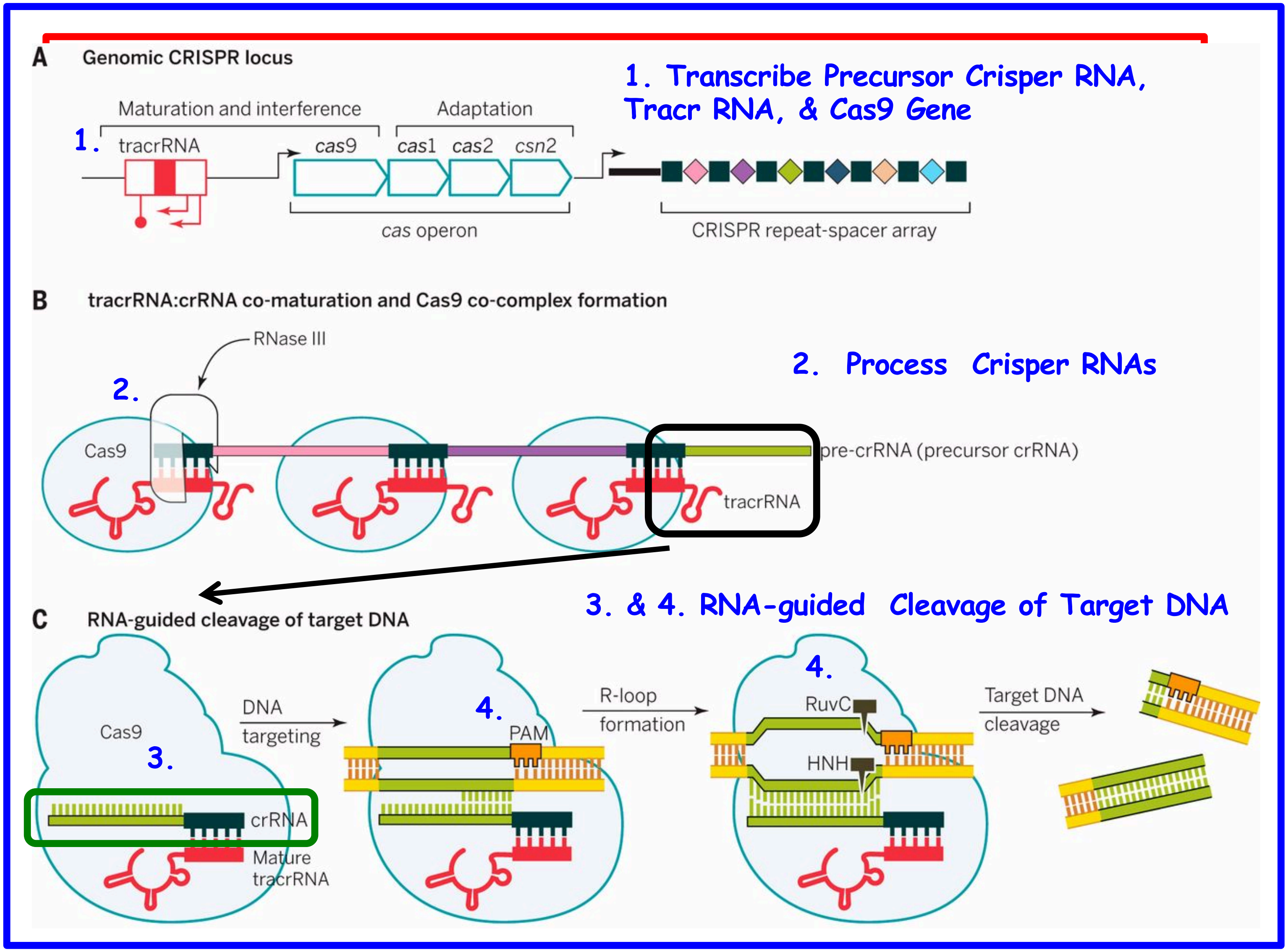

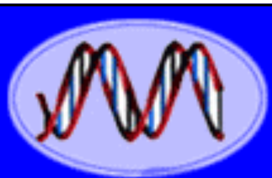

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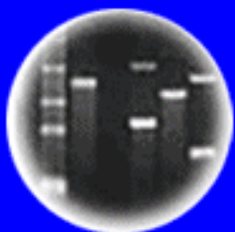

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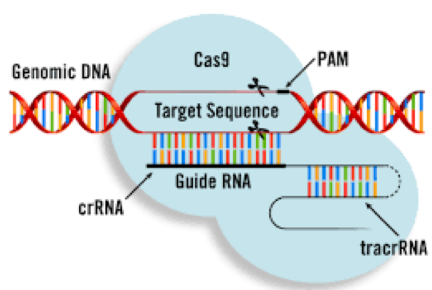
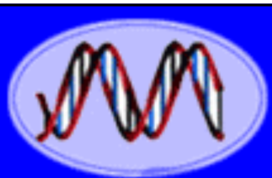
Using CRISPR-Cas9 Editing and Gene Drive To Knock-Out Mosquito Genes Required For Harboring the Malarial Plasmodium Parasite

Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang
CRISPR-Cas9 Editing (Molecular Typewriter)





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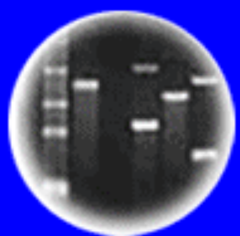

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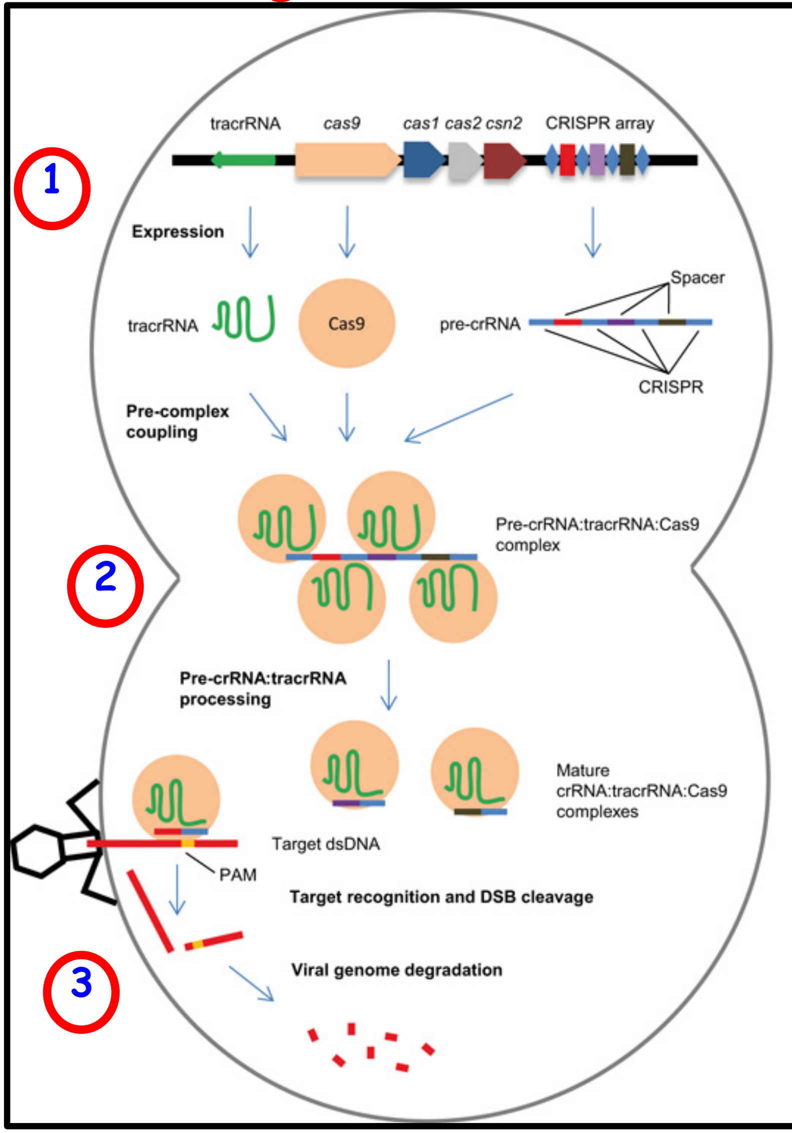
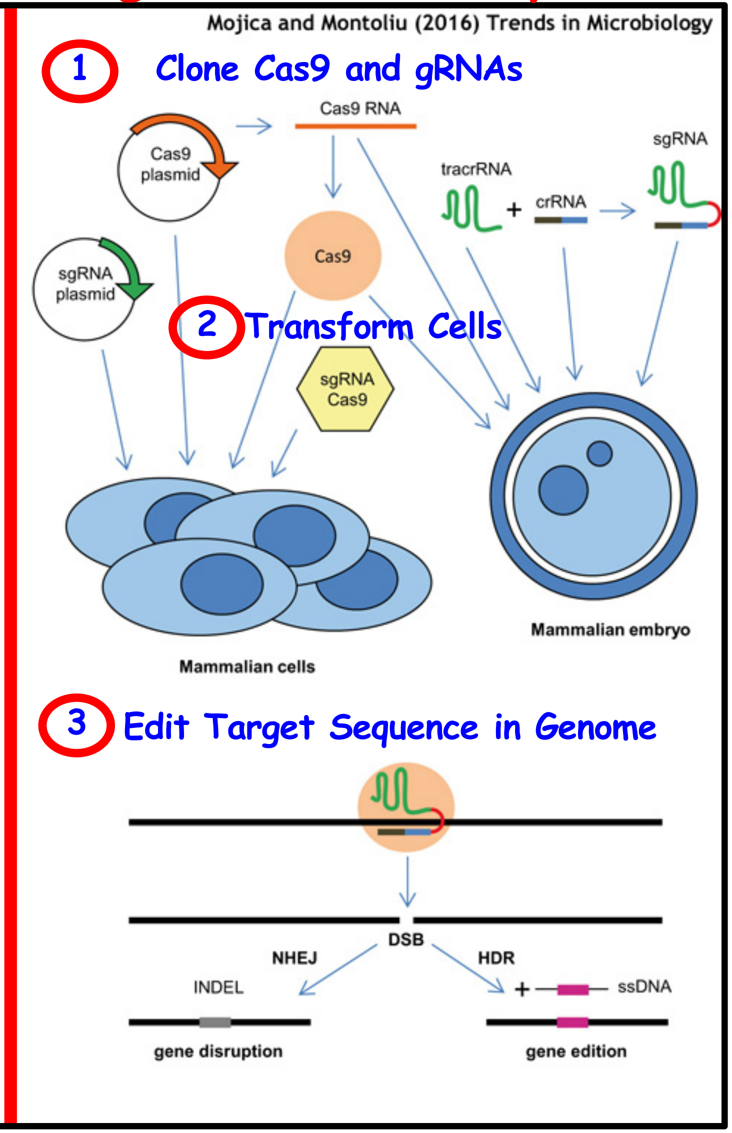
How To Use the CRISPR-Cas System For Editing Specific Genes

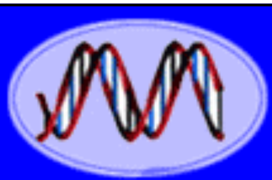
Endogenous Bacteria



Engineered Eukaryotes



Editing Can Either Mutate the Gene, Correct a Specific Defect, or Add DNA Sequences



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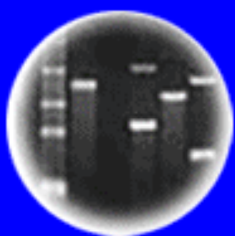

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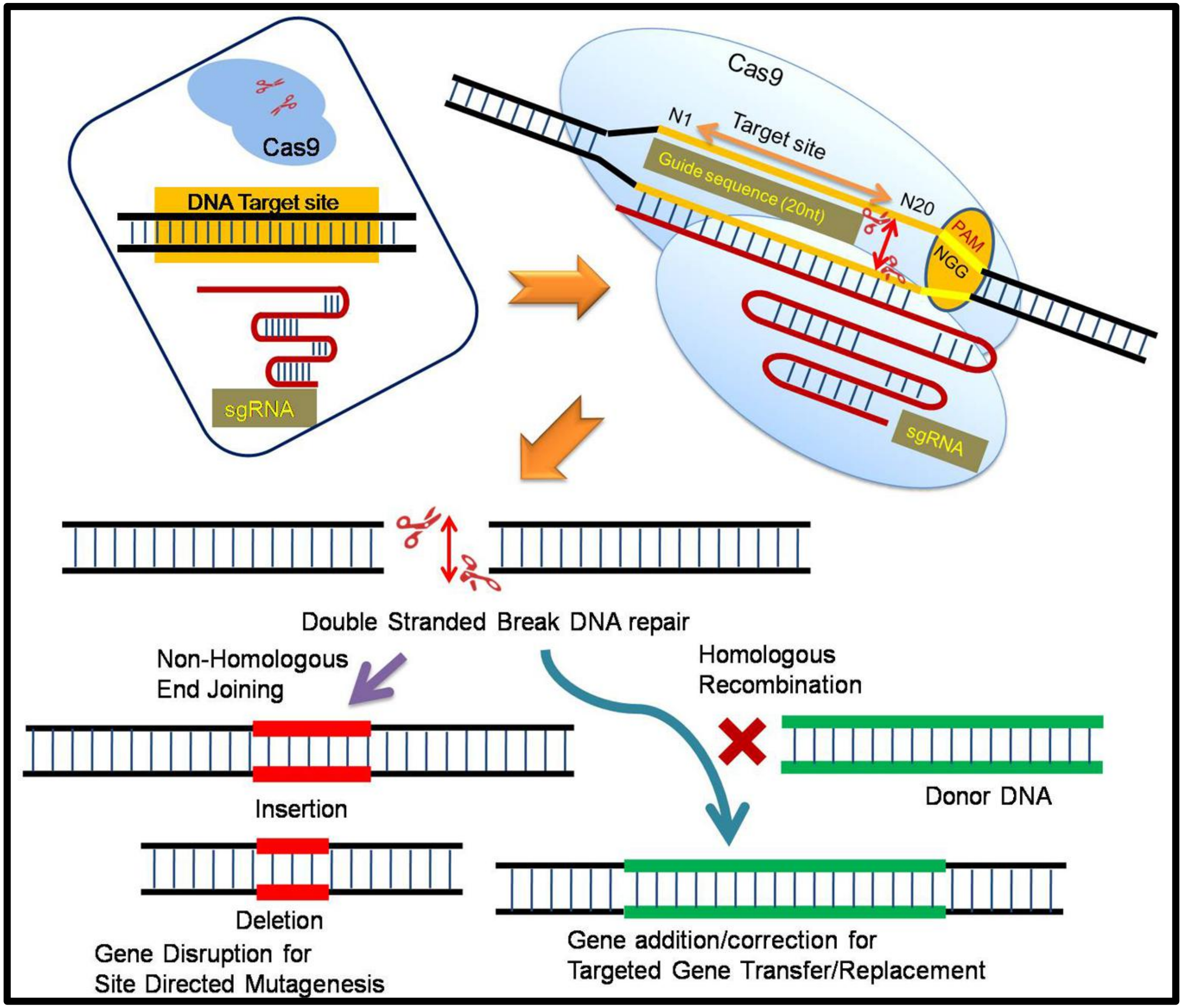
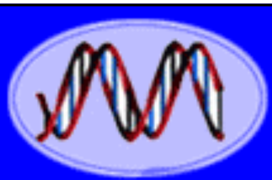


Sequence Specific Changes in a Complex Genome!!!!



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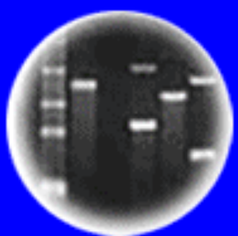

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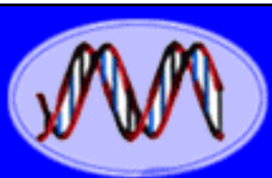
Advantages of Gene Editing Over "Cohen-Boyer" Genetic Engineering

- Simple Method to Edit, Correct, or Modify Any Endogenous Gene
- Multiple Genes Can Be Corrected at Once

Disadvantages of Gene Editing Over "Cohen-Boyer" Genetic Engineering

- Cannot Add Foreign Genes (e.g., GFP)
- Limited to Species-Specific Gene Corrections



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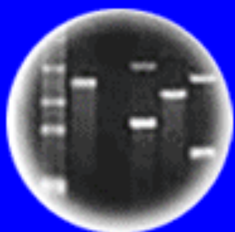

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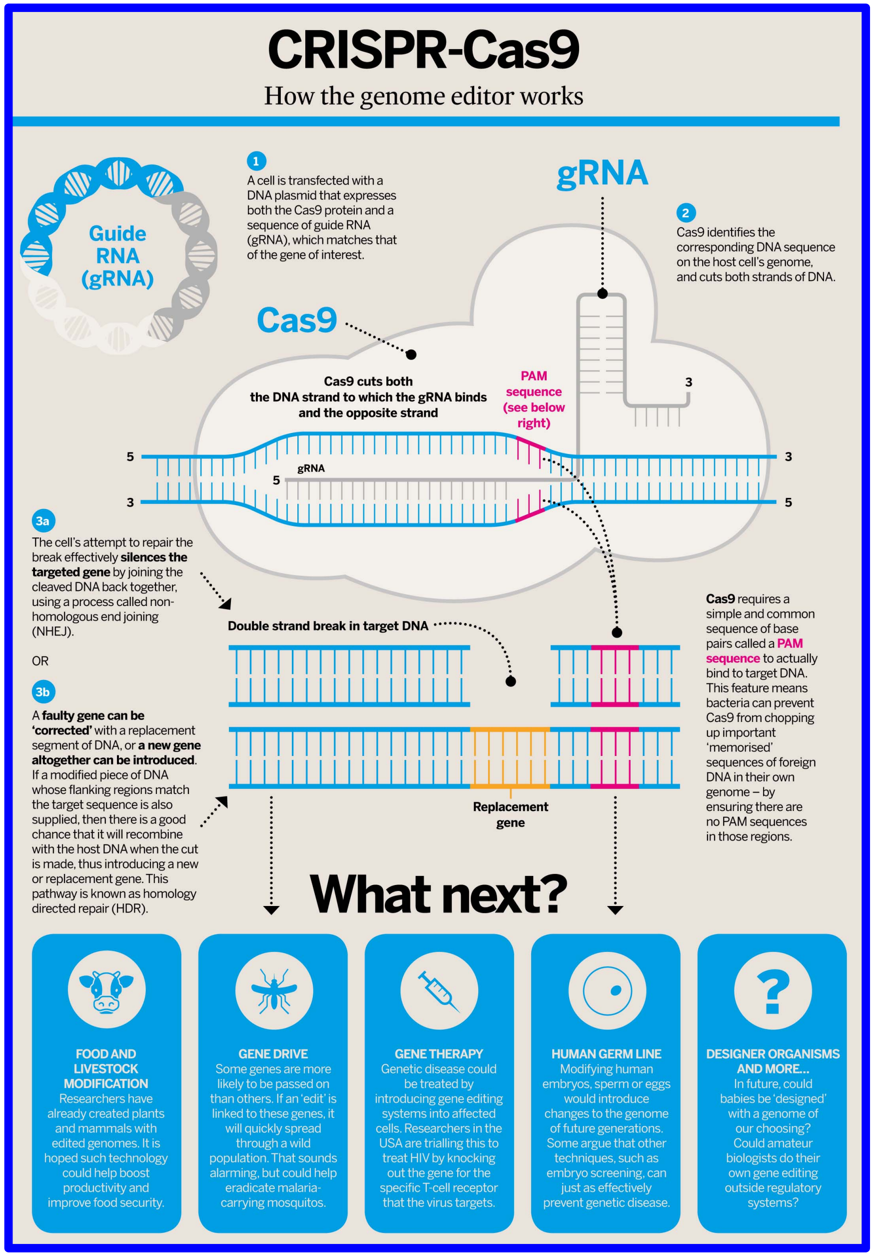
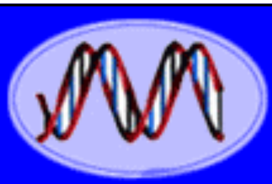
How Can Gene Editing Be Used in Genetic Engineering?



- 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!



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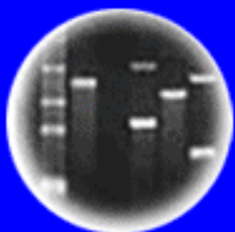

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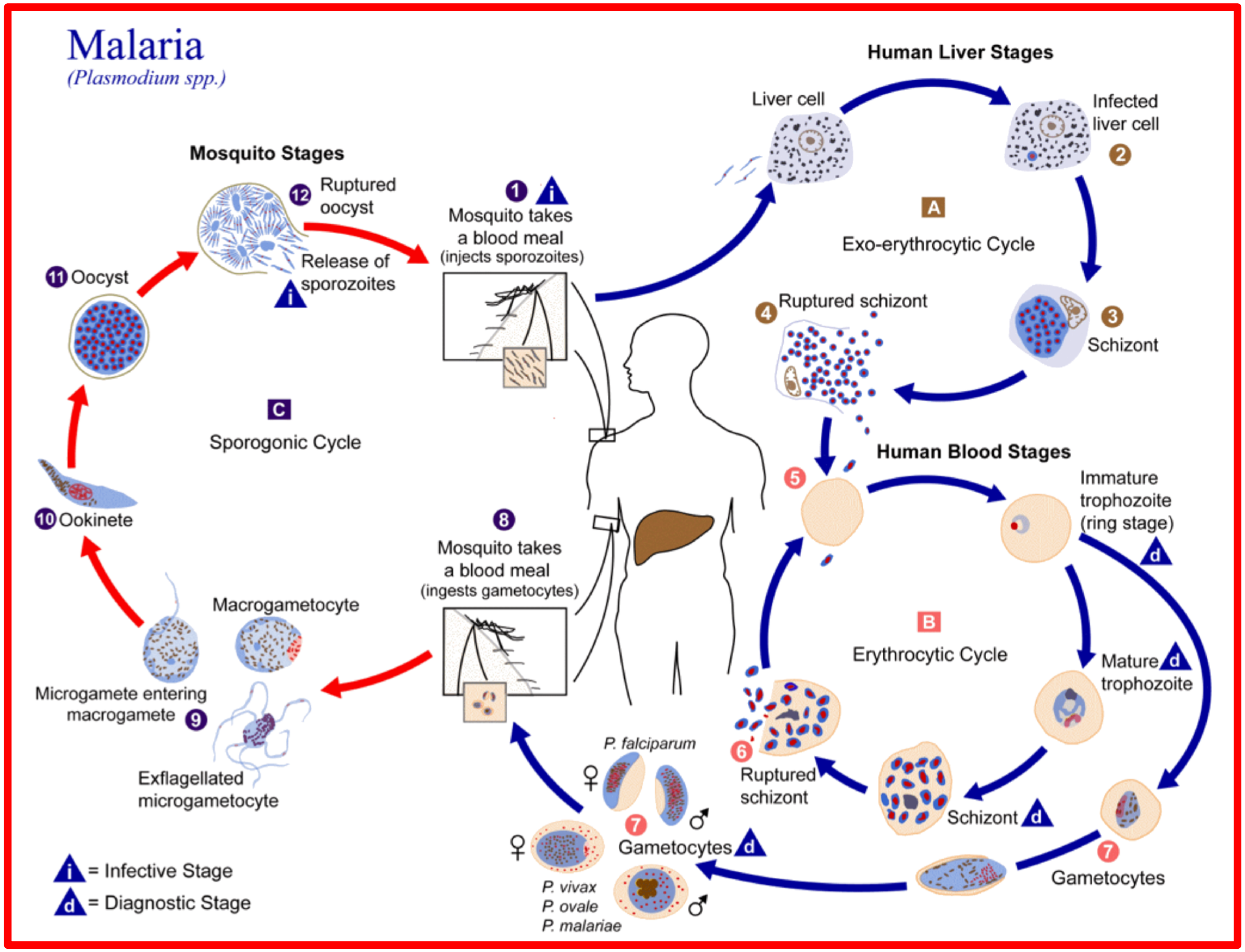
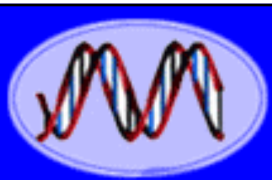
Using Gene Editing to Eliminate Mosquito-Transmitted Diseases



Specific Mosquito Genes Are Required For the Plasmodium Life Cycle If Mutated, Mosquitos Cannot Harbor the Malaria Parasite!!



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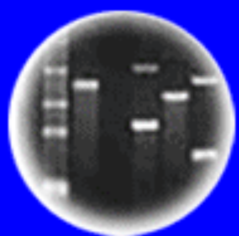

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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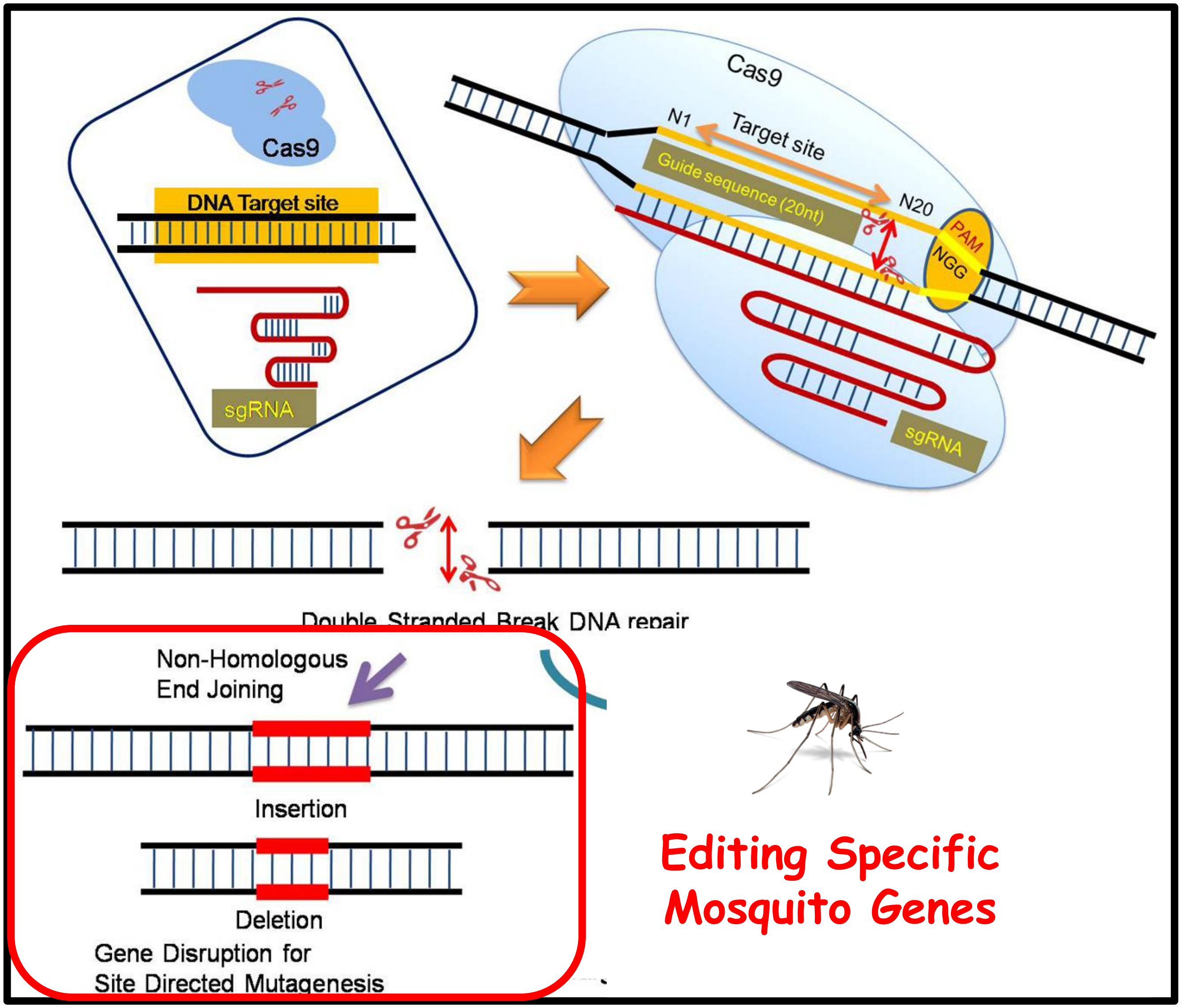
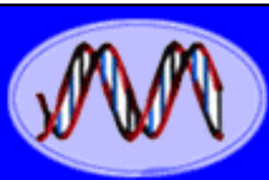
Editing Specific Mosquito Genes Using the CRISPR-Cas9 System Will Inhibit Infection With Plasmodium Parasites & Prevent Malaria!



Sequence Specific Changes in a Complex Genome!!!!



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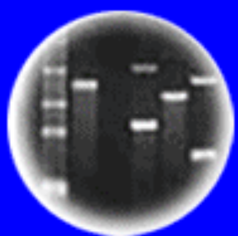

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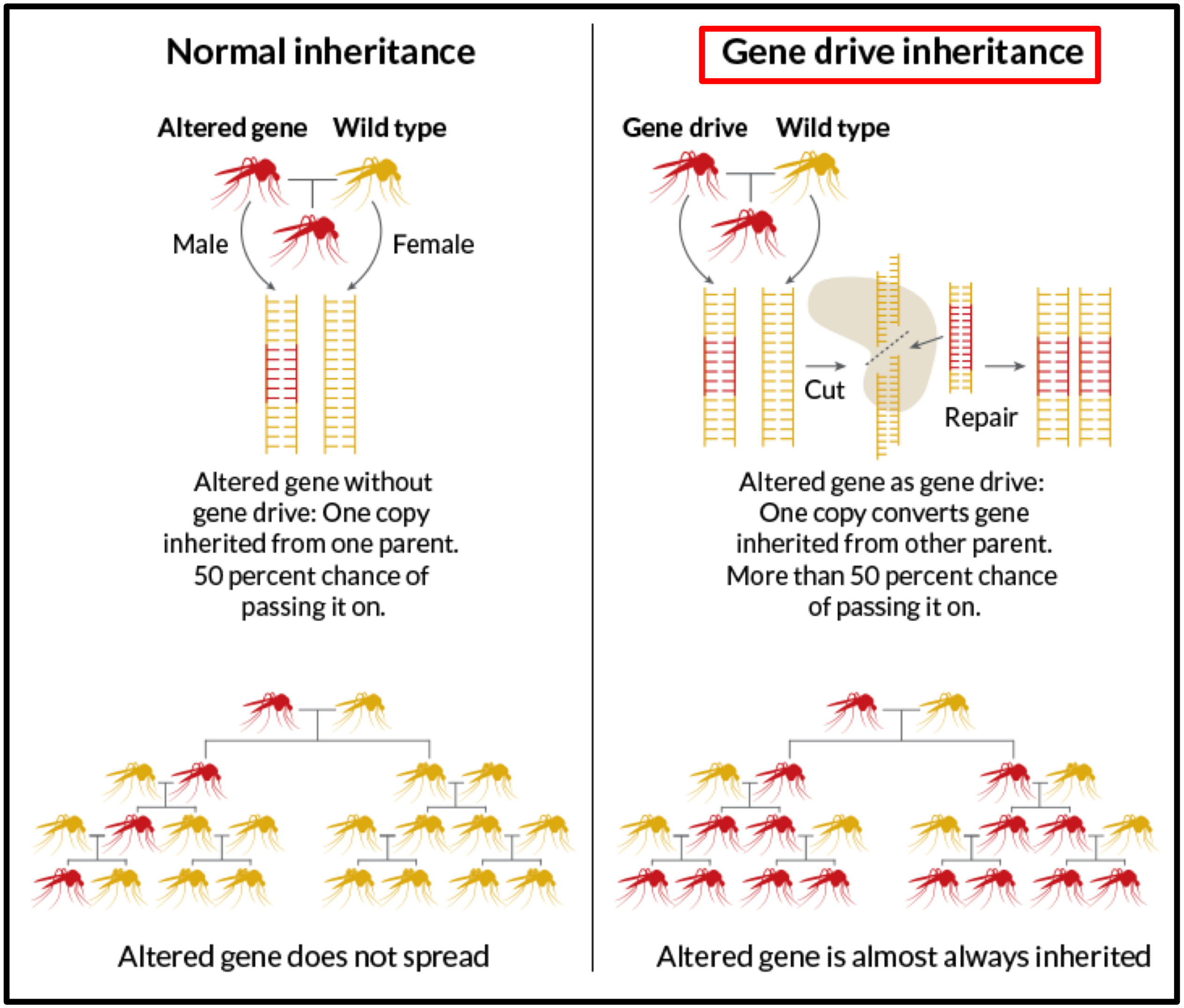
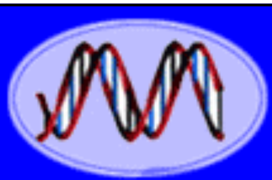
Genetic Engineering Mosquitos -"Gene Drive"
Spreading *Resistance to Plasmodium*
Throughout the Mosquito Population!



Mutate Plasmodium-Required Gene & Add Cas9-Guide RNA Into The Mosquito Genome

Autocatalytic Gene Editing!!



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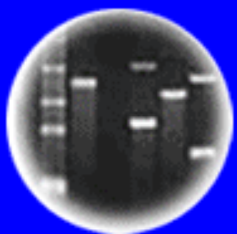

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Potential Gene Drive Applications

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

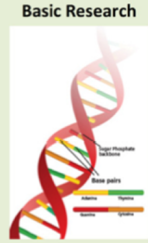
- Control or alter organisms that carry infectious diseases that threaten the survival of other species
- Eliminate invasive species that threaten native ecosystems and biodiversity
- Alter organisms that are threatened or endangered.

Agriculture

Fruit damage from spotted wing drosophila infestation
Image Source: US Department of Agriculture

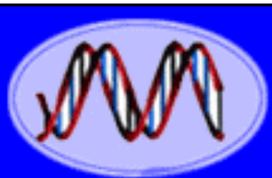
- Control or alter organisms that damage or carry crop diseases
- Eliminate weedy plants that compete with cultivated crops

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



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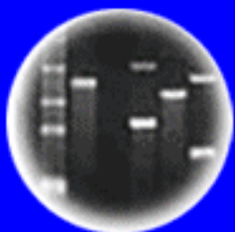

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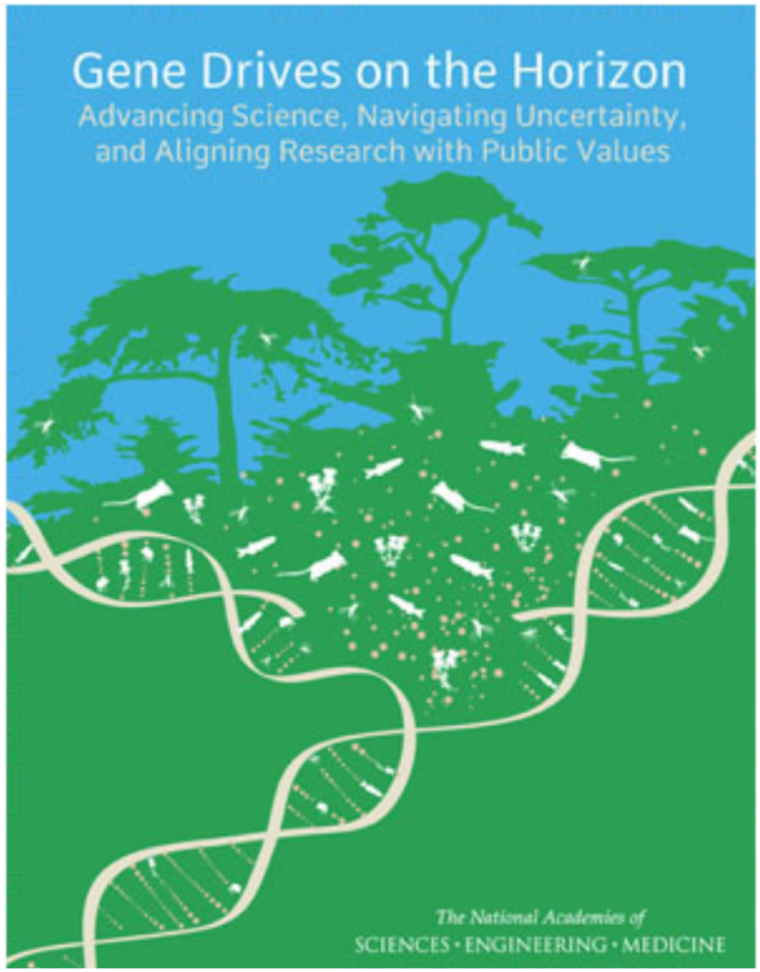
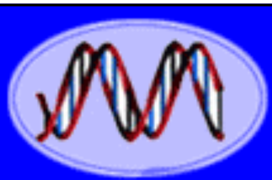
Potential Gene Risks & Benefits



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



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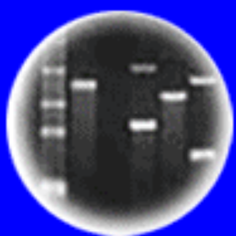

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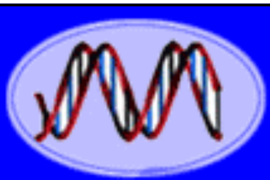
Recommendations For Using Gene Drive Systems

- 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



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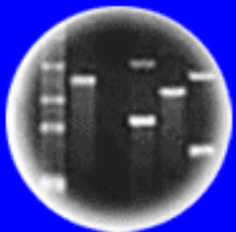

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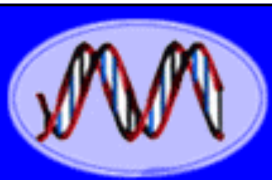
Other Uses Of CRISPR-Cas9 Editing



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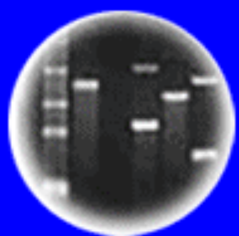

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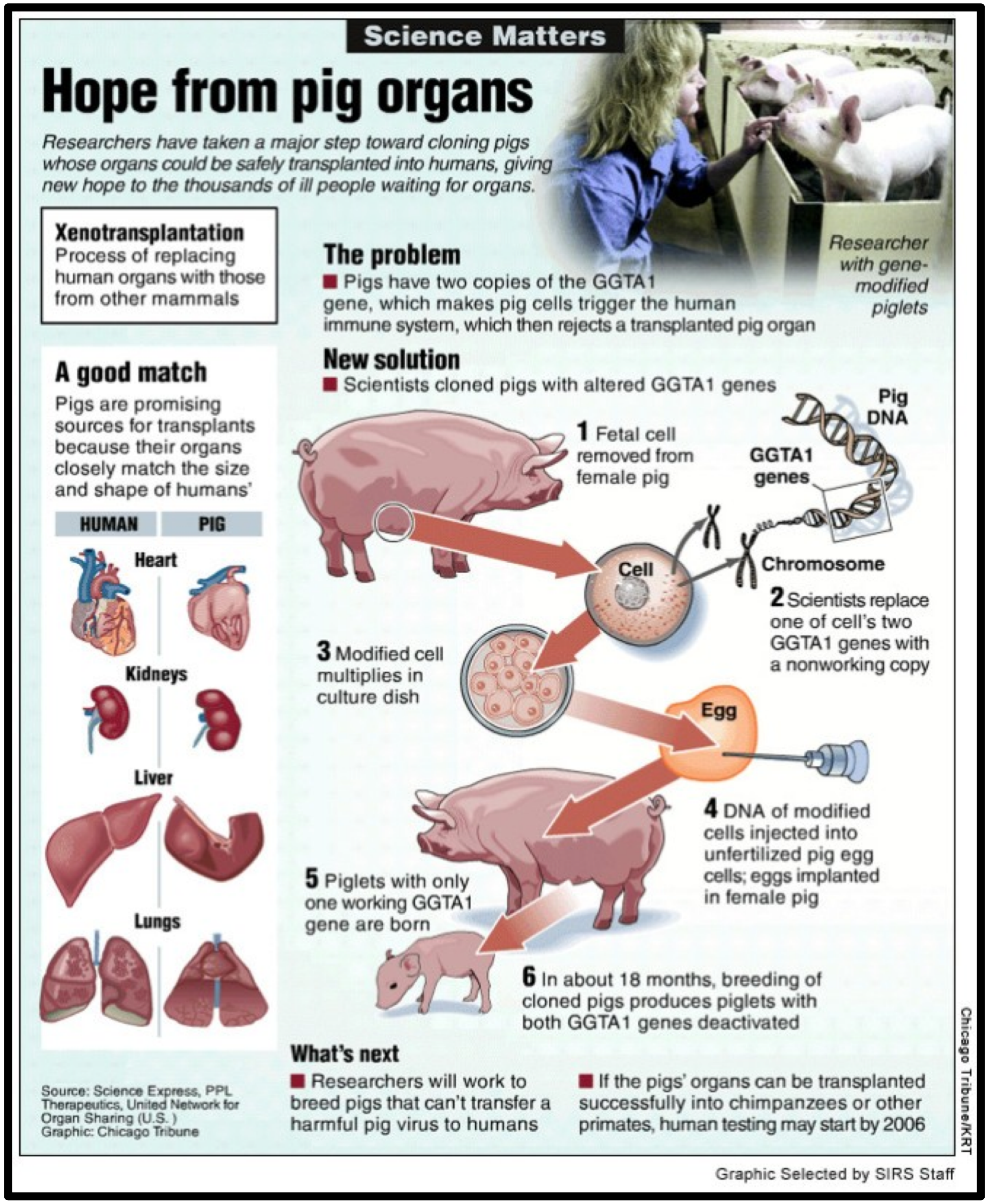
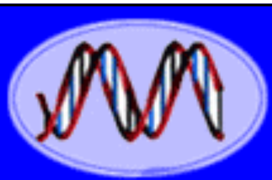
Removing Viral Sequences From Pig Genomes To Facilitate Human Pig Organ Transplants





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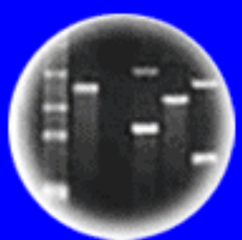

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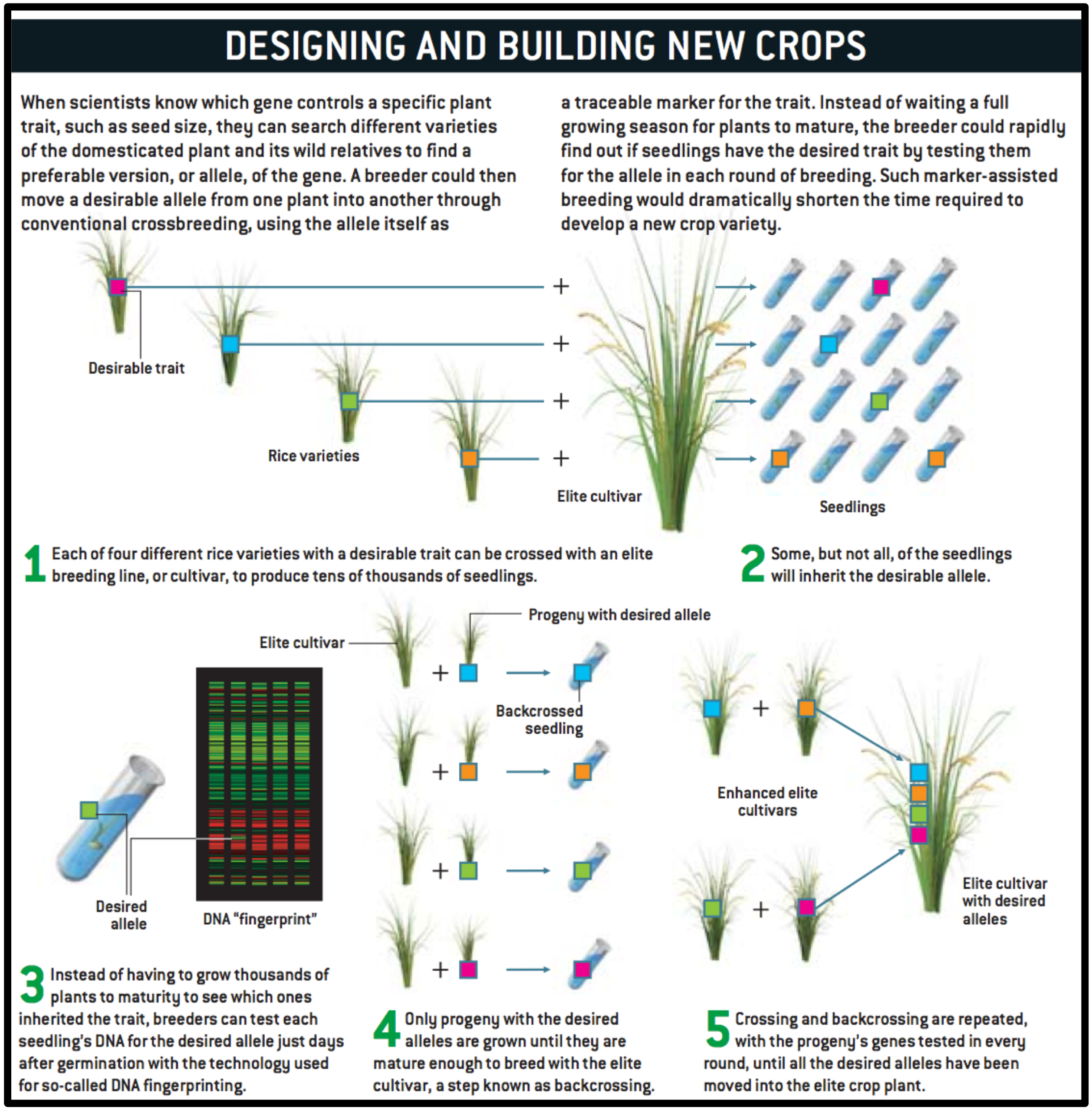
Using CRISPR-Cas9 Editing For Crop Improvement



Using Gene Editing to Improve Crop Plants

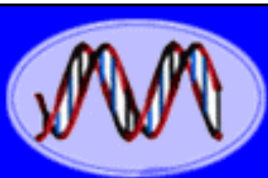
Geneticists Have Used CRISPR Gene Editing to Create Crops That Grow More Food

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



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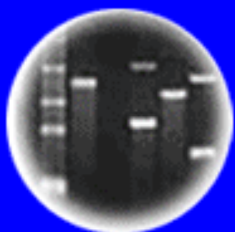

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CRISPR-Edited Crops Have Non-regulated Status in US

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

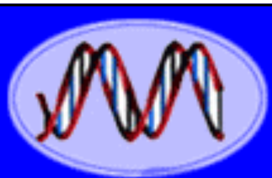
Section: News from Around the World

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



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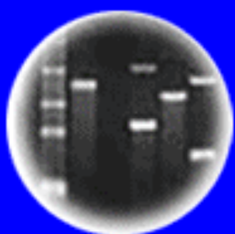

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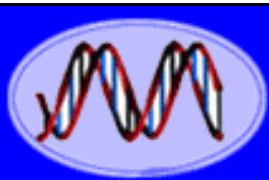
Using CRISPR-Cas9 Editing For Correcting Human Genetic Disorders



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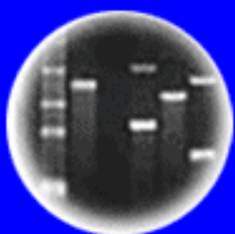

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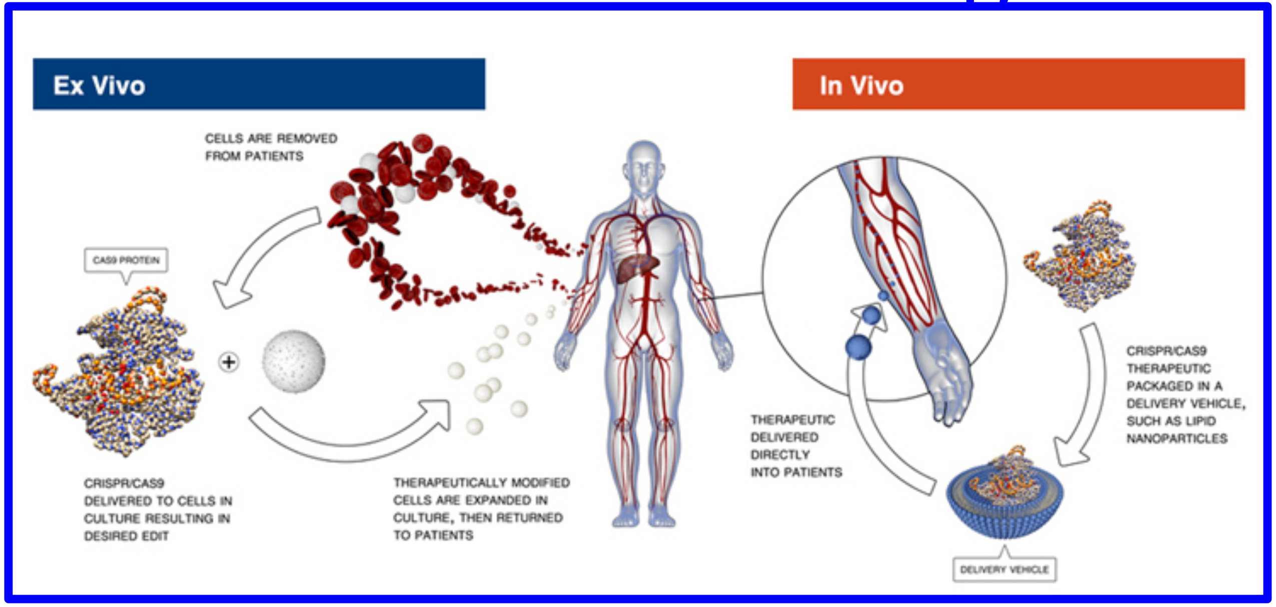
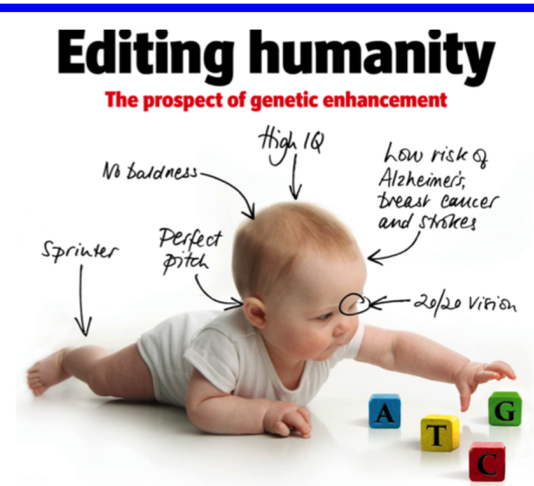
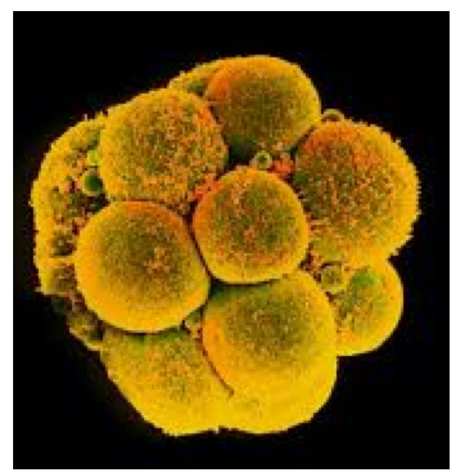
Somatic Cell Gene Therapy

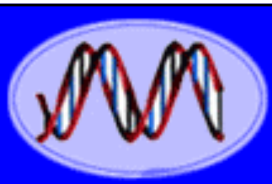


Germline Gene Therapy + Gene Enhancement





Using CRISPR-Cas9 Editing For Correcting Sickle Cell Anemia



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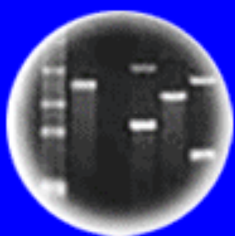

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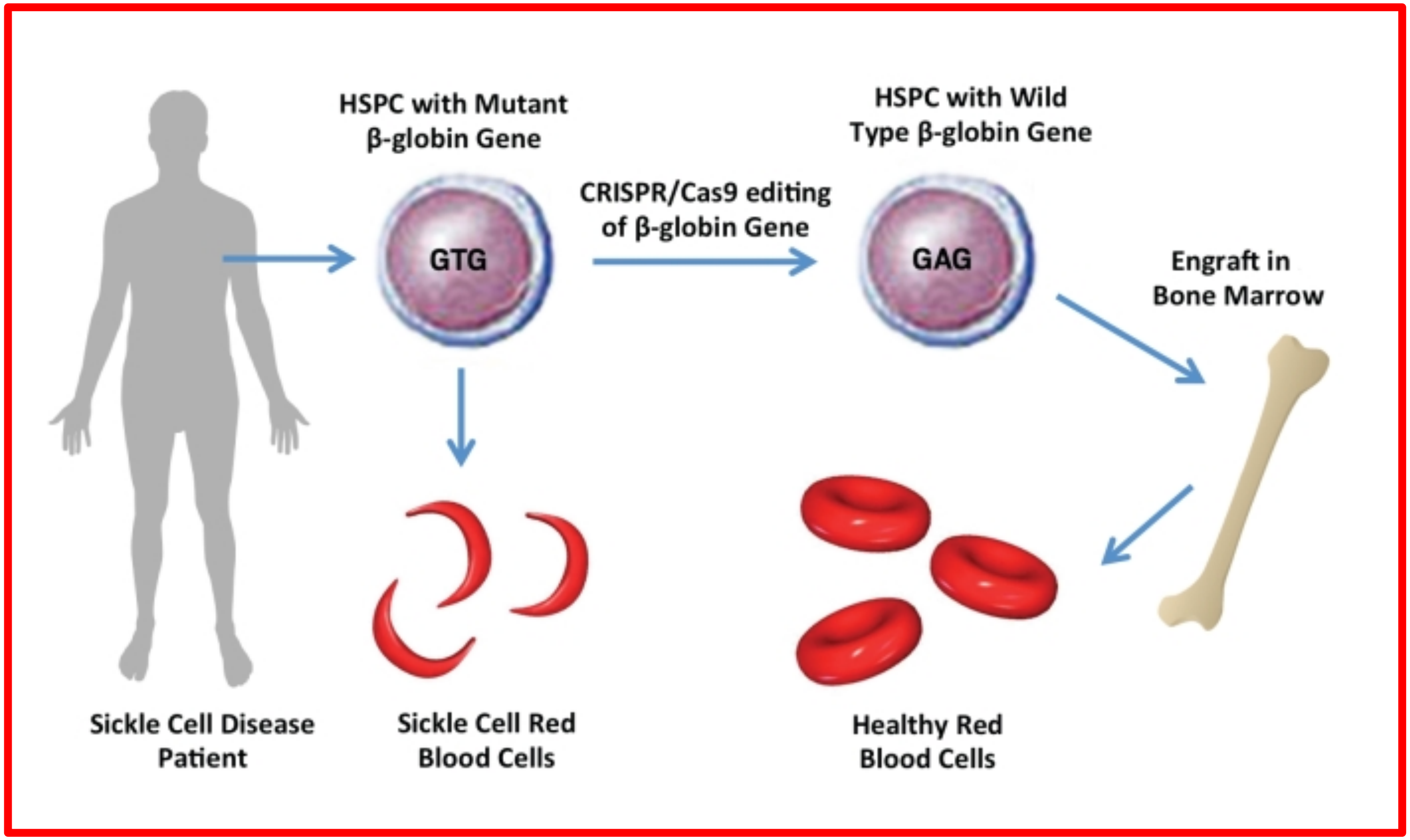
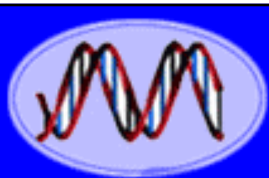


Using CRISPR-Cas9 to Edit Human Germline



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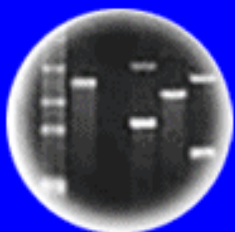

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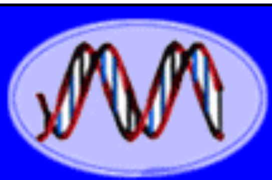
Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies

Chinese scientist's claim of gene-edited babies creates uproar

CRISPR-baby scientist fired by university

Scientist Who Edited Babies' Genes Is Likely to Face Charges in China



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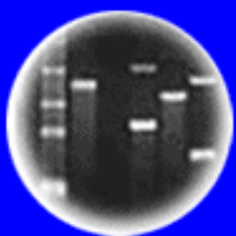

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INTERNATIONAL SUMMIT ON
HUMAN GENE EDITING

Recommendations For Using Human Gene Editing

1. Basic & Preclinical Research on Human Gene Editing Should Proceed Subject To Appropriate Legal and Ethical Rules and Oversight

2. Clinical Trials of Somatic Cell Gene Editing of Human Disease Genes Can Proceed Under Existing Gene Therapy Regulatory Frameworks

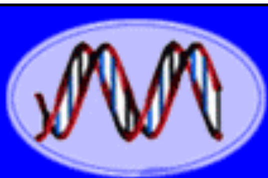
3. Germline Editing of Human Genes Poses Many Important Issues (e.g.,Difficulty of Predicting Harmful Effects, Permanent Change in Human Gene Pool, Permanent Genetic Enhancements Causing Social Inequalities, Changing Human Evolution), and it Would Be Irresponsible To Proceed Clinically Until These Issues Are Resolved

4. Need For International Standards and Norms Governing the Clinical Uses of Human Genome Editing Because There is One Human Genome Shared By All of Humanity

5. *Genome Editing For Purposes Other Than Treatment For Prevention of Human Disease Should Not Be Carried Out*

International Summit on Human Gene Editing, December, 2015 & Human Genome Editing Report, 2017, National Academy of Sciences

Discussion Reading For Week Ten



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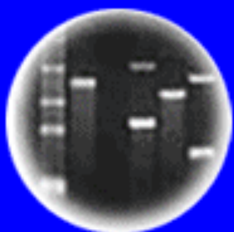

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