Recombinant DNA Technology

- **Genetic engineering, recombinant DNA technology, genetic modification/manipulation (GM) and gene splicing** are terms that are applied to the direct manipulation of an organisms genes.

- **Recombinant DNA** is a form of artificial DNA which is engineered through the combination or insertion of one or more DNA strands, thereby combining DNA sequences which would not normally occur together.

- **Cloning** is the process of creating an identical copy of something. In biology, it collectively refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. The term also encompasses situations whereby organisms reproduce asexually.

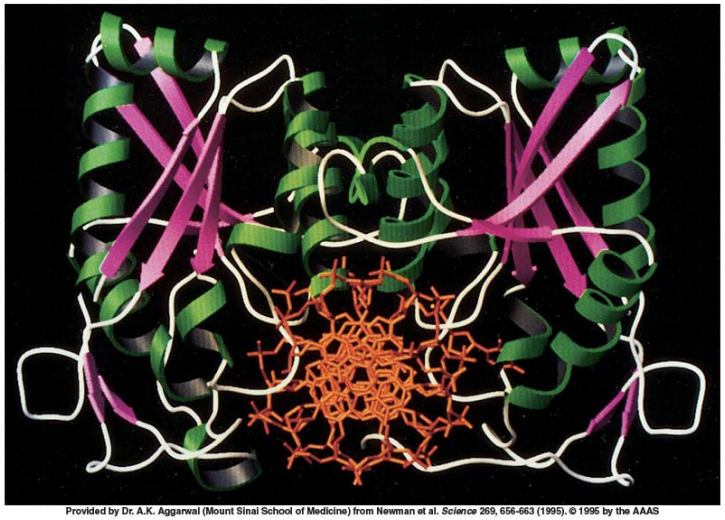
Recombinant DNA Technology

Applications of recombinant DNA techn.

* Molecular diagnostics
* Vaccines
* Bioremediation and biomass utilization
* Plant-technology
* Transgenic animals
* Gene therapy
* Commercial products
  - protein pharmaceuticals
  - restriction endonucleases
  - small biological molecules
  - antibiotics
  - biopolymers

DNA-manipulating enzymes

1. Nucleases
   - exonucleases
   - endonucleases, e.g. restriction enzymes
2. Ligases
3. Polymerases
4. DNA-modifying enzymes



Provided by Dr. A.K. Aggarwal (Mount Sinai School of Medicine) from Newman et al. *Science* 269, 656-663 (1995). © 1995 by the AAAS

These four main groups of enzymes are used to manipulate DNA *in vitro*.

A suitable aqueous environment is created (salt, pH, temp) for the enzyme.

Restriction enzymes

- bind to DNA at specific sequence called the recognition site
- cleave DNA at this site or a defined distance from it
- hundreds are commercially available
- Restriction and modification systems are very common in bacteria.
- Function:

  - exogenous DNA is cleaved

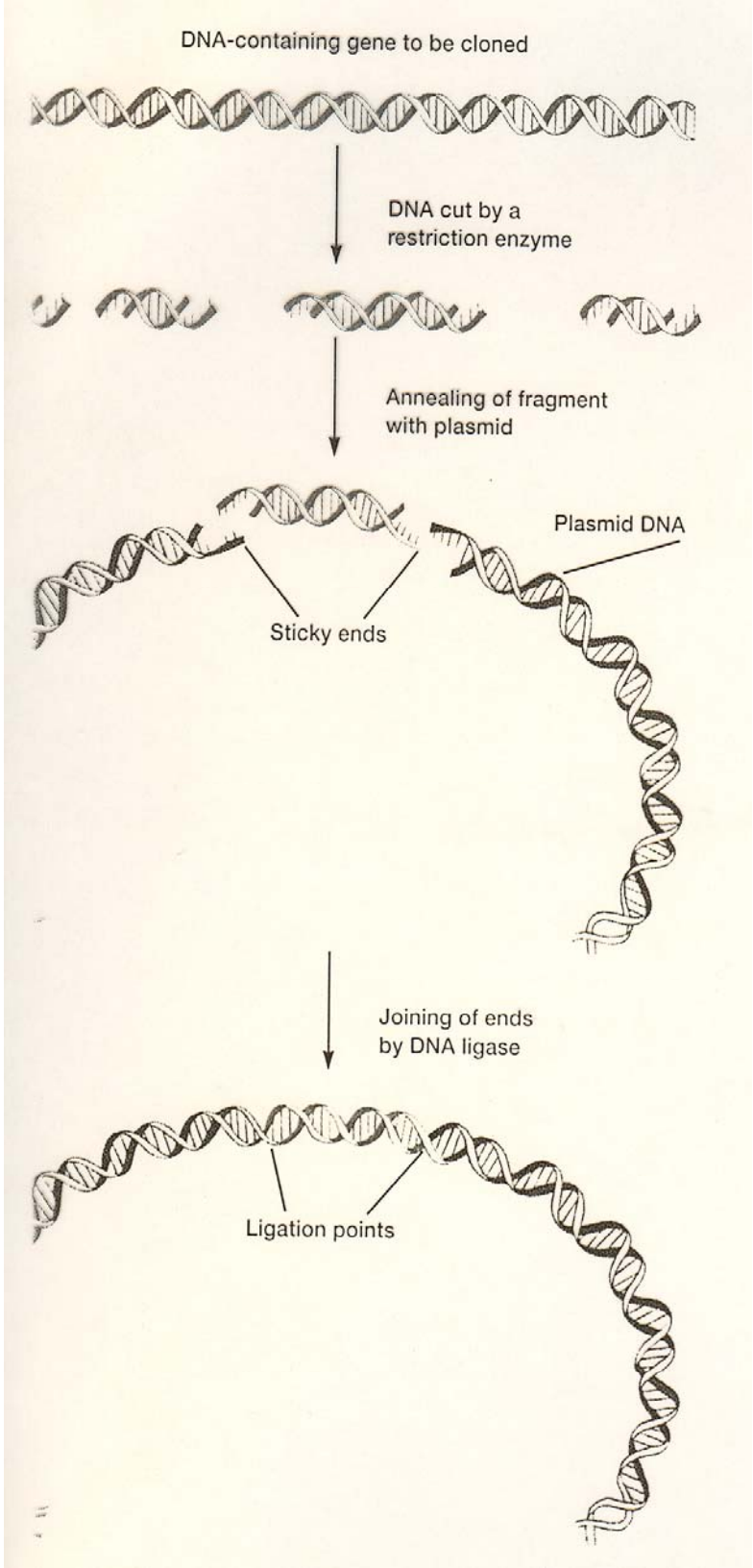
  - endogenous DNA is modified via methylation or glycosylation of A or C.

Class I type II enzymes

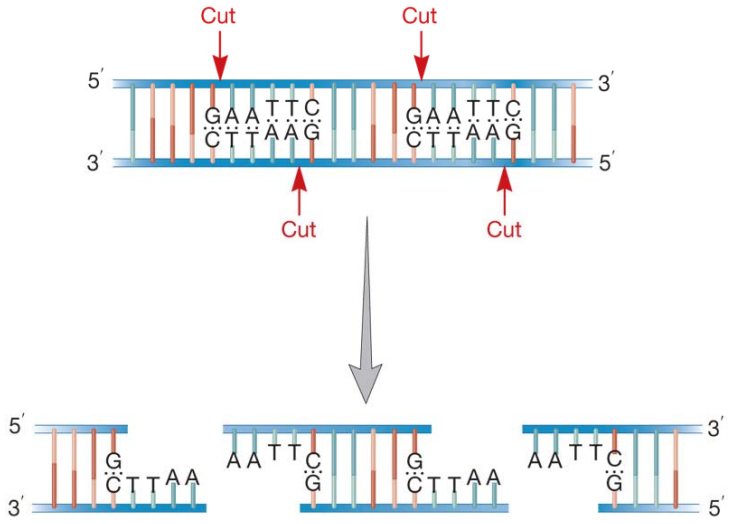
- Two different enzymes mediate cleavage and, e.g. methylation (binary system)
- A palindromic sequence that is 4 or 6 pg is often cleaved, resulting in “sticky ends” or “blunt ends”

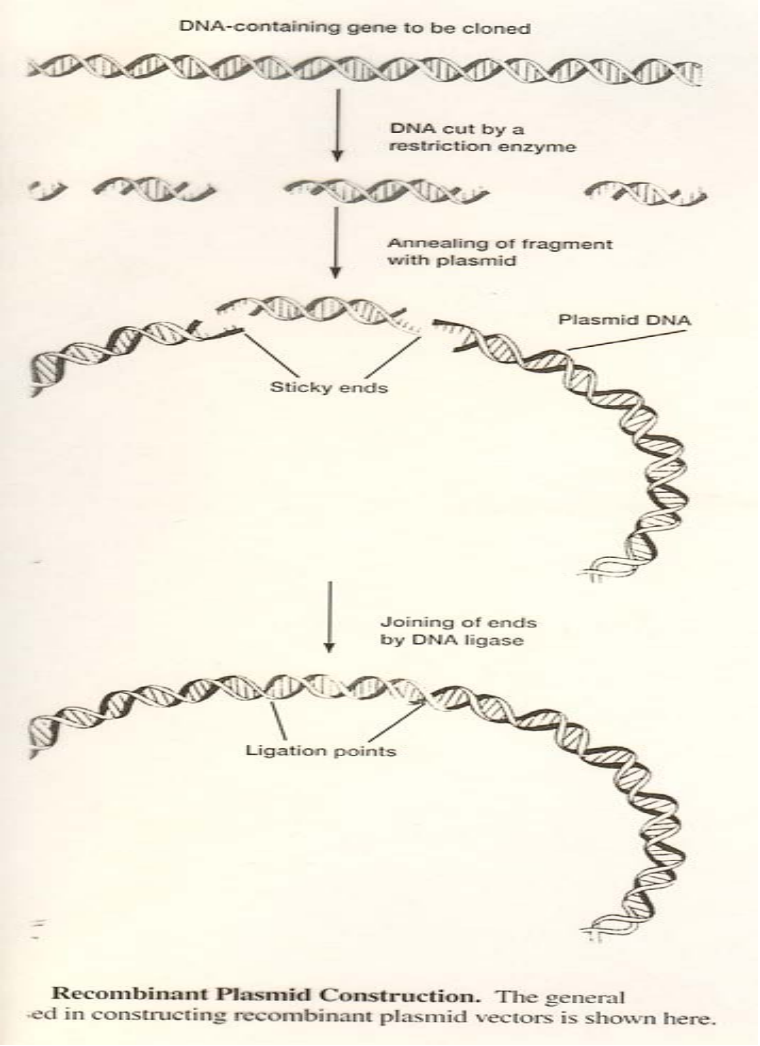
Class II (ATP-dependent, the same enzyme cleaves and methylates)

- Type I – cleave unmethylated DNA > 1000 bp from the binding site
- Type III – cleave about 25 bp from the binding site


DNA-containing gene to be cloned
DNA cut by a
restriction enzyme
Annealing of fragment
with plasmid
Plasmid DNA
Sticky ends
Joining of ends
by DNA ligase
Ligation points

Class I type II enzymes





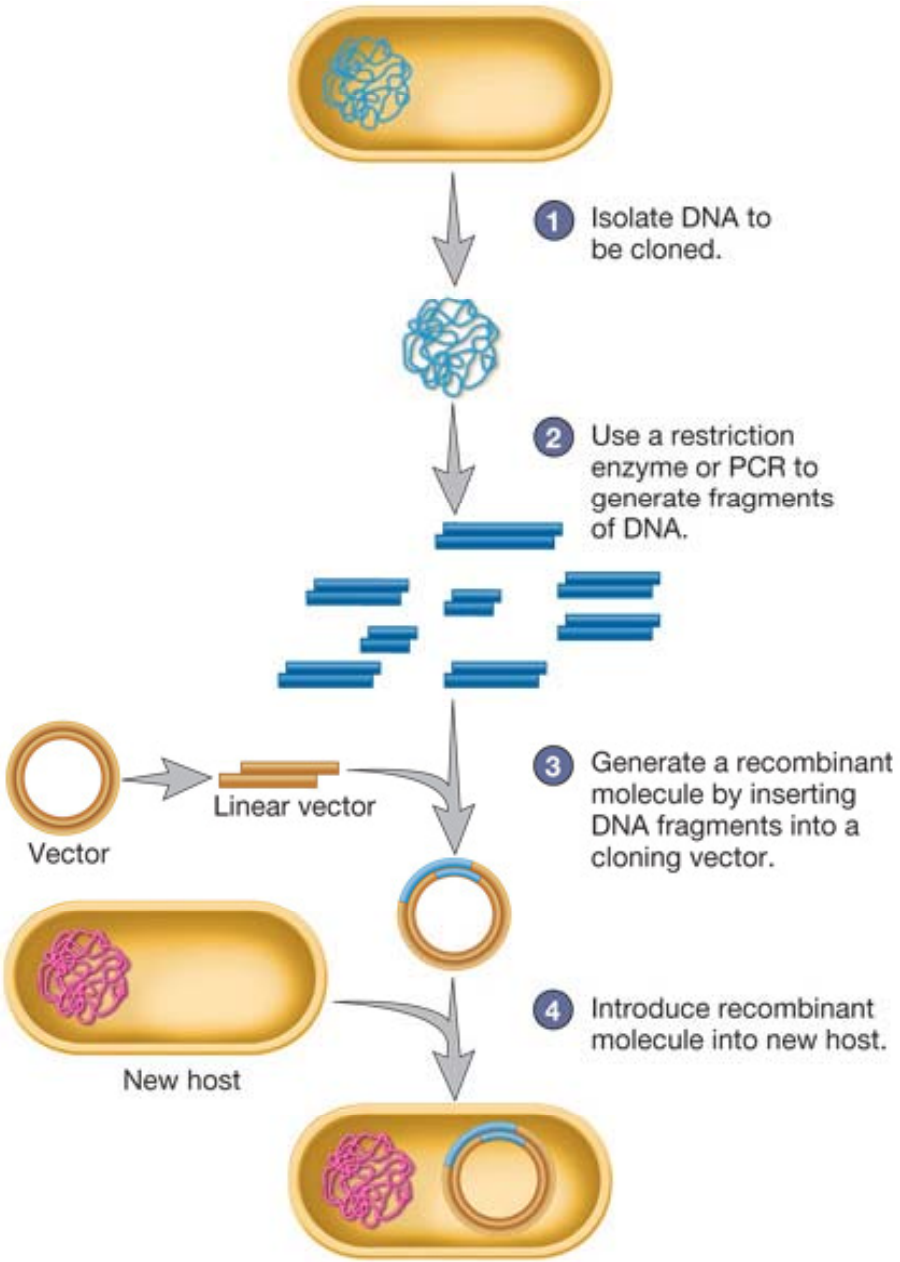
Recombinant Plasmid Construction. The general
ed in constructing recombinant plasmid vectors is shown here.

Restriction Endonucleases and Their Recognition Sequences

Table 14.2 Some Type II Restriction Endonucleases and Their Recognition Sequences

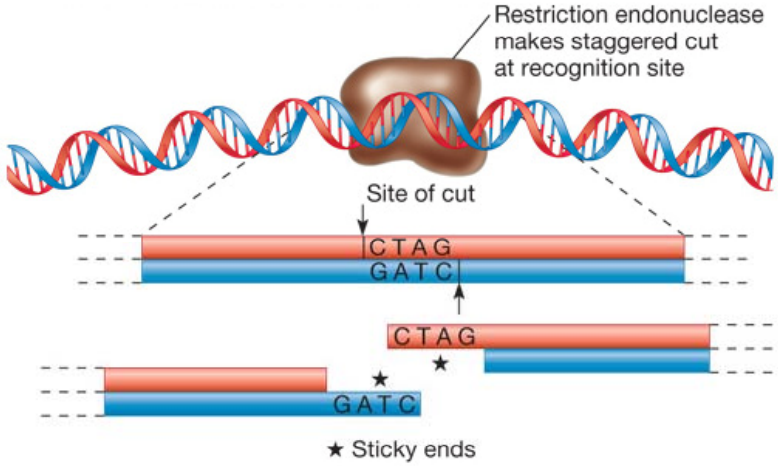
Enzyme	Microbial Source	Recognition Sequence[a]	End Produced
*Alu*I	*Arthrobacter luteus*	5′ AG↓CT 3′ 3′ TC↑GA 5′	5′ AG CT 3′ 3′ TC GA 5′
*Bam*HI	*Bacillus amyloliquefaciens* H	5′ G↓GATCC 3′ 3′ CCTAGG↑5′	5′ G GATCC 3′ 3′ CCTAG G 5′
*Eco*RI	*Escherichia coli*	5′ G↓AATTC 3′ 3′ CTTAA↑G 5′	5′ G AATTC 3′ 3′ CTTAA G 5′
*Hae*III	*Haemophilus aegyptius*	5′ GG↓CC 3′ 3′ CC↑GG 5′	5′ GG CC 3′ 3′ CC GG 5′
*Hin*dIII	*Haemophilus influenzae* d	5′ A↓AGCTT 3′ 3′ TTCGA↑A 5′	5′ A AGCTT 3′ 3′ TTCGA A 5′
*Not*I	*Nocardia otitidis-caviarum*	5′ GC↓GGCCGC 3′ 3′ CGCCGG↑CG 5′	5′ GC GGCCGC 3′ 3′ CGCCGG CG 5′
*Pst*I	*Providencia stuartii*	5′ CTGCA↓G 3′ 3′ G↑ACGTC 5′	5′ CTGCA G3′ 3′ G ACGTC 5′
*Sal*I	*Streptomyces albus*	5′ G↓TCGAC 3′ 3′ CAGCT↑G 5′	5′ G TCGAC 3′ 3′ CAGCT G 5′

Steps in Cloning a Gene

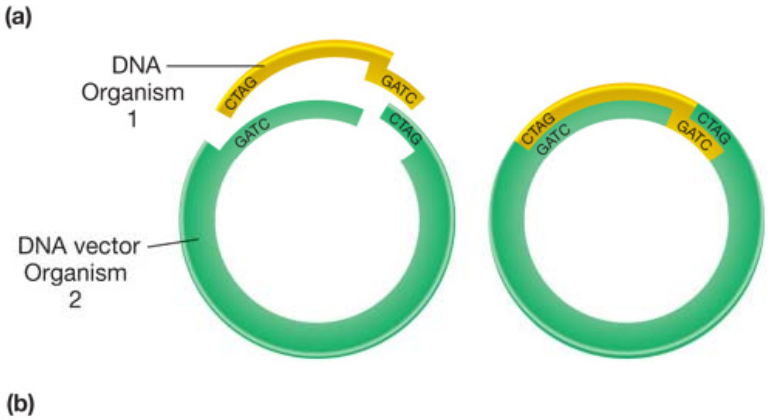


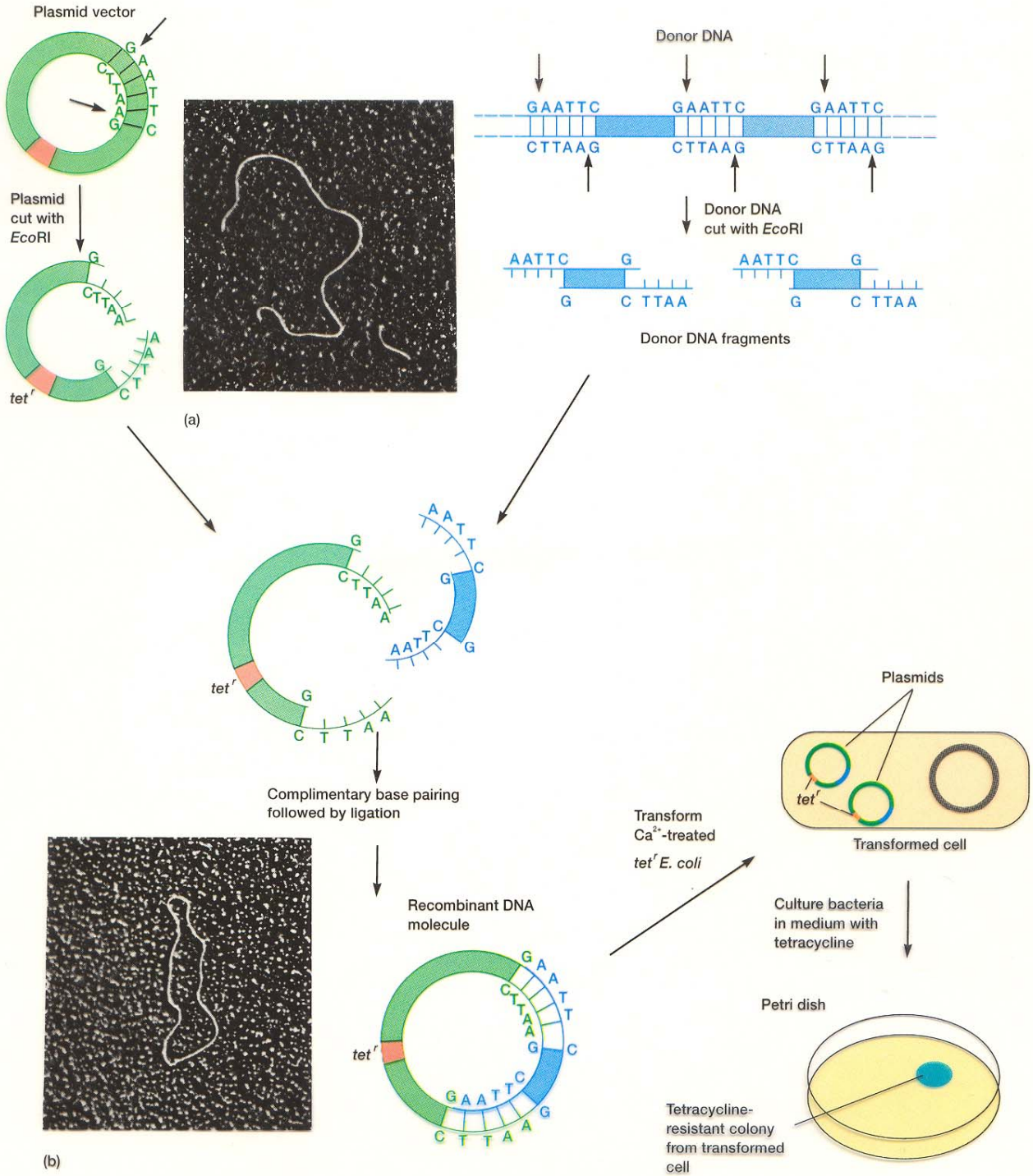
Recombinant DNA molecules

- Jackson, Symons, and Berg (1972)
  - generated first recombinant DNA molecules



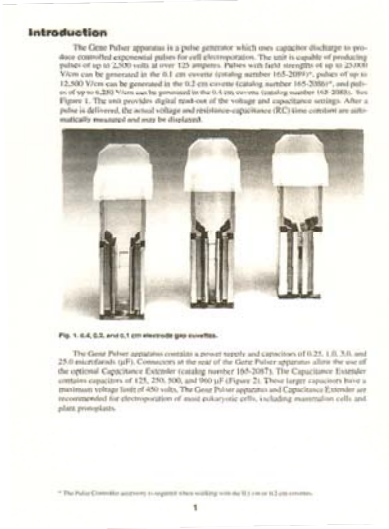
- Cohen and Boyer (1973)
  - produced first plasmid vector capable of being replicated within a bacterial host
    - vectors – carriers of foreign DNA




Plasmid vector
Plasmid cut with EcoRI
tet^r
(a)
Donor DNA
GAATTC
CTTAAG
Donor DNA cut with EcoRI
AATTC G
G C TTAA
Donor DNA fragments
Complimentary base pairing followed by ligation
Recombinant DNA molecule
(b)
Transform Ca2+-treated tet^r E. coli
Plasmids
Transformed cell
Culture bacteria in medium with tetracycline
Petri dish
Tetracycline-resistant colony from transformed cell

Inserting Recombinant DNA into Host Cells

- Electroporation
- microinjection
- gene gun
- Ti plasmid of *Agrobacterium tumefaciens* (used to introduce foreign DNA into plant genomes)


Introduction

The Gene Pulser apparatus is a pulse generator which uses capacitor discharge to produce controlled exponential pulses for cell electroporation. The unit is capable of producing pulses of up to 2,500 volts at over 125 amperes. Pulses with field strengths of up to 25,000 V/cm can be generated in the 0.1 cm cuvette (catalog number 165-2089)*, pulses of up to 12,500 V/cm can be generated in the 0.2 cm cuvette (catalog number 165-2086)*, and pulses of up to 6,250 V/cm can be generated in the 0.4 cm, cuvette (catalog number 165-2088). See Figure 1. The unit provides digital read-out of the voltage and capacitance settings. After a pulse is delivered, the actual voltage and resistance-capacitance (RC) time constant are automatically measured and may be displayed.

Fig. 1. 0.4, 0.2, and 0.1 cm electrode gap cuvettes.

The Gene Pulser apparatus contains a power supply and capacitors of 0.25, 1.0, 3.0, and 25.0 microfarads (μF). Connectors at the rear of the Gene Pulser apparatus allow the use of the optional Capacitance Extender (catalog number 165-2087). The Capacitance Extender contains capacitors of 125, 250, 500, and 960 μF (Figure 2). These larger capacitors have a maximum voltage limit of 450 volts. The Gene Pulser apparatus and Capacitance Extender are recommended for electroporation of most eukaryotic cells, excluding mammalian cells and plant protoplasts.

* The Pulse Controller accessory is required when working with the 0.1 cm or 0.2 cm cuvettes.

1

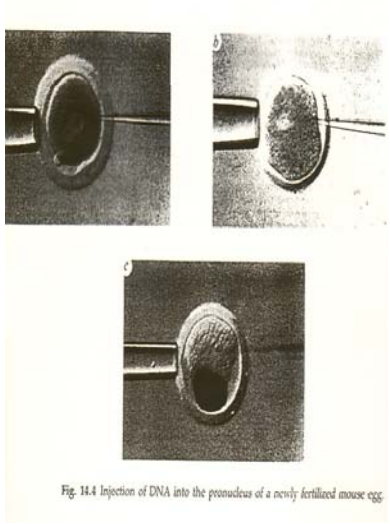
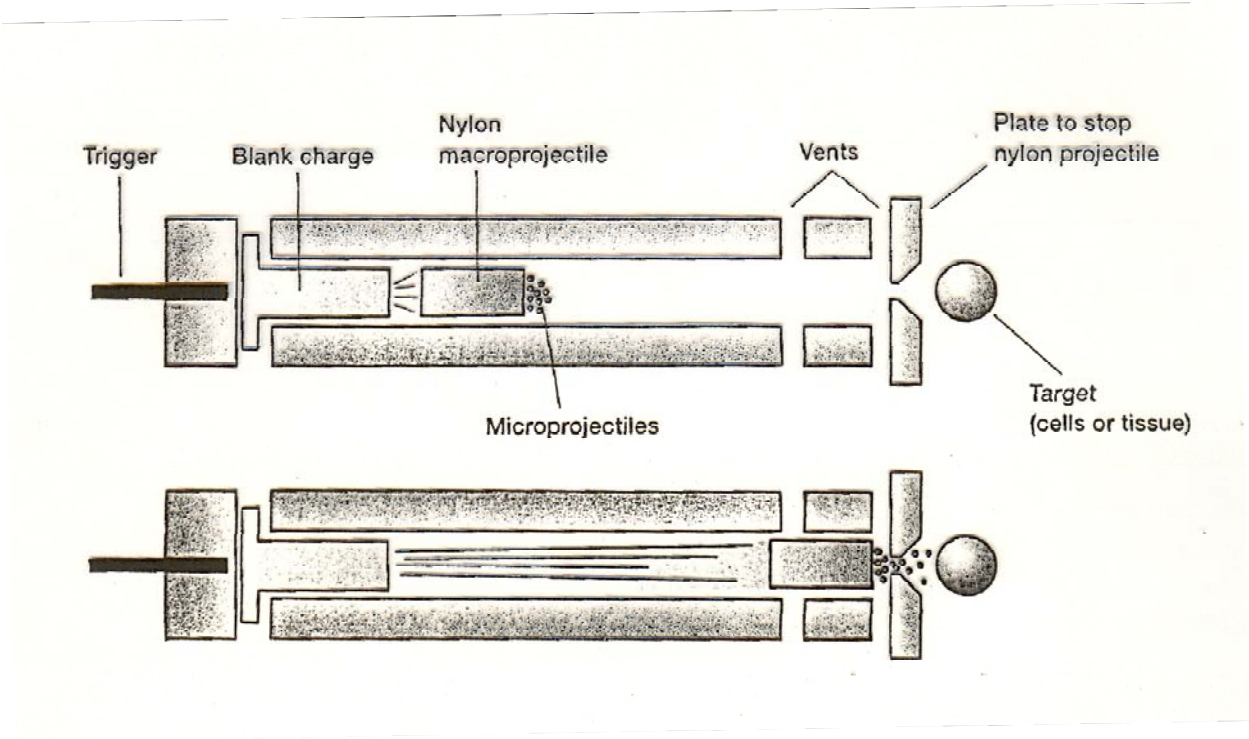


Fig. 14.4 Injection of DNA into the pronucleus of a newly fertilized mouse egg.

Gene gun

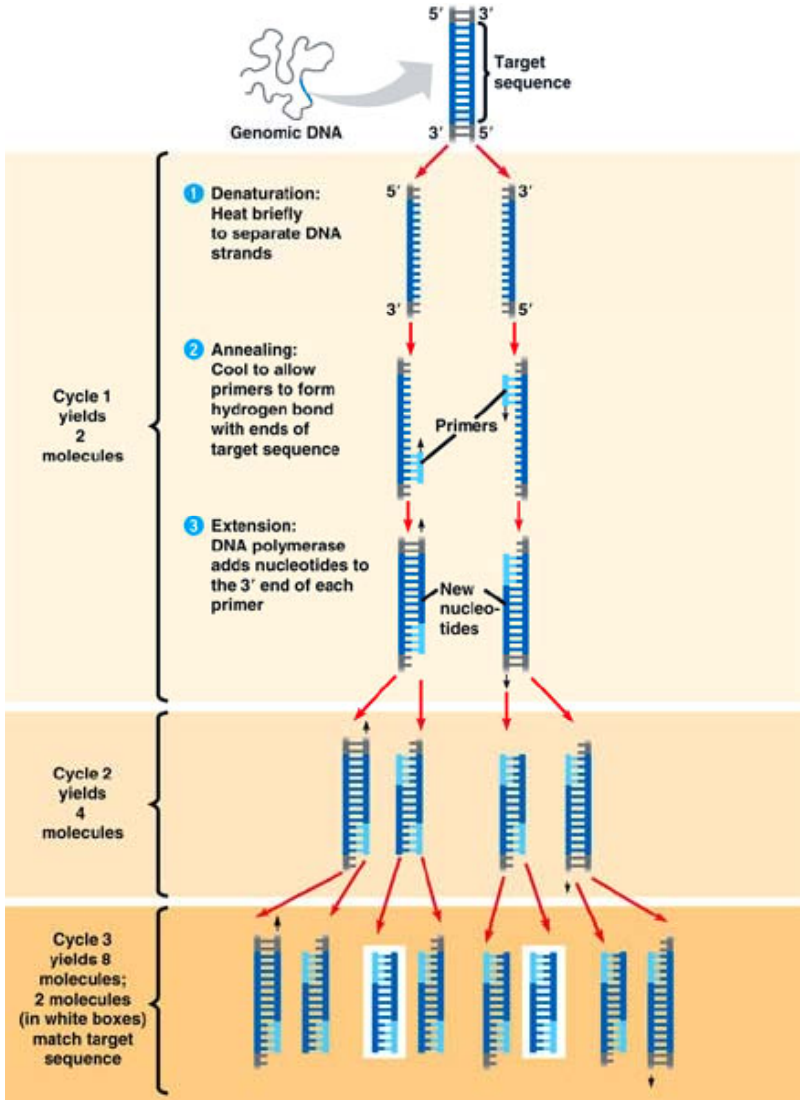

Trigger
Blank charge
Nylon
macroprojectile
Vents
Plate to stop
nylon projectile
Target
(cells or tissue)
Microprojectiles

PCR

- enables the rapid synthesis of many copies of a specific DNA fragment from a complex mixture of DNA and other cellular components

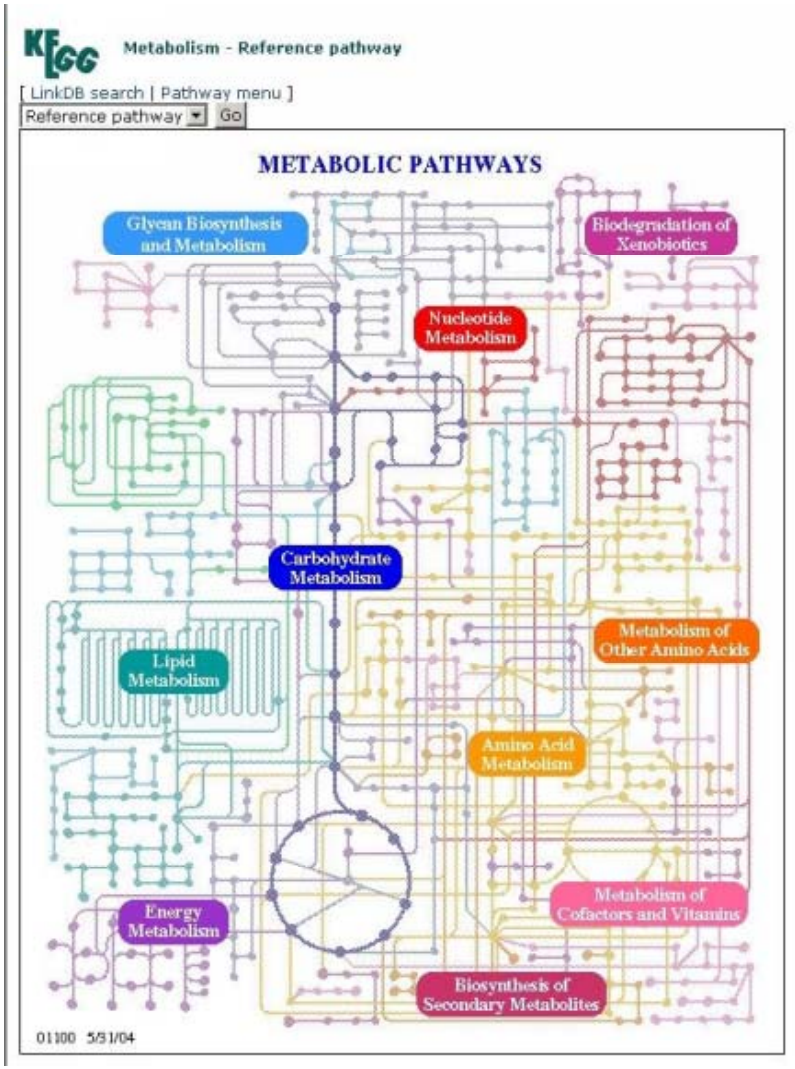
- reaction mix contains
  - primers
  - target DNA
  - thermostable DNA polymerase such as *Taq* polymerase
  - each of the four deoxyribonucleotide triphosphates

- thermocycler is the instrument used in the reaction
  - DNA is denatured
  - primers anneal to target DNA
  - copies of the target DNA are synthesized



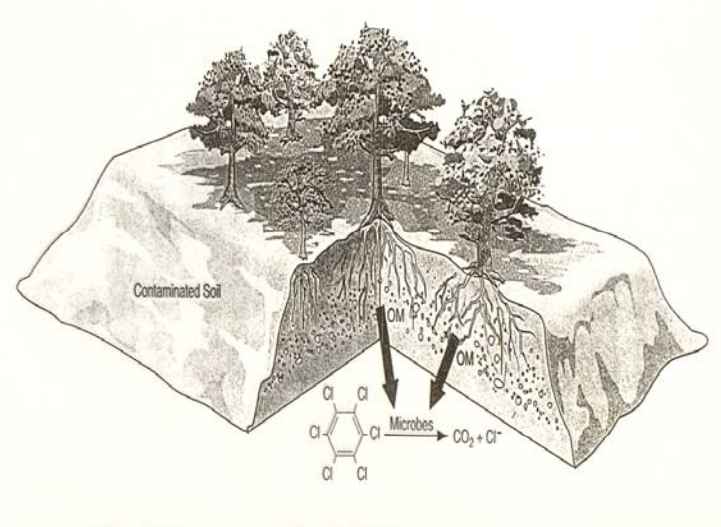
Metabolic Engineering

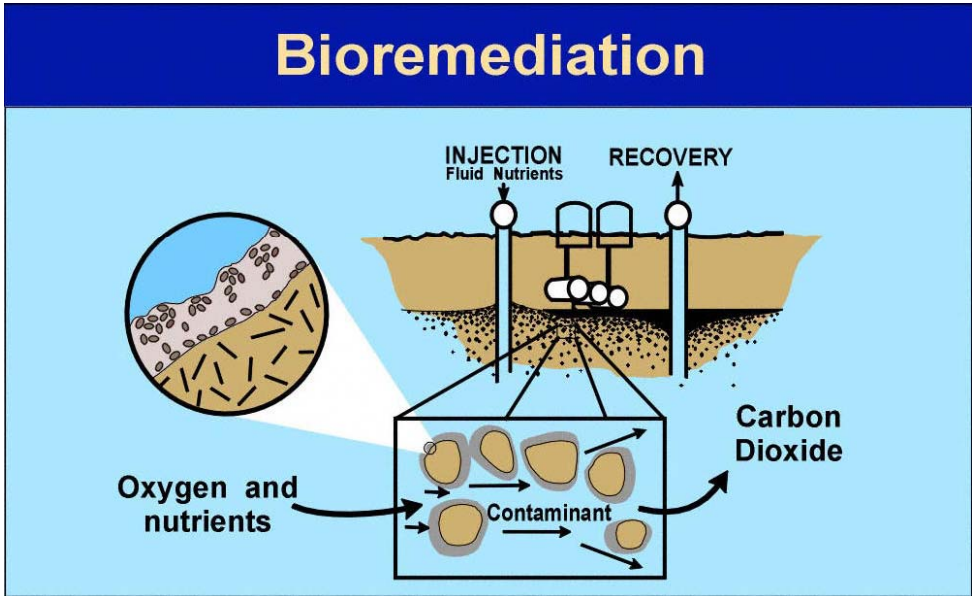
- **Metabolic Engineering** is a new approach to understanding and using metabolic processes. As the name implies, ME is the targeted and purposeful alteration of metabolic pathways found in an organism in order to better understand and use cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. Knowledge acquired from this research will benefit society in a number of ways, including the ability to modify biological pathways to produce biological substitutes for less desirable chemical processes; allowing greater agricultural production, permitting more efficient and safer energy production, and; providing better understanding of the metabolic basis for some medical conditions that could assist in the development of new cures.



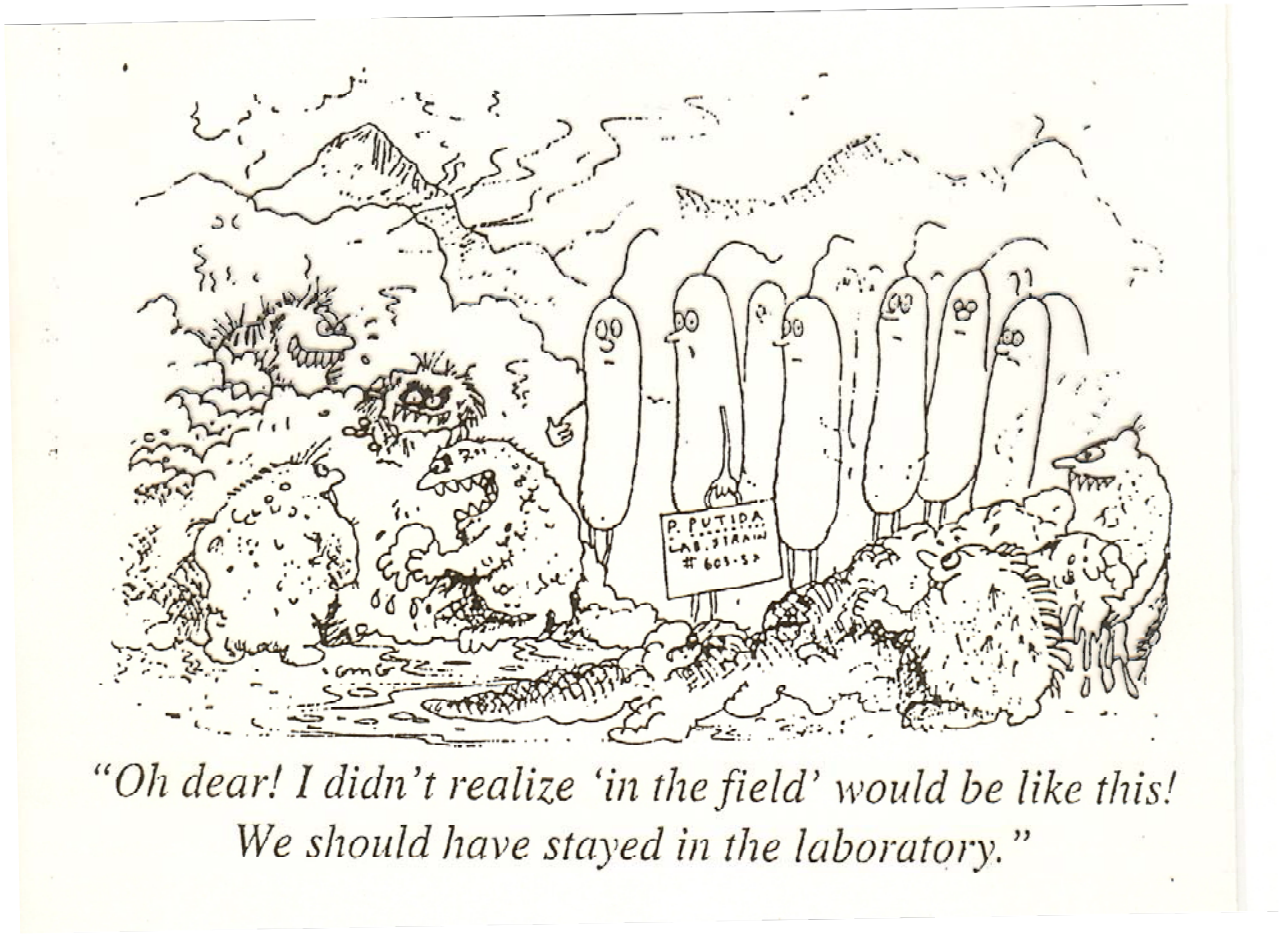
Bioremediation

- Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition.





Genetically Modified Organism (GMO)



"Oh dear! I didn't realize 'in the field' would be like this! We should have stayed in the laboratory."

An organism is "genetically modified", if its genetic material has been changed in a way that does not occur under natural conditions through cross-breeding or natural recombination - Article 2 of the EU Directive on the Deliberate Release into the Environment of Genetically Modified Organisms (2001/18/EG).

Gene Therapy

Acute myeloid leukemia	Fabry disease	Macular degeneration
AIDS	Gaucher disease	Malignant melanoma
Atherosclerosis	Hemophilia A	Neuroblastoma
Breast cancer	Hemophilia B	Osteoporosis
Cardiovascular disease	Hypercholesterolemia	Parkinson disease
Colon cancer	Leukemia	Renal cell carcinoma
Cystic fibrosis	Liver cancer	SCID
Emphysema	Lung cancer	Sickle-cell anemia

Figure 17.8 Diseases that are currently being considered for treatment with somatic cell gene therapies.

(I) Germline therapy (microinjection of DNA into a fertilized egg)

(II) Somatic cell therapy

- ex vivo gene therapy
- in vivo gene therapy

} non-functional protein

- antisense therapy or replacement of "gene" with a correct gene

} defective gene-product is responsible for the disease

Social Impact of Recombinant DNA technology

- there is scientific and philosophical concern about
  - the use of embryonic stem cells in gene therapy. This is currently a major controversial issue in the U.S.
  - the production of genetically engineered food
  - the possibility of the production of genetically engineered organisms by bioterrorists
  - and other issues