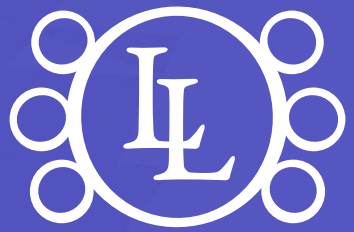


LOSCHMIDT
LABORATORIES

2. Introduction to Molecular Biotechnology

Outline

- Definition of biotechnology
- History of biotechnology
- Fundamentals of molecular biotechnology
- Basic concept of rDNA technology
- Methods of gene transfer
- Main fields of biotech applications
- Risks and positives

Definition of biotechnology

- **biotechnology** („biotech")

  bios – techne – logos

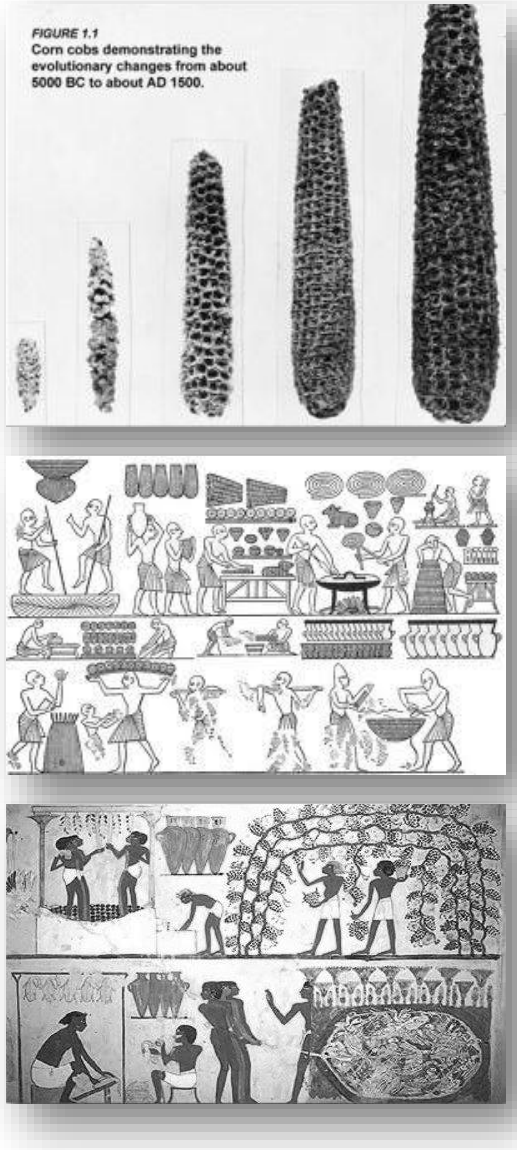
- **Kalr Ereky, 1917** – „biotechnology is a process by which raw materials could be biologically upgraded into socially useful products“

- „any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use“

  (The United Nations Convention on Biological Diversity, 1992)

History of biotechnology

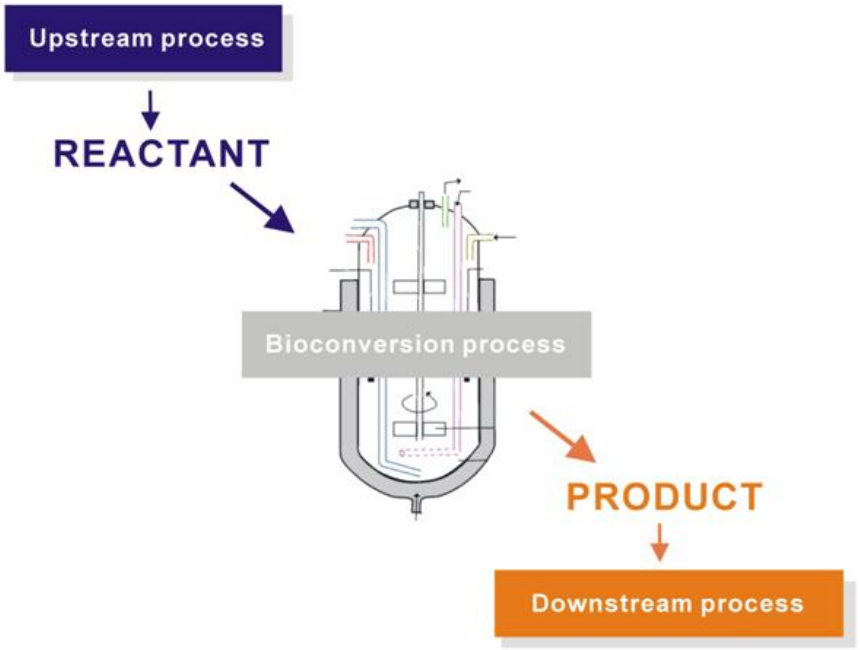
- a story that began long time ago
- 10,000 B.C. neolitic revolution
  cultivation and domestications
- 8,000 B.C. **fermented bread**
  (ancient Egypt)
- 8,000 B.C. **cheese making**
  (the Middle East)
- 6,000 B.C. **wine production**
  (Egypt and the Middle East)
- 5,000 B.C. **brewing**
  (ancient Egypt)
- developed without any knowledge about existence of cells, enzymes, genes



History of biotechnology

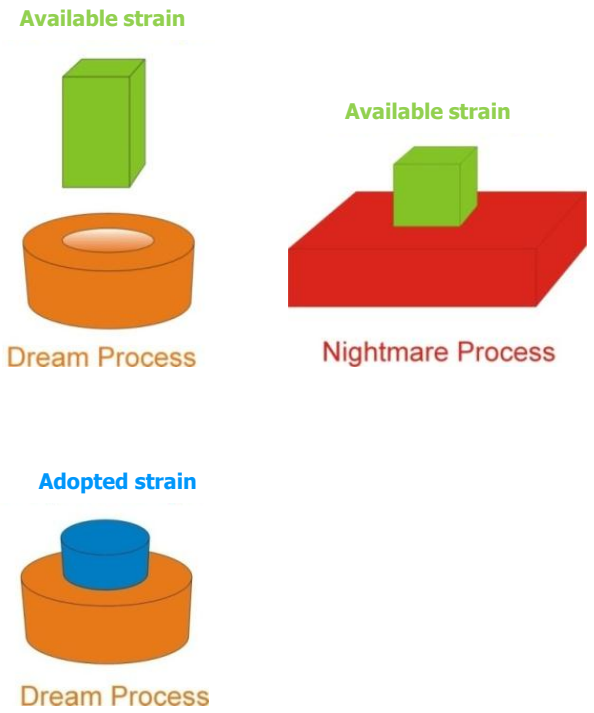
Traditional biotechnology

- 1970s biotechnology recognized as **scientific discipline** (interlink of chemical engineering, microbiology nad biochemistry)
- traditional biotechnology **based on fermentation**
- development focused on **process technology** (bioreactor design, upstream, downstream)



Traditional biotechnology

- 1970s biotechnology recognized as **scientific discipline** (interlink of chemical engineering, microbiology nad biochemistry)
- traditional biotechnology **based on fermentation**
- development focused on **process technology** (bioreactor design, upstream, downstream)
- **biotransformation component**
  - natural strains - far from optimum
  - difficult to optimise
  - induced mutagenesis and selection (chemical mutagens, UV radiation)
  - limited by inherited properties of the strain



Revolution in biotechnology

- 1973 Stanley Cohen and Herbert Boyer

  development of **recombinant DNA technology**

genetic engineering provided the means to create, rather than merely isolate, highly productive strains

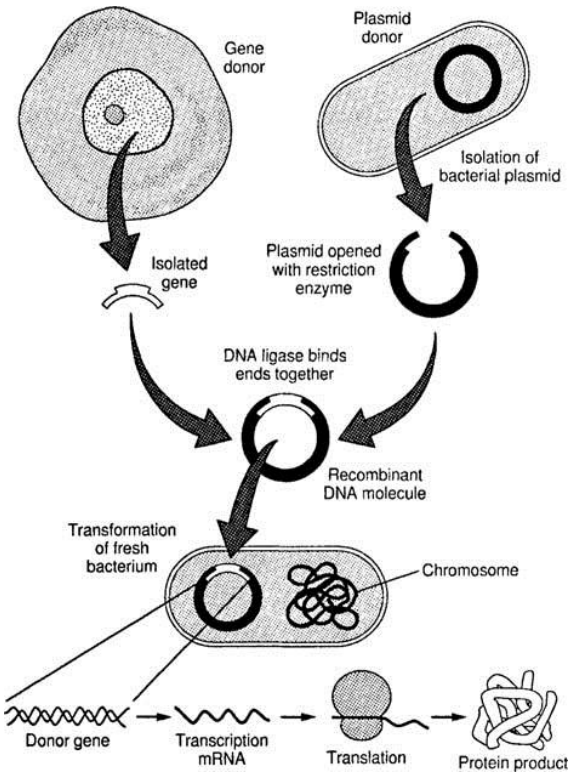
Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3240–3244, November 1973

Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122



Molecular biotechnology

- 1976 Herbert Boyer and Robert Swanson

Genentech
IN BUSINESS FOR LIFE

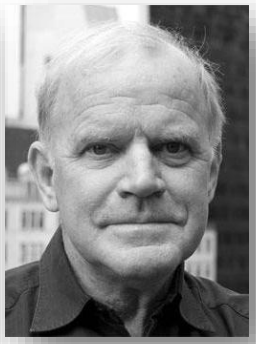
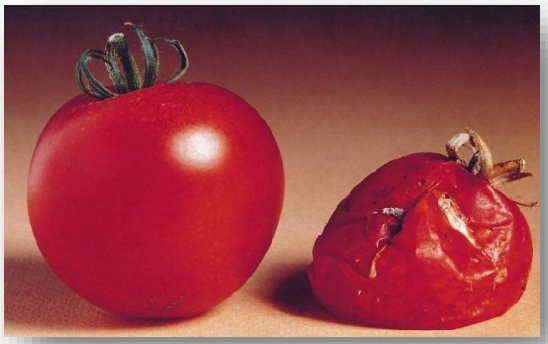
- 1978 production of **human insulin in *E. coli*** by Genentec (recombinant "human" insulin approved by FDA 1982)
- 1981 production of **recombinant growth hormone**
- 1987 production of **recombinant tissue plasminogen activator** used to dissolve blood clots during myocardial infarction
- 1980-83 about 200 **small biotechnological companies** founded in US

Molecular biotechnology

- 1974 Rudolf Jaenisch - **first transgenic mammal** (a mouse)

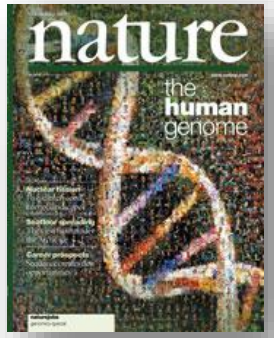
ANIMALS AND PLANTS ACT AS NATURAL BIOREACTORS

- 1982 first recombinant animal vaccine approved
- 1983 engineered Ti plasmid – **plant transformation**
- 1988 Kary Mullis - **PCR method** (**Nobel Prize in 1993**)
- 1994 **first genetically engineered food** aproved by FDA (tomato)

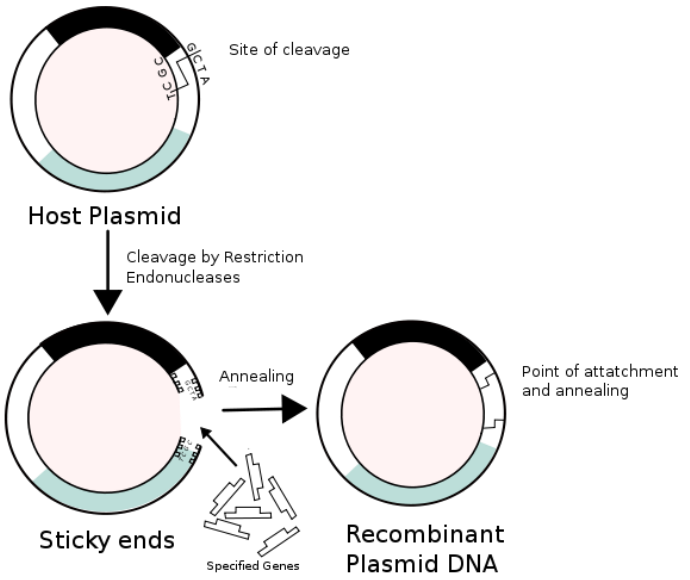
Molecular biotechnology

- 1995 **first genome** sequenced (bacterium *Haemophilus influenzae*)
- 1996 complete **eukaryotic DNA sequence**
- 1996 commercial planting of **GMO crops begins**
- 1997 Ian Wilmut – **nuclear cloning of a mammal**
- 1998 first **antisense drug** approved by FDA
- 1999 ***Drosophilia* genome** sequenced
- 2000 ***Arabidopsis* genome** sequenced
- 2000 development of „**golden rice**“
- 2001 **human genome** sequenced
- 2009 **first drug** produced in genetically engineered animal (a goat)



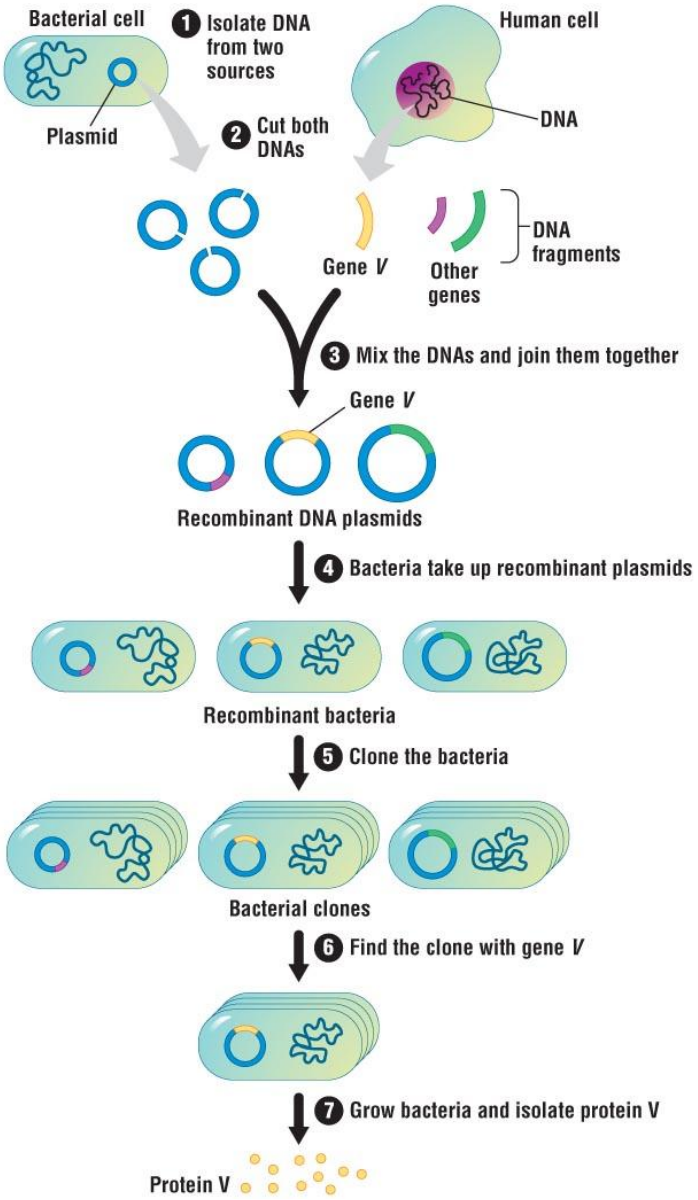
Molecular biotechnology

- **classical biotechnology** based on selective breeding
- **molecular biotechnology** (modern; „mol biotech“)
  revolutionary scientific discipline based on
  methods of gene manipulation *(Lecture 3)*
- the ability to transfer specific units of genetic information from one organism to another
- **recombinant DNA technology** (rDNA)
- **genetic engineering**
  enable create rather then isolate
  highly productive organisms



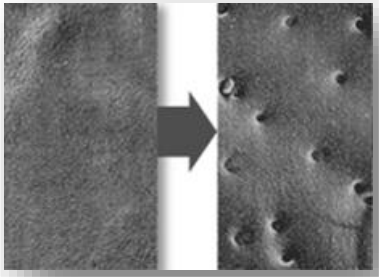
Concept of rDNA technology

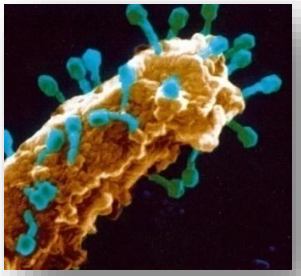
- ❑ **isolate** gene(s) of interest
- ❑ **modify** gene(s)
- ✓ *protein engineering (**Lecture 4**)*
- ❑ **ligate** gene(s) into a vector
- ❑ **transform** host organism
- ❑ **select** transformed cells
- ❑ **culture** host organism
- ❑ **application** of gene product

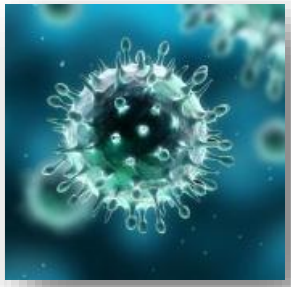


Techniques of DNA transfer

- **transformation** and **transfection**
- **direct methods**
  - electroporation (2.5 kV, 5 ms)
  - chemical transformation ($CaCl_2$)
  - heat shock (42°C)
  - micro-injection
  - biolistic delivery - „gene gun“
  - liposomal transfection
- **indirect methods**
  - transduction (bacteriophage)
  - viral and bacterial vectors







Mol. biotech applications

- **white** - industrial biotechnology (***Lecture 8***)
  - production of fine chemicals
  - production of proteins/enzymes
- **green** - agricultural biotechnology ***(Lecture 9)***
  - transgenic plants and animals
  - biofertilizers and biopesticides
- **red** - medical biotechnology ***(Lecture 10-11)***
  - developing new vaccines and drugs
  - tissue engineering and regenerative therapies
  - molecular diagnostics and pharmacogenomics
  - cell and gene therapy
- **grey** - environmental biotechnology ***(Lecture 12)***
  - biosensing and bioremediation

Pros and cons

- **safety and ethical concerns of molecular biotechnology**
  - do we have a right to move genes, creating new life forms,„playing God“?
  - will transgenic organisms be harmfull to other organism or environment?
  - should humans be genetically engineered?
- **positive aspects of molecular biotechnology**
  - opportunities to accuratelly diagnose, prevent and cure a wide range of infectious and genetic diseases
  - increase crop yield and resistence to insects and diseases, environmental stress (e.g., drought, heat, cold)
  - develop microorganisms that produce chemicals in sustainable manner
  - facilitate removal of pollutants and waste materials from environment