

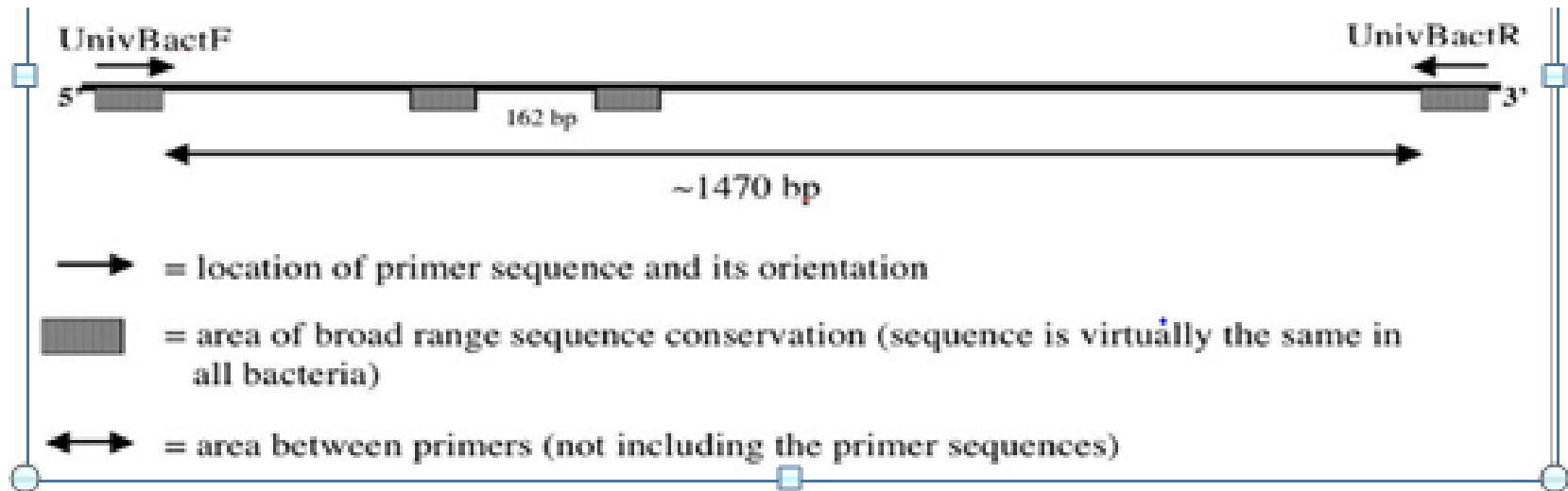
# DNA Sequencing and applications



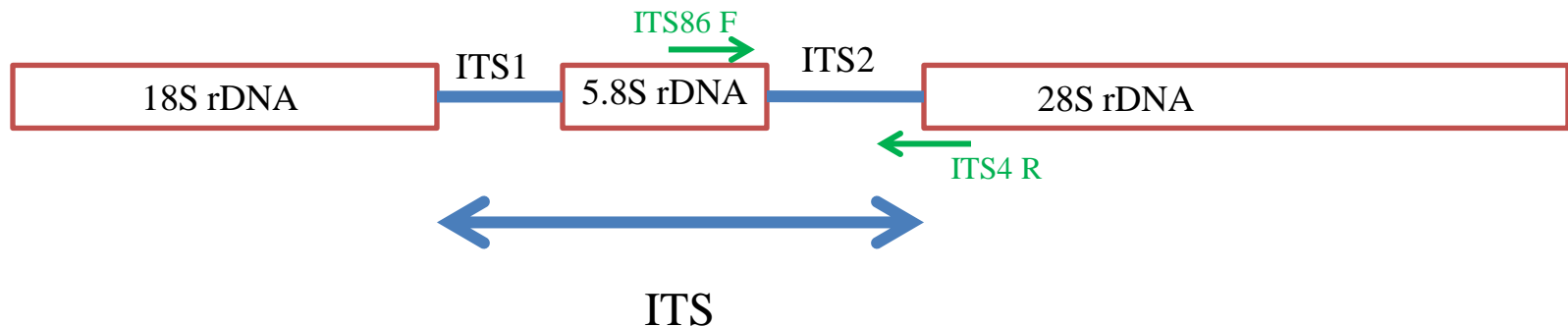
**Mohamed N. Seleem**

# Bacterial and Fungal identification

## Bacterial 16s rDNA



## Fungal ITS



**BLAST®**

Basic Local Alignment Search Tool

[Home](#)[Recent Results](#)[Saved Strategies](#)[Help](#)[► NCBI/ BLAST Home](#)

BLAST finds regions of similarity between biological sequences. [more...](#)

**New****DELTA-BLAST**, a more sensitive protein-protein search[Go](#)

## BLAST Assembled RefSeq Genomes

Choose a species genome to search, or [list all genomic BLAST databases](#).

- ☐ [Human](#)
- ☐ [Mouse](#)
- ☐ [Rat](#)
- ☐ [Arabidopsis thaliana](#)
- ☐ [Oryza sativa](#)
- ☐ [Bos taurus](#)
- ☐ [Danio rerio](#)
- ☐ [Drosophila melanogaster](#)
- ☐ [Gallus gallus](#)
- ☐ [Pan troglodytes](#)
- ☐ [Microbes](#)
- ☐ [Apis mellifera](#)

## Basic BLAST

Choose a BLAST program to run.

[nucleotide blast](#)

Search a **nucleotide** database using a **nucleotide** query  
*Algorithms: blastn, megablast, discontinuous megablast*

[protein blast](#)

Search **protein** database using a **protein** query  
*Algorithms: blastp, psi-blast, phi-blast, delta-blast*

[blastx](#)

Search **protein** database using a **translated nucleotide** query

[tblastn](#)

Search **translated nucleotide** database using a **protein** query

[tblastx](#)

Search **translated nucleotide** database using a **translated nucleotide** query

**blastn** [blastp](#) [blastx](#) [tblastn](#) [tblastx](#)

## Enter Query Sequence

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter accession number(s), gi(s), or FASTA sequence(s) ?

[Clear](#)

Query subrange ?

From

To

Or, upload file

[Browse...](#)

Job Title

Enter a descriptive title for your BLAST search ?

☐ Align two or more sequences ?

## Choose Search Set

Database

☐ Human genomic + transcript ☐ Mouse genomic + transcript ☒ Others (nr etc.):

Nucleotide collection (nr/nt)

Organism

Optional

Enter organism name or id--completions will be suggested

☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown. ?

Exclude

Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Entrez Query

Optional

Enter an Entrez query to limit search ?

**blastn** [blastp](#) [blastx](#) [tblastn](#) [tblastx](#)

## Enter Query Sequence

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter accession number(s), gi(s), or FASTA sequence(s)

[Clear](#)

Query subrange

From

To

Or, upload file

[Browse...](#)

Job Title

Enter a descriptive title for your BLAST search

☐ **Align two or more sequences**

## Choose Search Set

Database

☐ Human genomic + transcript ☐ Mouse genomic + transcript ☒ Others (nr etc.):

◆ 16S ribosomal RNA sequences (Bacteria and Archaea)

Organism

Optional

Enter organism name or id—completions will be suggested

☐ Exclude [+](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude

Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Entrez Query

Optional

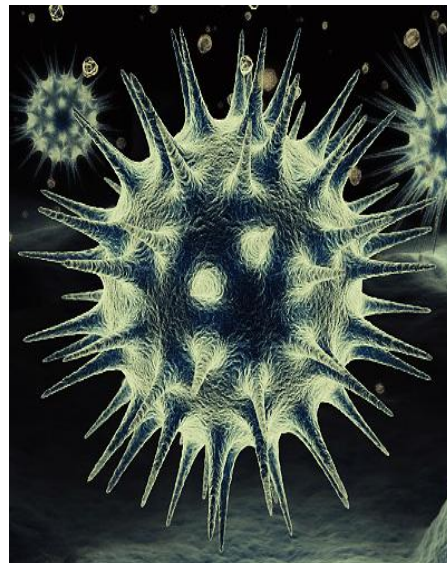
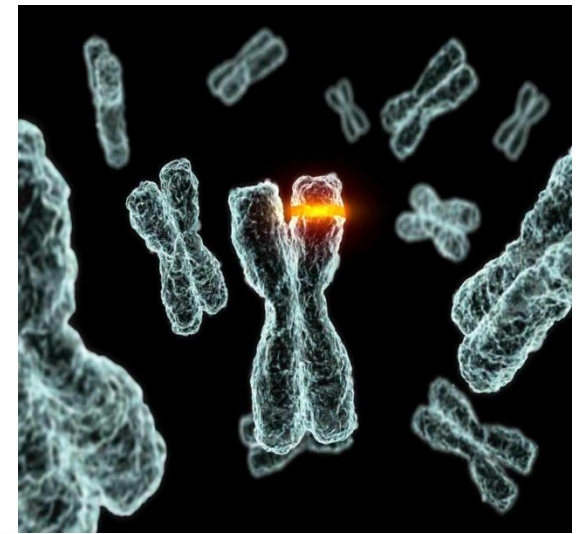
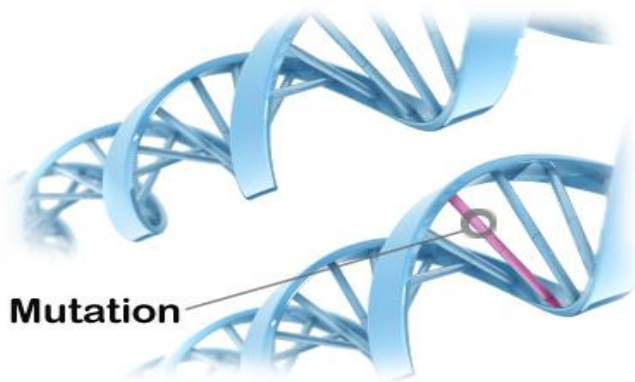
Enter an Entrez query to limit search

## Program Selection

Optimize for



# Mutation







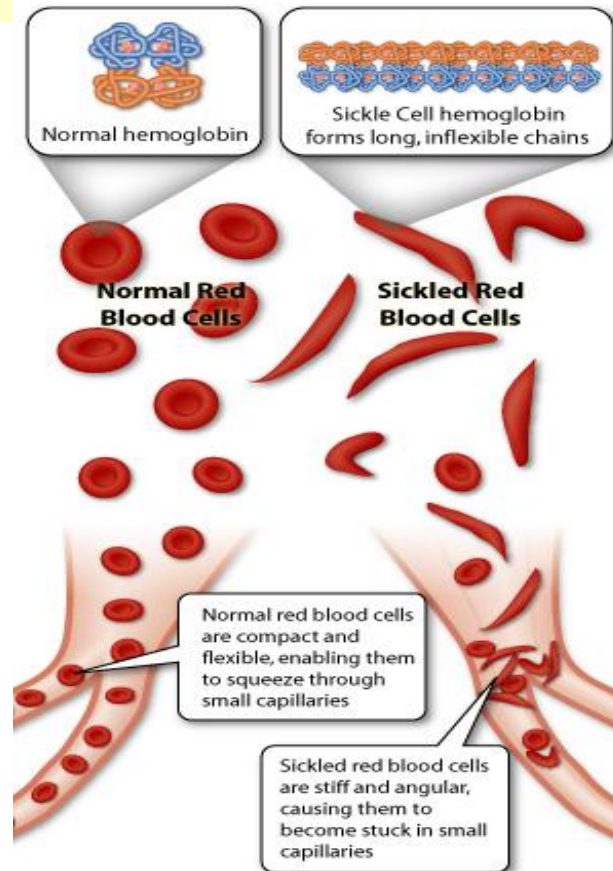
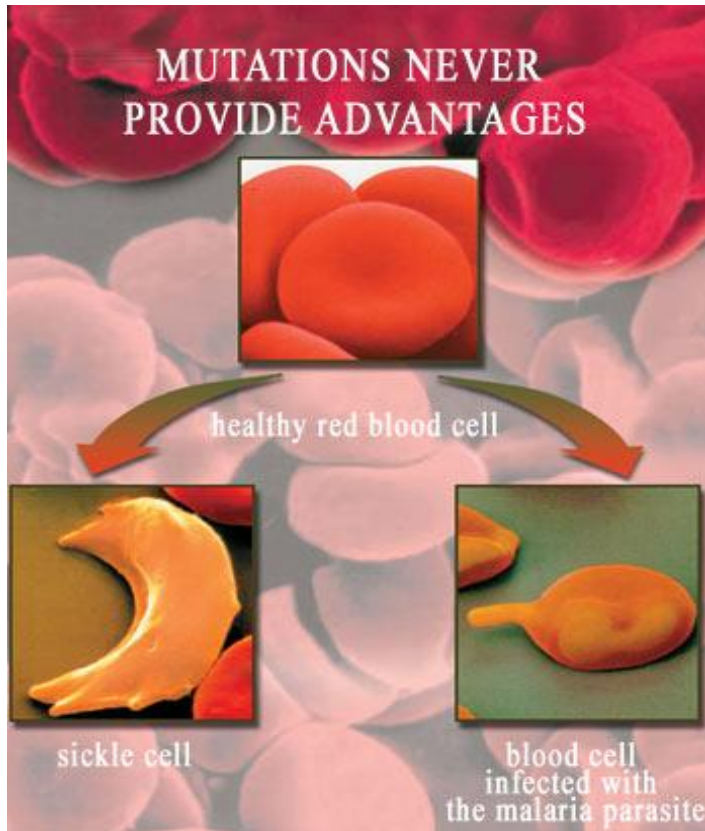
# Sickle Cell Anemia

## HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

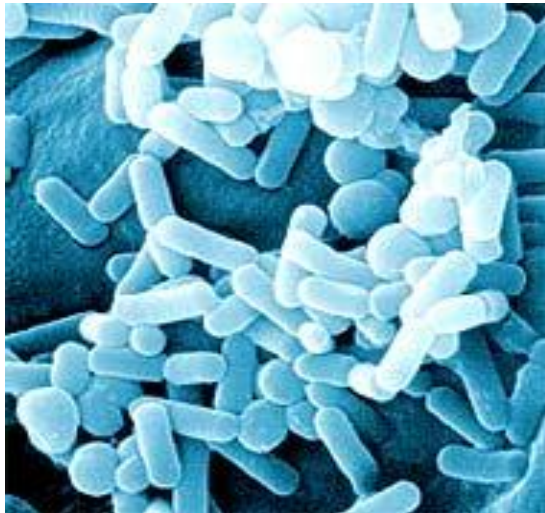
## HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9

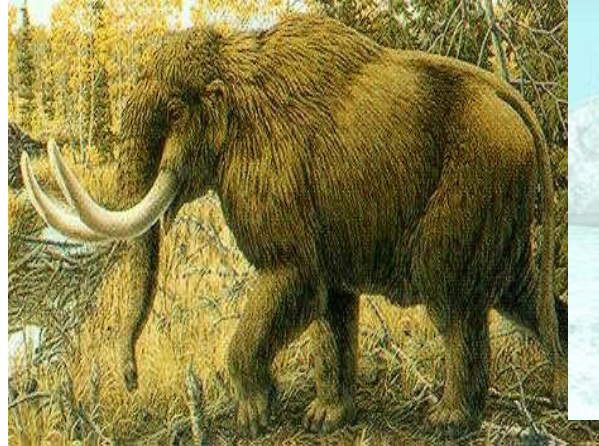


A  
T  
Glutamic  
-Valine

# *Studying evolution*

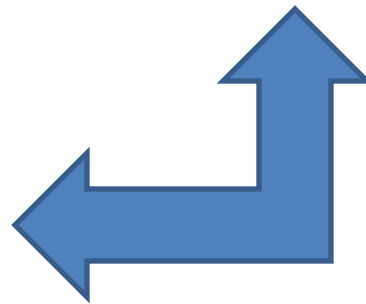


*Enterobacter cloacae*



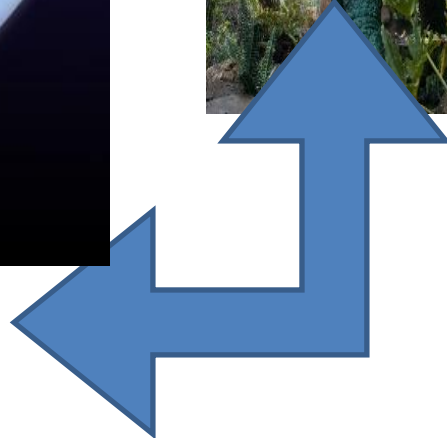
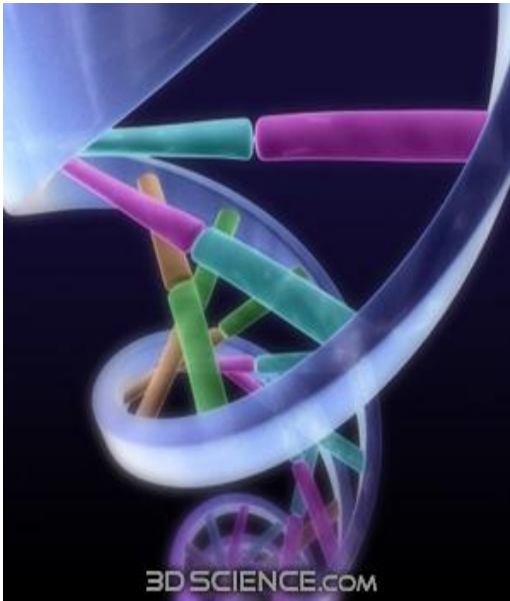
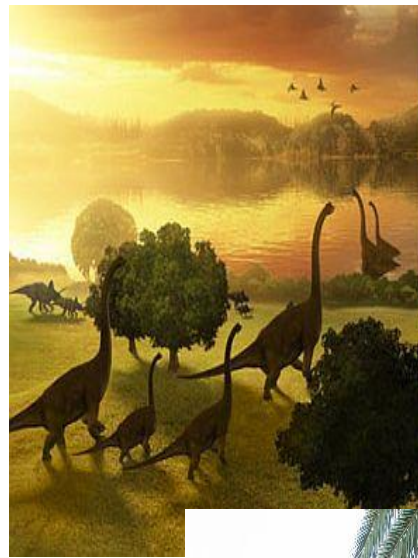
Mastodon

11,000  
years ago



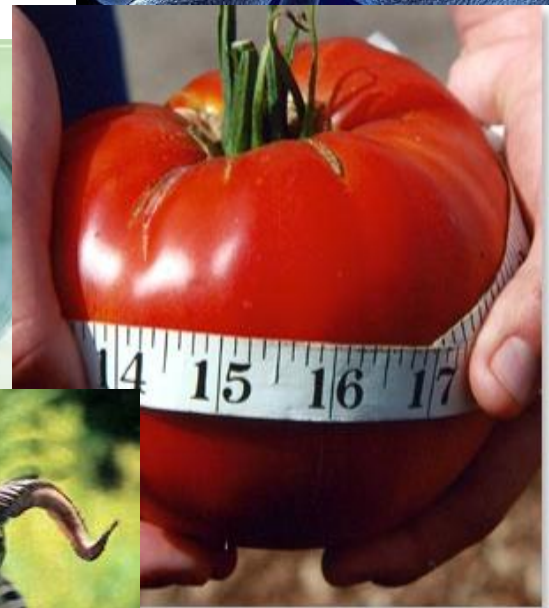
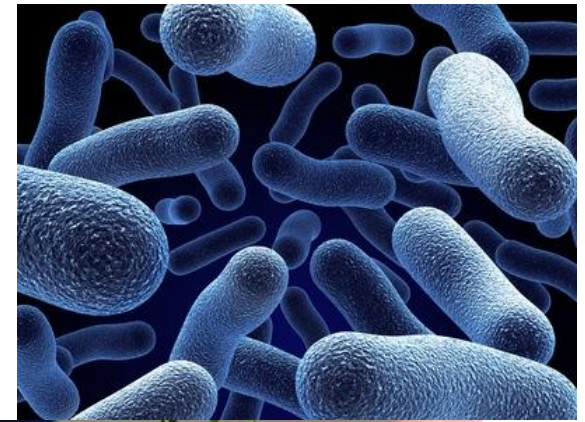


# Fossils





# *Genetic Engineering & Biotechnology*



# Non-Browning Apples



Polyphenol oxidase (PPO) is at the heart of enzymatic browning of both potatoes and apples. The browning reaction results from the oxidation of phenolic compounds in the fruit under the action of (PPO). Researchers pioneered a genetic modification to silence potatoes' PPO genes

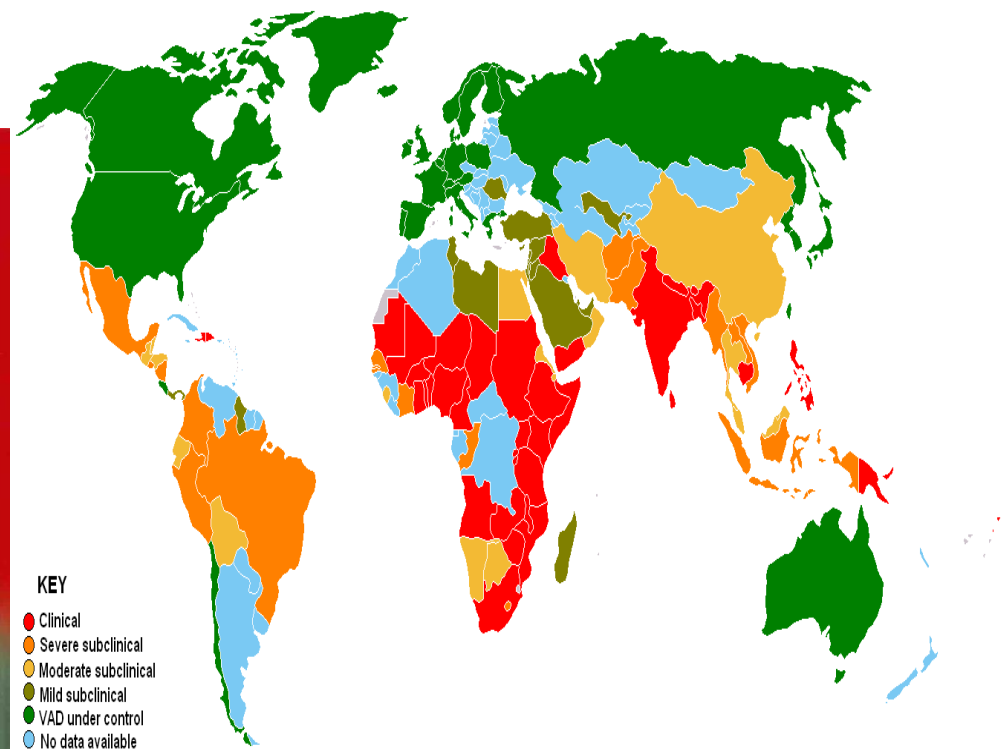


Arctic apples produce less than 10 percent of the Polyphenol oxidase (PPO) of their conventional counterparts – not enough to allow apples to brown.





Ingo Potrykus



Golden Rice

In 2005, a new variety called *Golden Rice 2*, which produces up to 23 times more beta-carotene than the original golden rice was announced



## Vitamin C

Citrus fruits, green peppers, strawberries, tomatoes, broccoli and sweet and white potatoes are all excellent food sources of vitamin C (ascorbic acid)

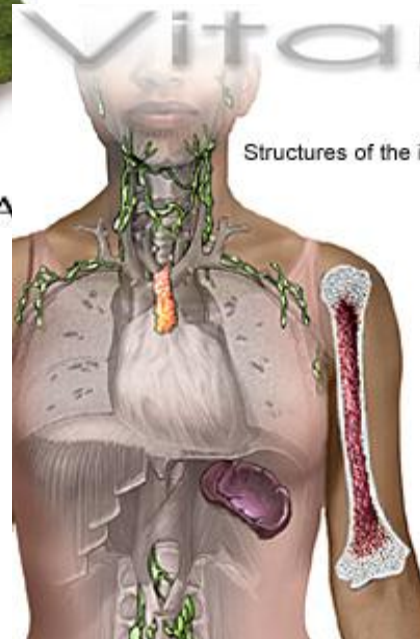


ADA



## Vitamin C

Structures of the immune system



Vitamin C promotes a healthy immune system, helps wounds heal, maintains blood vessels and connective tissue and aids in the absorption of iron

RDA: 60 mg

Water-soluble

ADAM.



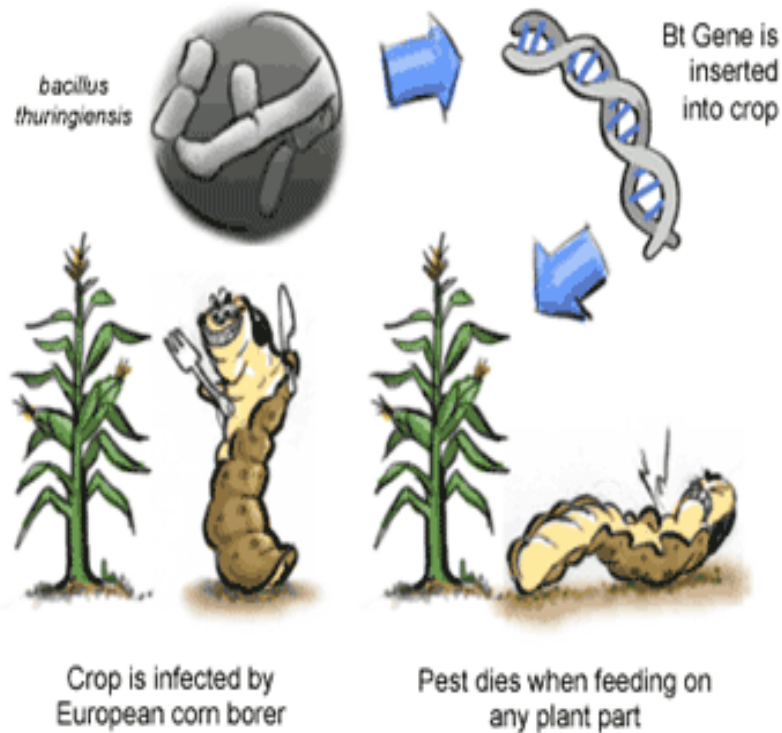
4XSalt

- Freeze resistance
- Drought resistance



*Arabidopsis thaliana*

# Friendly Insecticides



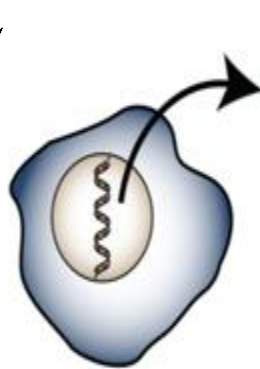
*Bacillus thuringiensis* bacteria produce a protein toxin that kills insect larvae pests and is 80,000 times more toxic than the typical chemical insecticide.



Pesticide reduced almost 90 percent in Australia since the introduction of insect-resistant Bt cotton



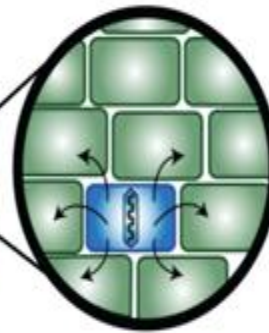
# Plant-Based Vaccine



...biological source of DNA which codes for an immunogenic protein of interest



Insertion of the antigenic DNA into the virus



Infection of the tobacco leaf with the virus and propagation of the virus throughout the plant stimulating the manufacture of the protein from the biological source

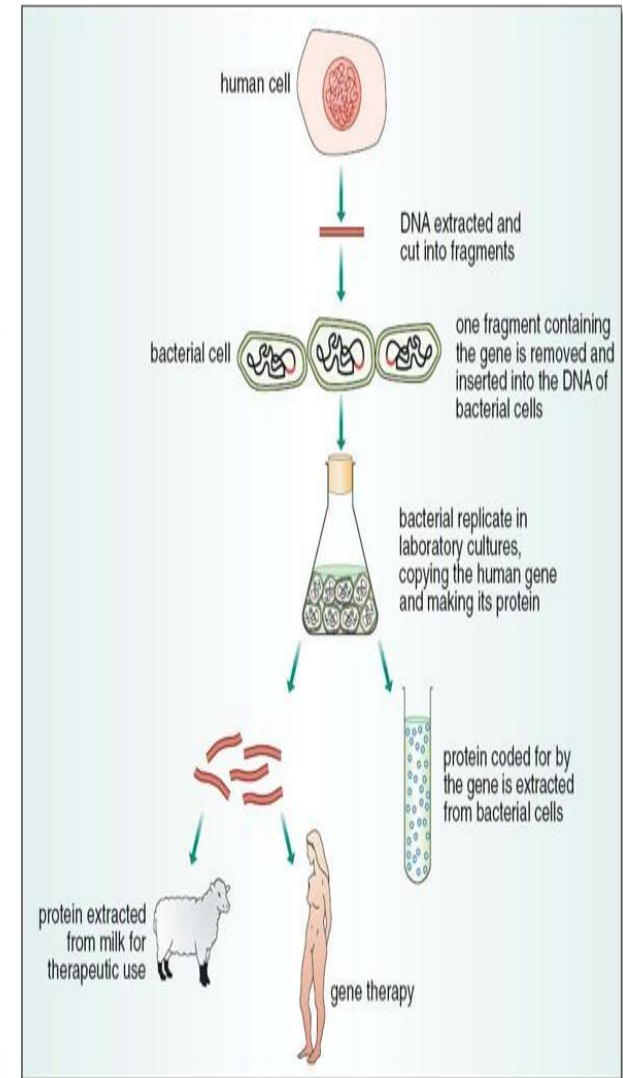
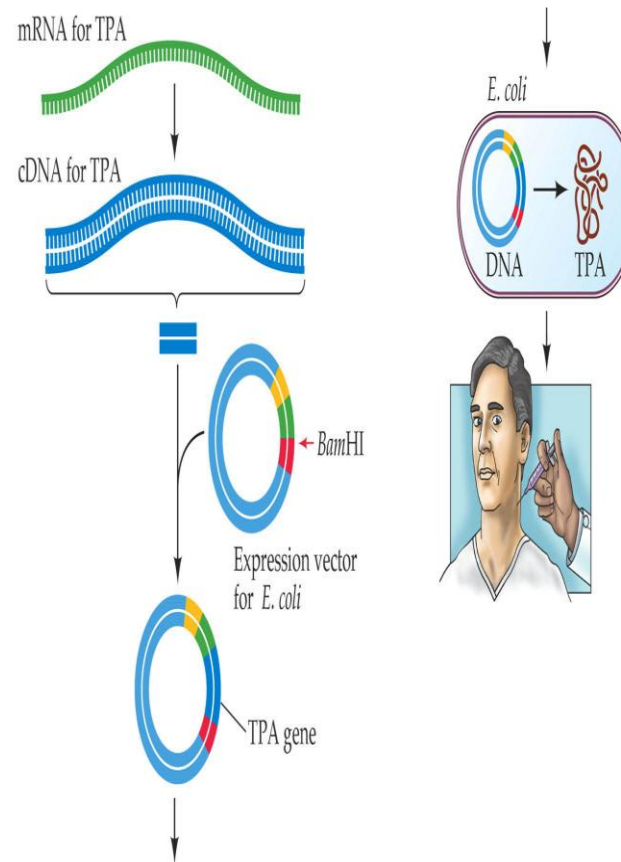


Isolation of the proteins produced by the plant to create a vaccine

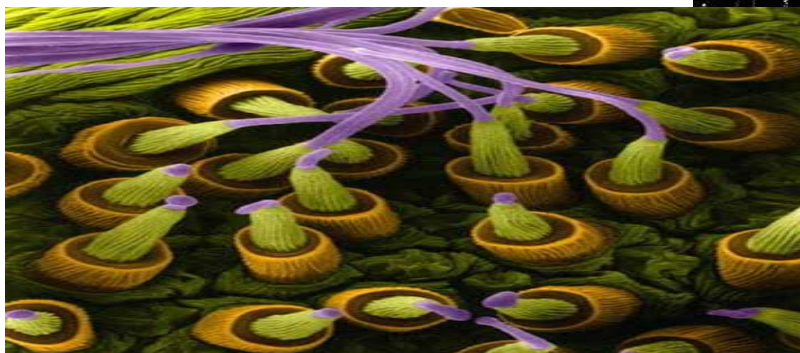




# Synthetic Insulin & Tissue plasminogen activator



# Spider Silk



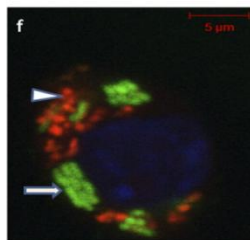
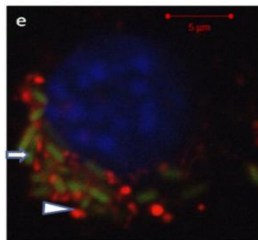
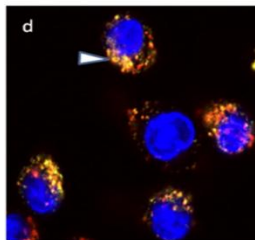
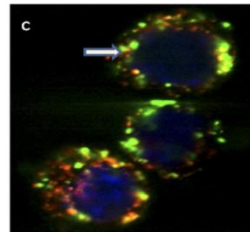
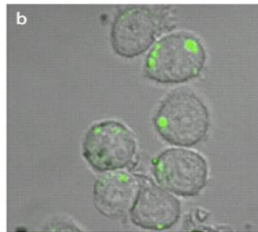
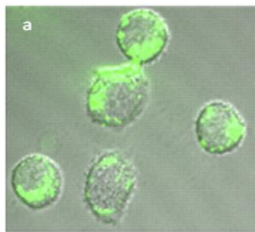
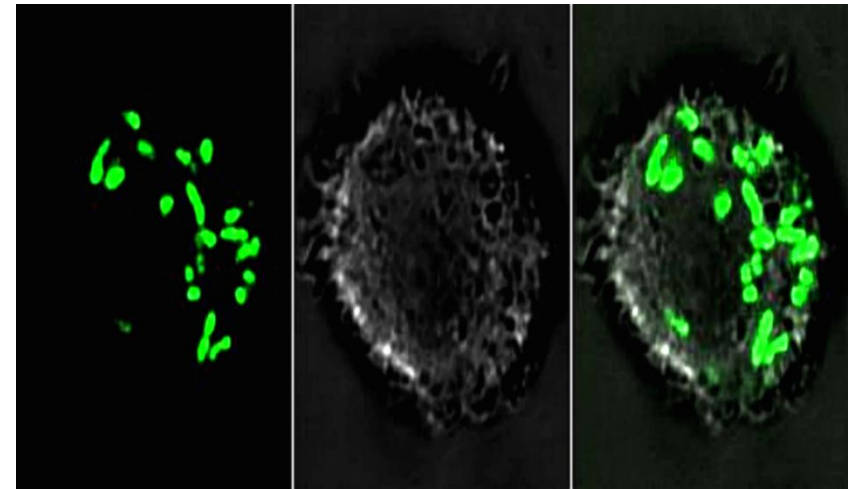
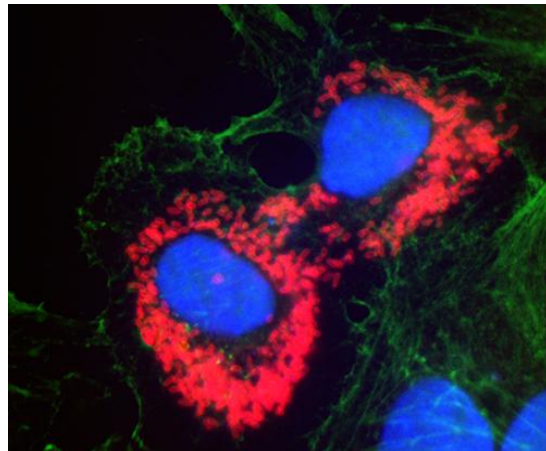
5X stronger than steel



# Tagging

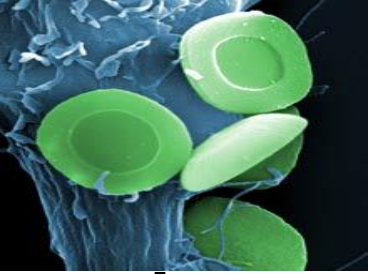
## Hide and seek: Game of Survival

Seleem et al., 2004



Confocal image of J774.A1 cells infected with *B. abortus* that has been transformed with vector expressing GFP after 4 hours of infection

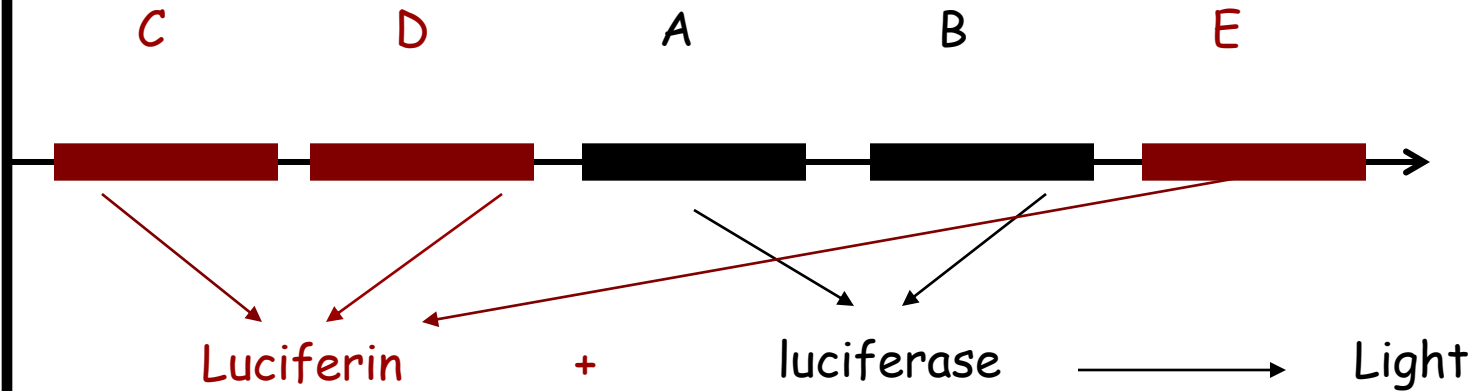




# Lux Model for drug testing

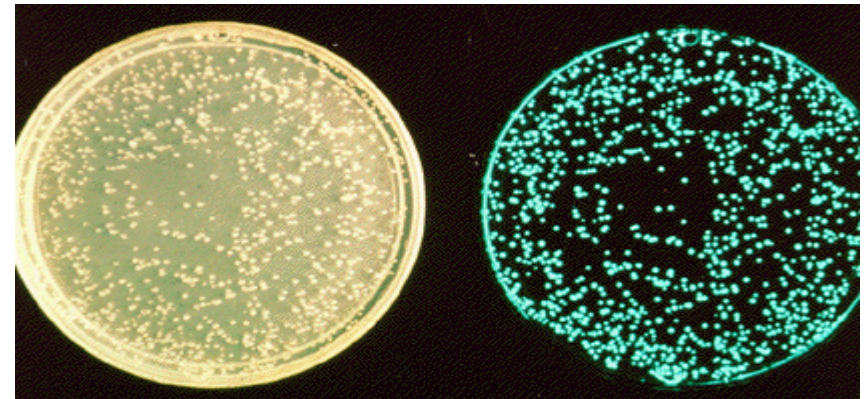
**Lux operon (*luxCDABE*)**  
*Photobacterium luminescens*

6.7 kb

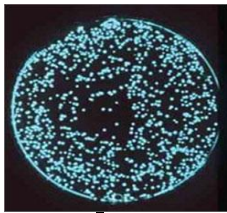


Just about 100% of a firefly's light is given off as light.

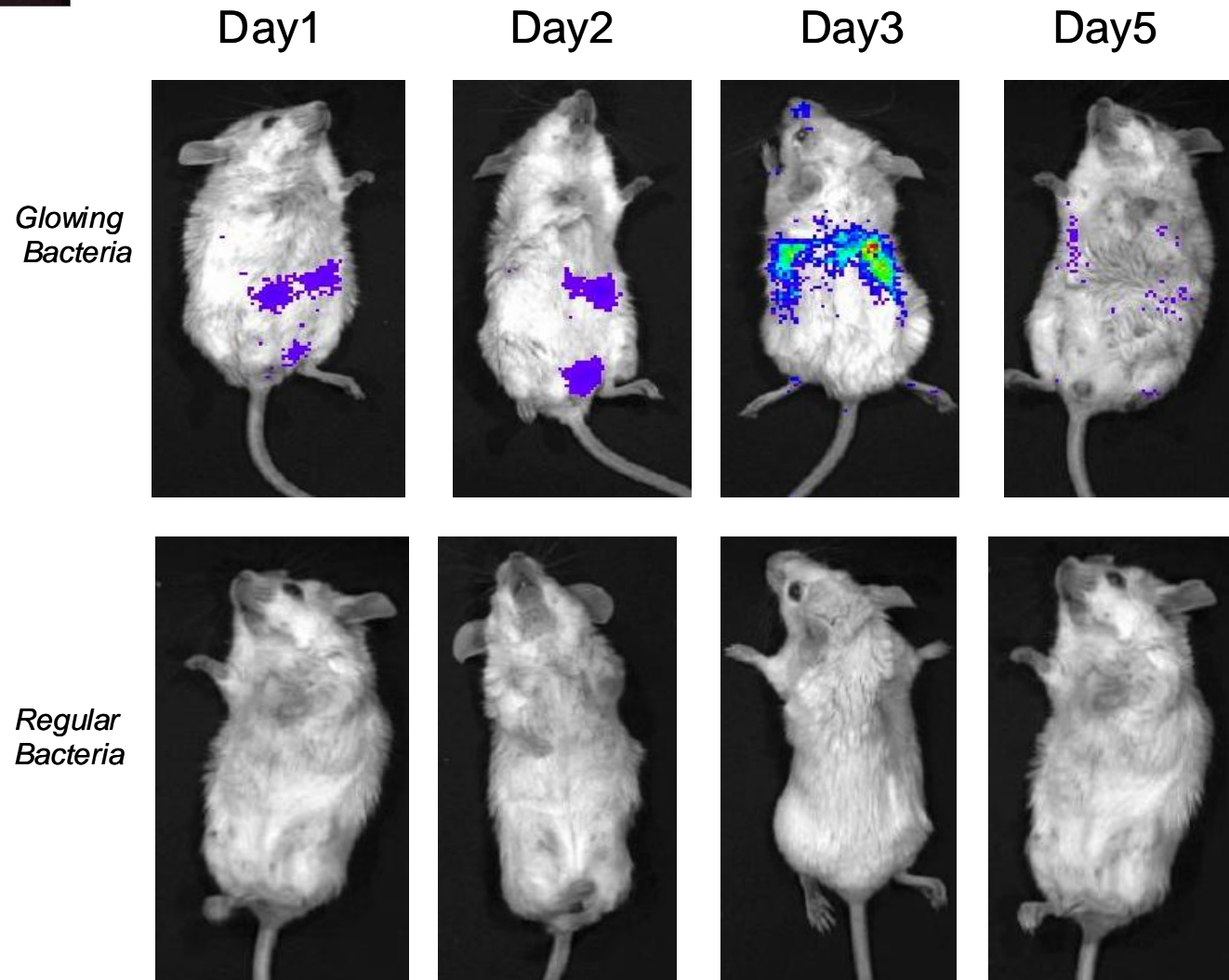
By comparison, a normal electric light bulb gives off only 10% of its energy as light, while 90% is wasted as heat.







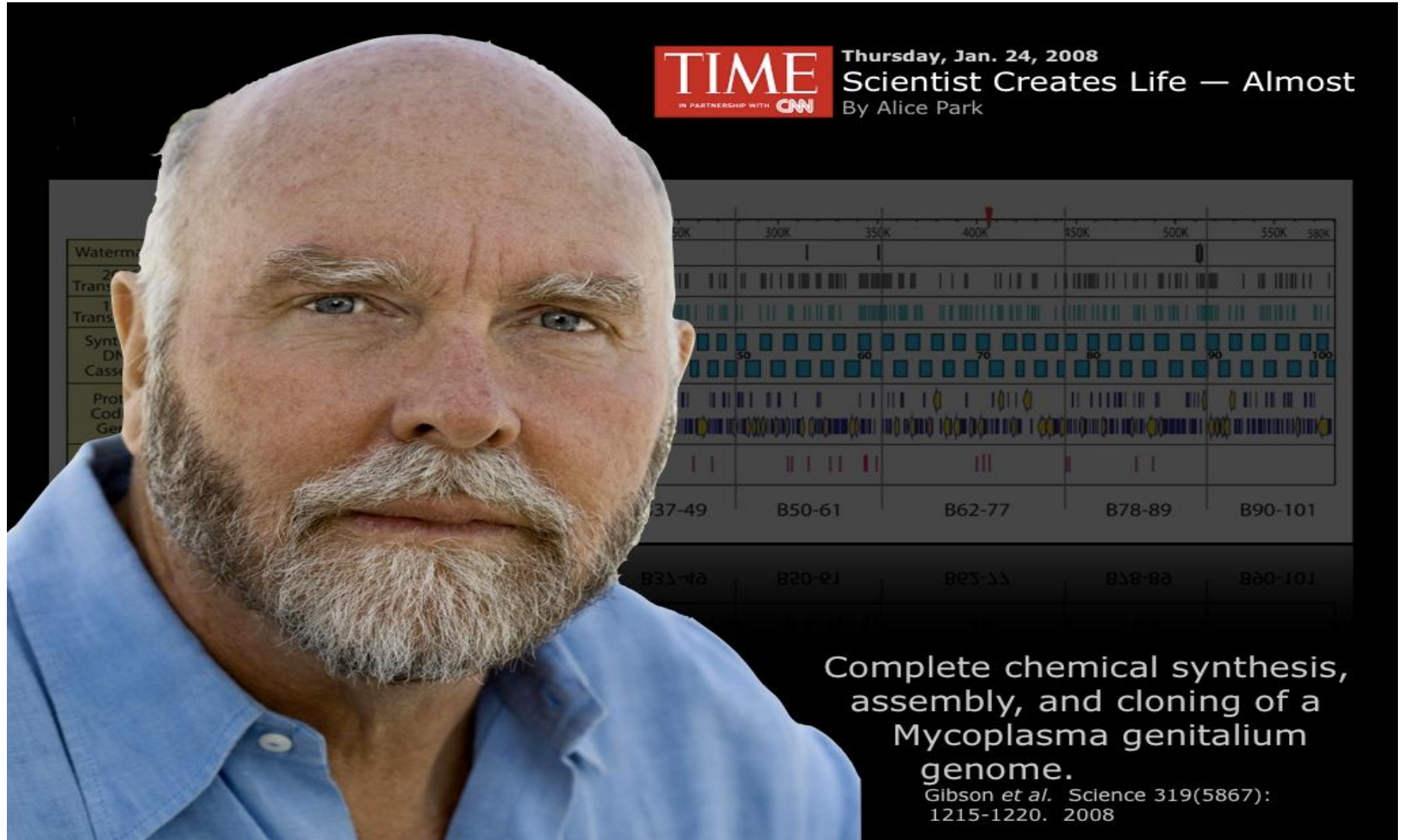
# Infected mice with *lux* bacteria



## 16.1 *Some Medically Useful Products of Biotechnology*

PRODUCT	USE
Colony-stimulating factor	Stimulates production of white blood cells in patients with cancer and AIDS
Erythropoietin	Prevents anemia in patients undergoing kidney dialysis and cancer therapy
Factor VIII	Replaces clotting factor missing in patients with hemophilia A
Growth hormone	Replaces missing hormone in people of short stature
Insulin	Stimulates glucose uptake from blood in people with insulin-dependent (Type I) diabetes
Platelet-derived growth factor	Stimulates wound healing
Tissue plasminogen activator	Dissolves blood clots after heart attacks and strokes
Vaccine proteins: Hepatitis B, herpes, influenza, Lyme disease, meningitis, pertussis, etc.	Prevent and treat infectious diseases

# *Synthetic life*



**TIME**  
IN PARTNERSHIP WITH **CNN**

Thursday, Jan. 24, 2008  
**Scientist Creates Life — Almost**  
By Alice Park

Watermark  
Trans  
Trans  
Synth  
DM  
Cass  
Prot  
Cod  
Gen

37-49 B50-61 B62-77 B78-89 B90-101

B33-48 B20-21 B25-33 B38-48 B40-101

Complete chemical synthesis,  
assembly, and cloning of a  
*Mycoplasma genitalium*  
genome.

Gibson *et al.* Science 319(5867):  
1215-1220. 2008



## *M. genitalium*

*Obligate intracellular bacterium*

*482 genes and 580,000 bp*

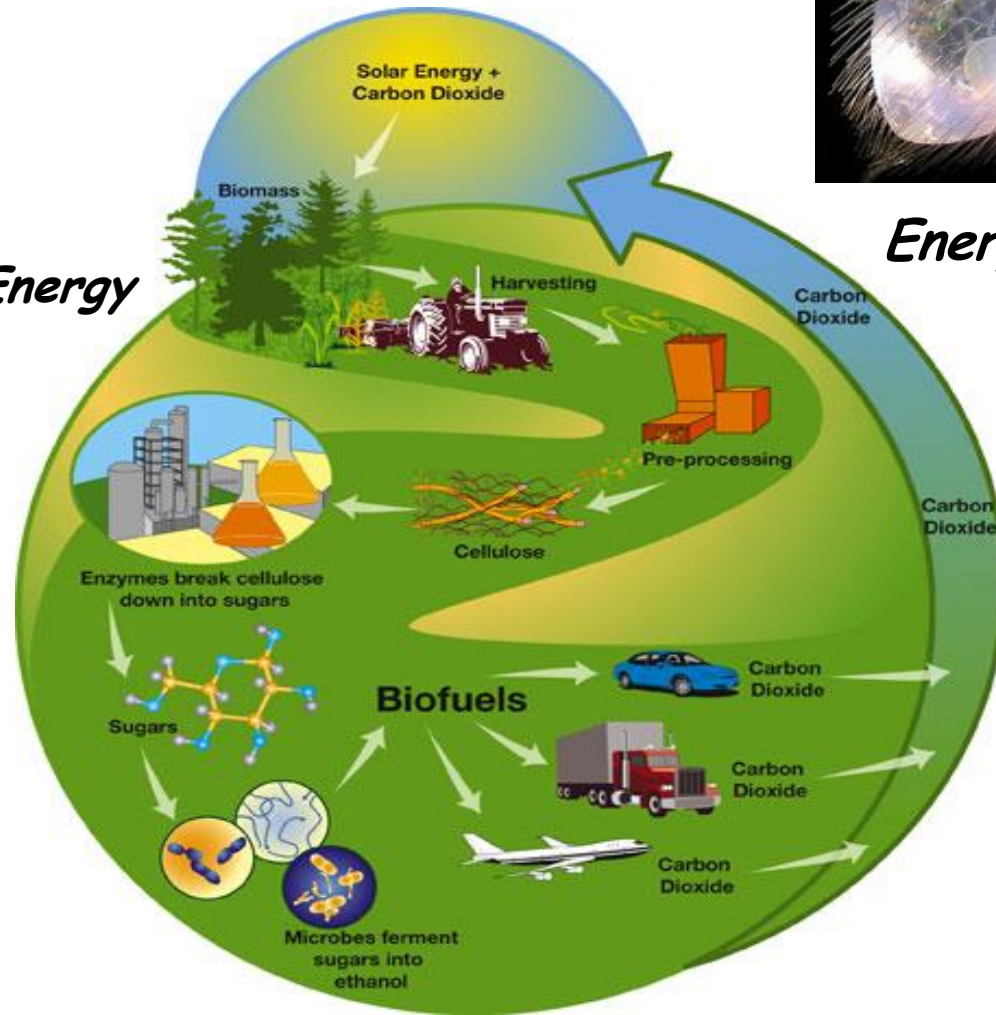
*Smallest genome*

# *382 genes essential for life*



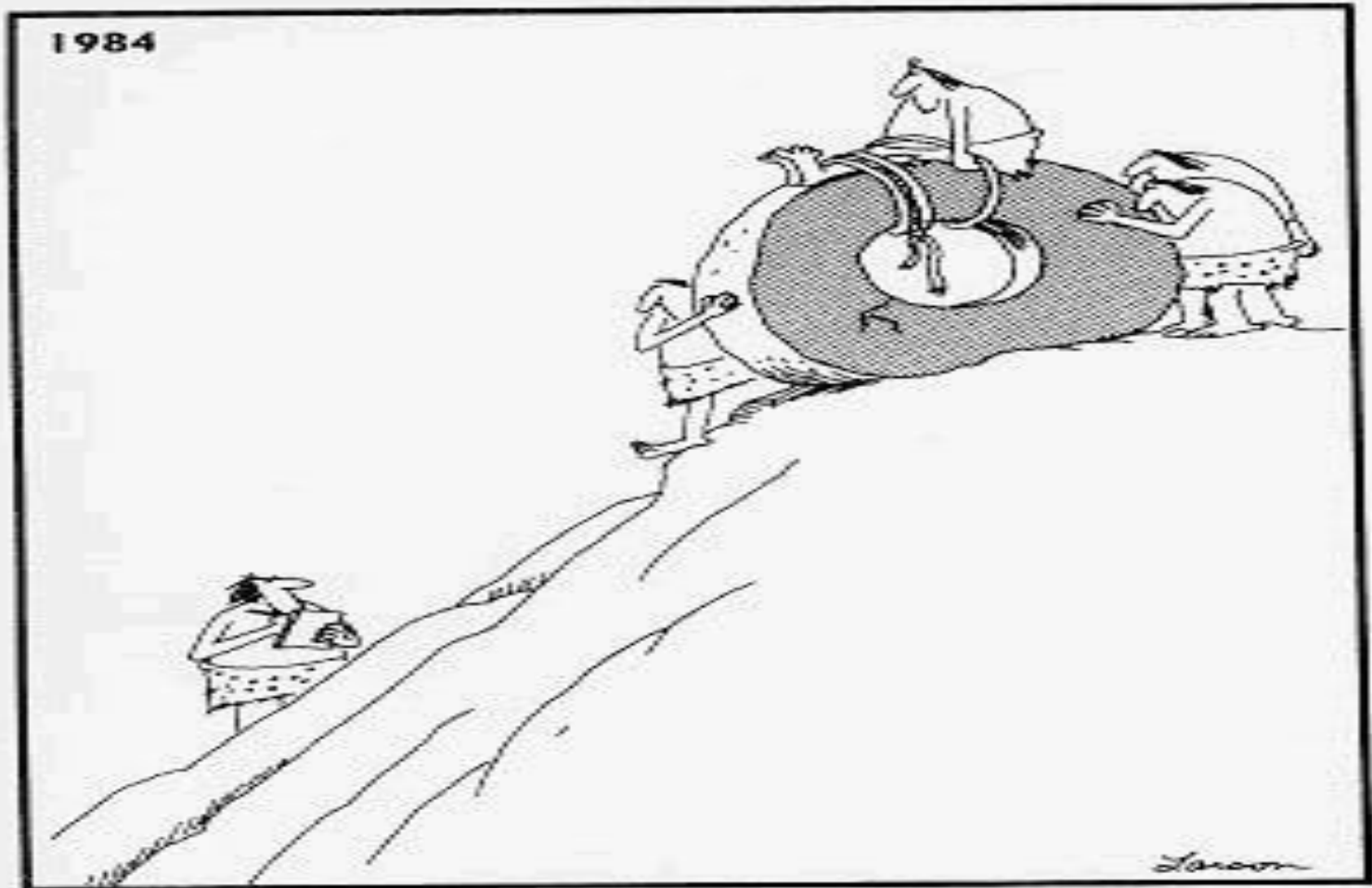
*Biological Alternative Energy*

*Energy Bug*





# Questions!!!



Early experiments in transportation