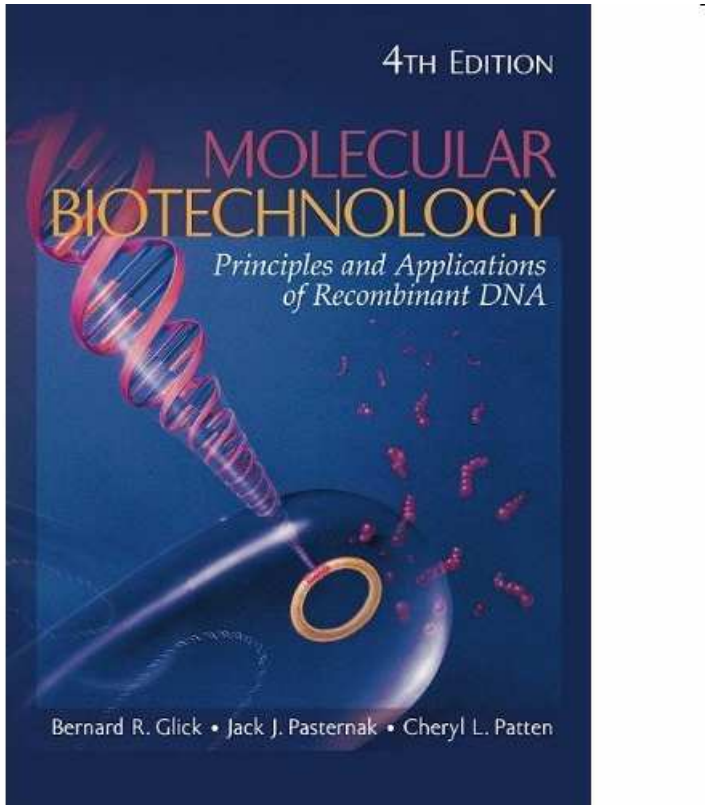
Genetic a protein engineering

- Book: Glick, Pasternak, Molecular Biotechnology, *Principles and application of recombinant DNA*
- 4th edition 2010



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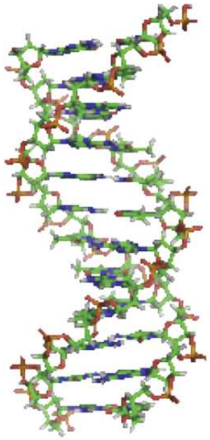
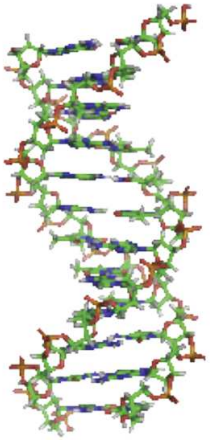
Lab
?

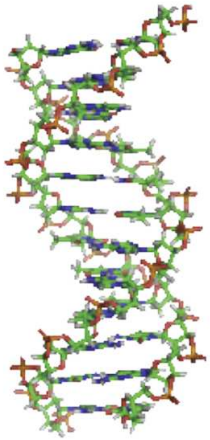
Exam
written, 20 questions, 90 minutes

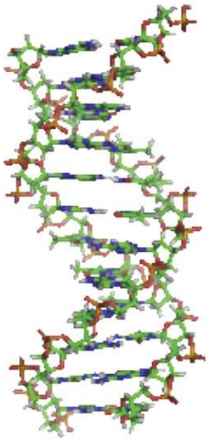
What is genetic engineering?



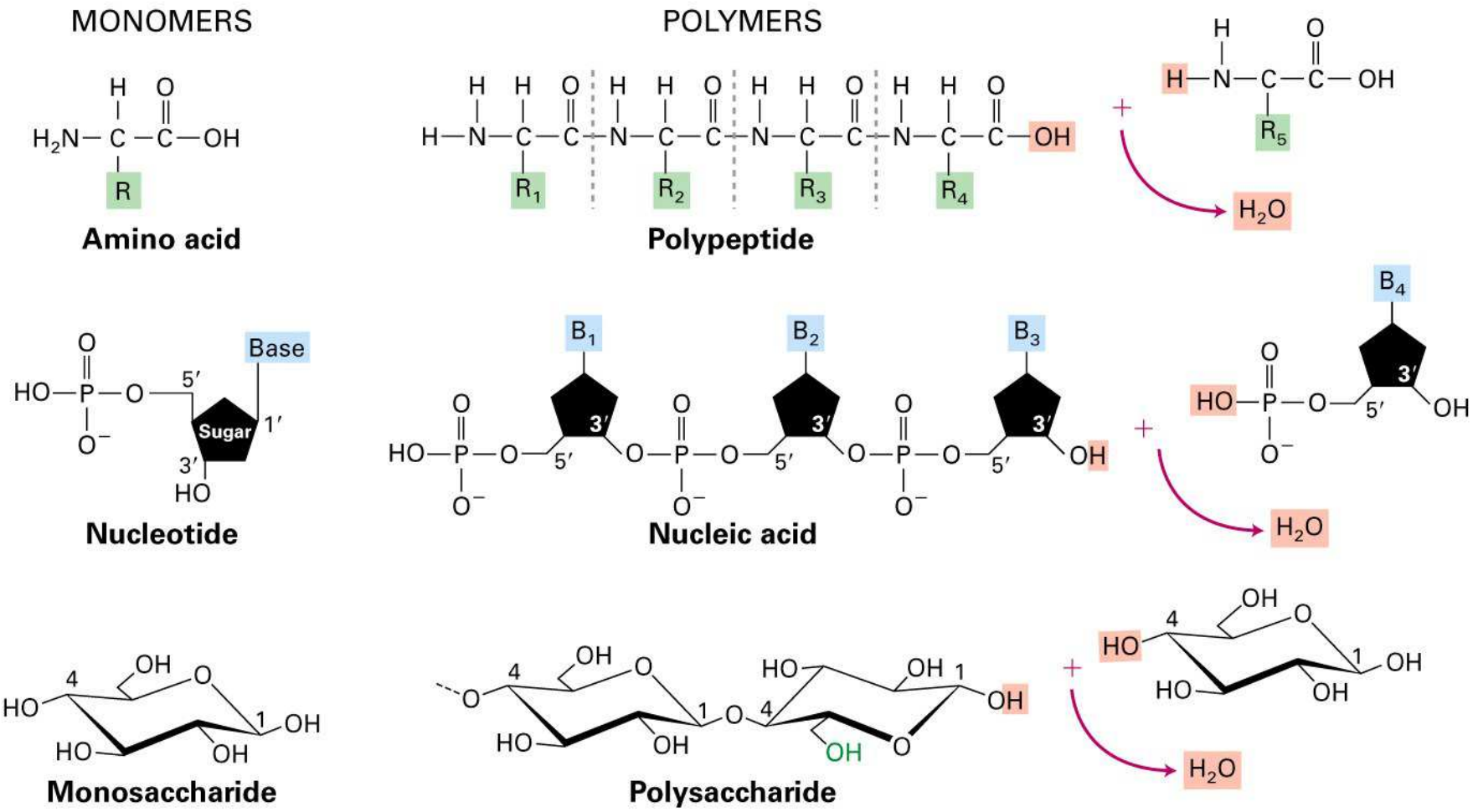


Genetic Engineering Is Manipulating DNA!



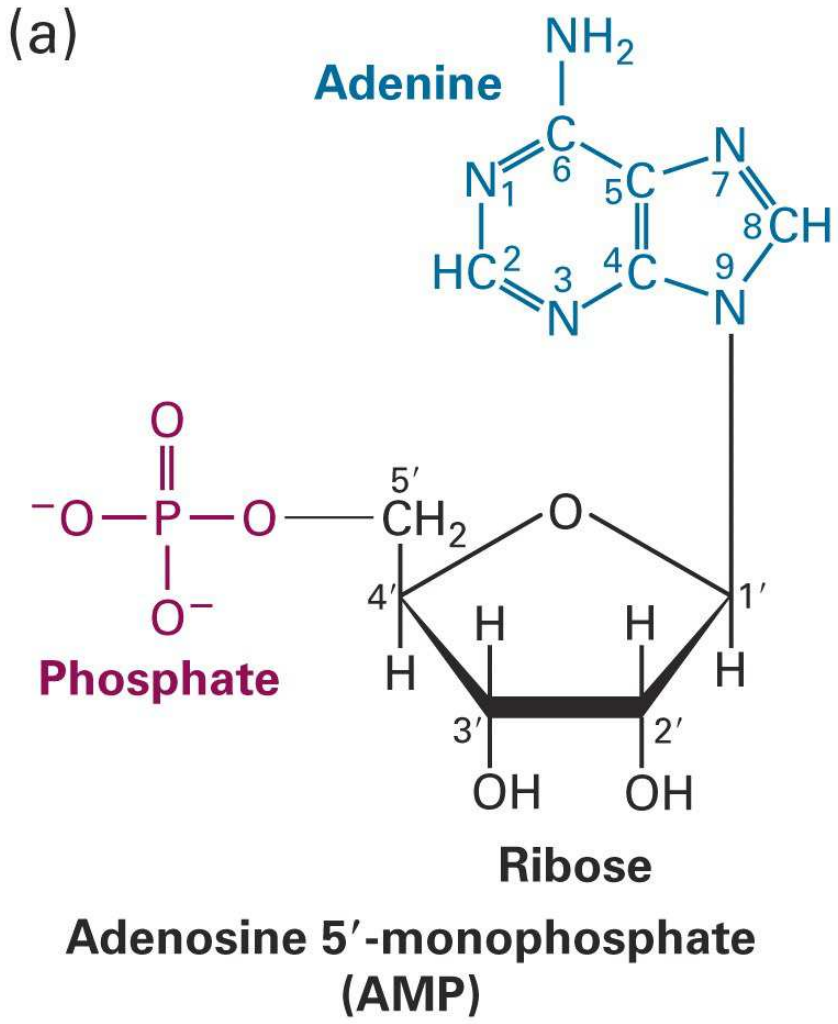


DNA?

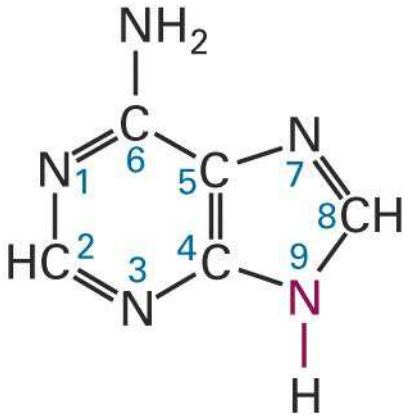


condensation
dehydration

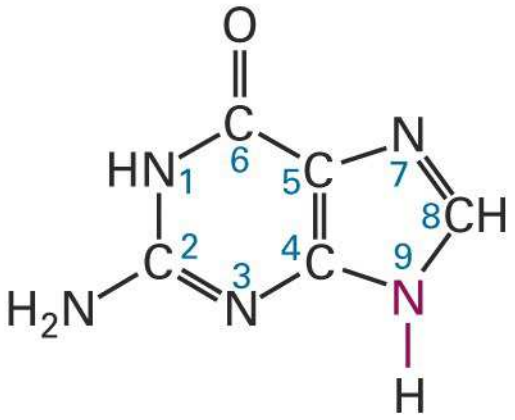
Nucleic Acids – DNA and RNA



PURINES

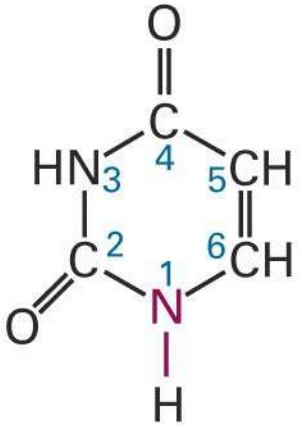


Adenine (A)

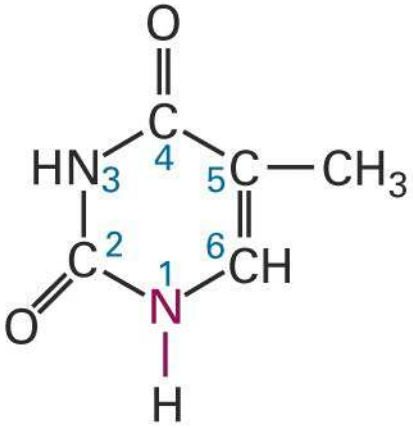


Guanine (G)

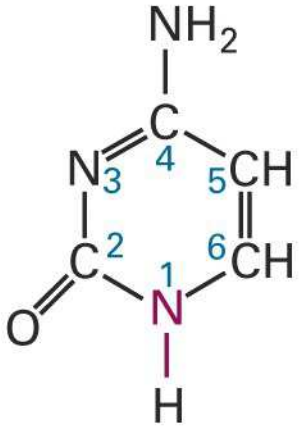
PYRIMIDINES



Uracil (U)

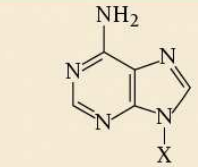
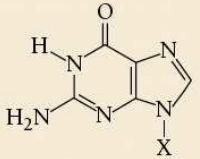
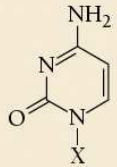
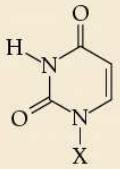
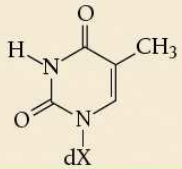


Thymine (T)



Cytosine (C)

Table 3-1 Names and Abbreviations of Nucleic Acid Bases, Nucleosides, and Nucleotides

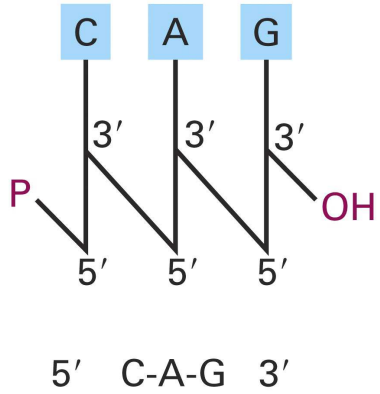
Base Formula	Base (X = H)	Nucleoside (X = ribose[a])	Nucleotide[b] (X = ribose phosphate[a])	
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP	DNA + RNA
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP	DNA + RNA
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP	DNA + RNA
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP	RNA
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP	DNA

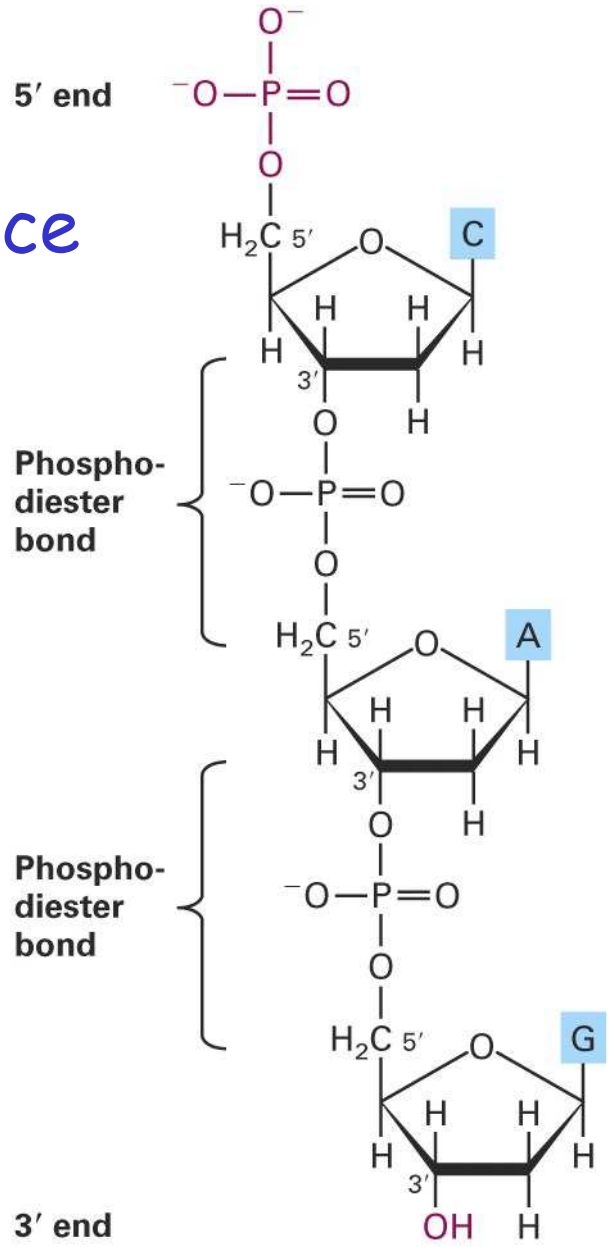
[a]The presence of a 2′-deoxyribose unit in place of ribose, as occurs in DNA, is implied by the prefixes "deoxy" or "d." For example, the deoxynucleoside of adenine is deoxyadenosine or dA. However, for thymine-containing residues, which rarely occur in RNA, the prefix is redundant and may be dropped. The presence of a ribose unit may be explicitly implied by the prefix "ribo."

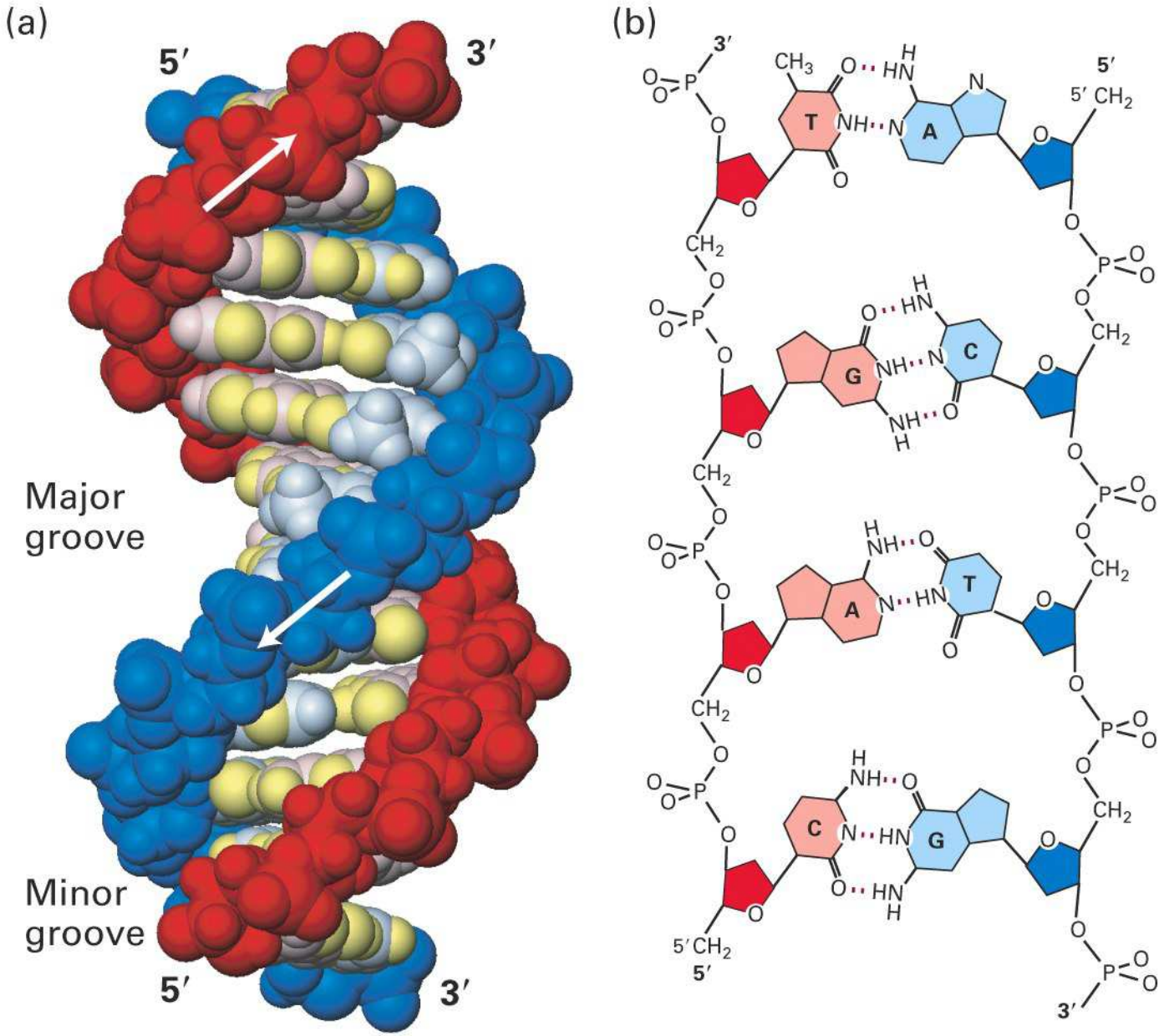
[b]The position of the phosphate group in a nucleotide may be explicitly specified as in, for example, 3′-AMP and 5′-GMP.

Table 3-1 Fundamentals of Biochemistry, 2/e

DNA structure -> sequence





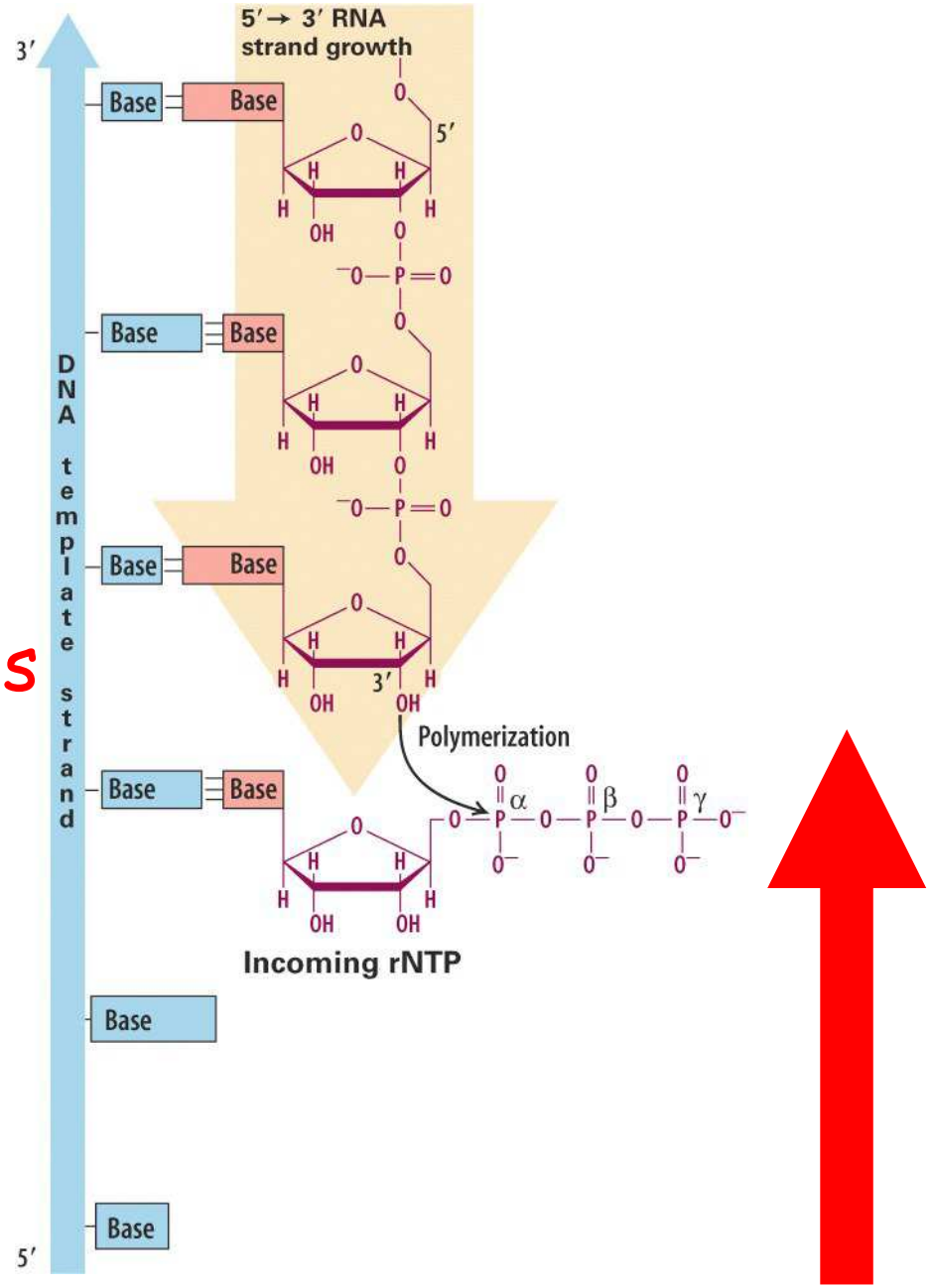

(a)
5′
3′
Major groove
Minor groove
5′
3′
(b)
3′
5′
5′ CH2
CH3
T
A
G
C
A
T
C
G
5′ CH2
5′
3′

Polymerase reaction:

5'-> 3'

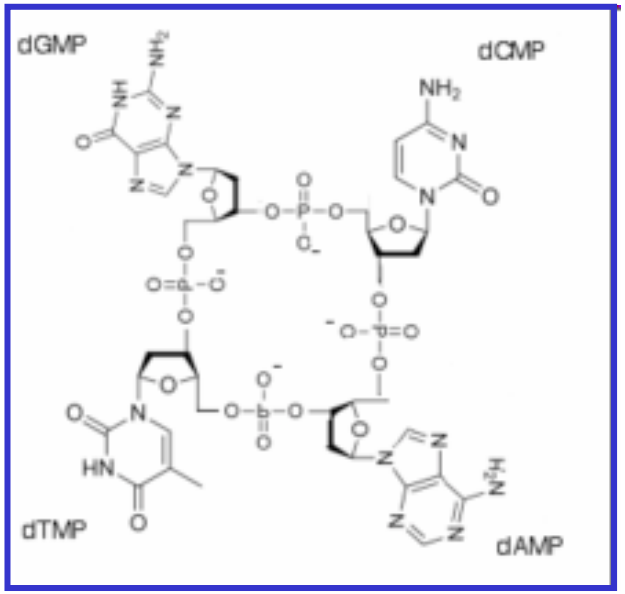
Chemical DNA synthesis

3'-> 5'



First DNA Structure

- By 1910 actual components known (nucleotides)
  - Phoebus Levene proposed a tetranucleotide structure for DNA

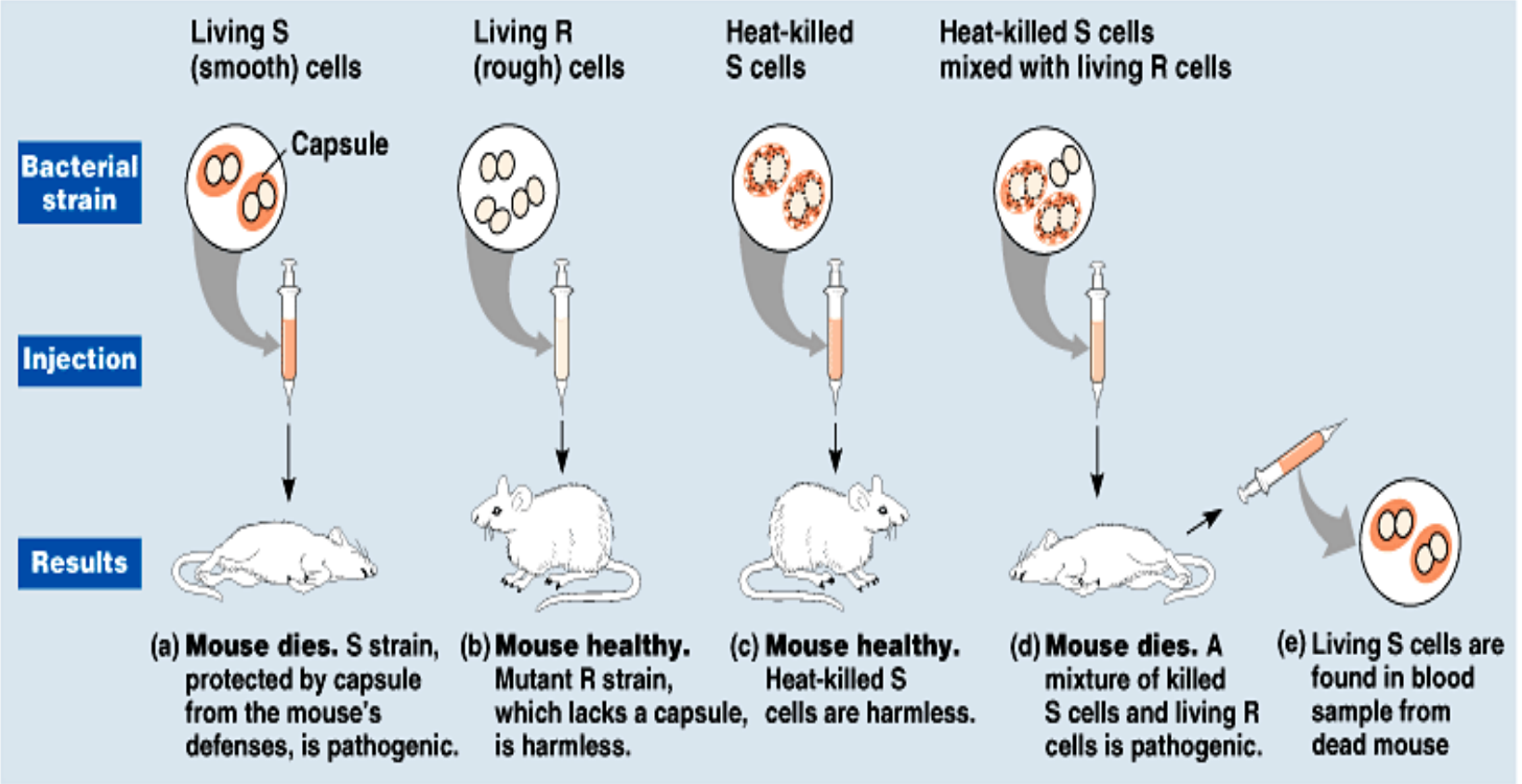


- Tetranucleotide repeat of *ATCG*
- Own data showed nucleotides not in 1:1:1:1 ratio
  - Differences "probably experimental error…"

So…

- If DNA was a single covalently bonded tetranucleotide structure then it couldn't easily encode information
- Proteins, on the other hand, had 20 different amino acids and could have lots of variation
- Most geneticists focused on "transmission genetics" and passively accepted proteins as being the likely genetic material

Frederick Griffith, 1928
Transformation of Bacteria



Heat-killed cells contain "transforming factor"

Avery, McCarty and MacLeod (1944)

- After 10 yrs of effort published work using Griffith's approach to assay for the genetic material
  - Used
    - Cell-free extract of S cells
    - From 75 liters of cell culture obtained 10-25 mg of "active factor
    - Proteases, RNases, DNases, etc.

    - Transforming factor is DNA

Erwin Chargaff

- 1949-1953
- Digested many DNAs and subjected products to chromatographic separation
- Results
  - A = T, C = G
  - A + G = C + T (purine = pyrimidine)
  - A + T does not equal C + G
    - Members of a species similar but different species vary in AT/CG ratio

TABLE 10.3 DNA BASE COMPOSITION DATA

(a) Chargaff's data*

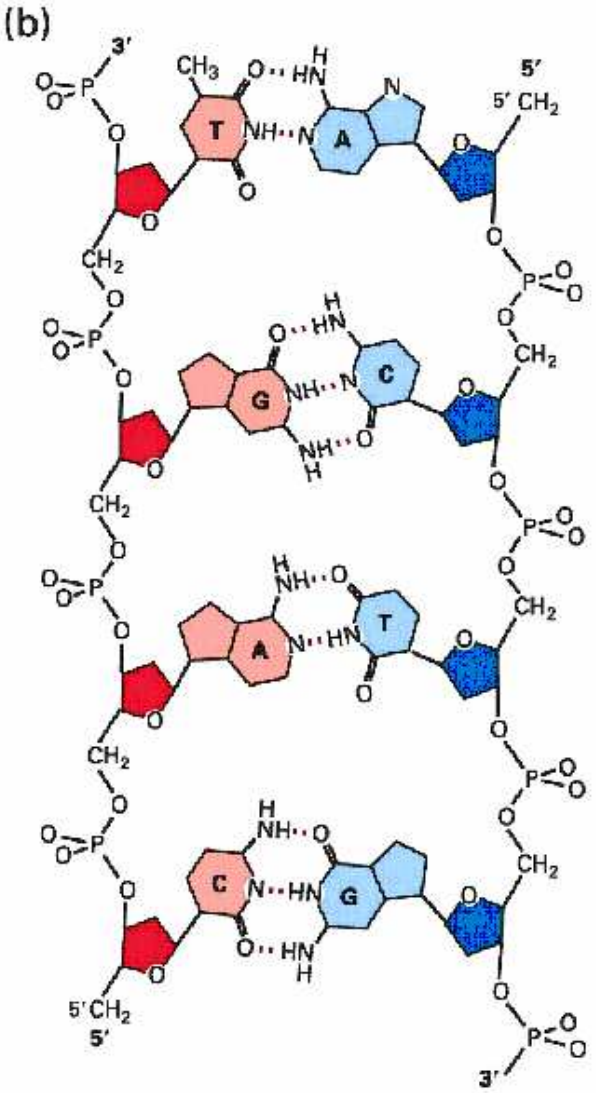
	Molar proportions[a]			
	1	2	3	4
Organism's/Source	A	T	G	C
Ox thymus	26	25	21	16
Ox spleen	25	24	20	15
Yeast	24	25	14	13
Avian tubercle bacilli	12	11	28	26
Human sperm	29	31	18	18

(b) Base compositions of DNAs from various sources

	Base composition				Base ratio	
	1	2	3	4	5	6
Source	A	T	G	C	A/T	G/C
Human	30.9	29.4	19.9	19.8	1.05	1.00
Sea urchin	32.8	32.1	17.7	17.3	1.02	1.02
E. coli	24.7	23.6	26.0	25.7	1.04	1.01
Sarcina lutea	13.4	12.4	37.1	37.1	1.08	1.00
T7 bacteriophage	26.0	26.0	24.0	24.0	1.00	1.00

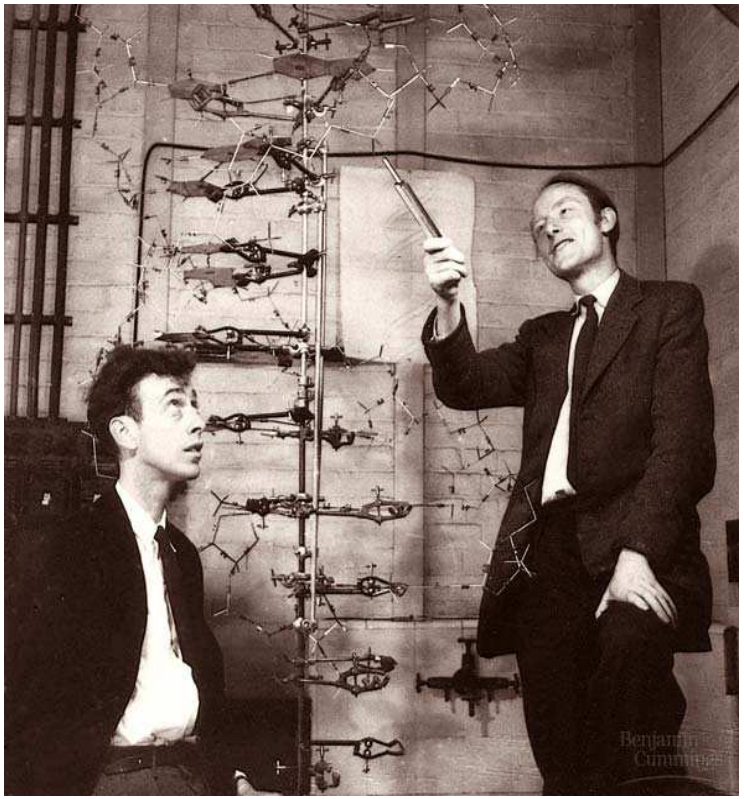
* *Source*: From Chargaff, 1950.

[a] Moles of nitrogenous constituent per mole of P. (Often, the recovery was less than 100 percent.)

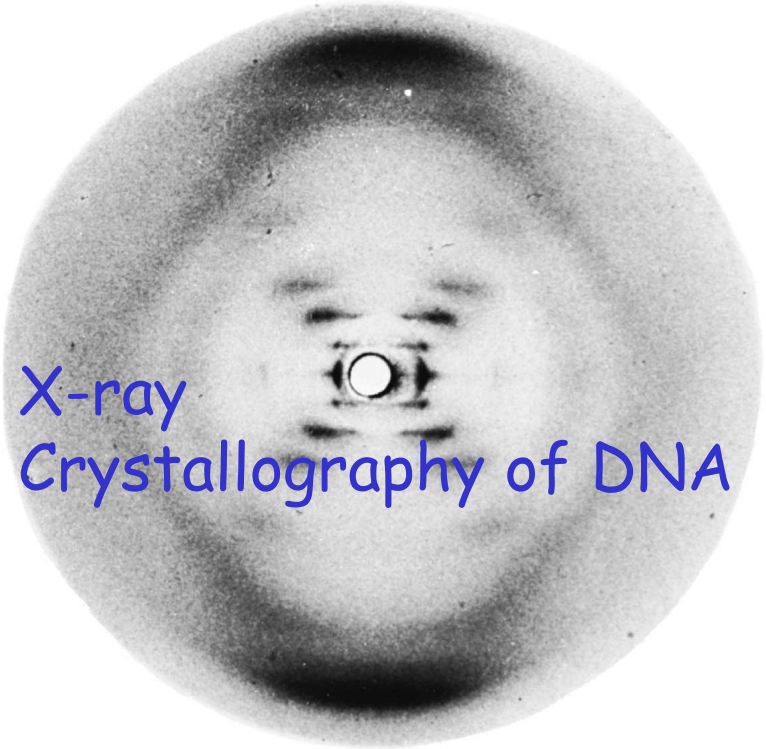


Watson and Crick

- 1953 propose double helix model
  - Right-handed double helix



Method?

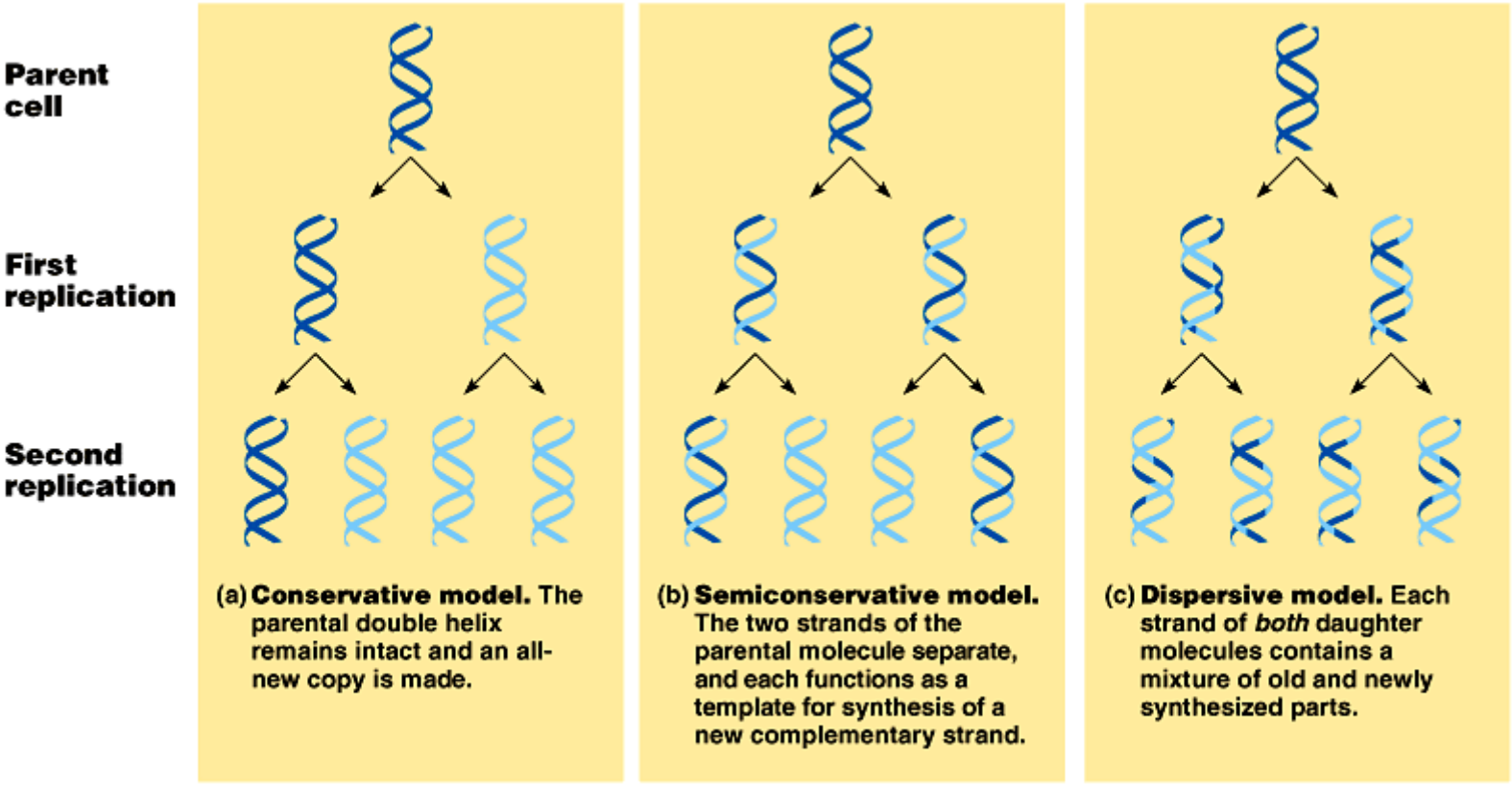


X-ray Crystallography of DNA

Impact

- Article in *Nature*
  - "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copy mechanism for the genetic material"
    - Second paper 2 months later describes semiconservative replication and that mutations must change bases in DNA (information encoded in the bases and their order)
- DNA became the genetic material…

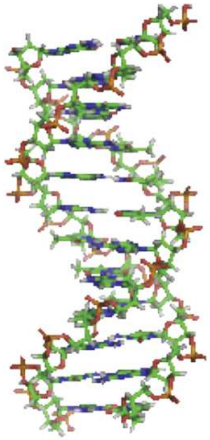
DNA Replication

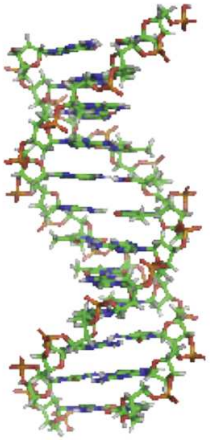


Nobel Prize in 1962.

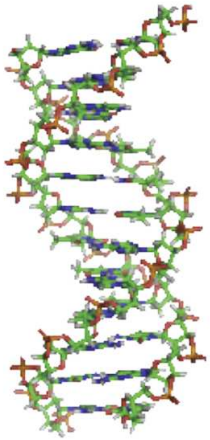
The Nobel Committee noted that the discovery had "no immediate practical application, but determining the molecular structure of the substance that is responsible for the forms that life takes is a discovery of tremendous importance".

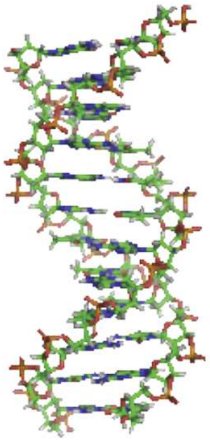
The understatement of the year?





We Live in
The Age of DNA!





We Live in the Era of....

- Genes & DNA
- Genomics & Genome Sequencing
- Genetic Engineering of Microbes, Plants, & Animals
- Biotechnology Using Genetic Engineering Technology
- Synthetic Microbes Made by "Man"
- Personalized Genomes and Ability to Identify Any Individual Using DNA
- Mammalian Reproduction, Stem Cells & Cloning

And the SYNTHESIS of These Technologies!!

Genetic Engineering Can Now Be Used To Synthesize Entire Chromosomes From Chemicals and Create Synthetic Microbes in a Test Tube

2 JULY 2010 VOL 329 SCIENCE www.sciencemag.org

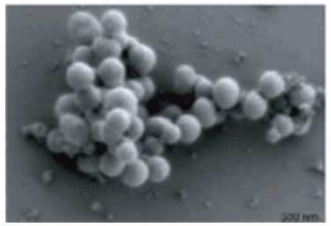
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

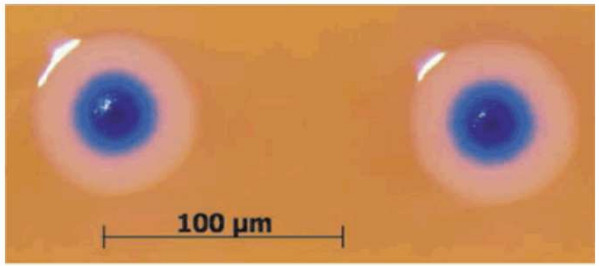
May 20, 2010

Researchers Say They Created a 'Synthetic Cell'

By NICHOLAS WADE

The genome pioneer J. Craig Venter has taken another step in his quest to create synthetic life, by synthesizing an



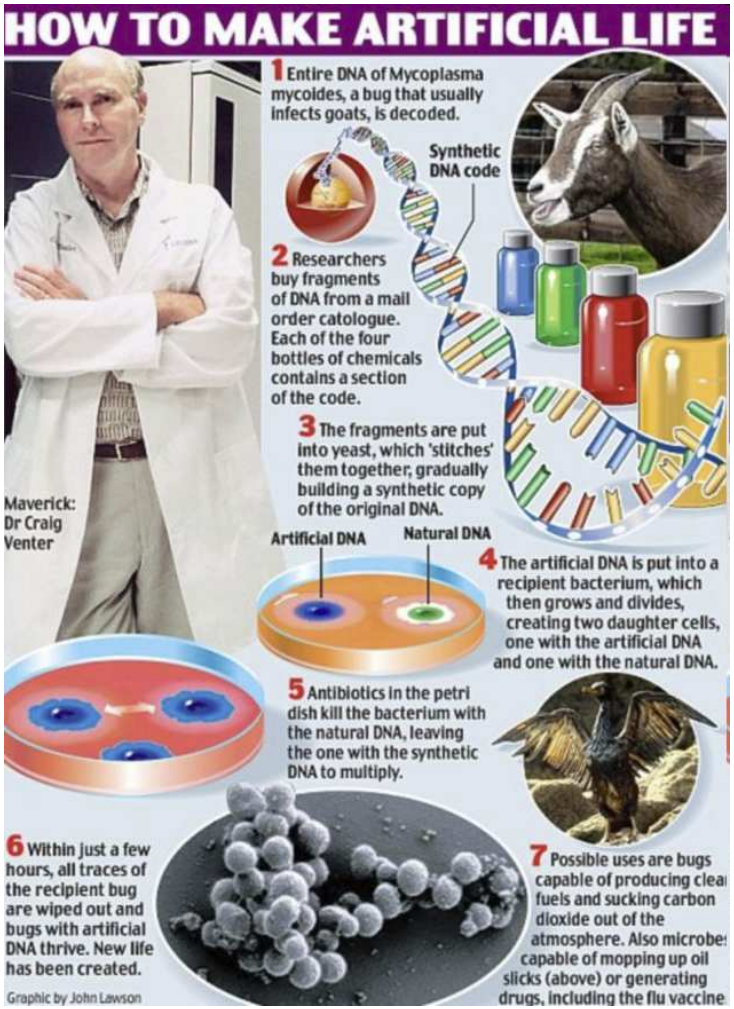


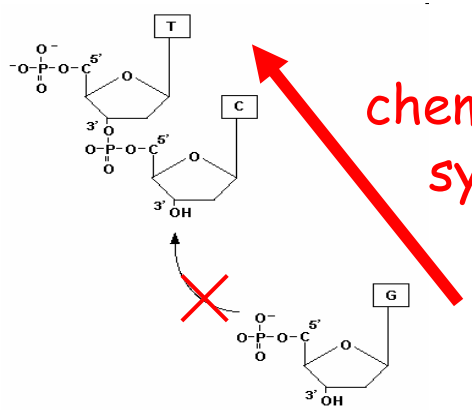
J. Craig Venter
Mycoplasma genitalium

Why did they choose this Pathogenic organism?

582,970 bp genome
Low GC content ~ 30%

Chemical gene and chromosome synthesis

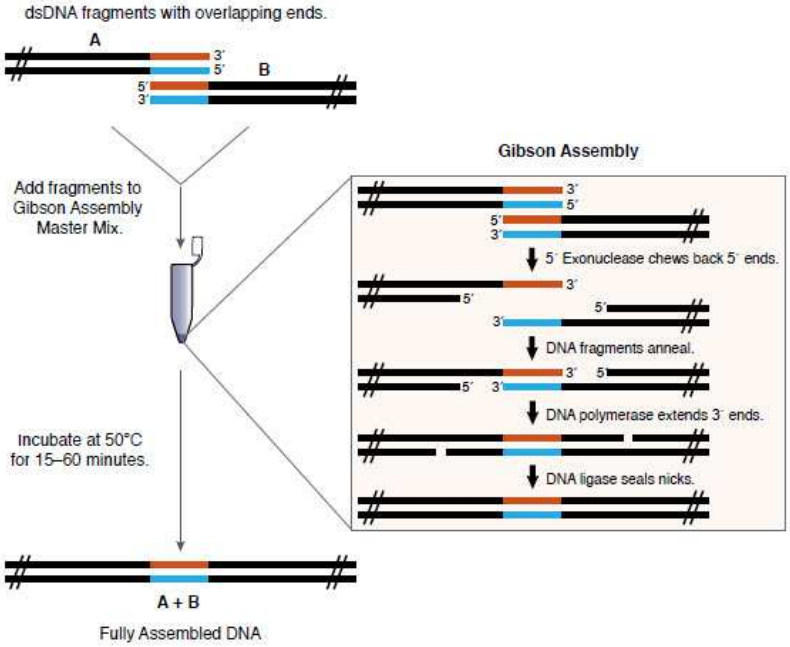


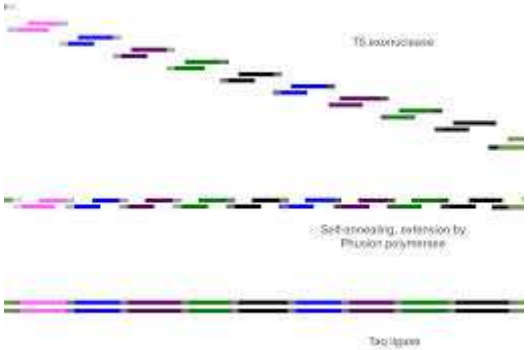


Gene synthesis from $0.35/bp

Scientist accused of playing God

Gibbson assembly





Example of synthetic biology

Taq ligase

DNA can be used to look into the past

RESEARCH ARTICLE

Science, May 7, 2010 (328, 710-722)

A Draft Sequence of the Neandertal Genome

From a 45,000 Year-Old Bone

Reconstruction by Kennis & Kennis / Photograph by Joe McNally

For the first time, a Neanderthal female peers from the past in a reconstruction informed by both fossil anatomy and ancient DNA. At least some of her kind carried a gene for red hair and pale skin.

2014

1 Genetic variants in 846 people of non-African heritage, 176 people from sub-Saharan Africa, and a 50,000-year-old Neanderthal

Areas with reduced Neanderthal ancestry tend to cluster on the X chromosome – hybrid infertility

This suggests that when ancient humans met and mixed with Neanderthals, the two species were at the edge of biological incompatibility

2 Whole-genome sequencing data from 379 Europeans and 286 East Asians to identify Neanderthal lineages that persist in the modern DNA

Non-African humans inherit ~1 to 3% of their genomes from Neandertal ancestors. Evidence that Neanderthal skin genes made Europeans and East Asians more evolutionarily fit

1 Sriram Sankararaman et al. The genomic landscape of Neanderthal ancestry in present-day humans. *Nature*,

2 Benjamin Vernot and Joshua M. Akey. Resurrecting Surviving Neandertal Lineages from Modern Human Genomes. *Science*

Vol 456|20 November 2008|doi:10.1038/nature07446 nature

Nature, November 2008

LETTERS

Sequencing the nuclear genome of the extinct woolly mammoth

Think About Bringing a Woolly Mammoth Back to Life!!

Webb Miller[1], Daniela I. Drautz[1], Aakrosh Ratan[1], Barbara Pusey[1], Ji Qi[1], Arthur M. Lesk[1], Lynn P. Tomsho[1], Michael D. Packard[1], Fangqing Zhao[1], Andrei Sher[2]‡, Alexei Tikhonov[3], Brian Raney[4], Nick Patterson[5], Kerstin Lindblad-Toh[5], Eric S. Lander[5], James R. Knight[6], Gerard P. Irzyk[6], Karin M. Fredrikson[7], Timothy T. Harkins[7], Sharon Sheridan[7], Tom Pringle[8] & Stephan C. Schuster[1]

Dna can be used to "bring back the dead"!!

PNAS

November 11, 2008

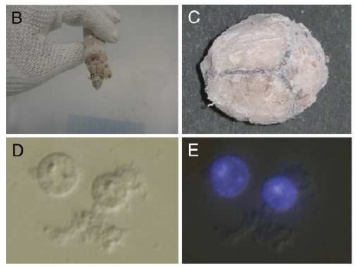
Production of healthy cloned mice from bodies frozen at −20°C for 16 years

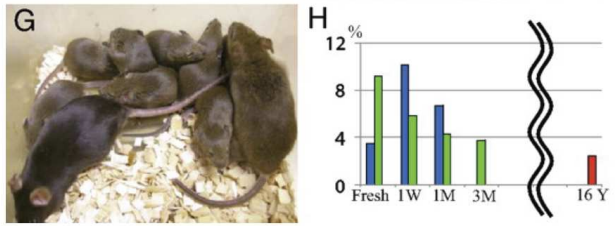
Think of the possibilities!

Sayaka Wakayama[a], Hiroshi Ohta[a], Takafusa Hikichi[a], Eiji Mizutani[a], Takamasa Iwaki[b], Osami Kanagawa[c], and Teruhiko Wakayama[a,1]

[a]RIKEN, Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Kobe, 650-0047, Japan; [b]Jikei University School of medicine, Tokyo 105-8461, Japan; and [c]RIKEN, Research Center for Allergy and Immunology, 1-7-22, Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045, Japan

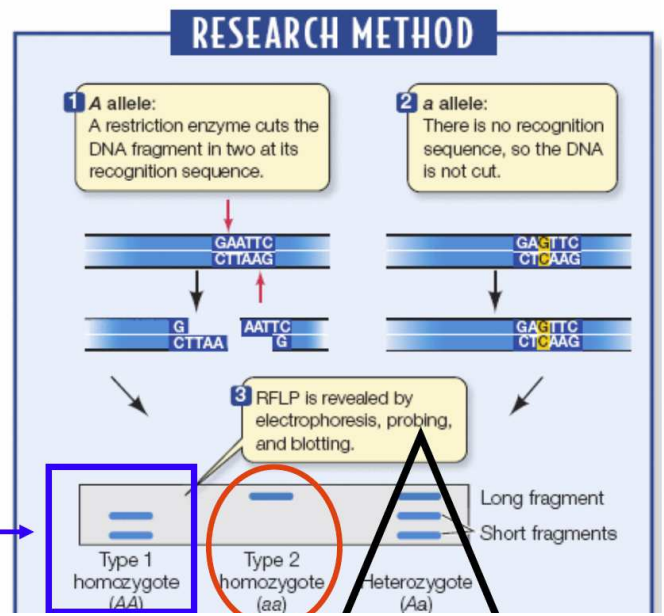
How Know a Clone or Genetically Identical Individual - DNA!





By Using DNA Fingerprints to Identify Individuals & Genes

DNA Fingerprints →



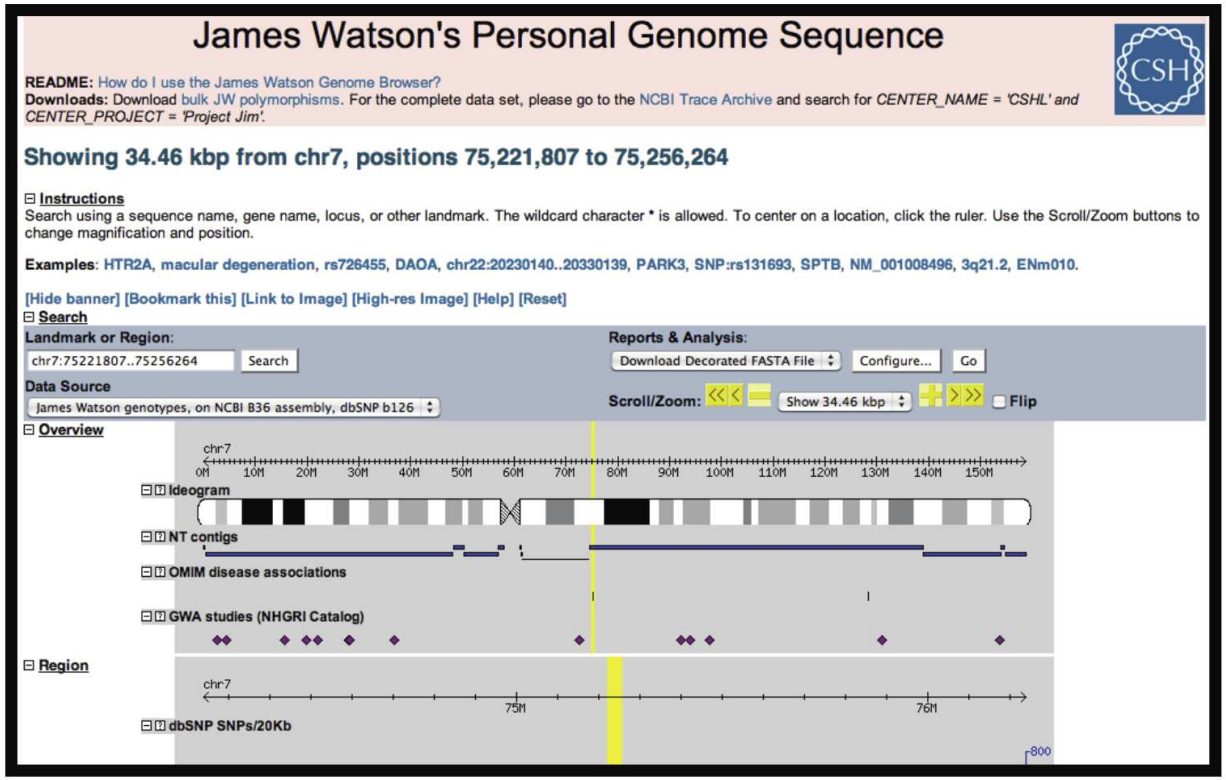
What is YOUR DNA Fingerprint?

Example of SNP

The Complete Genome of Individuals Can Now Be Sequenced Very Inexpensively ($10,000)!!

Genome of DNA Pioneer Is Deciphered

By NICHOLAS WADE
Published: May 31, 2007



The Era of Personalized Genomes is Here!

The goal is $1,000 per genome

ARTICLE Nature, October 10, 2010

doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

The 1000 Genomes Project aims to provide a deep characterization of human genome sequence variation as a foundation for investigating the relationship between genotype and phenotype. Here we present results of the pilot phase of the project, designed to develop and compare different strategies for genome-wide sequencing with high-throughput platforms. We undertook three projects: low-coverage whole-genome sequencing of 179 individuals from four populations; high-coverage sequencing of two mother-father-child trios; and exon-targeted sequencing of 697 individuals from seven populations. We describe the location, allele frequency and local haplotype structure of approximately 15 million single nucleotide polymorphisms, 1 million short insertions and deletions, and 20,000 structural variants, most of which were previously undescribed. We show that, because we have catalogued the vast majority of common variation, over 95% of the currently accessible variants found in any individual are present in this data set. On average, each person is found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in inherited disorders. We demonstrate how these results can be used to inform association and functional studies. From the two trios, we directly estimate the rate of *de novo* germline base substitution mutations to be approximately 10^{-8} per base pair per generation. We explore the data with regard to signatures of natural selection, and identify a marked reduction of genetic variation in the neighbourhood of genes, due to selection at linked sites. These methods and public data will support the next phase of human genetic research.

- Sequenced Genomes of ~900 individuals
- From Seven Different Global Populations
- Found 250-300 Loss-Of-Function Mutations (KOs) Per Person
- 10^{-8} bp Mutations per Generation (30 per Genome)

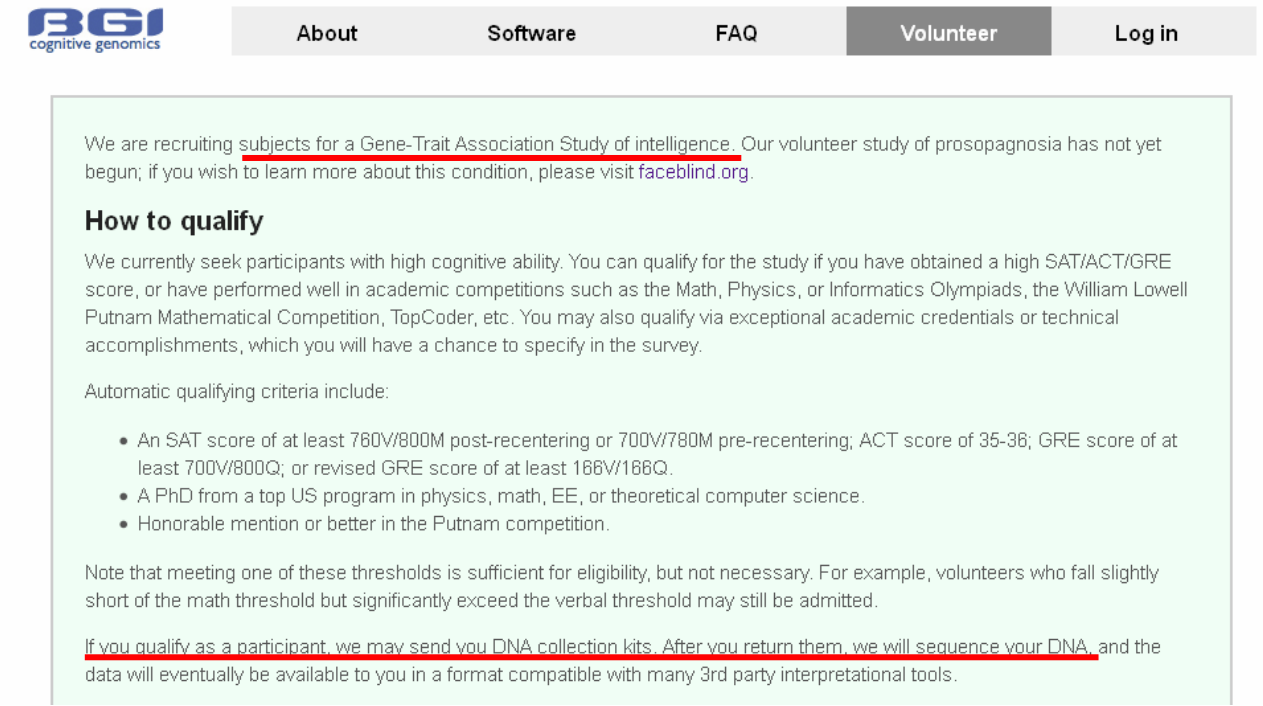
BGI

BGI produces 10% to 20% of the world's genetic information.

BGI

China's BGI to Sequence 2,200 Geniuses In Search For "Smart" Genes

(movies)



SNP Single Nucleotide Polymorphism in humans

- A precise position along a chromosome where the DNA of different people may vary. Generally two alternate alleles are found at a particular SNP. At least 2,000,000 SNPs are now known and there may be over 30,000,000 in the human genome.
- The importance of SNPs comes from their ability to influence disease risk, drug efficacy and side-effects, tell you about your ancestry, and predict aspects of how you look and even act.
- Mostly not determined by sequencing –Illumina chip - movie

Example - Rs1815739

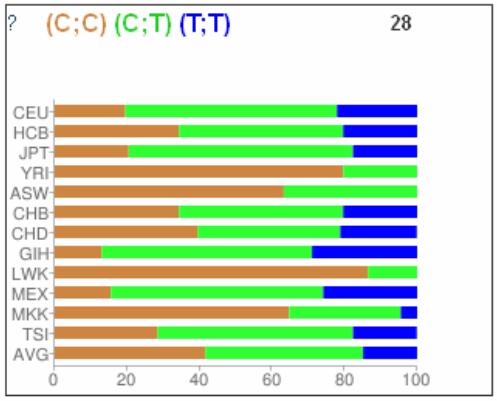
- This SNP, in the ACTN3 gene, encodes a premature stop codon in a muscle protein called alpha-actinin-3. The polymorphism alters position 577 of the alpha-actinin-3 protein. Normal – C, truncated - T
- (T;T) is under-represented in elite strength athletes, consistent with reports indicating that alpha-actinin-3 deficiency appears to impair muscle performance.

muscle performance

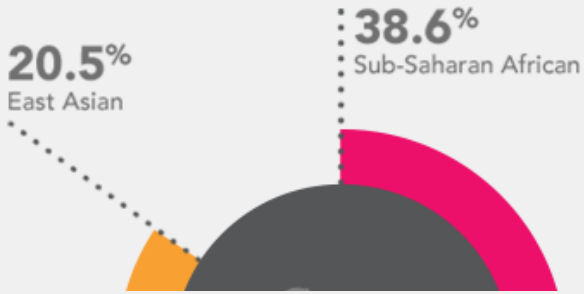
Orientation plus

Stabilized plus

Geno	Mag	Summary
(C;C)	2.2	Better performing muscles. Likely sprinter.
(C;T)	2.1	Mix of muscle types. Likely sprinter.
(T;T)	2.3	Impaired muscle performance. Likely endurance athlete.



23 pairs of chromosomes. One unique you.



Find out what pe
around the worl
Europe and mor
regions such as

Neanderthal DNA lives on in us.

Even though Neanderthals vanished about 40,000 years ago, their DNA lives on in us. Based on research, they interbred with humans around 60,000 years ago and we are able to tell you what percentage of your genome came from Neanderthals.

We estimate how much of your DNA is from Neanderthals and compare it to other users.

Modern Human

Flatter faces

Slender, with longer limbs

Advanced artistic abilities and tool making

Neanderthals

Large noses

Wide, robust bodies

Larger eye sockets, likely better vision

Ever wonder who's related to you? You'll likely discover dozens or even hundreds of people who share DNA and ancestors. The matches you'll get can range from close family to distant cousins.

In rare cases, participation in DNA Relatives may reveal that you are related to someone whom you didn't expect, or that you are not related to someone in the way that you expected. Consider this before you opt in to this feature.

ILT
Absent
Absent
Absent
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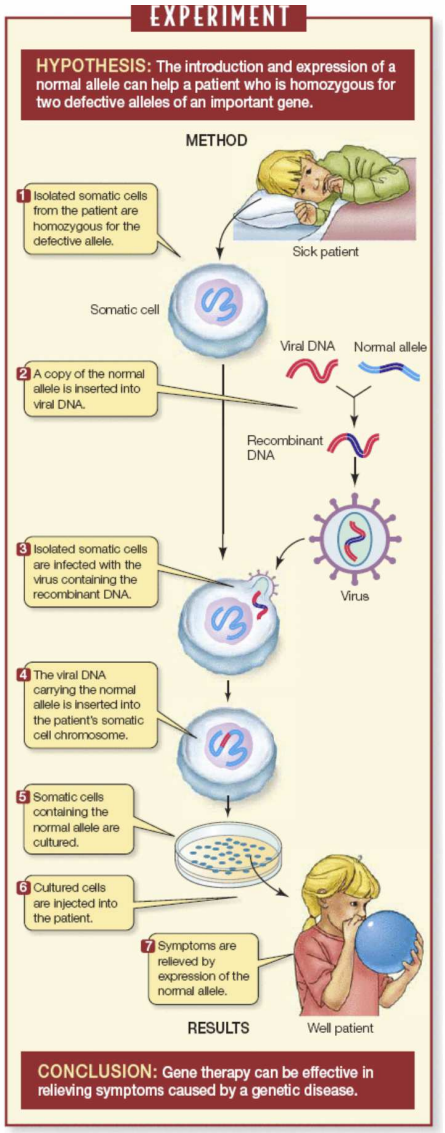
traits
ow you

How About Genetically Engineered Humans?

Humans Have Been Genetically Engineered To Cure a Lethal Genetic Disease (SCID)



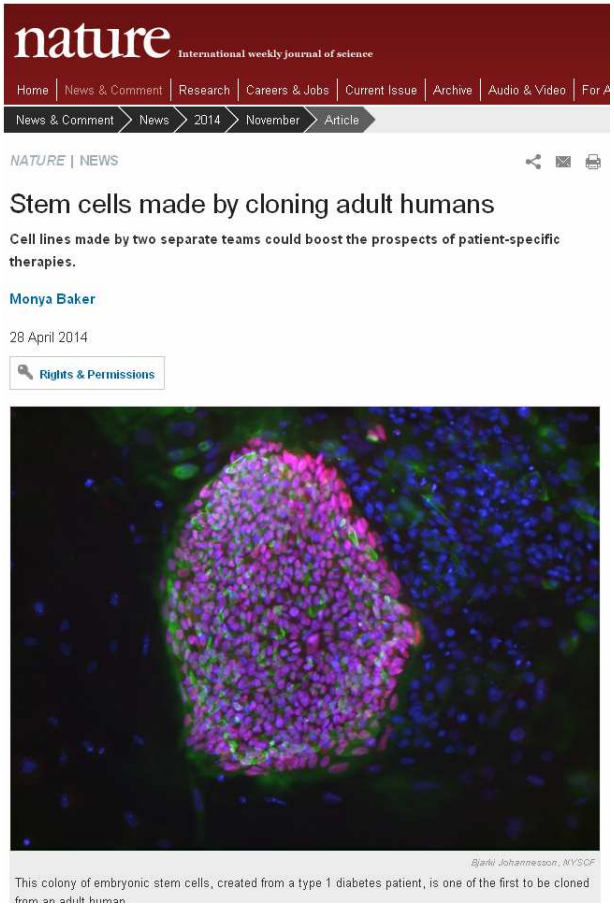
Severe combined immunodeficiency

Adenosine DeAminase ADA gene
Breaking DNA metabolites
ADA deficiency – metabolites acumulate
-killing lyphocytes

Several Teenagers Are Alive Because They Have Been Engineered With an ADA Gene That They Were Not Born With!!!

ADA engineered T lymphocytes
Gene Therapy

How about Human Cloning?



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NATURE | NEWS

Stem cells made by cloning adult humans

Cell lines made by two separate teams could boost the prospects of patient-specific therapies.

Monya Baker

28 April 2014

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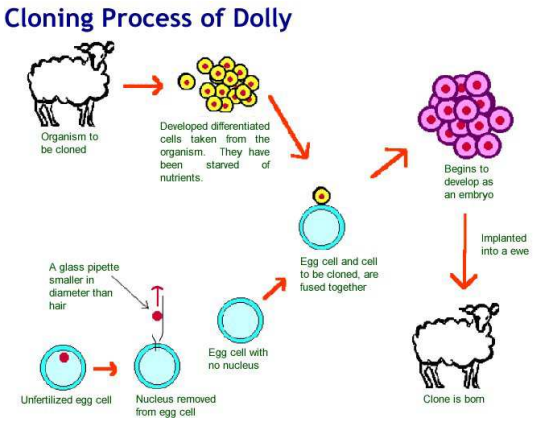
Bjarki Johannesson, NYSCF

This colony of embryonic stem cells, created from a type 1 diabetes patient, is one of the first to be cloned from an adult human

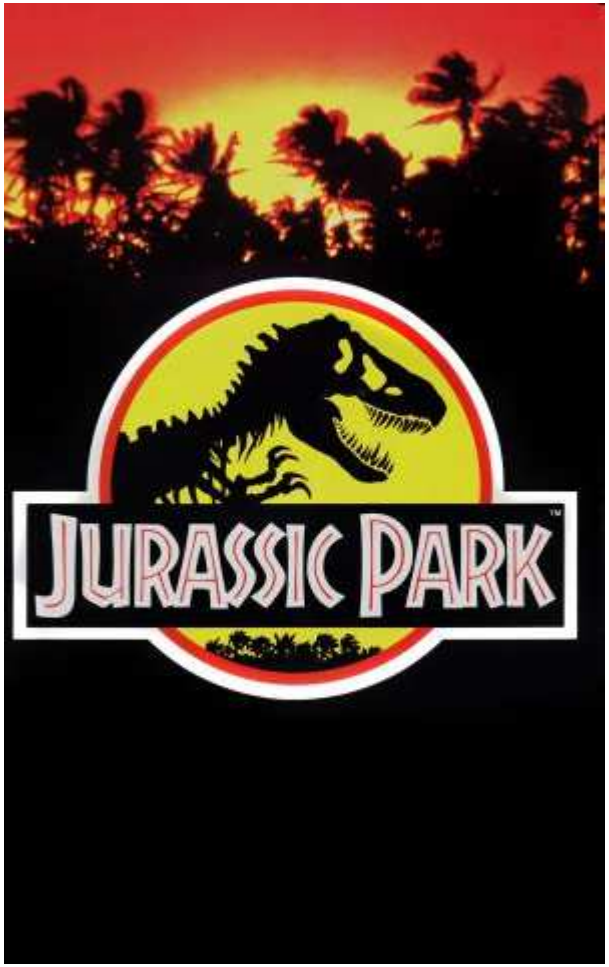
2014



1996

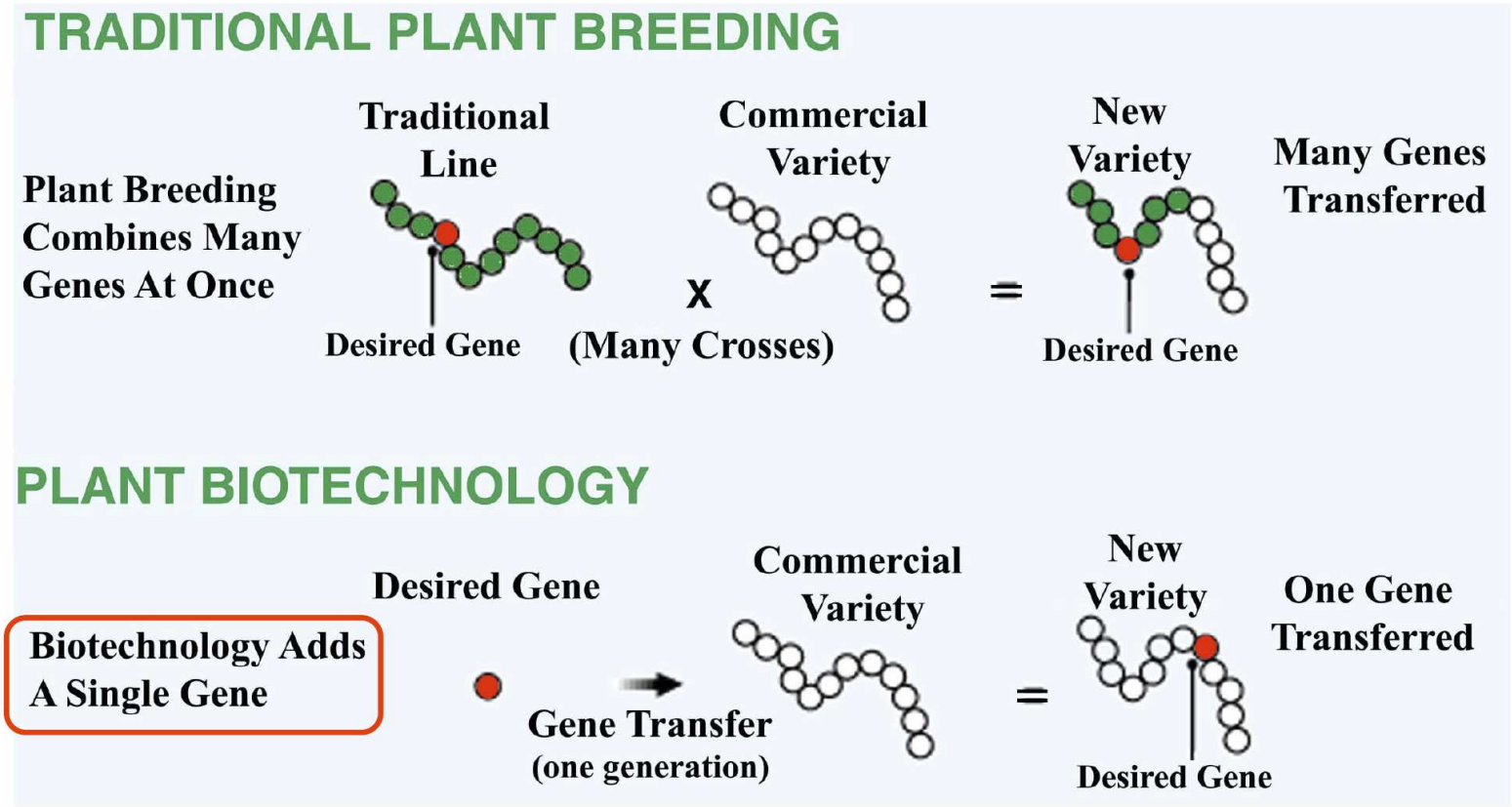
There Are NO Genetic Limitations to What Can Be Done Using Genetic Engineering

-Sequencing genomes of ancient organisms
-Chemical genome synthesis
-Animal (and human) cloning
-Reviving the dead organisms



Is genetic engineering a new technology?

Classical vs. Molecular Genetic Engineering



There is principally nothing new about genetic engineering

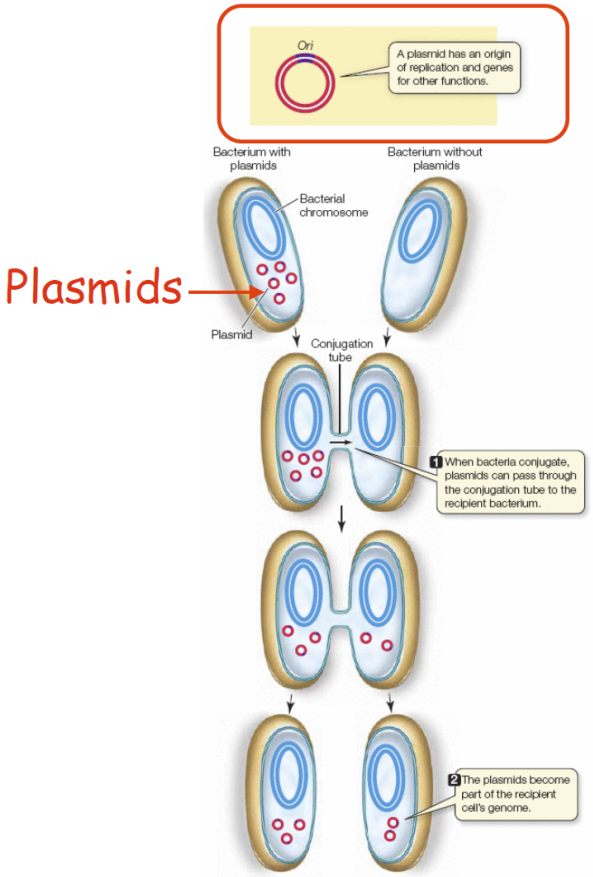
How Was Genetic Engineering Invented?
&
How Did It Lead To All of These Remarkable Advances With DNA?

Genetic engineering started in a Hawai delicatesen in 1972

With An Unexpected "Eureka" Moment Dealing With Two Unrelated Areas of Study:

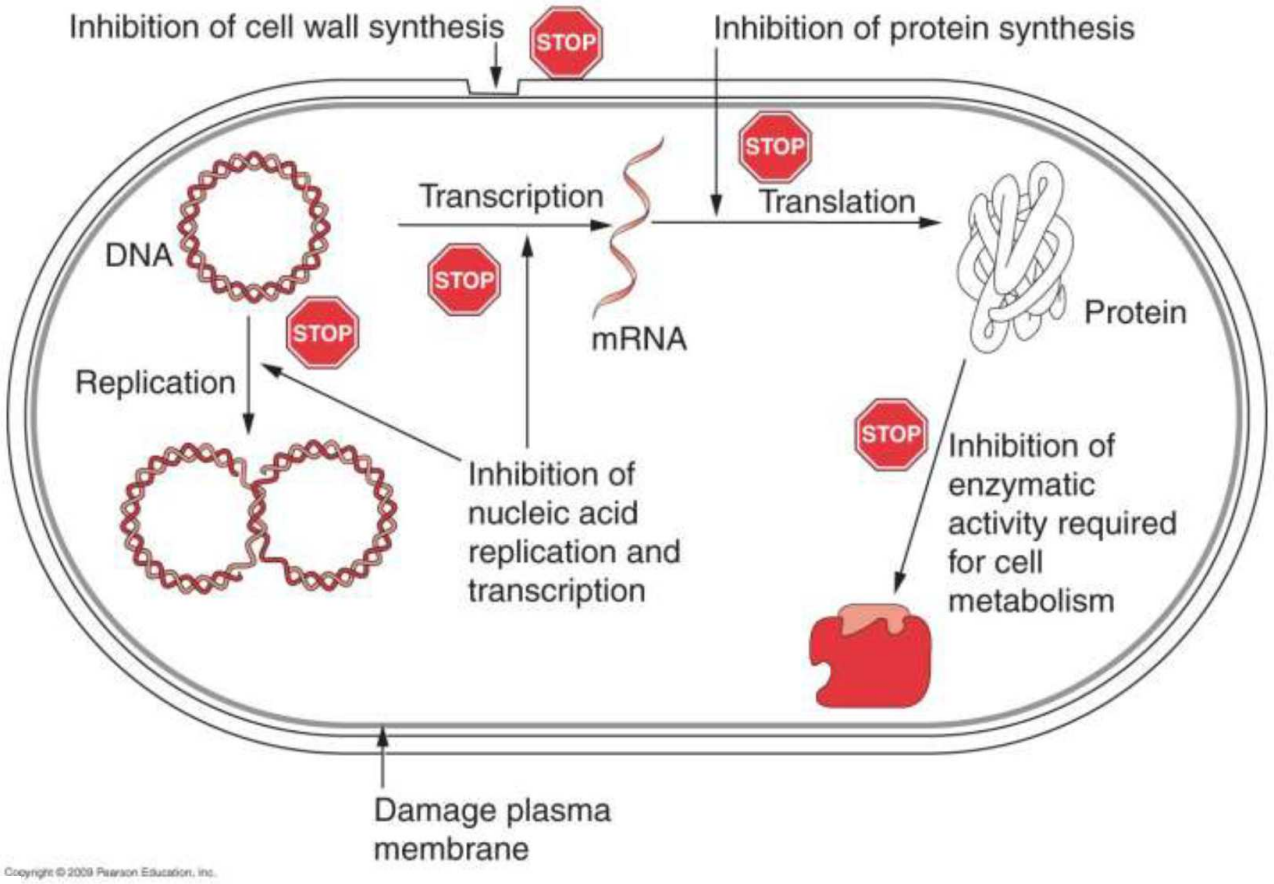
1. **The Mechanism of Bacterial Antibiotic Resistance** — Herbert Boyer
2. **How Novel Enzymes That Protect Bacteria From Destruction By Viruses "Cut" DNA Into Pieces** — Stanley Cohen

Plasmids Are Circular Self-Relicating DNA Molecules in Bacterial Cells That Carry Antibiotic Resistance Genes



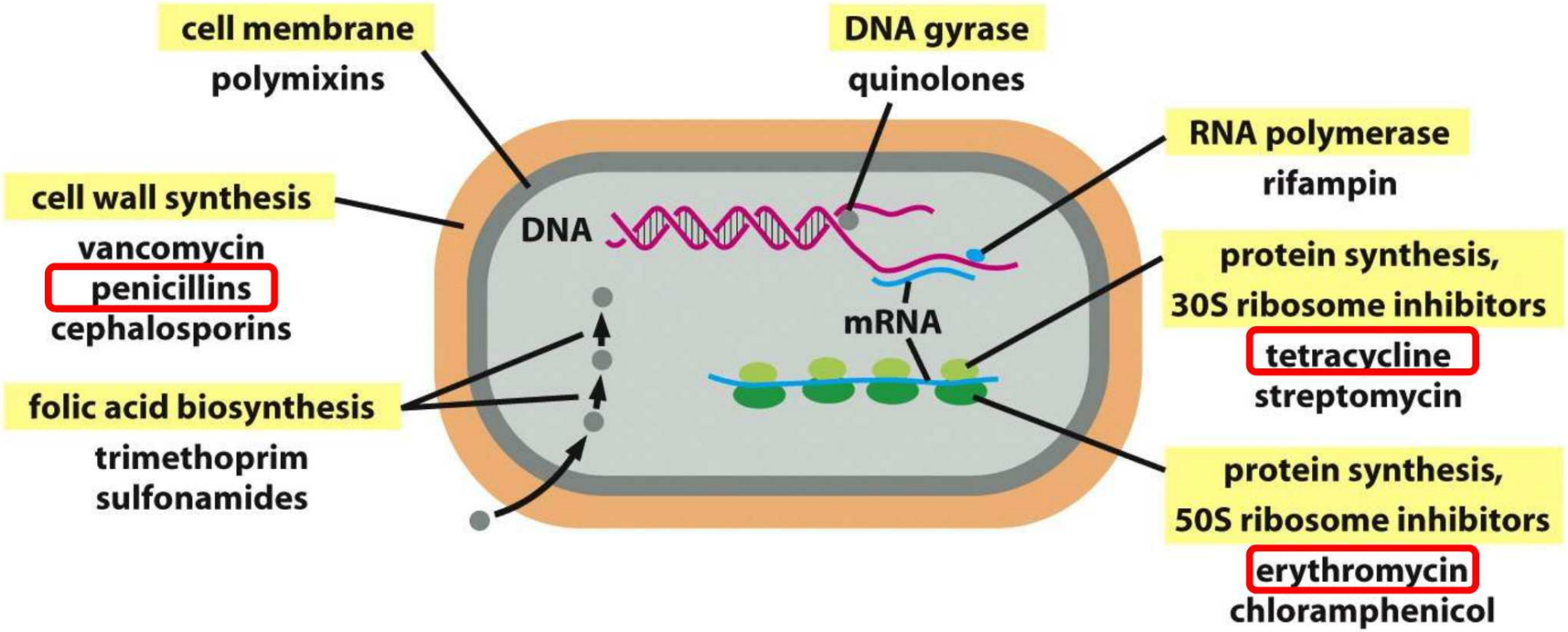
Plasmids Defend Bacteria Against Antibiotics!

How Do Antibiotics Kill Bacterial Cells?

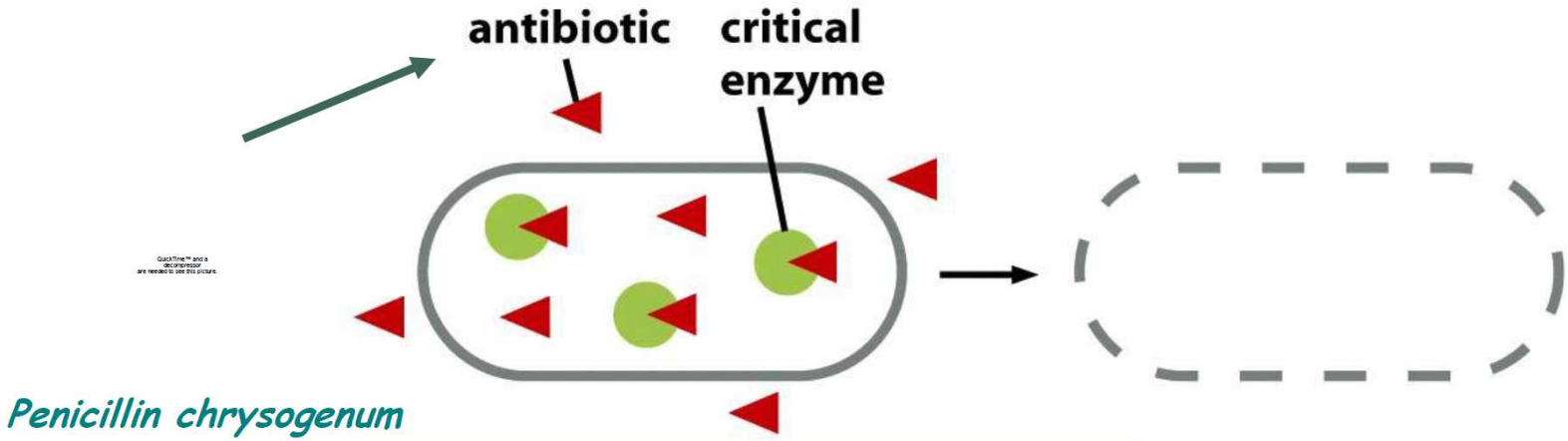


Inhibiting Basic Microbial Cell Processes

Selected Antibiotics and Their Cell Targets

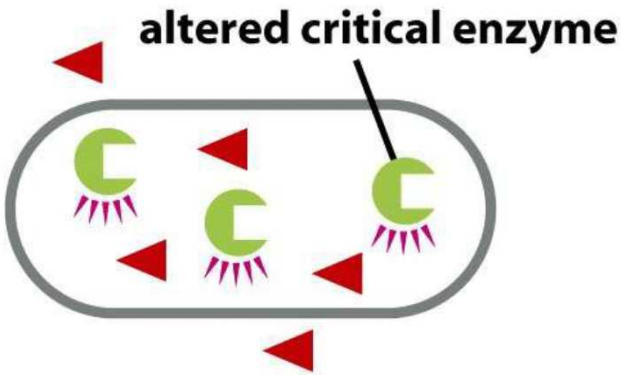


How Do Bacterial Plasmid Antibiotic Resistance Genes Work?



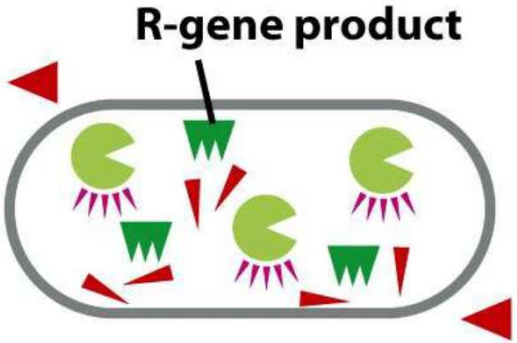
Penicillin chrysogenum

(A) antibiotic kills wild-type bacterium



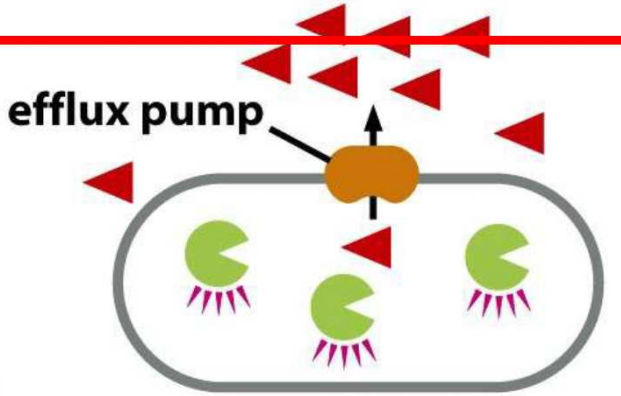
(B) antibiotic resistance

Bacterial Enzyme Alters Target Enzyme So Antibiotic Can't Affect It



(C) antibiotic resistance

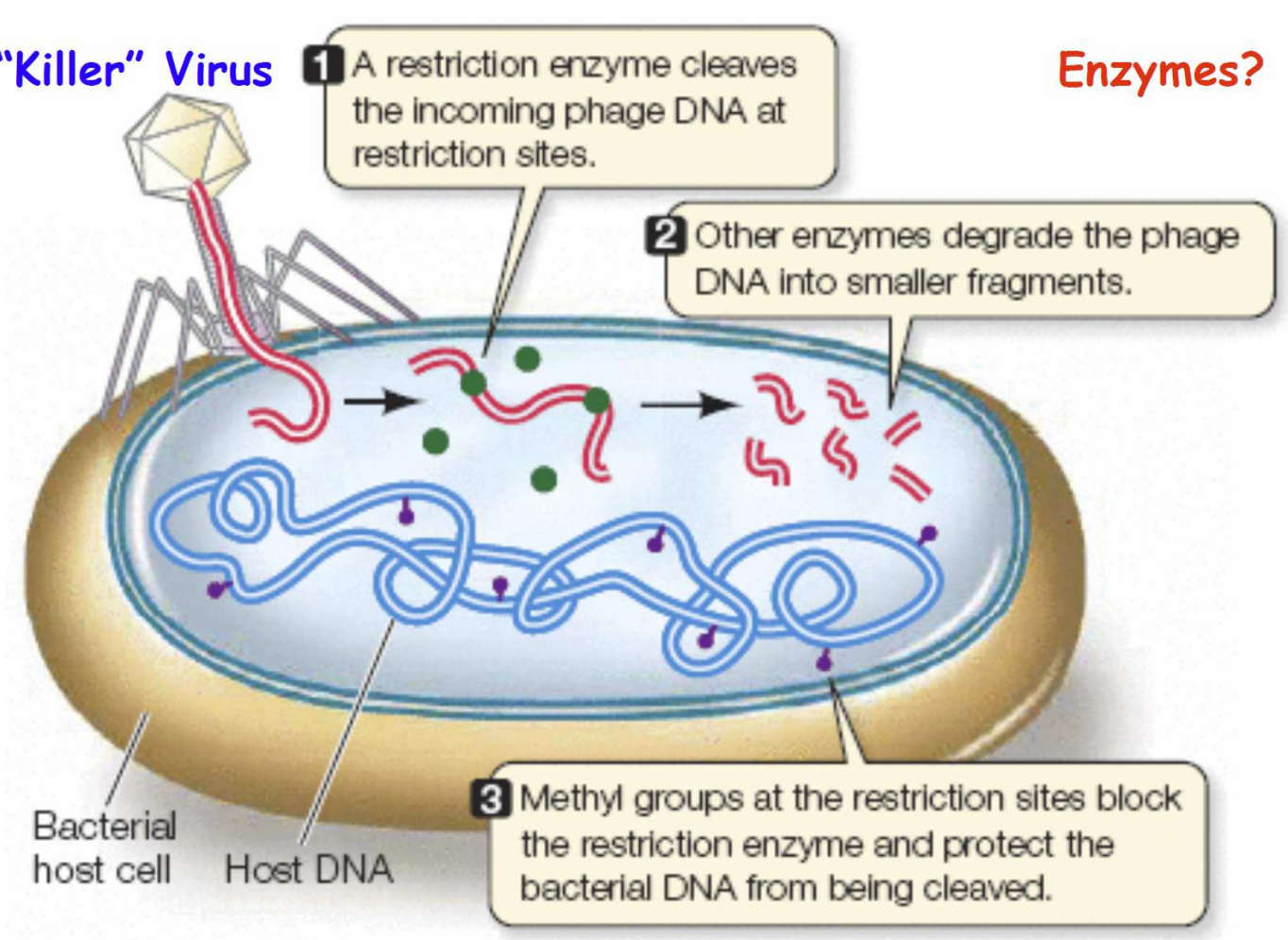
Bacterial Protein Alters Antibiotic Making it Inactive



(D) antibiotic resistance

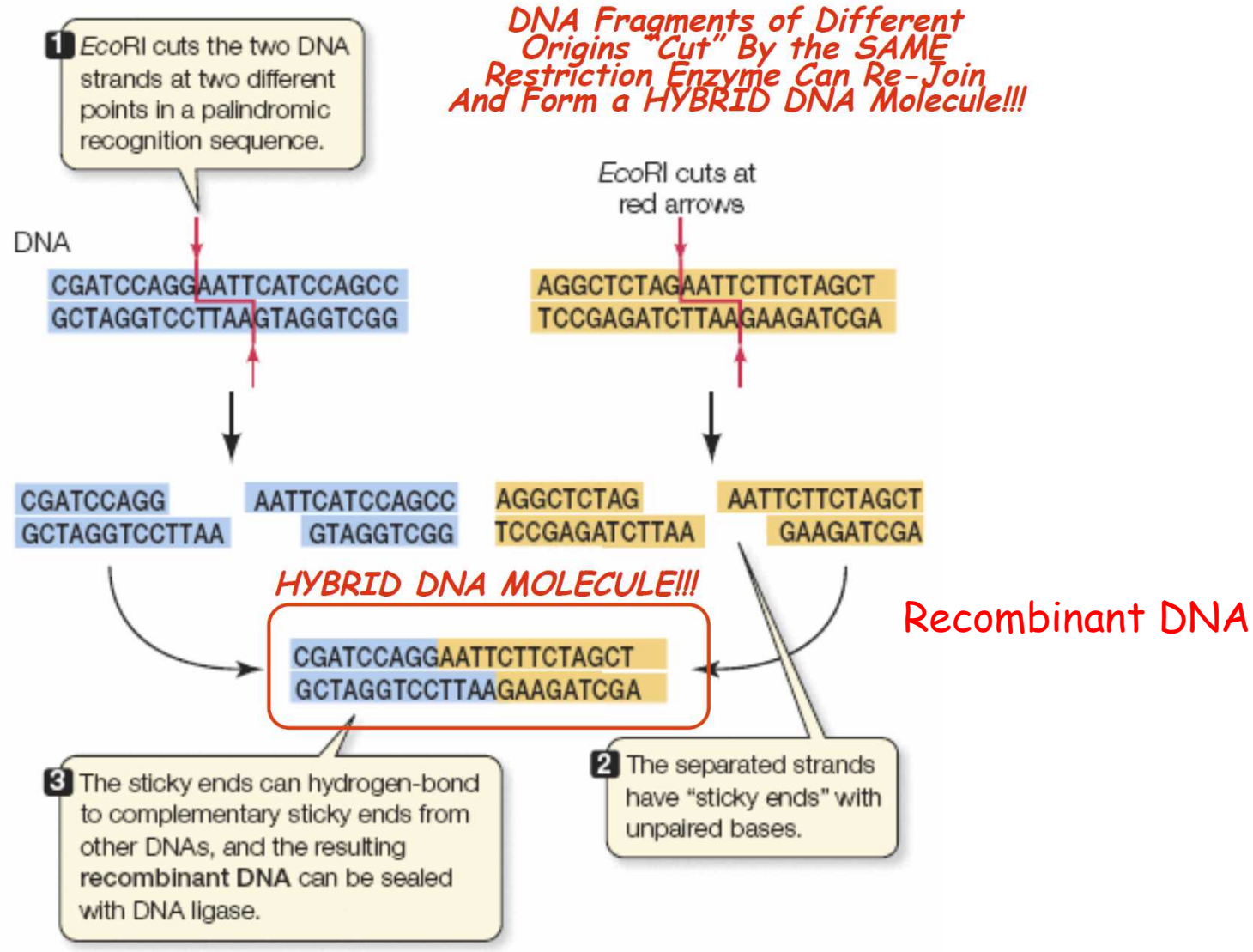
Bacterial Protein Pumps Antibiotic Out of Cell

Restriction Enzymes Are Proteins That "Cut" DNA Into Pieces

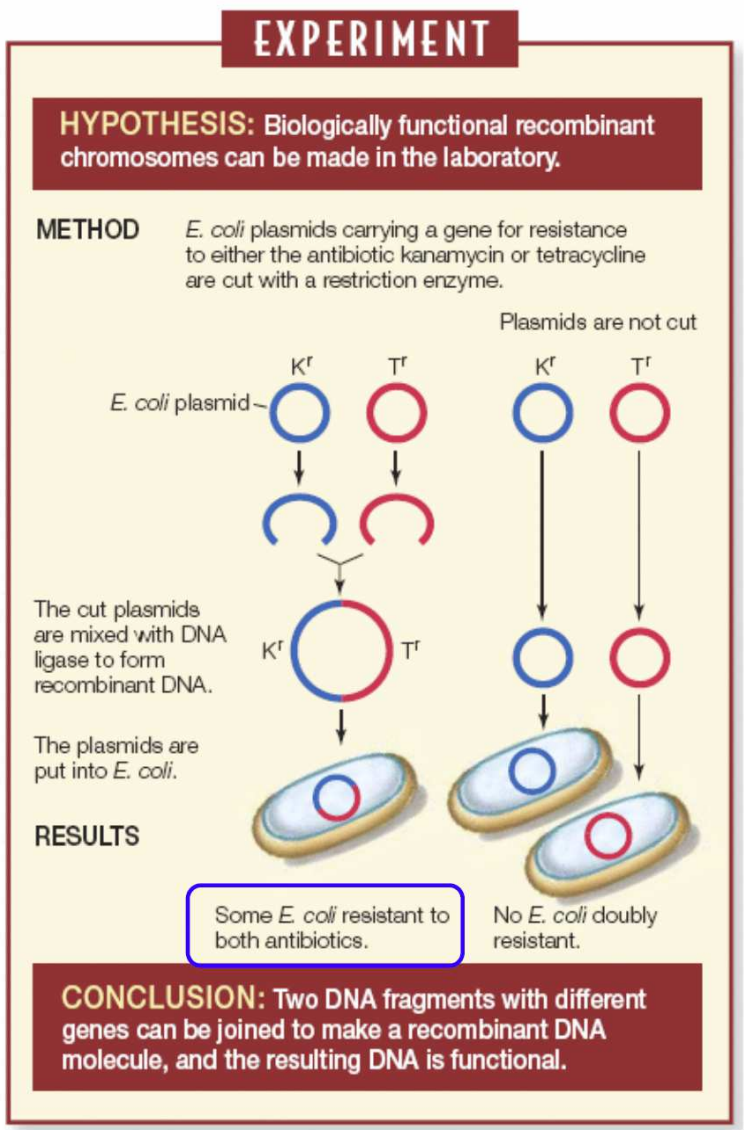


Restriction Enzymes Protect Bacteria From "Killer" Viruses!

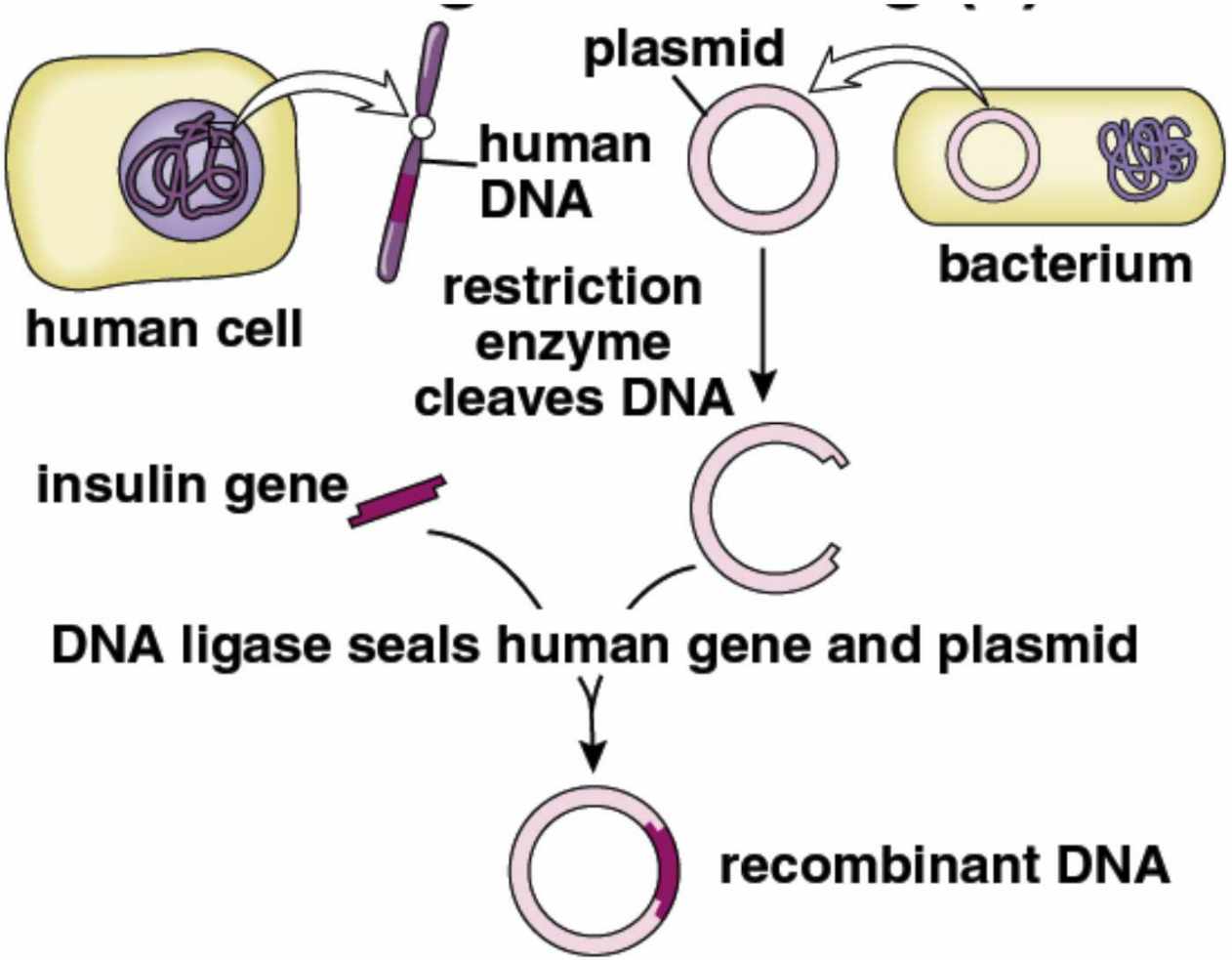
Restriction Enzymes Are Proteins That "Cut" DNA Into Pieces At Specific Sequences



Genetic Engineering Technology Can Combine DNA (Genes) From Different Sources Leading to New Gene Combinations!!



The Human Insulin Gene Can Be Separated From Other Human Genes and Cloned in Bacteria Using Recombinant DNA Methods!



Leading to a REVOLUTION in Technology and Making it Possible For the First Time to Isolate, Manipulate, and Study Genes

The Genes of Any Organism Can Be Isolated, Combined With Those of Another Organism, and Made to Function Normally in New Cellular Environments!

For Example: Human Genes in Bacteria, Bacterial Genes in Plants, Jellyfish Genes in Monkeys, etc., etc., etc., etc.

The Era Of DNA Manipulation Means……

1. Specific DNA/Genes Can Be Isolated From Any Organism
2. DNA Segments of Any Kind From Any Organism Can Be Combined
3. Isolated Genes Can Be Re-Inserted Into the Chromosomes of Any Organism and Made to Work
4. Genes and Genomes Can Be Synthesized and Made To Work in Any Organism

There Are No Genetic Limits. All Biological Organisms Use the Same Genetic Rules. The Implications Are Enormous!!

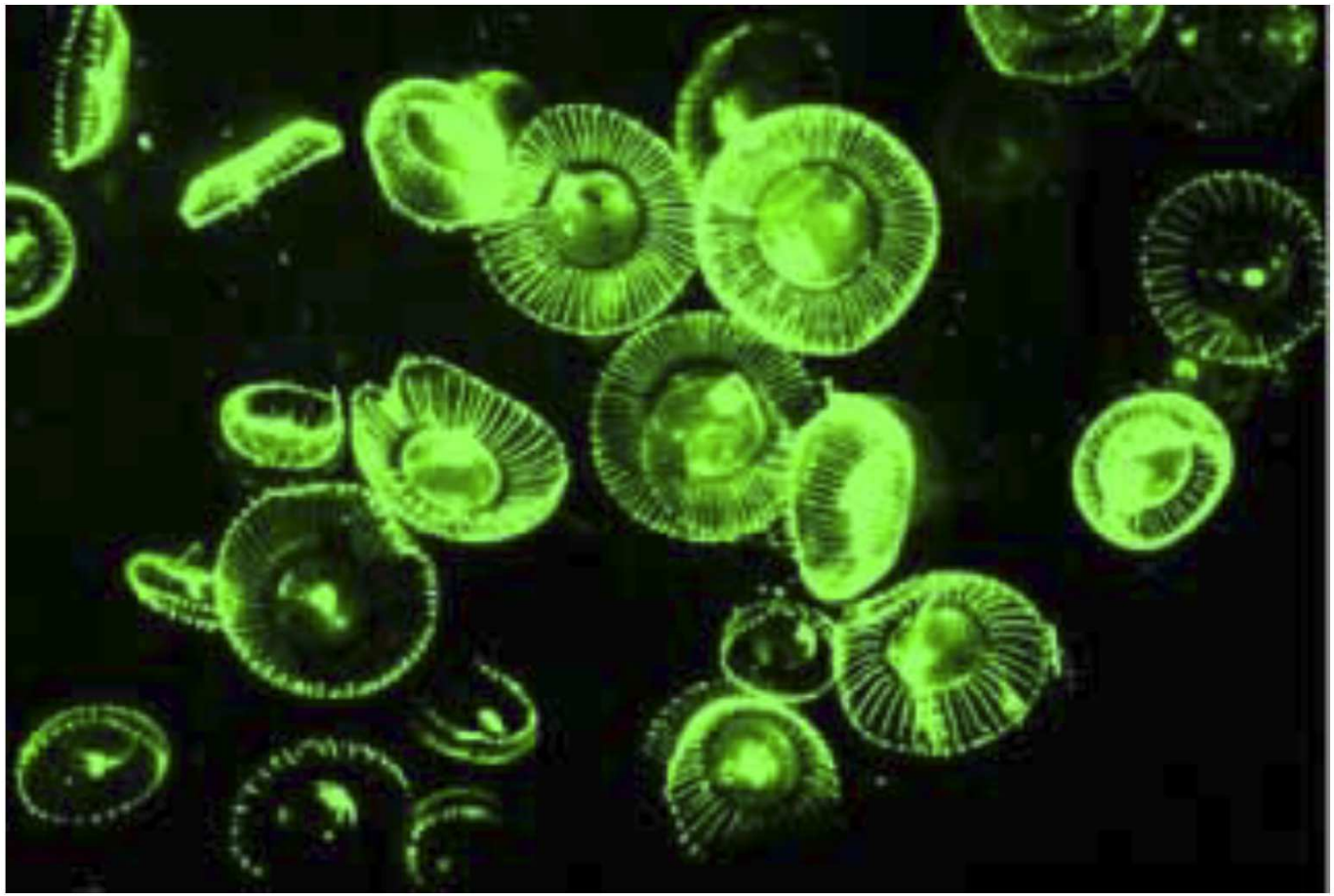
"Why" Clone Genes From An Organism's Genome?

1. PURIFY Individual Genes From the Genome (e.g.,one of 25,000 human genes)
2. AMPLIFY The Gene to Obtain Enough DNA For Study
3. Use the Cloned Gene To:
   a) Study Gene Structure & Function (THE Major Use!)
   b) Use to Convert Cells Into Factories To Make Drugs and Pharmaceuticals
   c) Use to Diagnose Genetic Diseases
   d) Use to Identify Individuals (e.g., paternity, forensics)
   e) Use to Correct Genetic Disease
   f) Use to Engineer New Crops and Farm Animals
   g) Synthesize New Genomes and Many Other Uses

Genetic Engineering Has Lead to New Knowledge About How Cells and Genes Function and Has Lead to Applications That Have Improved Our Lives!!

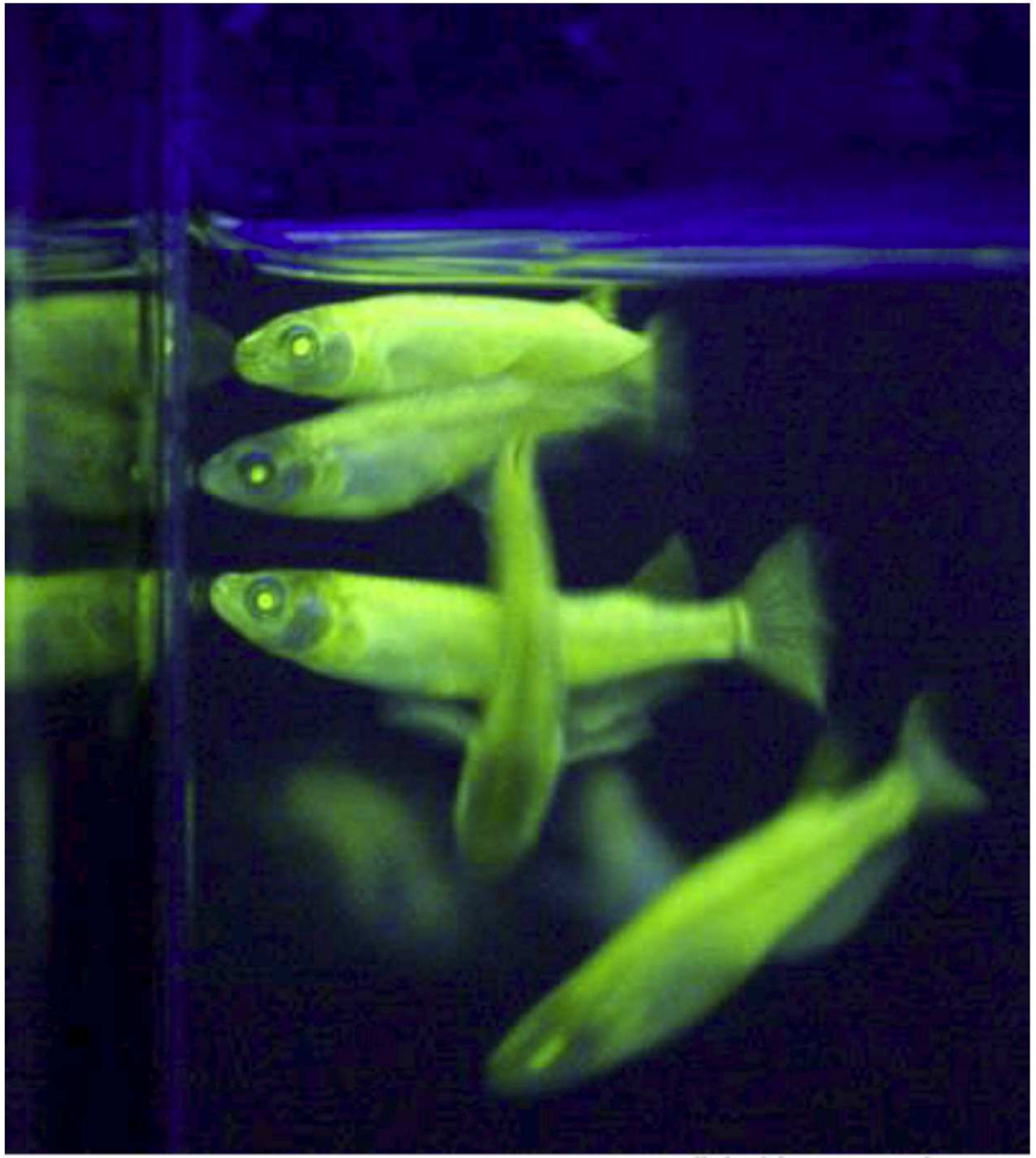
What can be done?

Using a Jellyfish Gene to Make Animals and Plants Glow!!!!



Green Fluorescence Protein

A "GloFish!!!!!"



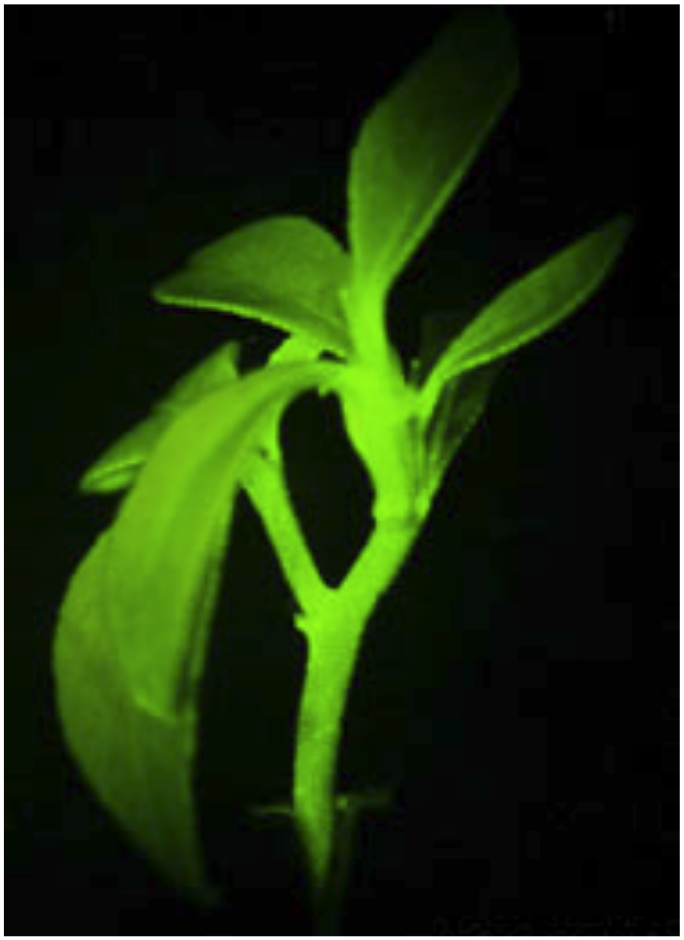
GloFish Fluorescing With Different Colors!!

How About a GloFly!

What About "GloMice!!!"

What About Glo Monkeys, Cats and Pigs!!

And a GloPlant With the Same Jellyfish Gene!!!



Genetic code is universal, gene from one organism can be expressed in another – some limitations