HC70A & SAS70A Winter 2014
Genetic Engineering in Medicine, Agriculture, and Law

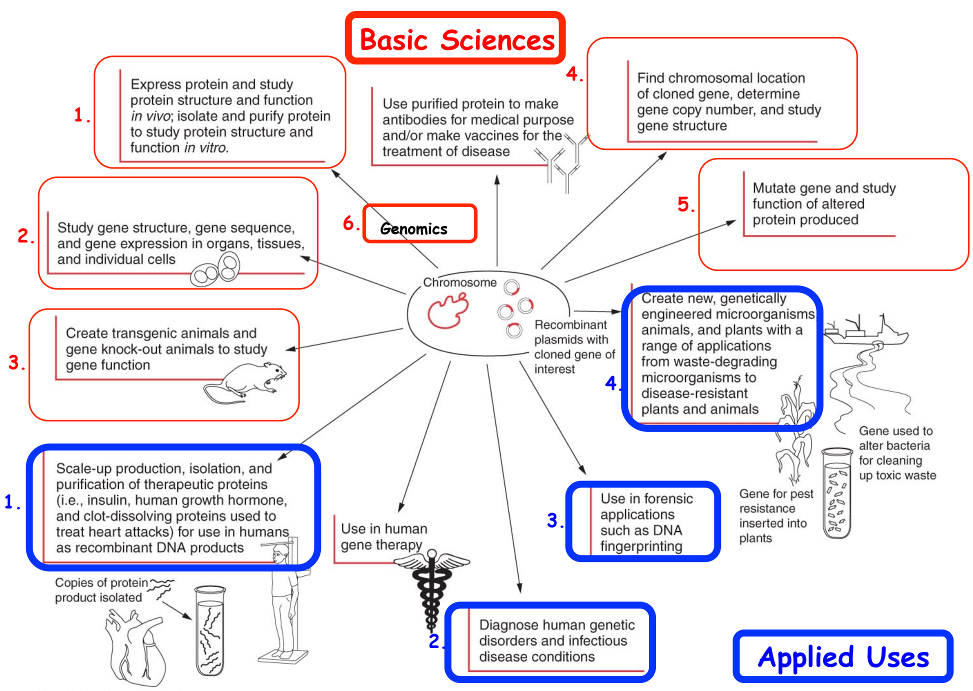
Professors Bob Goldberg. John Harada, & Channapatna Prakash

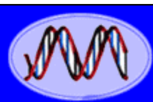
Lecture 6

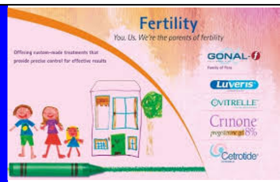
Twenty-First Century Genetic Engineering Applications

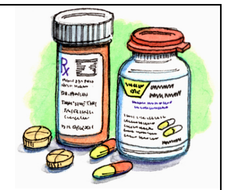
UCLA TUSKEGEE UNIVERSITY UCDAVIS UNIVERSITY OF CALIFORNIA

There Are Numerous Applications of Genetic Engineering – Many Have Been Discussed in Class To Date









Drugs & Vaccines

Recombinant Drugs Made In Bacteria And Mammalian Cells To Treat Human & Animal Diseases

Mammalian Cells Why?

E. coli

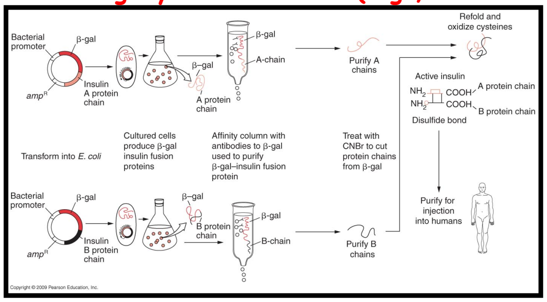
Table 10.1 Some recombinant proteins that have been approved for human use in either the United States or the European Union

Compound	Company	Disorder
Factor VIII	Baxter Healthcare, Genetics Institute, Centeon, Bayer	Hemophilia A
Factor VIIa	Novo Nordisk	Some forms of hemophilia
Factor IX	Genetics Institute	Hemophilia B
Hirudin	Ciba Novartis, Europharm, Hoechst Marion Roussel	Venous thrombosis, heparin-associated thrombocytopenia
Tissue plasminogen activator	Genentech	Acute myocardial infarction
Truncated tissue plasminogen activator	Galenus Mannheim, Boehringer Mannheim/Centocor	Acute myocardial infarction
Insulin	Eli Lilly, Novo Nordisk, Hoechst AG	Diabetes mellitus
Insulin analogues	Eli Lilly, Novo Nordisk, Aventis	Diabetes mellitus
Human growth hormone	Eli Lilly, Genentech, Biotechnology General, Pharmacia, Upjohn, Novo Nordisk, Serono Laboratories	Growth hormone deficiency in children
Human growth hormone analogue	Genentech	Growth hormone deficiency in children
Human growth hormone	Serono Laboratories	AIDS-associated catabolism and wasting
Glucagon	Novo Nordisk	Hypoglycemia
Thyrotrophin-α	Genzyme	Thyroid cancer
Follicle-stimulating hormone	Ares-Serono, Organon	Anovulation and superovulation
Erythropoietin	Amgen, Ortho Biotech, Boehringer-Mannheim	Anemia
Platelet-derived growth factor	Ortho-McNeil Pharmaceuticals, Janssen-Cilag	Lower-extremity diabetic neuropathic ulcers
DNase I	Genentech	Cystic fibrosis
β-Glucocerebrosidase analogue	Genzyme	Gaucher disease
IFN-α_{2a}	Hoffmann-La Roche, Schering-Plough	Hairy cell leukemia, hepatitis B and C
Synthetic type 1 IFN-α	Amgen, Yamanouchi Europe	Chronic hepatitis C
IFN-α_{2b}	Schering-Plough	Hairy cell leukemia, genital warts, hepatitis B and C
IFN-β_{1b} analogues	Schering AG, Berlex Laboratories, Chiron	Multiple sclerosis
IFN-β_{1a}	Biogen, Ares-Serono	Relapsing multiple sclerosis
IFN-γ_{1b}	Genentech	Chronic granulomatous disease
IL-2 analogue	Chiron	Renal cell carcinoma
IL-11 analogue	Genetics Institute	Prevention of chemotherapy-induced thrombocytopenia

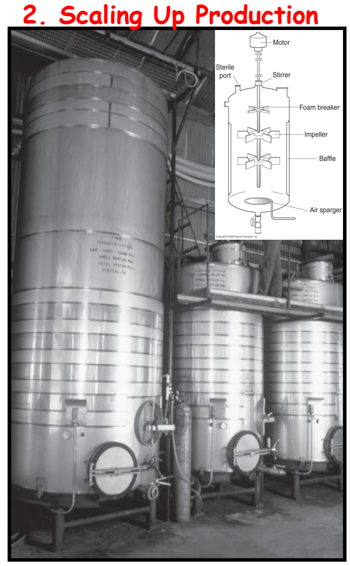
Abbreviations: IFN, interferon; IL, interleukin.

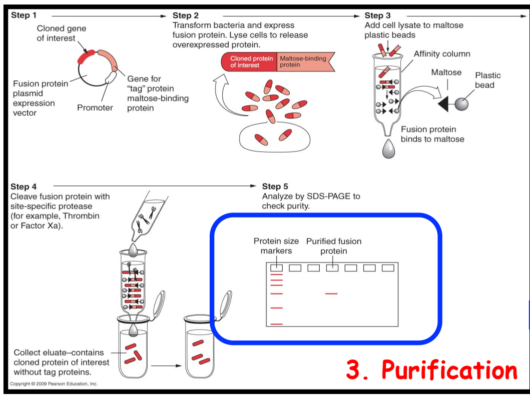
Manufacturing Recombinant Drugs Requires Industrial-Scale Facilities For Growing Genetically Engineered Cells

1. Cloning Synthetic cDNAs (e.g., Insulin cDNA)



2. Scaling Up Production





3. Purification

4. FDA Approval & Sale

Must Have FDA Approval of Recombinant Drug Production Process, & Drug Safety, and Use

Table 12.1 PRIMARY FEDERAL REGULATORY AGENCIES IN THE UNITED STATES

Regulatory Oversight of Biotechnology Products	
Agency	**Product Regulated**
U.S. Department of Agriculture	Plants, plant pests (including microorganisms), animal vaccines
Environmental Protection Agency	Microbial/plant pesticides, other toxic substances, microorganisms, animals producing toxic substances
Food and Drug Administration	Food, animal feeds, food additives, human and animal drugs, human vaccines, medical devices, transgenic animals, cosmetics
Major Laws that Empower Federal Agencies to Regulate Biotechnology	
Law	**Agency**
The Plant Protection Act	USDA
The Meat Inspection Act	USDA
The Poultry Products Inspection Act	USDA
The Eggs Products Inspection Act	USDA
The Virus Serum Toxin Act	USDA
The Federal Insecticide, Fungicide, and Rodenticide Act	EPA
The Toxic Substances Control Act	EPA
The Food, Drug, and Cosmetics Act	FDA, EPA
The Public Health Service Act	FDA
The Dietary Supplement Health and Education Act	FDA
The National Environmental Protection Act	USDA, EPA, FDA

Source: www.fda.gov.

Federal Food, Drug, and Cosmetic Act

Draft Year: 1938 Amendment Years: 1954, 1958

FDR signed the Food, Drug, and Cosmetic Act on 25 June 1938.

➢The FFDCA is a national act

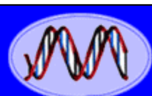
The act requires…

- ✓ **Pre-market safety approval of all new drugs**
- ✓ **Prohibited false therapeutic claims for drugs**
- ✓ **Drugs labeled with adequate directions for safe use**
- ✓ **Cosmetics and medical devices under FDA's jurisdiction**
- ✓ **Drugs be labeled with adequate directions for safe use.**

➢The FDA is responsible for regulations and enforcement

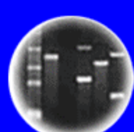
(Left)

FDA employee inspecting fish for pollutants or toxins



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

How Can the FDA Regulate Drug Approval?

"We the People of the United States, in order to form a more perfect Union, establish justice, insure domestic tranquility, provde for the common defense, promote the General Welfare……"

Article I - Section 8.1 of the US Constitution

The Congress shall have the Power:

[1] "To lay and collect Taxes, Duties, Imposts, and Excises, to pay the Debts and provide for the common Defense and general Welfare of the United States; but all Duties, Imposts, and Excises shall be uniform throughout the United States"

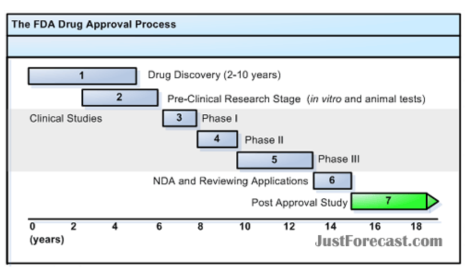
Key Concept: Provide For the General Welfare-Which Can Apply to Almost Everything Dealing With Science, Health, Medicine, Agriculture, and Safety!

Drug Testing For FDA Approval is a LONG and COSTLY Process

TABLE 7.1 FOOD AND DRUG ADMINISTRATION REQUIRED TESTING PHASES FOR DRUG APPROVAL

FDA Phase testing involves the use of animals for pre-clinical testing before allowed in humans. If the new drug candidate has proven to be non-toxic and has benefit, then it can be awarded and Investigational New Drug (IND)status. If it is successful in the three phases of human testing it can receive a New Drug Application (NDA) and likely approval for marketing. The FDA continues evaluating the NDA for another 2.5 years, resulting a total of about 12 years for a successful drug approval.

	Preclinical Testing		Phase I	Phase II	Phase III		FDA		Phase IV
Years	3.5		1	2	3		2.5	12 total	
Tested on	Animals in the lab		20–80 healthy volunteers	100–300 patient volunteers	1,000–3,000 patient volunteers			$1 Billion!!!	
Purpose	Assess safety and biological activity	File IND at FDA	Determine safety and dosage	Evaluate effectiveness and look for side effects	Verify effectiveness, monitor adverse reactions from long-term use	File NDA at FDA	Review process/ approval		Additional testing after approval required by FDA
Success rate	5,000 compounds evaluated			5 enter trials			1 approved		



What If There Was No Patent Protection For Drugs?

1. Article I - Section 8.8

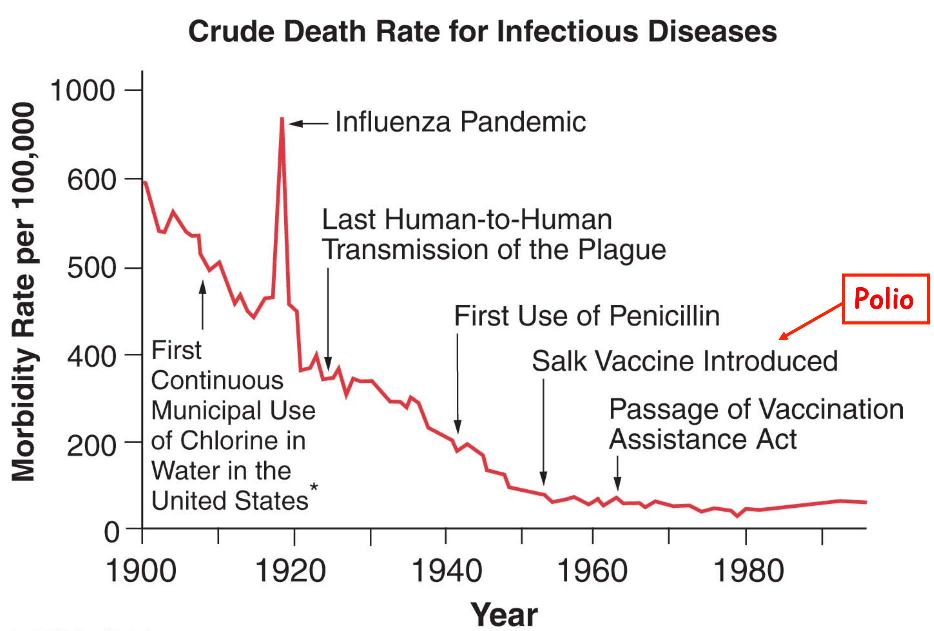
The Congress shall have the Power:

[8] "To Promote the Progress of Science and the useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their Writings and Discoveries"

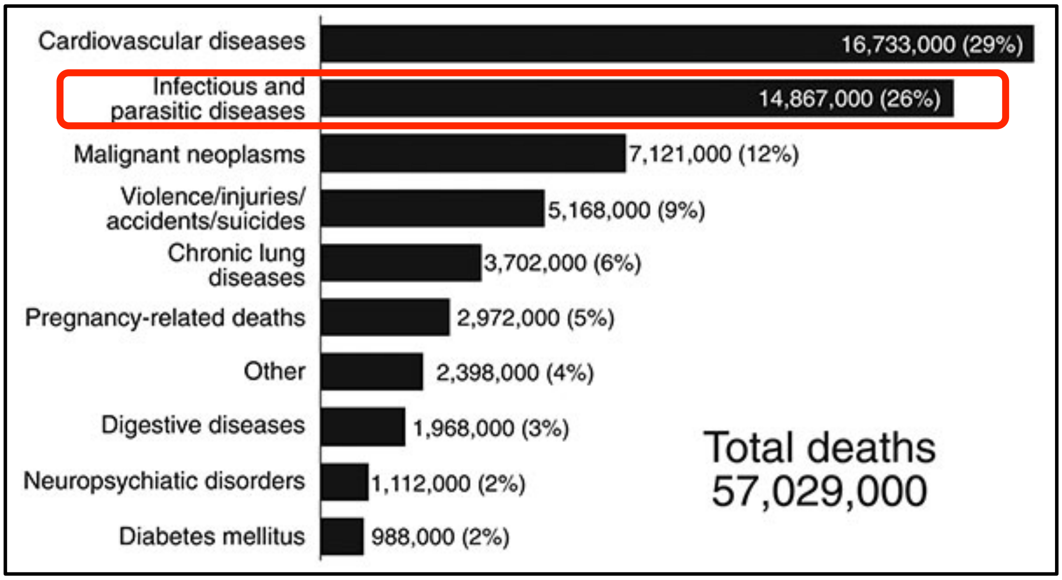
Keyword: Inventors not Science.
Wanted to Promote Economic Development & Promote a National Economics Policy Grounded in Property Rights.
That is, Entrepreneurship!

PATENTS!!

Over the Past 50 Years Vaccines and Antibiotics Have Been Essential in Combating Infectious Diseases



One Fourth of the Annual Deaths Word-Wide Are Caused By Infectious Diseases



Bacterial Diseases That Are Vaccine Targets

TABLE 28.1 Important Human Bacterial Diseases

Disease	Pathogen	Vector/Reservoir	Epidemiology
Anthrax	*Bacillus anthracis*	Animals, including processed skins	Bacterial infection that can be transmitted through contact or ingestion. Rare except in sporadic outbreaks. May be fatal.
Botulism	*Clostridium botulinum*	Improperly prepared food	Contracted through ingestion or contact with wound. Produces acute toxic poison; can be fatal.
Chlamydia	*Chlamydia trachomatis*	Humans, STD	Urogenital infections with possible spread to eyes and respiratory tract. Increasingly common over past 20 years.
Cholera	*Vibrio cholerae*	Human feces, plankton	Causes severe diarrhea that can lead to death by dehydration; 50% peak mortality if untreated. A major killer in times of crowding and poor sanitation; over 100,000 died in Rwanda in 1994 outbreak.
Dental caries	*Streptococcus mutans, Streptococcus sabrinus*	Humans	A dense collection of these bacteria on the surface of teeth leads to secretion of acids that destroy minerals in tooth enamel; sugar alone will not cause caries.
Diphtheria	*Corynebacterium diphtheriae*	Humans	Acute inflammation and lesions of respiratory mucous membranes. Spread through respiratory droplets. Vaccine available.
Gonorrhea	*Neisseria gonorrhoeae*	Humans only	STD, on the increase worldwide. Usually not fatal.
Hansen disease (leprosy)	*Mycobacterium leprae*	Humans, feral armadillos	Chronic infection of the skin; worldwide incidence about 10–12 million, especially in southeast Asia. Spread through contact with infected individuals.
Lyme disease	*Borrelia burgdorferi*	Ticks, deer, small rodents	Spread through bite of infected tick. Lesion followed by malaise, fever, fatigue, pain, stiff neck, and headache.
Peptic ulcers	*Helicobacter pylori*	Humans	Originally thought to be caused by stress or diet, most peptic ulcers now appear to be caused by this bacterium; good news for ulcer sufferers because it can be treated with antibiotics.
Plague	*Yersinia pestis*	Fleas of wild rodents: rats and squirrels	Killed one-fourth of the population of Europe in the fourteenth century; endemic in wild rodent populations of the western United States today.
Pneumonia	*Streptococcus, Mycoplasma, Chlamydia, Haemophilus*	Humans	Acute infection of the lungs; often fatal without treatment. Vaccine for streptococcal pneumonia available.
Tuberculosis	*Mycobacterium tuberculosis*	Humans	An acute bacterial infection of the lungs, lymph, and meninges. Its incidence is on the rise, complicated by the development of new strains of the bacterium that are resistant to antibiotics.
Typhoid fever	*Salmonella typhi*	Humans	A systemic bacterial disease of worldwide incidence. Fewer than 500 cases a year are reported in the United States. Spread through contaminated water or foods (such as improperly washed fruits and vegetables). Vaccines are available for travelers.
Typhus	*Rickettsia typhi*	Lice, rat fleas, humans	Historically a major killer in times of crowding and poor sanitation; transmitted from human to human through the bite of infected lice and fleas. Peak untreated mortality rate of 70%.

DTaP Vaccine – Diptheria, Tetanus, Pertussis

Corynebacterium diptheriae, Closteridium tetani, & Bordetella pertussis

All students entering, advancing or transferring into 7th grade need proof of an adolescent whooping cough booster immunization (called "Tdap")

Bacterial Diseases That Are Vaccine Targets

Kids who didn't get whooping cough vaccine a cause of outbreak, scientists say

By Mary MacVean

2:18 PM PDT, September 30, 2013

Children who did not get vaccinated against whooping cough are one of the causes of the 2010 outbreak of the illness, when more cases were reported than in any year since 1947, researchers say.

Researchers who looked at the geography of the cases suggest that clusters of "nonmedical exemptions" to immunizations were one of several factors in the California outbreak. They reported their findings Monday in the journal Pediatrics.

In California in 2010, there were 9,120 cases of the illness that's also called pertussis – one-third of all the U.S. cases. Los Angeles had 1,000 of those cases. Whooping cough is a respiratory ailment marked by bouts of coughing that are accompanied by a noise that can frighten parents – hence the name.

DTaP Vaccine – Diptheria, Tetanus, Pertussis

Corynebacterium diptheriae, Closteridium tetani, & Bordetella pertussis

All students entering, advancing or transferring into 7th grade need proof of an adolescent whooping cough booster immunization (called "Tdap")

How Can There Be a Law REQUIRING Vaccinations?

Viral Diseases That Are Vaccine Targets

TABLE 27.1 Important Human Viral Diseases

Disease	Pathogen	Genome	Vector/Epidemiology
Chicken pox	Varicella zoster	Double-stranded DNA	Spread through contact with infected individuals. No cure. Rarely fatal. Vaccine approved in U.S. in early 1995.
Hepatitis B (viral)	Hepadnavirus	Double-stranded DNA	Highly infectious through contact with infected body fluids. Approximately 1% of U.S. population infected. Vaccine available. No cure. Can be fatal.
Herpes	Herpes simplex virus	Double-stranded DNA	Blisters; spread primarily through skin-to-skin contact with cold sores/blisters. Very prevalent worldwide. No cure. Exhibits latency—the disease can be dormant for several years.
Mononucleosis	Epstein–Barr virus	Double-stranded DNA	Spread through contact with infected saliva. May last several weeks; common in young adults. No cure. Rarely fatal.
Smallpox	Variola virus	Double-stranded DNA	Historically a major killer; the last recorded case of smallpox was in 1977. A worldwide vaccination campaign wiped out the disease completely.
AIDS	HIV	(+) Single-stranded RNA (two copies)	Destroys immune defenses, resulting in death by infection or cancer. As of 2005, WHO estimated that 40 million people are living with AIDS; 4.1 million new HIV infections were predicted and 2.8 million deaths were expected. More than 25 million have died from AIDS since 1981.
Polio	Enterovirus	(+) Single-stranded RNA	Acute viral infection of the CNS that can lead to paralysis and is often fatal. Prior to the development of Salk's vaccine in 1954, 60,000 people a year contracted the disease in the U.S. alone.
Yellow fever	Flavivirus	(+) Single-stranded RNA	Spread from individual to individual by mosquito bites; a notable cause of death during the construction of the Panama Canal. If untreated, this disease has a peak mortality rate of 60%.
Ebola	Filoviruses	(−) Single-stranded RNA	Acute hemorrhagic fever; virus attacks connective tissue, leading to massive hemorrhaging and death. Peak mortality is 50–90% if untreated. Outbreaks confined to local regions of central Africa.
Influenza	Influenza viruses	(−) Single-stranded RNA (eight segments)	Historically a major killer (20–50 million died during 18 months in 1918–1919); wild Asian ducks, chickens, and pigs are major reservoirs. The ducks are not affected by the flu virus, which shuffles its antigen genes while multiplying within them, leading to new flu strains.
Measles	Paramyxoviruses	(−) Single-stranded RNA	Extremely contagious through contact with infected individuals. Vaccine available. Usually contracted in childhood, when it is not serious; more dangerous to adults.
SARS	Coronavirus	(−) Single-stranded RNA	Acute respiratory infection; an emerging disease, can be fatal, especially in the elderly. Commonly infected animals include bats, foxes, skunks, and racoons. Domestic animals can be infected.
Rabies	Rhabdovirus	(−) Single-stranded RNA	An acute viral encephalomyelitis transmitted by the bite of an infected animal. Fatal if untreated. Commonly infected animals include bats, foxes, skunks, and racoons. Domestic animals can be infected.

Using Genetic Engineering To Produce Vaccines Can Play a Big Role in Combating Bioweapons

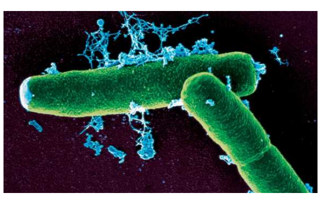
TABLE 5.5 POTENTIAL BIOLOGICAL WEAPONS

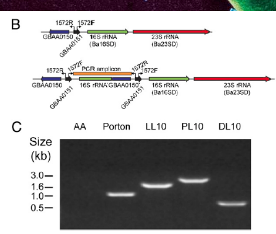
Agent	Disease Threat and Common Symptoms
Brucella (bacteria)	Different strains of *Brucella* infect livestock such as cattle and goats. They can cause brucellosis in animals and humans. Prolonged fever and lethargy are common symptoms. The disease can be mild or life-threatening.
Bacillus anthracis (bacterium)	Anthrax. Skin form (cutaneous) produces skin-surface lesions that are generally treatable. Inhalation anthrax initially produces flu-like symptoms leading to pulmonary pneumonia, which is usually fatal.
Clostridium botulinum (bacterium)	Botulism. Caused by ingestion of food contaminated with *C. botulinum* or its toxins. Varying degrees of paralysis of the muscular system created by botulinum toxins are typical. Respiratory paralysis and cardiac arrest often cause death.
Ebola virus or Marburg virus	Both are highly virulent viruses that cause hemorrhagic fever. Symptoms include severe fever, muscle/joint pain, and bleeding disorders.
Francisella tularensis (bacterium)	Tularemia. Lung inflammation can cause respiratory failure, shock, and death.
Influenza viruses (a large, highly contagious group)	Influenza (flu). Severity and outcome depend largely on the strain of the virus.
Rickettsia (several bacteria strains)	Different strains cause diseases such as Rocky Mountain spotted fever and typhus.
Variola virus	Smallpox. Chills, high fever, backache, headache, and skin lesions.
Yersinia pestis (bacterium)	Bubonic plague. High fever, headache, painful swelling of lymph nodes, shock, circulatory collapse, organ failure, and death within days after infection in a majority of cases.

October 18, 2001

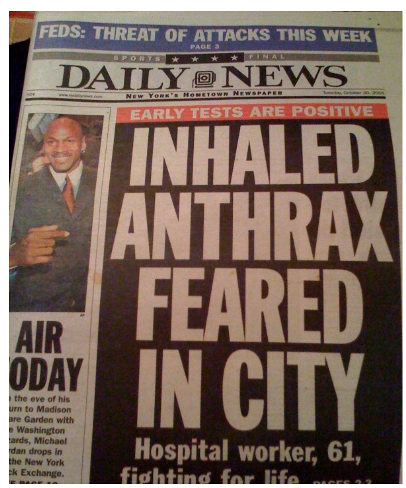
A NATION CHALLENGED: THE ANTHRAX THREAT

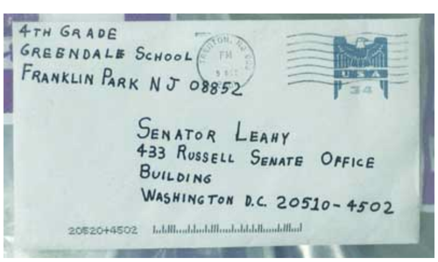
A NATION CHALLENGED: THE ANTHRAX THREAT; TESTS SHOW ANTHRAX EXPOSURE IN AT LEAST 30 CAPITAL WORKERS

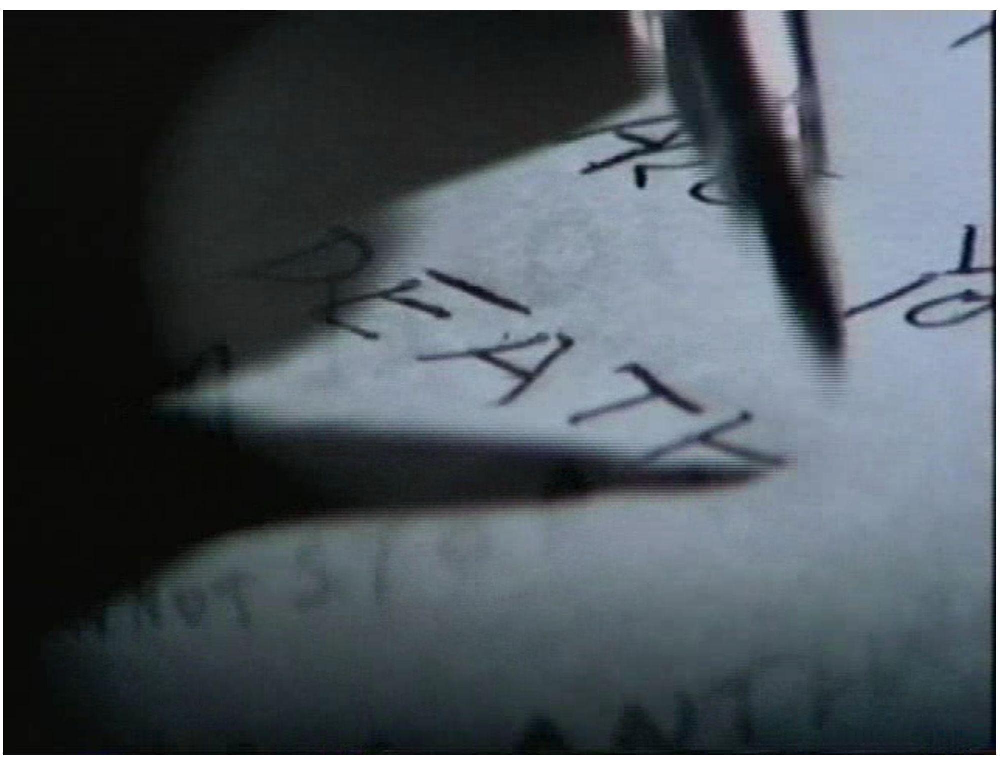


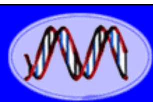


DNA Fingerprints Can Be Used To Find the Source of the Strain






DEATH



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Studies of deadly H5N1 bird flu mutations test scientific ethics

Dutch scientists have created a version of the deadly H5N1 bird flu that's easily transmitted. In an unprecedented move, a U.S. board asks that some details of the research not be published.

By Eryn Brown, Los Angeles Times

10:21 PM PST, December 26, 2011

WHO: Bird flu research raises safety questions

By FRANK JORDANS, Associated Press – 1 hour ago 0

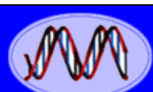
GENEVA (AP) — The World Health Organization is warning that dangerous scientific information could fall into the wrong hands after U.S. government-funded researchers engineered a form of the deadly H5N1 bird flu virus more easily transmissible between humans.

In Dramatic Move, Flu Researchers Announce Moratorium on Some H5N1 Flu Research, Call for Global Summit

by David Malakoff and Martin Enserink, with reporting by Gretchen Vogel and Jon Cohen on 20 January 2012, 12:42 PM | 4 Comments

Controversial H5N1 influenza work likely to resume

International experts hammer out the future of their field at a top-level workshop on how to fund certain types of work on bird flu.



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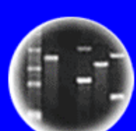

DNA Fingerprinting

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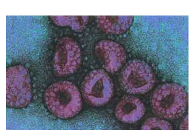
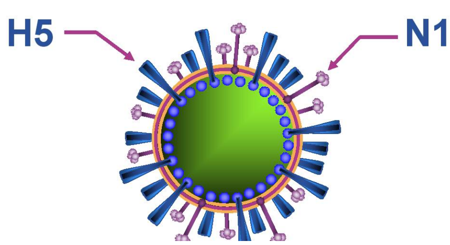
Plants of Tomorrow

Bird Flu Paper Is Published After Debate

By DONALD G. McNEIL Jr.
Published: June 21, 2012 | 27 Comments

Synthetic Generation of Influenza Vaccine Viruses for Rapid Response to Pandemics
Philip R. Dormitzer *et al.*
Sci Transl Med **5**, 185ra68 (2013);
DOI: 10.1126/scitranslmed.3006368





Influenza A and B viruses have 2 surface glycoproteins: hemagglutinin, which is necessary for the virus to attach to host-cell membranes, and neuraminidase, an enzyme involved in viral penetration and release of virus from infected cells.

Should Results Showing Which DNA Sequences in the Bird Flu Genome Can Be Changed to Allow Airborne Ferret to Ferret Viral Transmission Be Published?

a. yes
b. no

<u>Note:</u> H5N1 bird flu rarely infects humans and is not naturally transmitted from human to human. However, it can cause up to 50% death rate in humans that it infects!

Edward Jenner Using Cowpox to Vaccinate a Child Against Smallpox

In 1776 George Washington Lost 1,000 Men to Battle
And 100,000 Men to Smallpox!

Washington Had His Army Innoculated With a Small Amount of Fluid From a Smallpox Victim and the Smallpox Rate Went Down

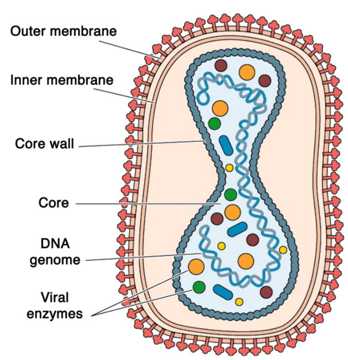
Smallpox Was One of the First Biological Warfare Agents-Having Been Used For Centuries

Responsible For 300-500 MILLION Deaths in the 20th Century

Smallpox is the Only Human Infectious Disease That Has Been Eradicated Globally

186,000 bp dsDNA Genome

A



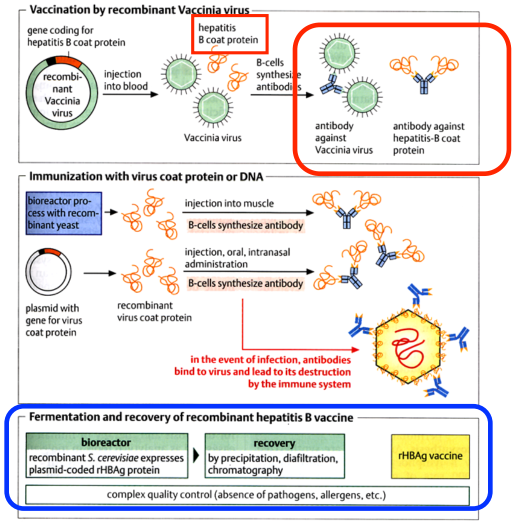
The Last Reported Case of Smallpox in US was in Was Reported in 1949 & in World in 1977 – Smallpox Vaccinations Are No Longer Given. Smallpox Virus Destroyed in 1980! What About Monkeypox?

Using Genetic Engineering To Make Vaccines

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

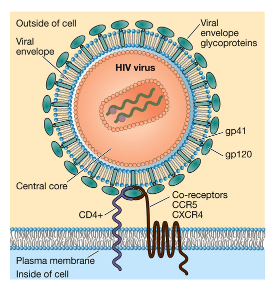
Recombinant vaccines (selection)

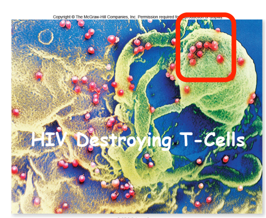
		antigen	status
viruses	hepatitis B	surface antigens	registered
	Herpes simplex type 2	surface antigens	clinical studies
	rabies vaccine	surface antigens	not registered
	yellow fever virus	surface antigens	preclinical studies
	AIDS virus	surface antigens	clinical studies
bacteria	*Streptococcus pneumoniae*	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



Recombinant Vaccines Are Being Developed To Combat Many Pathogens

Pathogenic agent	Disease(s)
Viruses	
Varicella-zoster virus	Chicken pox
Cytomegalovirus	Infection in infants and immunocompromised patients
Dengue virus	Hemorrhagic fever
Hepatitis A virus	High fever, liver damage
Hepatitis B virus	Long-term liver damage
Herpes simplex virus type 2	Genital ulcers
Influenza A and B viruses	Acute respiratory disease
Japanese encephalitis virus	Encephalitis
Parainfluenza virus	Inflammation of the upper respiratory tract
Rabies virus	Encephalitis
Respiratory syncytial virus	Upper and lower respiratory tract lesions
Rotavirus	Acute infantile gastroenteritis
Yellow fever virus	Lesions of heart, kidney, and liver
Human immunodeficiency virus	AIDS
Bacteria	
Vibrio cholerae	Cholera
E. coli enterotoxin strains	Diarrheal disease
Neisseria gonorrhoeae	Gonorrhea
Haemophilus influenzae	Meningitis, septicemic conditions
Mycobacterium leprae	Leprosy
Neisseria meningitidis	Meningitis
Bordetella pertussis	Whooping cough
Shigella strains	Dysentery
Streptococcus group A	Scarlet fever, rheumatic fever, throat infection
Streptococcus group B	Sepsis, urogenital tract infection
Streptococcus pneumoniae	Pneumonia, meningitis
Clostridium tetani	Tetanus
Mycobacterium tuberculosis	Tuberculosis
Salmonella typhi	Typhoid fever
Parasites	
Onchocerca volvulus	River blindness
Leishmania spp.	Internal and external lesions
Plasmodium spp.	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma spp.	Sleeping sickness
Wuchereria bancrofti	Filariasis





But a Vaccine To The AIDS Virus Remains Elusive!!

Genomics Can Provide Valuable Information About Pathogen Protein Targets For Vaccine Production

TABLE 5.4 EXAMPLES OF MEDICALLY IMPORTANT VIRAL GENOMES THAT HAVE BEEN SEQUENCED

Virus	Human Disease or Illness	Year Sequenced
Ebola virus	Ebola hemorrhagic fever	1993
Hepatitis A virus	Hepatitis A	1987
Hepatitis B virus	Hepatitis B	1984
Hepatitis C virus	Hepatitis C	1990
Herpes simplex virus, type I	Cold sores	1988
Human immunodeficiency virus (HIV-1)	Acquired immunodeficiency syndrome (AIDS)	1985
Human papillomavirus	Cervical cancer	1985
Human poliovirus	Poliomyelitis	1981
Human rhinovirus	Common cold	1984
Influenza A virus		
• Subtype H5N1 (Avian flu)	Severe flu	2007
• Subtype H5N1 (Swine flu)	Severe flu	2009
Severe acute respiratory coronavirus (SARS-CoV)	Severe acute respiratory syndrome (SARS)	2003
Variola virus	Smallpox	1992

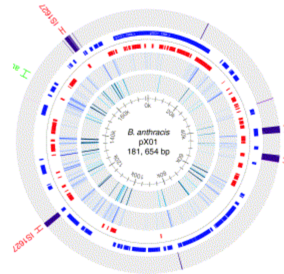
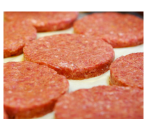


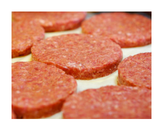
TABLE 5.3 SELECTED MICROBIAL GENOMES

Bacterium	Human Disease Condition (megabases, mB)	Approximate Genome Size	Approximate Number of Genes
Bacillus anthracis	Anthrax	5.23	5,000
Borrelia burgdorferi	Lyme disease	1.44	853
Chlamydia trachomatis	Eye infections, genitourinary tract infections (e.g., pelvic inflammatory disease)	1.04	896
Escherichia coli O157:H7	Severe food-borne illness (diarrhea)	4.10	5,283
Haemophilus influenzae	Serious infections in children (eye, throat, and ear infections, meningitis)	1.83	1,746
Helicobacter pylori	Stomach (gastric) ulcers	1.66	1,590
Listeria monocytogenes	Listeriosis (serious food-borne illness)	2.94	2,853
Mycobacterium tuberculosis	Tuberculosis	4.41	3,974
Neisseria meningitidis (MC58) infections	Meningitis and blood	2.27	2,158
Pseudomonas aeruginosa	Pneumonia, chronic lung infections	6.30	5,570
Rickettsia prowazekii	Typhus	1.11	834
Rickettsia conorii	Mediterranean spotted fever	1.30	1,374
Streptococcus pneumoniae	Acute (short-term) respiratory infection	2.16	2,236
Yersinia pestis	Plague	4.65	4,012
Vibrio cholerae	Cholera (diarrheal disease)	4.00	3,885

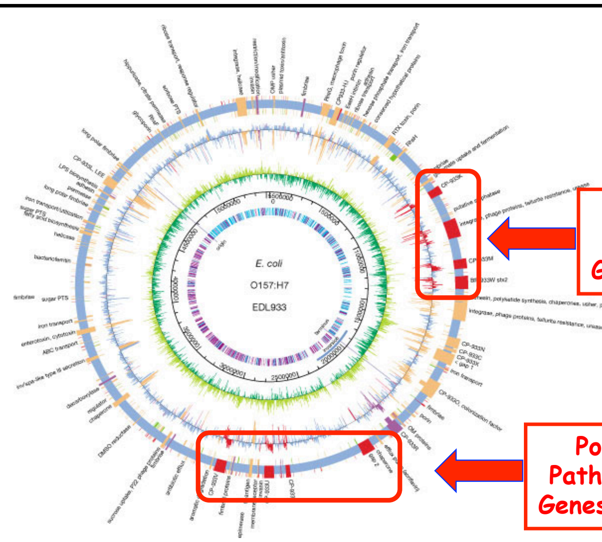
Comparison of Pathogenic and Non-Pathogenic *E. coli* Genomes



E. coli O157:H7 was first recognized as a foodborne pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis (bloody diarrhea) associated with consumption of contaminated hamburgers (Riley, et al., 1983). The following year, Shiga toxin (Stx), produced by the then little-known E. coli O157:H7, was identified as the real culprit.



75,000 Illnesses & 650 Deaths Due To E. coli H0157 & 72 Million Illnesses & 5,000 Deaths Due to All Foodborne Diseases In US!!! A BIG PROBLEM



Potential Pathogenicity Genes/Proteins

Potential Pathogenicity Genes/Proteins

http://www.cdc.gov/ncidod/eid/Vol5no5/mead.htm

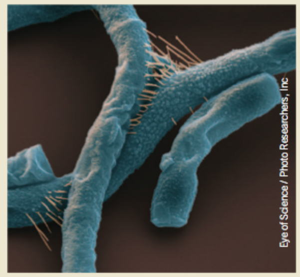
Nature **409**, 529-533 (25 January 2001)
November 2000

Understanding Pathogen Genes and Infection Process Leads to New Drugs
For Example – A New Anthrax Drug Just Released

Anthrax drug first antibacterial mAb to win approval

Officials of the US Food and Drug Administration (FDA) in mid-December approved ABthrax, or raxibacumab, for use in patients with inhalational anthrax. This approval is the first under the agency's 'animal rule', established for evaluating the efficacy of products that would be unethical or impossible to test (except for safety) in humans. ABthrax is a human monoclonal antibody (mAb), licensed for use as an adjunct to conventional antibiotics such as Cipro (ciprofloxacin).

This is also the first time FDA approved a mAb for an antibacterial indication, according to Steven Projan, a senior vice president at MedImmune in Gaithersburg, Maryland. "This should signal a new era in pathogen-specific drugs for the prevention and/or treatment of bacterial infections for bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, where there are already monoclonal antibodies in clinical trials," he says.



Bacillus anthracis is the causative agent of anthrax, which affects both humans and animals.

ABthrax interferes with the binding of a key antigen of *Bacillus anthracis*, the bacterial pathogen responsible for anthrax—a potentially deadly infection, particularly when it involves the lungs and becomes systemic. Spores of this pathogen can be used as a bioterror agent—in 2001, spores deliberately distributed through the US Postal Service led to 5 deaths amid 17 cases of anthrax—or in biological warfare. The mAb was developed by Rockville, Maryland–based Human Genome Sciences, a biotech company that GlaxoSmithKline of London acquired last August (*Nat. Biotechnol.* **30**, 815, 2012).

Under a contract from 2005, FDA allowed the US Department of Health and Human Services to purchase and stockpile ABthrax under Project BioShield and within its Biomedical Advanced Research and Development Authority (BARDA). Until full approval came in 2012, however, the mAb was subject to FDA emergency use authority (EUA), according to Amesh Adalja, senior associate at the Center for Biosecurity, a nonprofit organization of the University of Pittsburgh Medical School, in Baltimore. "FDA approval [of ABthrax] makes it easier for physicians to use the product," he says.

Nature Biotechnology
January, 2013

Synthetic Biology Can Be Used to Rapidly Synthesize Vaccines

VACCINES

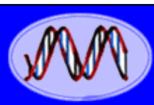
Synthetic Generation of Influenza Vaccine Viruses for Rapid Response to Pandemics

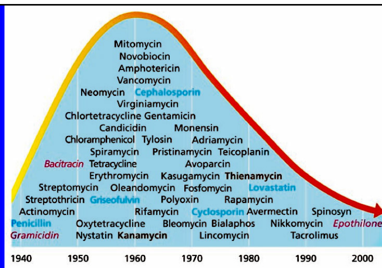
Synthetic Biologists Engineer A Custom Flu Vaccine In A Week

A synthetic biology method proves its chops.

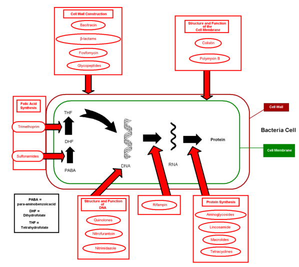
Synthetic Biology Could Speed Flu Vaccine Production

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



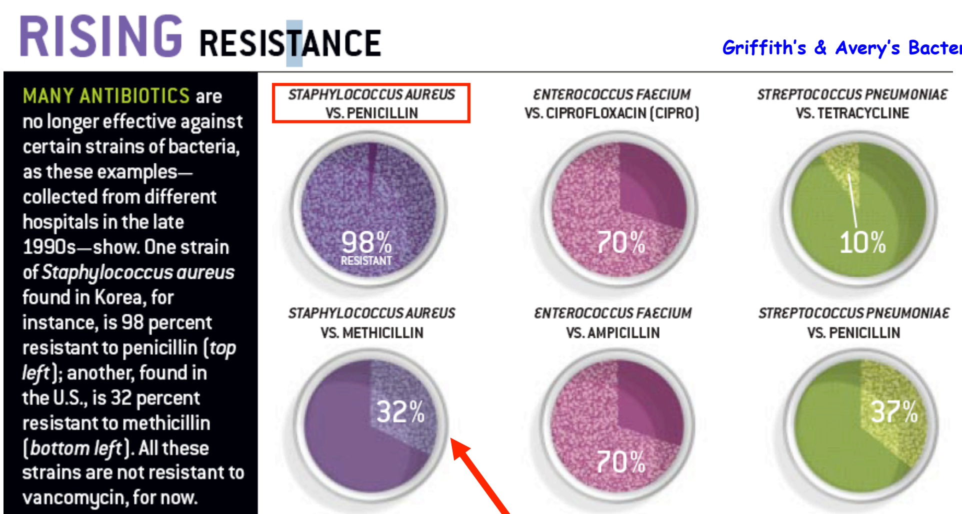


Antibiotics



Antibiotic Resistance Is Also A Major Problem in Combating Pathogens

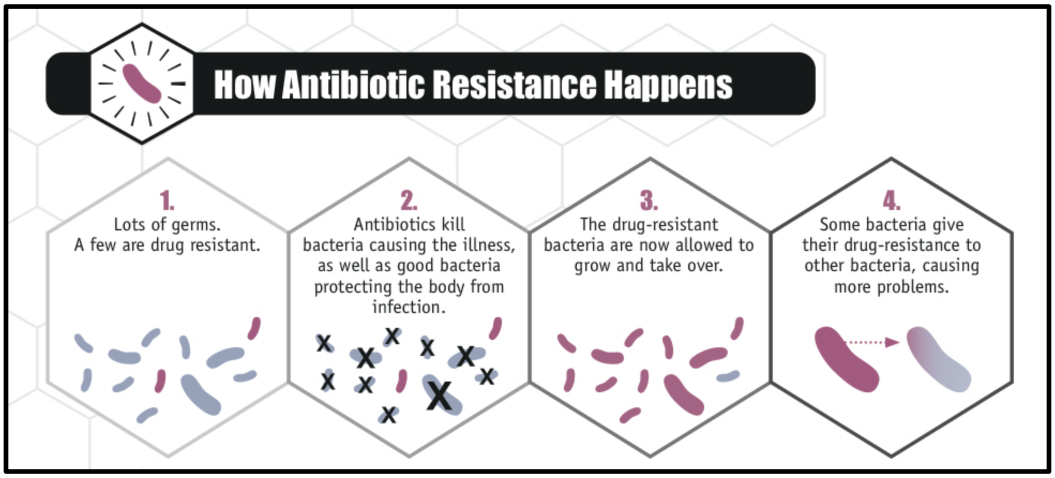
Griffith's & Avery's Bacteria



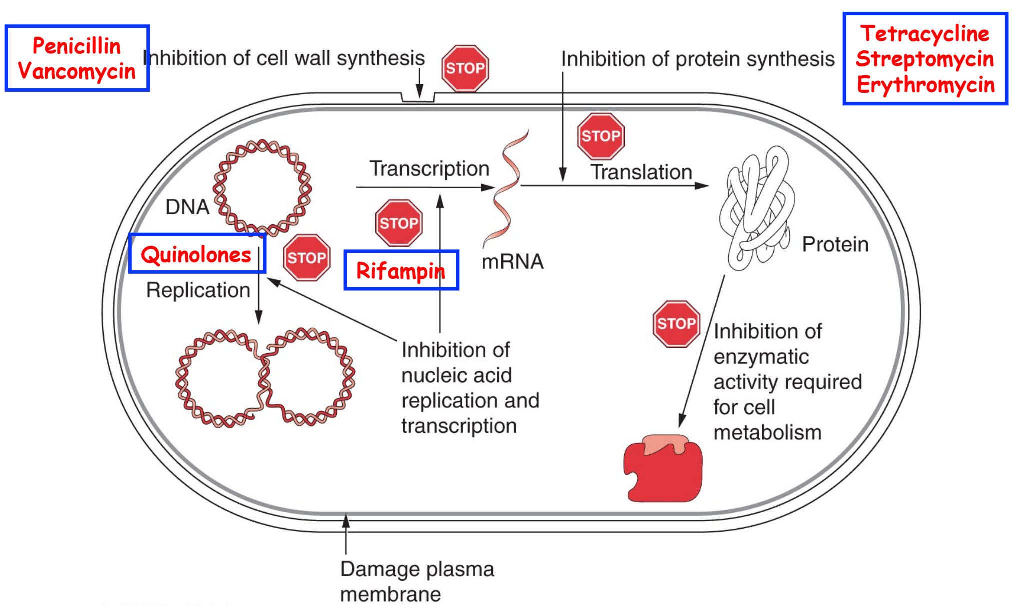
Methicillin Resistant
Staphlococcus aureus
MRSA!!

Scientific American, May, 2001

Antibiotic Resistance Is Also A Major Problem in Combating Pathogens



A Review.........How Do Antibiotics Kill Bacterial Cells?



By Inhibiting Basic Microbial Cell Processes

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

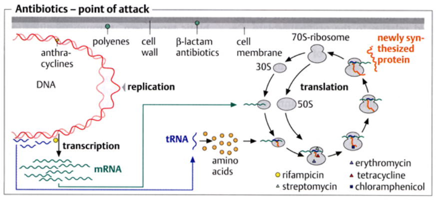
Systemic antibiotics (2001)

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

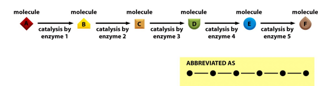
Classification by chemical structure

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



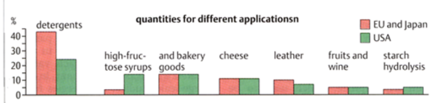
By Modifying Pathways Leading to Antibiotics In Bacterial Cells. But Need To Know Genes/Proteins in Pathway & By Finding Their Targets In Pathogens As Well



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 isomerization	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

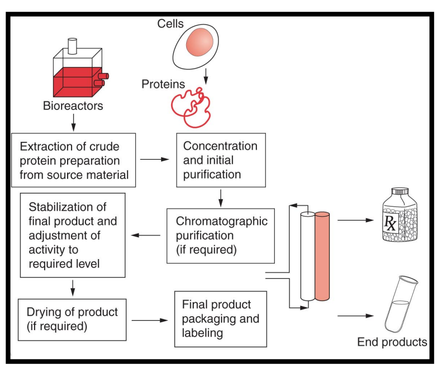


process/application	enzyme cost per unit quantity (US $)
starch liquefaction	ca. $ 2 per t starch
glucose from starch	$ 3.5 per t starch
isomerization of glucose	$ 6 per t starch
HFS in USA	$ 6–7 per t starch
ethanol	$ 1 per t starch
beer	$ 0.1 per 100 L
bakery goods USA	$ 0.1 per 100 kg flour
bakery 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 1 000 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

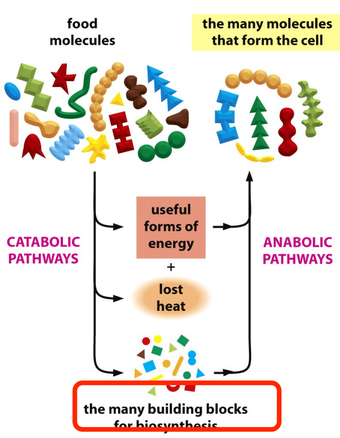
1. higher product quality
2. improved taste
3. better yields
4. reduced process costs
5. better filtration
6. better conservation
7. improved working conditions, reduced environmental load

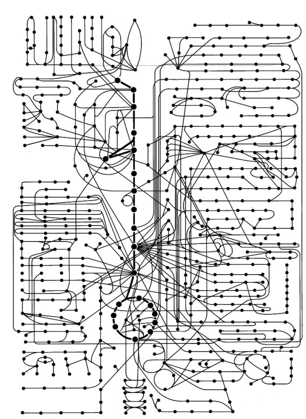
Specific Proteins and/or Metabolic Pathways Can Be Improved and/or Manipulated By Recombinant DNA!

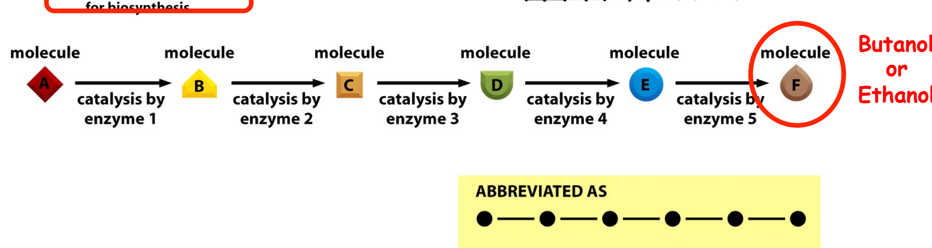


Industrial & Food Products

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]

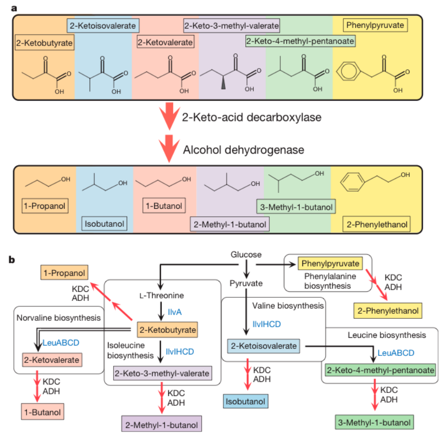


Figure 1 | Production of higher alcohols through the synthetic non-fermentative pathways. a, Various 2-keto acid precursors lead to corresponding alcohols through 2-ketoacid decarboxylase and alcohol dehydrogenase. **b**, The synthetic networks for the non-fermentative alcohol production in engineered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

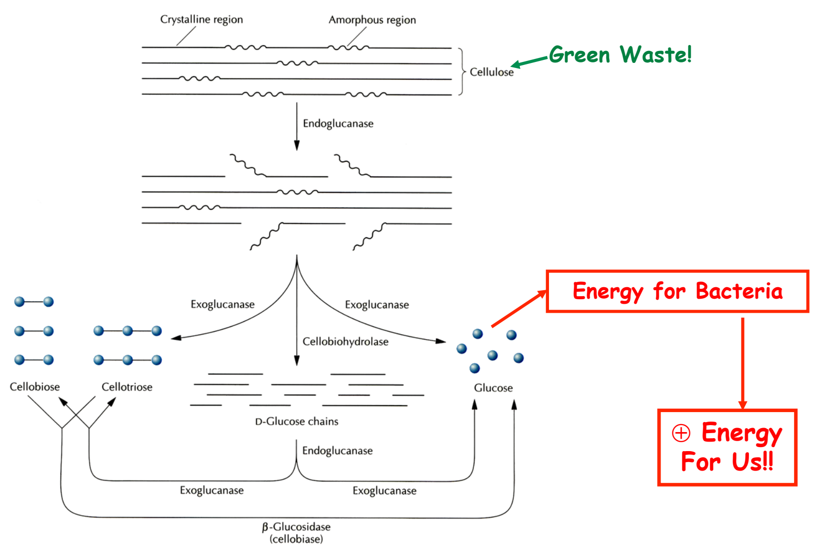


Figure 13.27 Enzymatic biodegradation of cellulose. Cellulose hydrolysis begins with the cleavage of β-1,4-linkages within the accessible amorphous regions of the cellulose chains by endoglucanase(s). This reaction is followed by the removal of oligosaccharides from the reducing ends of the partially 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

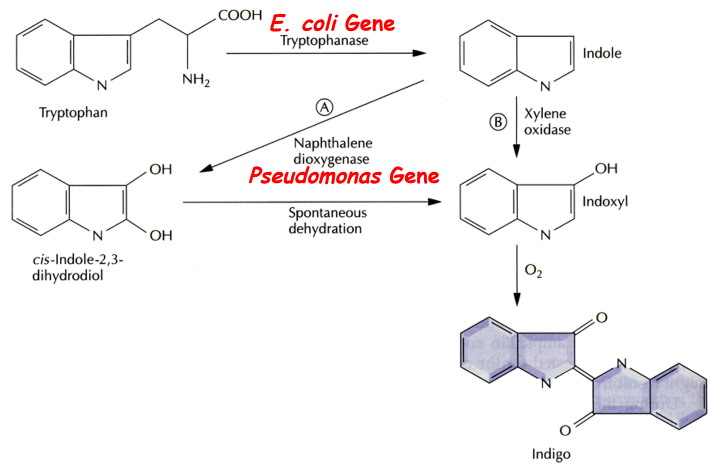
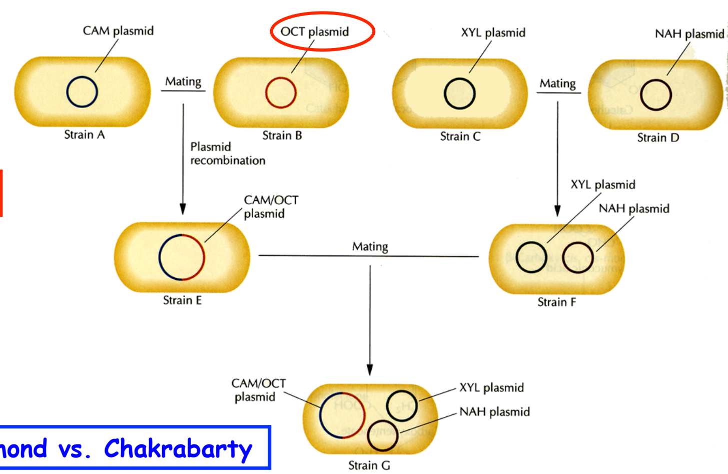


Figure 12.8 Indigo biosynthesis from tryptophan in genetically engineered *E. coli*. Tryptophanase is an *E. coli* enzyme. In pathway A, the naphthalene dioxygenase is derived from the NAH plasmid; in pathway B, the xylene oxidase is from the TOL plasmid. *E. coli* transformants that synthesize indigo 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

Pseudomonas



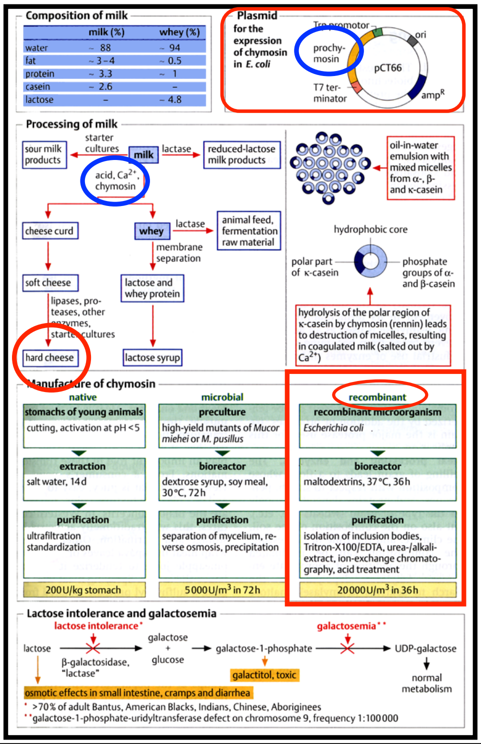
A Landmark Decision- Diamond vs. Chakrabarty

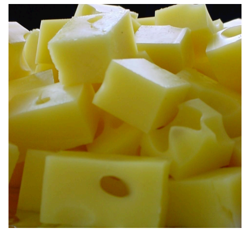
Chakrabarty US Patent 4,259,444 1981
Genetically Engineered Microorganisms Are "Inventions"

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.

Recombinant Chymosin Is Used To Make Cheese





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

Is Cheese A GMO?

FDA Approval of Cheese Made With Recombinant Chymosin

Extraordinary precautions were taken before chymosin, made by recombinant DNA technology, was marketed. Regulators ensured that no toxins of any kind had been introduced and that no live recombinant organisms were present. Indeed, the product contained nothing but pure chymosin. Cheese made with it is completely indistinguishable from that produced with animal rennet. In any case, chymosin itself is degraded during cheese making and none is left in the finished product. Today, in North America, over 80 percent of all cheese is made using chymosin produced by recombinant DNA technology. Cheese makers no longer have to worry about a shortage of calf stomachs and turophiles can satisfy their critical tastebuds. Thanks to biotechnology they can "say cheese" and smile.

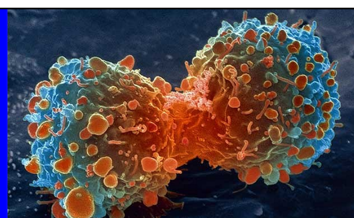
Chymosin In Cheese Making

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- ∴ <u>GRAS</u>- Generally Regarded As Safe & No Labeling Needed — Because Not An Additive & Not Different From Non-Recombinant Chymosin!!

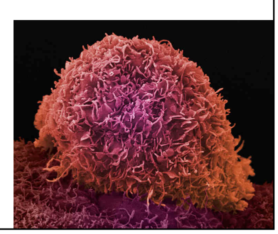
Is Cheese Made Using Recombinant Chymosin a GMO?

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

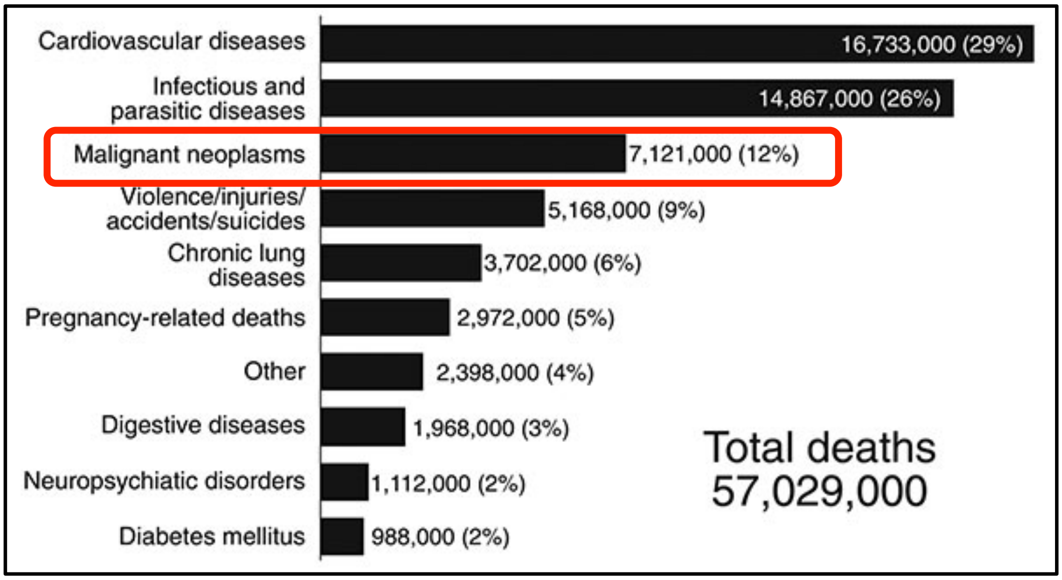
Why No Fuss?



Cancer Treatment & Discovery



Over 10% of Annual Deaths Word-Wide Are Caused By Various Types of Cancer



Frequency of Different Cancer Types

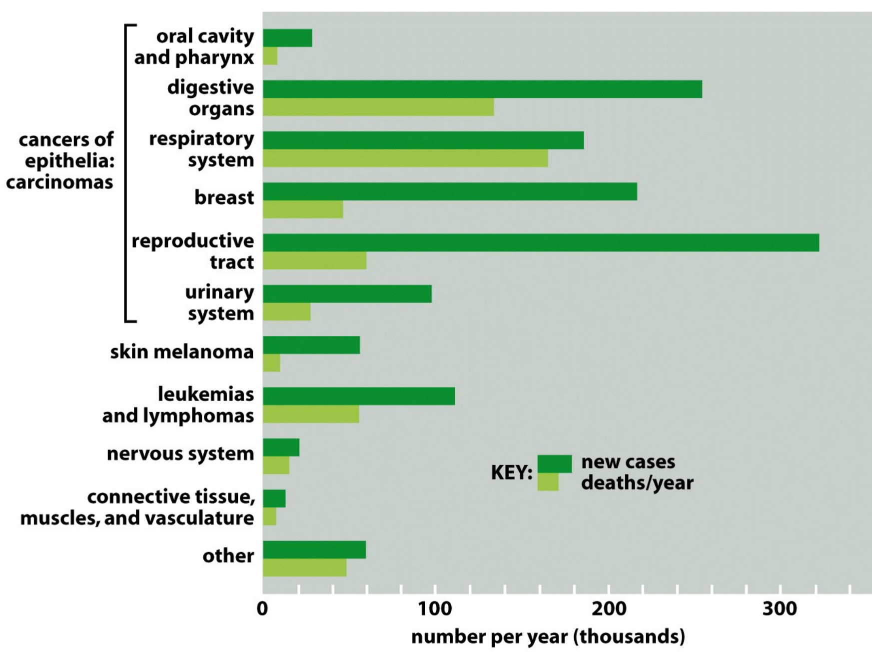


Figure 20-2 *Molecular Biology of the Cell* (© Garland Science 2008)

The Frequency of Cancer Increases With Age

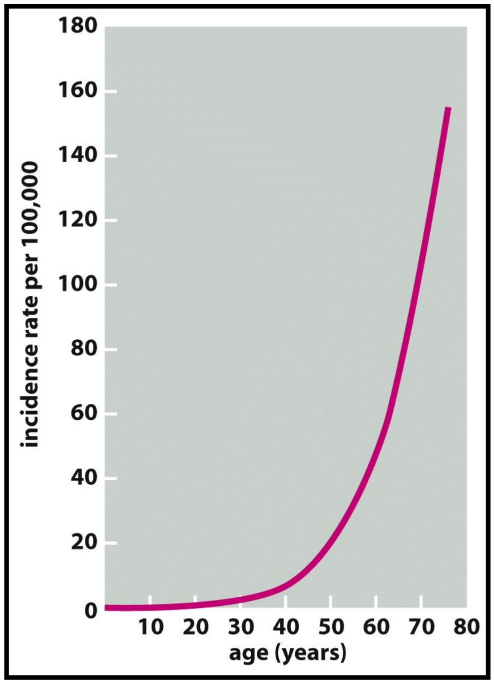
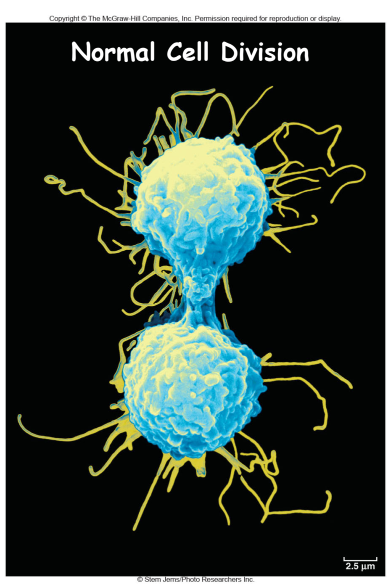
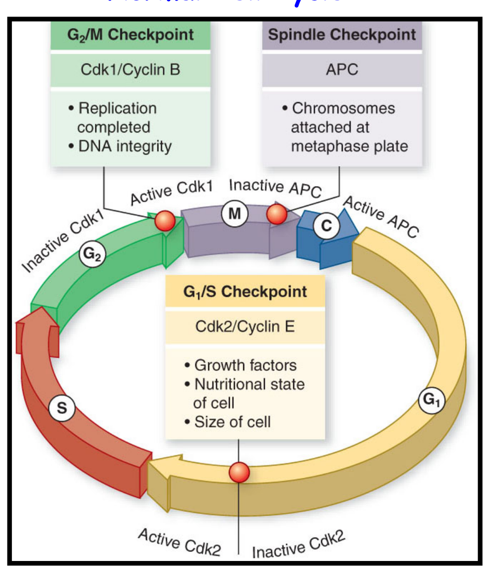


Figure 20-7 *Molecular Biology of the Cell* (© Garland Science 2008)

Vaccines Can Also Be Made To Treat Cancer - Checking Abnormal Cell Division



Normal Cell Cycle



Check Points Controlling Cell Division

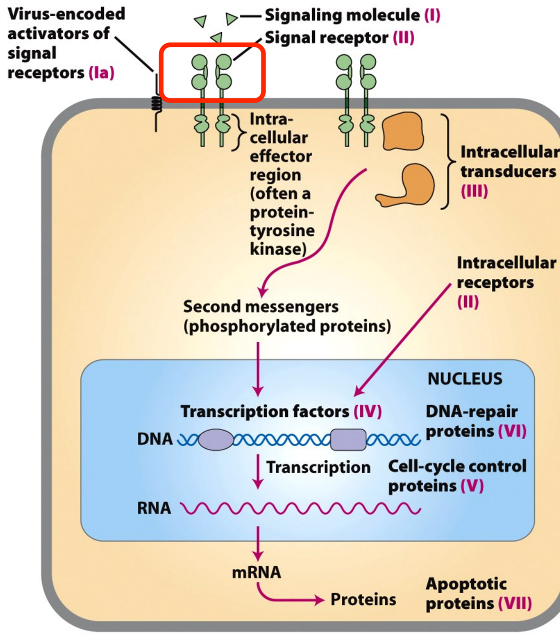


Figure 25-11
Molecular Cell Biology, Sixth Edition

Mutations in Check Point Genes/Proteins Lead To Cancer – Cancer is a "Gene Mutation" Disease

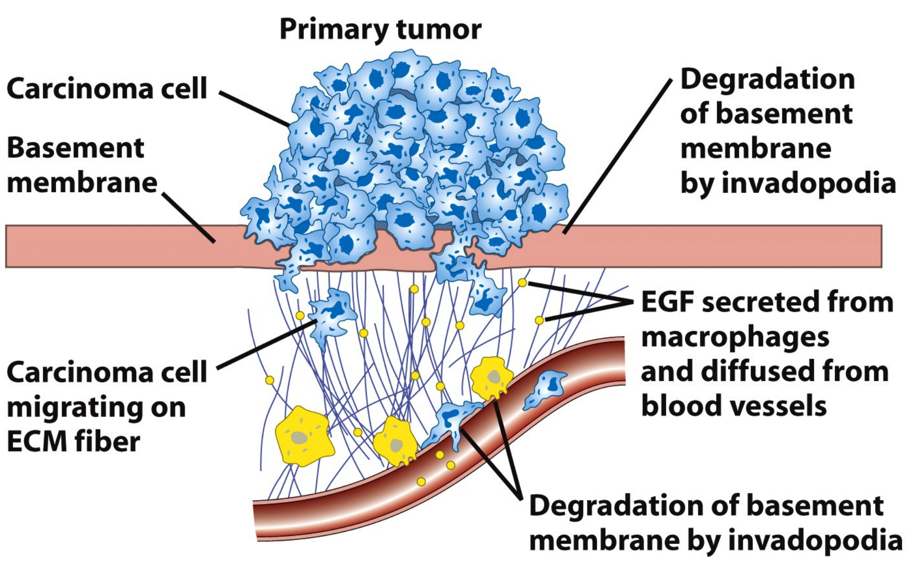
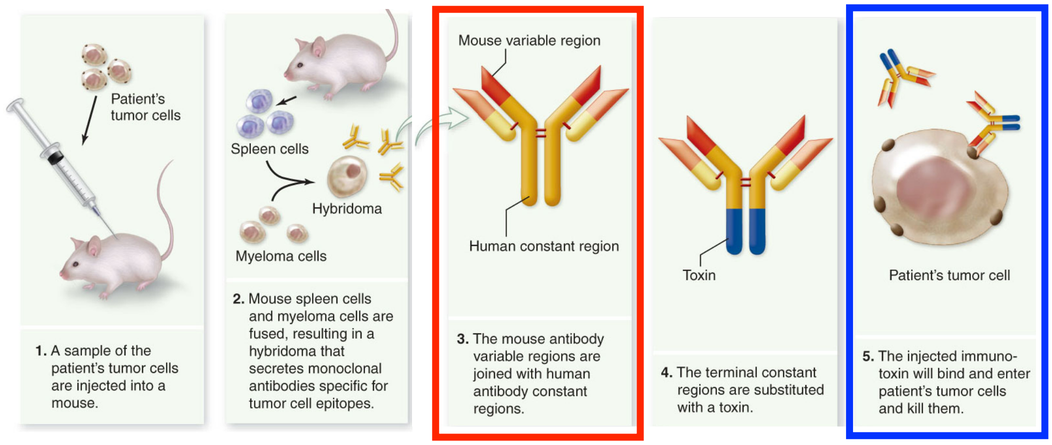
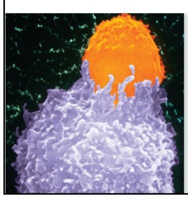


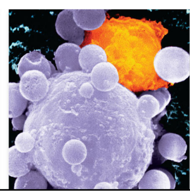
Figure 25-3a
Molecular Cell Biology, Sixth Edition

Recombinant Vaccines Are Being Developed To Fight Cancer

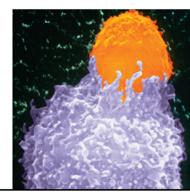


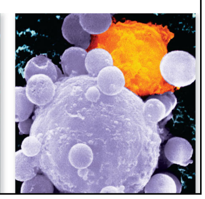
Genetic Engineering Step



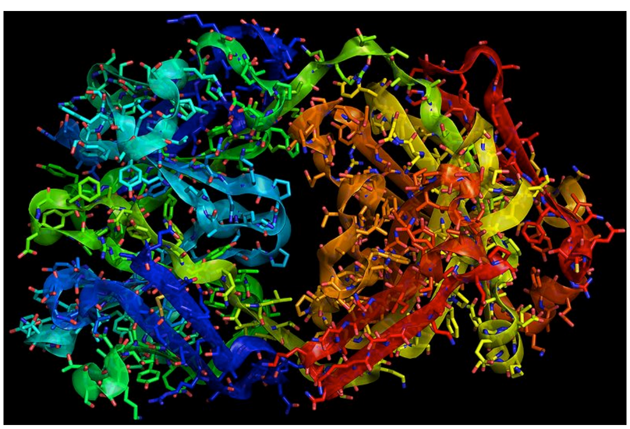


Cancer Cell Being Destroyed By T-Cell Containing Cancer-Cell-Specific Antibody

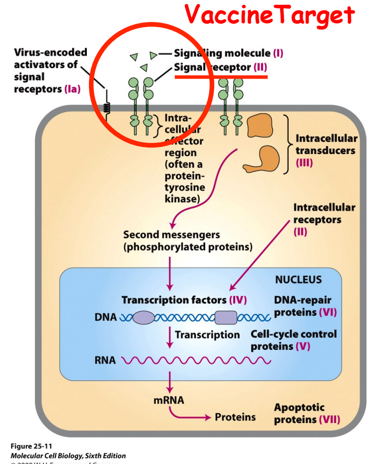




Using Herceptin® to Treat Breast Cancer



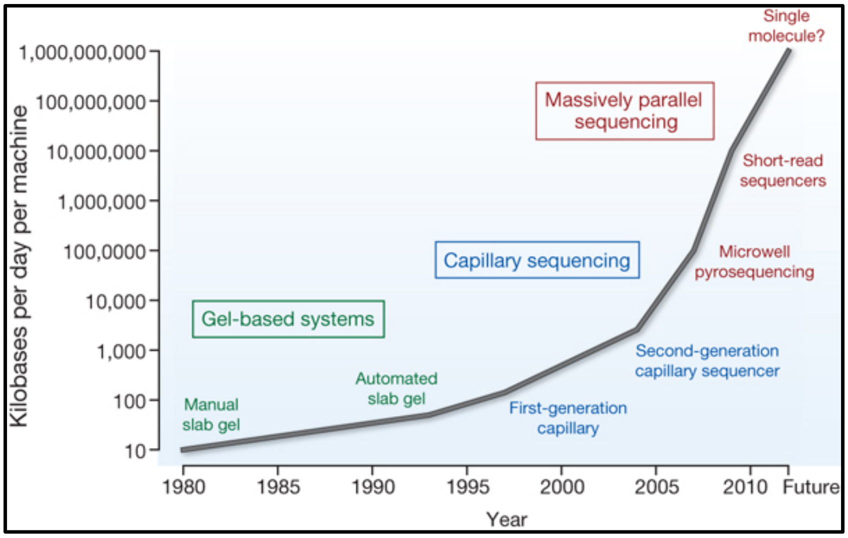
VaccineTarget



Trastuzumab® or Herceptin®

Dr. Dennis Slamon, UCLA Jonsson Cancer Treatment Center

The Cancer Genome Project Is Mapping Tumor-Specific Genes To Find Drug Targets



Sequencing Costs Have Dropped Exponentially Allowing the Genome Sequence of Specific Tumors – As They Progress – To Be Sequenced In Order To Identify The Mutated Genes Causing the Cancer

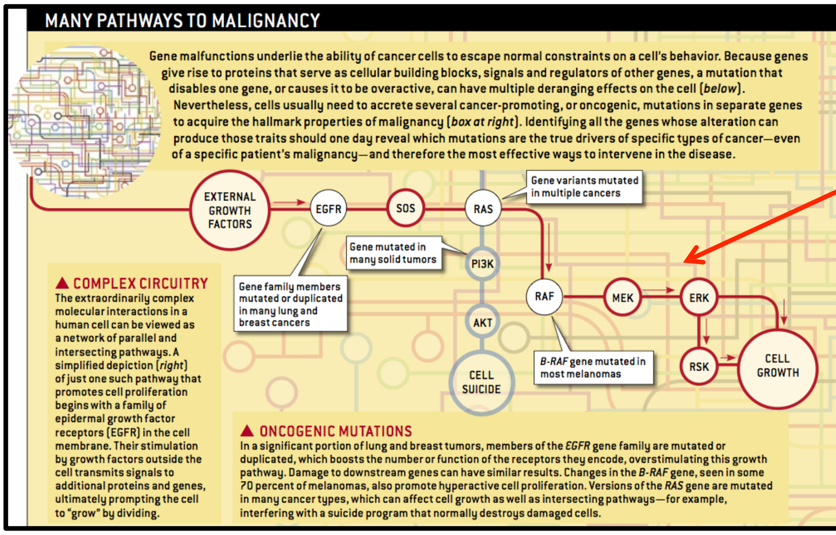
A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance[1], Philip J. Stephens[1], Sarah O'Meara[1,2], David J. McBride[1], Alison Meynert[3], David Jones[1], Meng-Lay Lin[1], David Beare[1], King Wai Lau[1], Chris Greenman[1], Ignacio Varela[1], Serena Nik-Zainal[1], Helen R. Davies[1], Gonzalo R. Ordoñez[1], Laura J. Mudie[1], Calli Latimer[1], Sarah Edkins[1], Lucy Stebbings[1], Lina Chen[1], Mingming Jia[1], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Adam Butler[1], Jon W. Teague[1], Jonathon Mangion[2], Yongming A. Sun[4], Stephen F. McLaughlin[5], Heather E. Peckham[5], Eric F. Tsung[5], Gina L. Costa[5], Clarence C. Lee[5], John D. Minna[6], Adi Gazdar[6], Ewan Birney[3], Michael D. Rhodes[4], Kevin J. McKernan[5], Michael R. Stratton[1,7], P. Andrew Futreal[1] & Peter J. Campbell[1,8]

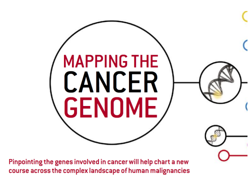
Cancer is driven by mutation. Worldwide, tobacco smoking is the principal lifestyle exposure that causes cancer, exerting carcinogenicity through >60 chemicals that bind and mutate DNA. Using massively parallel sequencing technology, we sequenced a small-cell lung cancer cell line, NCI-H209, to explore the mutational burden associated with tobacco smoking. A total of 22,910 somatic substitutions were identified, including 134 in coding exons. Multiple mutation signatures testify to the cocktail of carcinogens in tobacco smoke and their proclivities for particular bases and surrounding sequence context. Effects of transcription-coupled repair and a second, more general, expression-linked repair pathway were evident. We

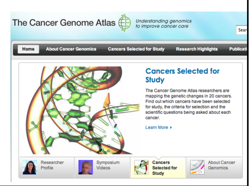
A comprehensive catalogue of somatic mutations from a human cancer genome

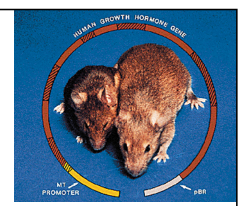
The Cancer Genome Project Is Mapping Tumor-Specific Genes To Find Drug Targets



Possible Drug Targets

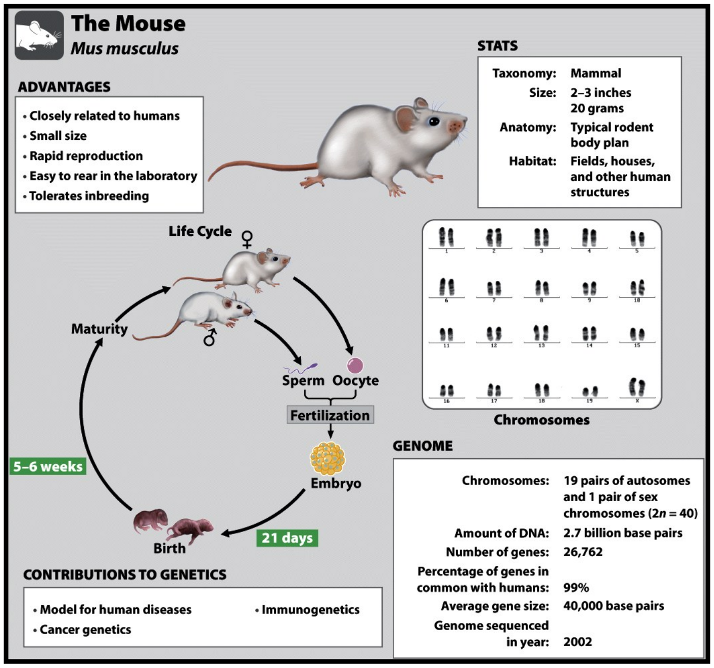




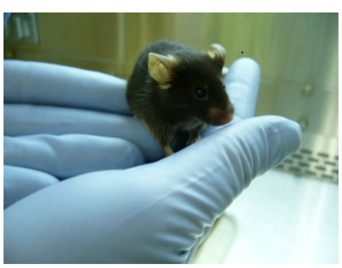


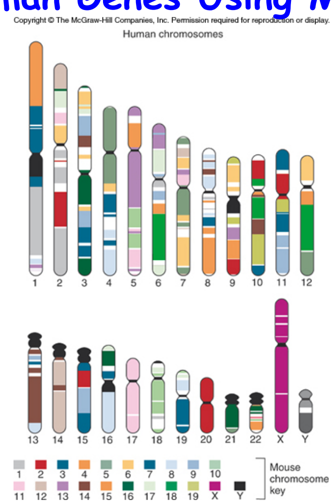
Animals (and Plants) Can Also Be Engineered For Applied Purposes

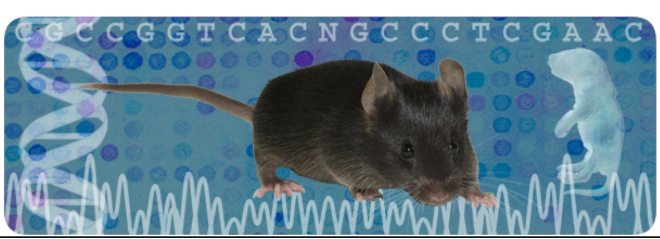
Using the Mouse as a Model to Determine the Function of Human Genes



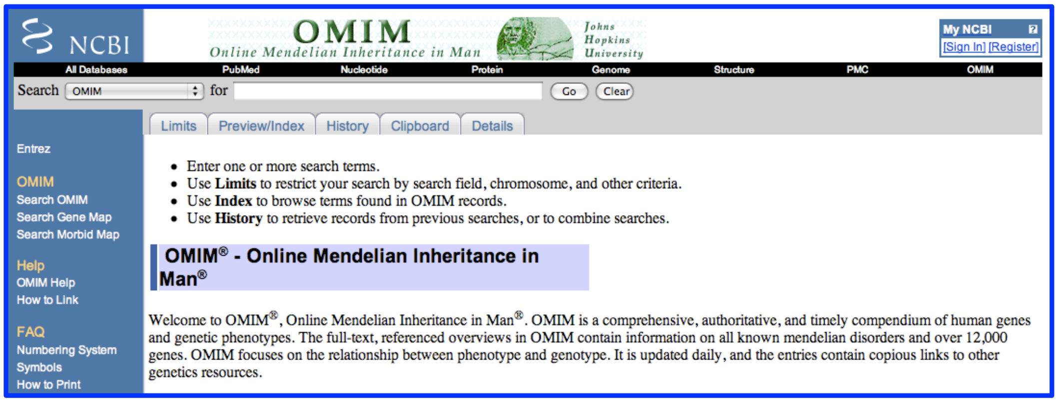
Human and Mouse Genomes 99% Similar ∴ Can Study Human Genes Using Mouse as a Model







How Many Human Disease Genes Have Been Identified?

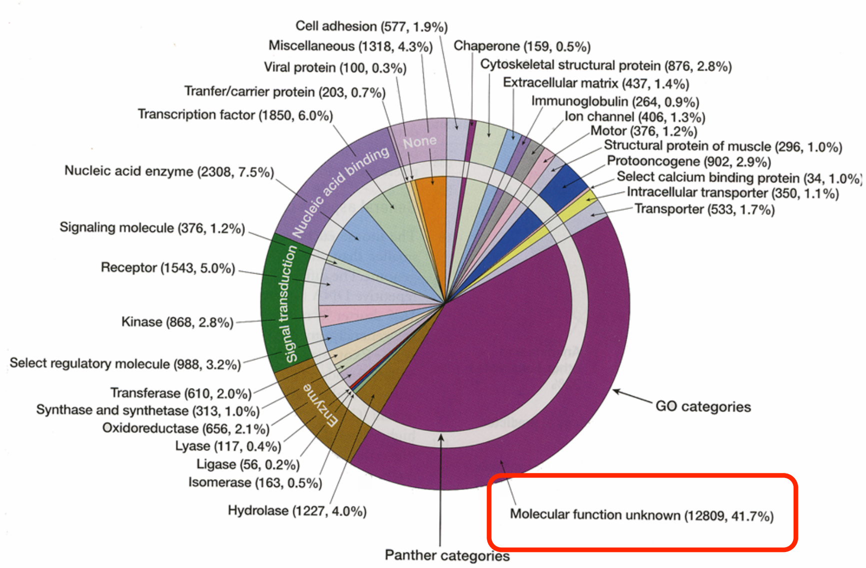


There are ~25,000 Genes in The Human Genome

1. ~4034 Genes Correlate With a Disease Phenotype
2. The Molecular Basis of These Genetic Diseases Are Known (e.g., Sickle Cell Anemia, Hemophilia A)
3. ~1717 Disease Genes – Molecular Basis Unknown

OMIM 2/13/14

The Human Genome Contains ~25,000 Different Genes



Do Not Know Functions of Most Human Genes!
How Find?

Genetic Engineering Mice and Other Mammals

1. Egg DNA Injection

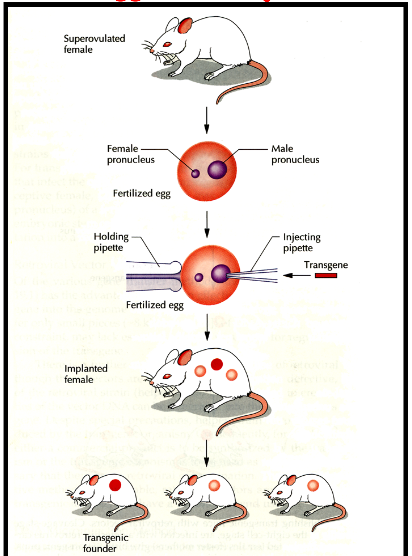


Figure 19.2 Establishing transgenic mice by DNA microinjection. Eggs are obtained from donor females that have been induced to superovulate and then mated with males. Purified samples of the transgene construct are microinjected into the male pronucleus of a fertilized egg. Implanted females (foster mothers) give birth to transgenic pups from which transgenic lines can be established.

2. Embryo Stem Cell DNA Transformation

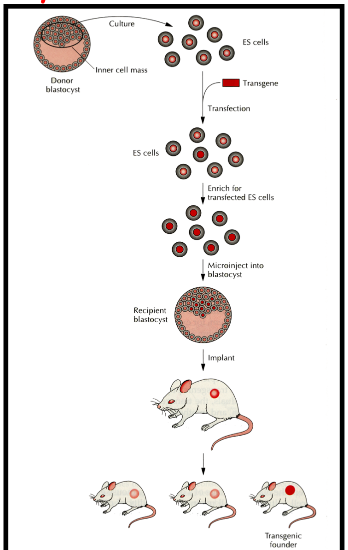
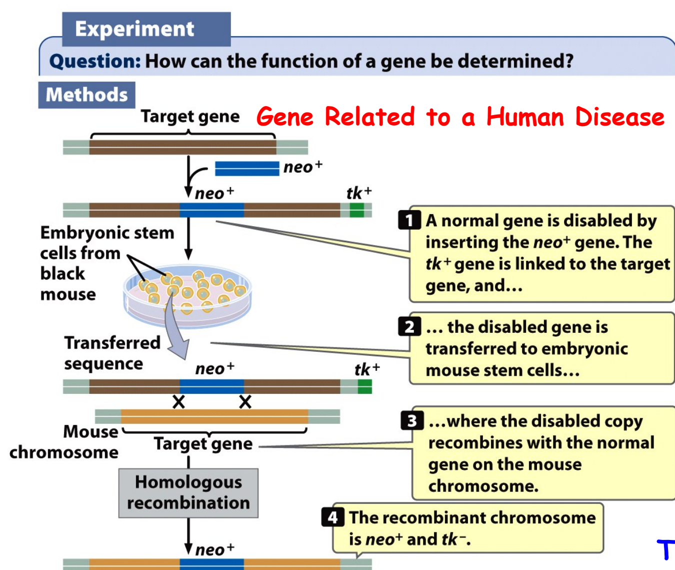


Figure 19.4 Establishing transgenic mice with genetically engineered ES cells. An ES cell culture is initiated from the inner cell mass of a mouse blastocyst. The ES cells are transfected with a transgene. After growth, the transfected cells are identified by either the positive-negative selection procedure or PCR analysis. Populations of transfected cells can be cultured and inserted into blastocysts, which are then implanted into foster mothers. Transgenic lines can be established by crosses from founder mice that carry the transgene in their germ lines.

Both Methods Alter the Germ Line (i.e., Genes are Inherited)

Mouse Genes Related to Those in the Human Genome Can be "Knocked Out" In Order to Determine Their Functions



Gene Related to a Human Disease Gene

Figure 19-35 part 1
Genetics: A Conceptual Approach, Third Edition

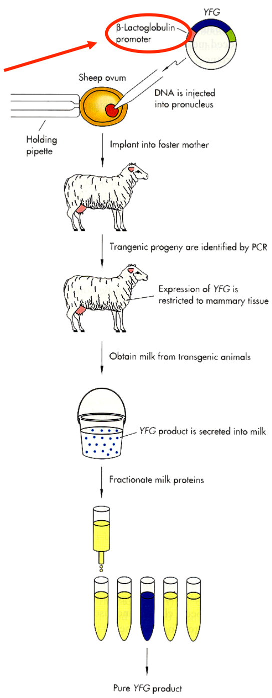
Insertional Mutagenesis (Similar to Mutating the *β-gal* gene Producing White Colonies)

Mutant Gene

Transfer to Mouse & Observe Phenotype

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

Where is Switch Active?



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 fermentors + 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

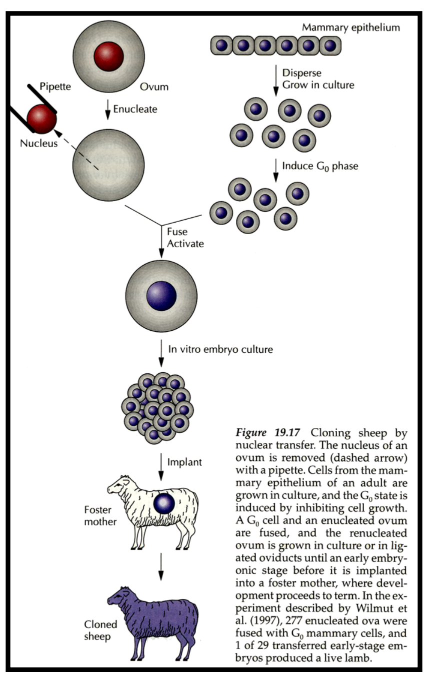


Figure 19.17 Cloning sheep by nuclear transfer. The nucleus of an ovum is removed (dashed arrow) with a pipette. Cells from the mammary epithelium of an adult are grown in culture, and the G_0 state is induced by inhibiting cell growth. A G_0 cell and an enucleated ovum are fused, and the renucleated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

Examined Data From Seven Generations of Genetically Engineered Goats

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.

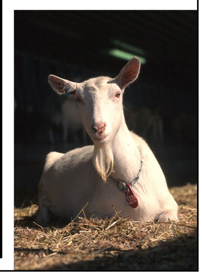
MODIFYING THE DNA A human gene that produces the blood protein antithrombin is inserted into a short strand of goat DNA.

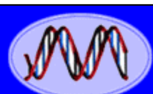
IMPLANTING THE DNA The modified DNA is injected into the nucleus of a fertilized goat egg, which is then implanted into a female.

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.

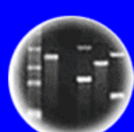
Sources: GTC Biotherapeutics





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

If Cloning Humans Was 100% Safe and "Normal" Humans Could be Produced at the Same Percentage as Doing It "Naturally," I Would Not Object To Individuals Cloning Themselves, Parents, Children, or Whomever They Wanted to Clone:

a. Yes
b. No

Making Recombinant Human Proteins in Animals

Table 19.3 Some exogenous proteins that have been expressed in the mammary glands of transgenic animals

- Antithrombin III
- Calcitonin
- Erythropoietin
- Factor IX
- Factor VIII
- Fibrinogen
- Glucagon-like peptide
- Granulocyte colony-stimulating factor
- Growth hormone
- Hemoglobin
- Human serum albumin
- Insulin
- Insulin-like growth factor 1
- Interleukin 2
- Lactoferrin
- Lysozyme
- Monclonal antibodies
- Nerve growth factor β
- Protein C
- Superoxide dismutase
- Tissue plasminogen activator
- α1-Antitrypsin
- α-Glucosidase
- α-Lactalbumin

Advantages over Bacteria?

Table 19.2 Milk production and estimated recombinant protein yields from organisms used for the expression of transgenes in mammary glands

Organism	Annual milk yield (liters)	Estimated recombinant protein per female (kg/yr)
Rabbit	5	0.02
Pig	300	1.5
Sheep	500	2.5
Goat	900	4
Cow	10,000	60 !!!

Making Transgenic Mammals is an Inefficient Process

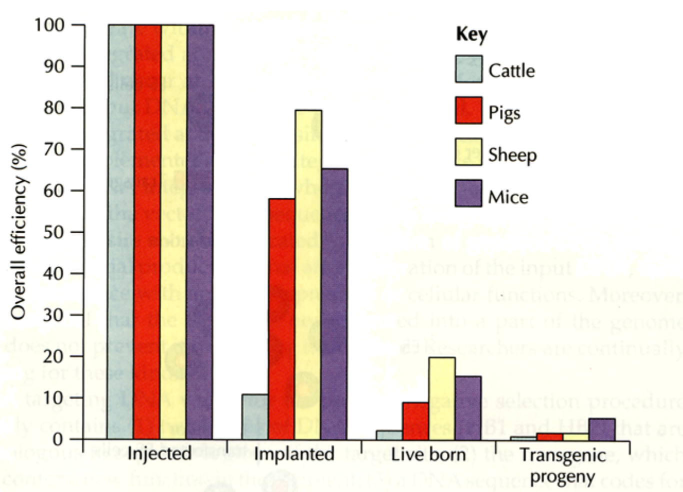


Figure 19.3 Overall efficiency of the transgenesis process after DNA microinjection. All the fertilized eggs (100%) of cattle, pigs, sheep, and mice are inoculated with a transgene, but the success of implantation and giving birth to offspring is much lower, and only 5% or fewer of the treated eggs become transgenic progeny.

Hypothesis For Inefficiency?

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.

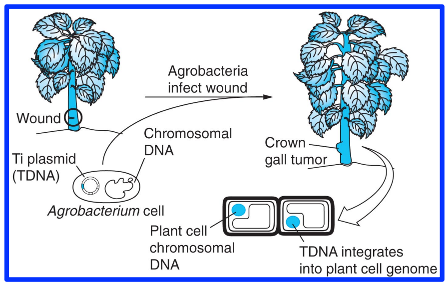
PLANTS IN THE PIPELINE

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?



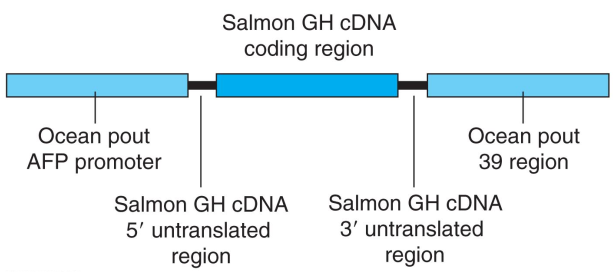
Other Transgenic Animals Have Been Generated

TABLE 2.1 State of the art of transgenic technology for selected organisms.

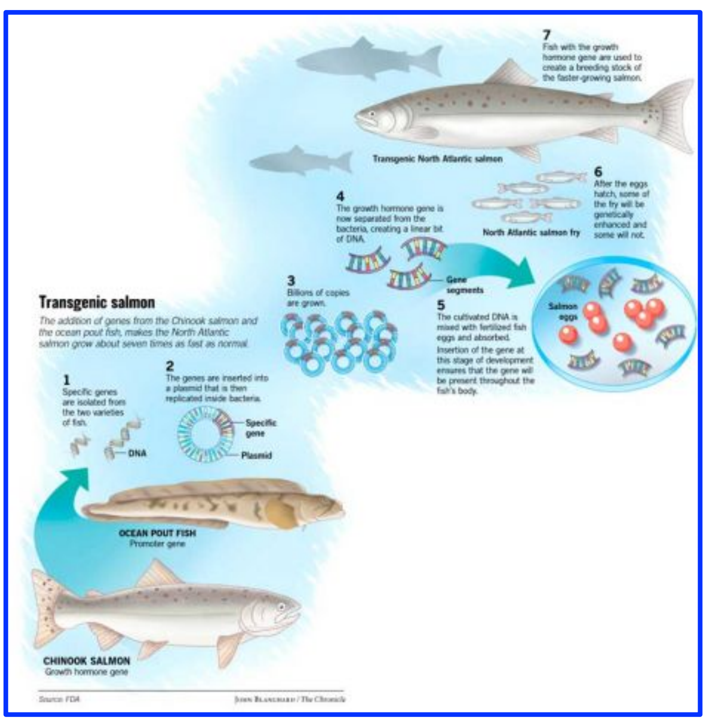
Organism	Transfection	Viral vectors	Transposon	ES cells	Nuclear transfer
Mouse	4[a]	2	1	4[a]	2
Cow	3	1	0	0	2
Sheep	3	0	0	0	2
Goat	3	0	0	0	2
Pig	3	0	0	0	2
Rabbit	3	0	0	1	0
Chicken	1	2	1	0	0
Altlantic salmon	3	0	0	0	0
Channel catfish	2	0	0	0	0
Tilapia	3	0	0	0	0
Zebrafish	1	0	0	1	1
Crustaceans	1	1	0	0	0
Mollusks	1	1	0	0	0
Drosophila	2	2	2	2	0
Mosquito	1	0	2	0	0

NOTE:
0: No significant progress.
1: Has been accomplished experimentally (proof of concept).
2: Routine experimental use.
3: Commercialization sought.
4: Widespread production.
[a] For experimental uses.
See (Dove, 2000)

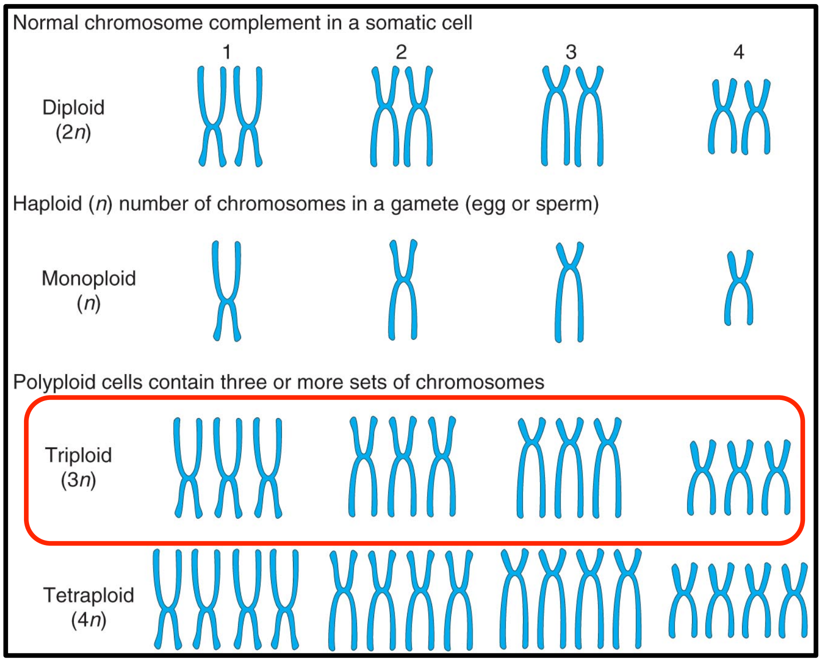
Genetic Engineering Fast Growing Salmon



Producing Genetically Engineered Salmon



Genetically Engineered Salmon Are Sterile



Producing Genetically Engineered Salmon

FOOD SCIENCE

Politics holds back animal engineers

Funds and approvals lag for transgenic livestock in US.

OFF THE TABLE
A brief history of some of the genetically engineered food animals submitted to the US Food and Drug Administration (FDA) for review. No such animal has yet been approved.

Animal	Purpose	Created	History
Salmon	Grows to market size faster than conventional salmon	1989 (Massachusetts)	1995 FDA receives application 2008 Fish farm moved to Panama 2010 Cleared by FDA scientific advisory panel
Pig	Produces more milk to nurse healthier young	1993 (Illinois)	1999 FDA receives application
Goat	Milk has human lysozymes to treat diarrhoeal disease	1999 (California)	2003 Funding denied by USDA 2008 FDA receives application 2011 Research moved to Brazil
Pig	Efficiently digests plant phosphorus, reducing pollution	1999 (Ontario, Canada)	2007 FDA receives application 2012 Pigs killed owing to lack of commercial interest
Cow, sheep, goat, pig	Increased muscle mass without reduced fertility	2010 (Texas)	2009 FDA receives application

FDA expected to approve Genetically Modified Salmon

AQUAADVANTAGE SALMON | JANUARY 3, 2013 | BY: MARK WACHTLER |

How To Assess Risk?

TABLE 2-1 Systematic Risk Assessment and Management

Step	Key questions
Hazard identification	What event posing harmful consequences could occur?
Risk analysis	How likely is the hazard? What would be the harms from realization of the hazard, and how severe are they, taking into account social values? What is the risk assessment as shown on a matrix of risk (likelihood of harm) plotted against severity of harm; see Figure 2-1, above)? Each cell of the matrix should be accompanied by a qualitative assessment of the response and a quantification of assurance needed to reduce harm if the cell's conditions were to occur. How well established is the knowledge used to identify the hazard, estimate its risk, and predict harms?
Risk reduction planning and implementation	What can be done (including bioconfinement and other confinement) to reduce risk, either by reducing the likelihood or mitigating the potential harms? Are there steps that can be taken to prepare for remediation?
Risk tracking (monitoring)	How effective are the implemented measures for risk reduction? Are they as good as, better than, or worse than planned? What follow-up, corrective action, or intervention will be pursued if findings are unacceptable? Did the intervention adequately resolve the concern?
Remedial action	What remedial action should be taken?
Transparency and public participation	How transparent should the entire process be? How much and what type of participation should there be in the steps above (and in risk characterization) by the public at large, by experts, and by interested and affected parties?

NRC Report — Biological Confinement of Genetically Engineered Organisms (2004)

What Are The Issues?

MYTH 1: *Transgenic salmon grow much larger than other salmon - so much so that they could gain a mating advantage or outcompete native salmon for food or space.*

FACT: Transgenic salmon grow **faster** than other salmon but they do not grow any larger by the time they reach maturity.

FACT: Male salmon do not gain a mating advantage because of size. In fact, "precocious parr," only 6 inches in length, father about one-fifth of each new generation before they go to sea. Studies of escaped farmed salmon, which are almost always larger than wild fish, have found them to mate successfully only **3 percent** as often as native salmon.

FACT: Farmed salmon are trained to eat fish feed -- small, dry pellets that look exactly like the "dog chow" we feed our family pets. If they escape, they look for something similar. Most don't find it. More than 85 percent of the farm escapees caught off British Columbia and Alaska had **no food** in their bellies. In a 1999 study, the Washington State Department of Ecology found farm escapees to be **eating tree bark** in local rivers, because it apparently looked like fish feed. Transgenic salmon may forage even more poorly because they lack the critical swimming speed to pursue prey, deplete their energy reserves more quickly and expose themselves to predators more often in the search for food.

MYTH 2: *If transgenic salmon do breed successfully with native fish, their novel gene will escape into the wild gene pool and destroy native salmon populations. Researchers at Purdue University found that only 60 transgenic salmon could drive a wild population to extinction.*

FACT: The U.S. Food and Drug Administration will not approve the use of transgenic salmon unless they can be demonstrated to be **sterile**. Aqua Bounty Farms has stipulated that it will market **only sterile, all female transgenic salmon**. There can be no gene flow to wild salmon because sterile fish cannot reproduce.

And will be grown in cages far from natural salmon habitats!

FACT: Muir and Howard, the Purdue scientists who proposed the "Trojan Gene Hypothesis," did not study transgenic salmon. They designed a mathematical model based on the behavior of Japanese medaka, a small, freshwater fish that matures in 56 days and breeds daily until it dies. Salmon take three, five and even ten years to mature and most breed only once in their lifetimes. Sterile salmon do not breed at all.

MYTH 3: *Sterilization is not 100 percent effective so we can't be sure that transgenic salmon will really be sterile.*

FACT: Triploidy produces complete, 100 percent sterilization in female salmon because it prevents the development of the ovaries needed to produce eggs. The only uncertainties about the technique have been raised in the context of male salmon, grass carp and oysters. There is no scientific debate over the complete sterility of triploid female salmon.

FACT: Scientists can test for triploidy by scanning blood or embryonic fluids in a flow cytometer. The sterility of every batch of transgenic salmon eggs can be verified before they ever leave the hatchery.

MYTH 4: *Transgenic salmon are voracious predators that will consume all the available food in an ecosystem and will prey on native juveniles.*

FACT: Transgenic salmon actually consume **less food** per pound of weight gained because they process their food 10 to 30 percent more efficiently.

FACT: Transgenic salmon may be highly prone to starvation in the natural environment as they learn to identify and hunt for wild food. They maintain a higher metabolic level for a longer period of time in food deprivation studies, and deplete their energy reserves more quickly than do standard salmon.

FACT: Any food competition would occur in the marine environment because sterile transgenic salmon cannot produce the juveniles that occupy freshwater habitat. In the marine life stages, transgenic salmon would compete with older native salmon of about the same size. Because food availability is not limiting in the marine environment, transgenic salmon would gain no advantage from their higher feeding motivation.

FACT: Sterile female salmon do not engage in spawning behaviors and almost never return to freshwater habitat after they begin to feed at sea. Native juveniles are confined to freshwater habitat. Any predation risk would, therefore, be lower than now occurs in conventional salmon aquaculture. There is no evidence of predation by current farm escapees on native juveniles.

MYTH 5: *Transgenic salmon produce antifreeze proteins and excessive amounts of growth hormone.*

FACT: Transgenic salmon produce no antifreeze proteins. Only the molecular "switch" from the antifreeze gene is used.

FACT: Transgenic salmon produce the same amount and kind of circulating growth hormone as wild-type salmon, but they produce it through the entire year.

GM salmon: FDA's assessment of environmental risks

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. FDA–2011–N–0899]

Draft Environmental Assessment and Preliminary Finding of No Significant Impact Concerning a Genetically Engineered Atlantic Salmon; Availability

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

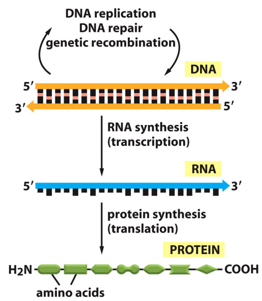
SUMMARY: The Food and Drug Administration (FDA, the Agency) is announcing the availability for public comment of the Agency's draft environmental assessment (EA) of the proposed conditions of use specified in materials submitted by AquaBounty Technologies, Inc., in support of a new animal drug application (NADA) concerning a genetically engineered (GE) Atlantic salmon. Also available for comment is the Agency's preliminary finding of no significant impact (FONSI) for those specific conditions of use.

Both documents -- an environmental assessment and preliminary "finding of no significant impact," known by the policy wonks as a FONSI -- will be published Dec. 26 in the Federal Register and be available for public comment for 60 days. 12/26/12

The assessment focused on the environmental questions. Food safety came earlier. Back in 2010, the FDA concluded that the salmon "is as safe as food from conventional salmon, and there is a reasonable certainty of no harm from consumption." For example, the flesh of the fish contain no more growth hormone than regular Atlantic salmon, the FDA said -- a concern of opponents to the fish because of the manner in which they were genetically engineered.

How Genes Work & What Are Genes In Context of…

Thinking About The Consequences of GMOs



Need Science-Based Questions & Science-Based Solutions-NOT OPINIONS!

1. What is a Gene?
2. What is the Anatomy of a gene?
3. How Does the Gene Replicate?
4. How Does the Gene Direct Synthesis of a Protein?
5. Does the Gene Work Independently of other Genes?
6. What is the Sequence & Structure of the Protein?
7. How does it work in cell?
8. Does the Protein Structure imply any Potential "Harm"?
9. Does the Gene Change the Organism? Fitness?
10. Is the Environment Harmed?
11. Does the "Benefit" Outweigh the Cost?