InterTech

presents

# (Basic) Laboratory Techniques (in Molecular Biology)

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  - point mutation
  - chromosome mutation
- arrays
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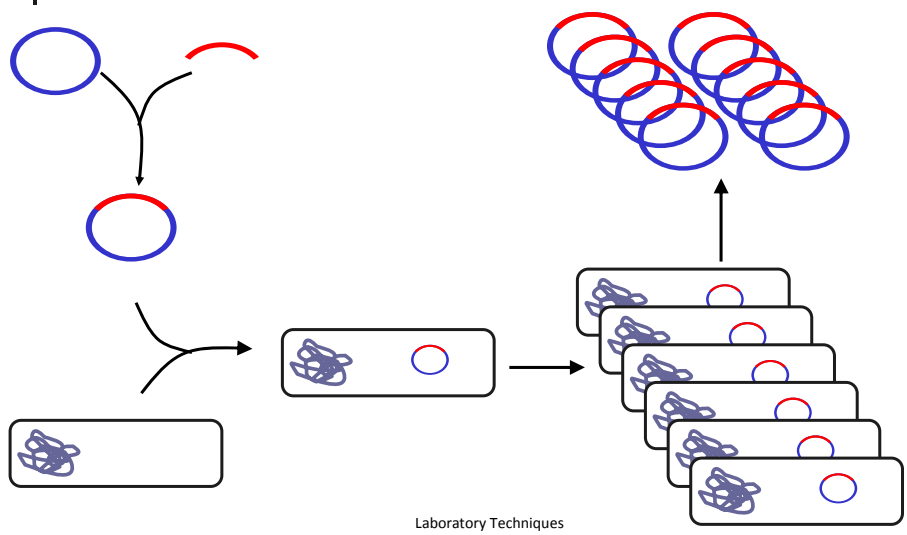
  - protein array

DNA cloning

## DNA cloning overview



# *cut and paste* DNA

- join two DNA molecules
  - insert : usually smaller
  - vector : has origin of replication

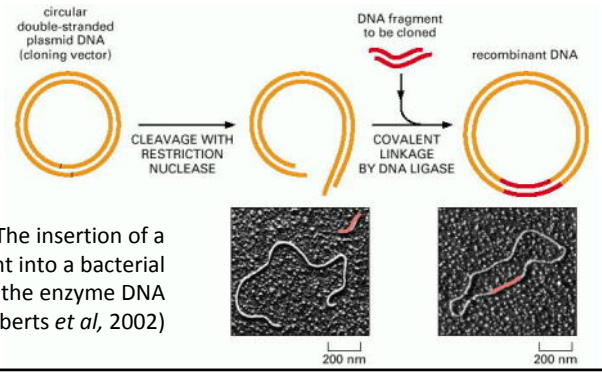



**Figure 8-30.** The insertion of a DNA fragment into a bacterial plasmid with the enzyme DNA ligase. (Alberts *et al,* 2002)

# *cut and paste* DNA : vectors

- bacteria : plasmids
  - restriction sites
  - resistance gene
  - origin of replication

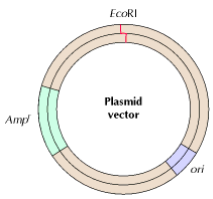
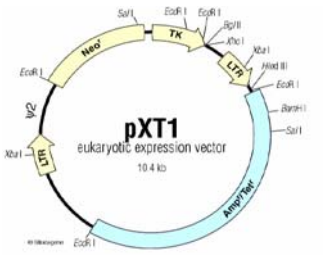


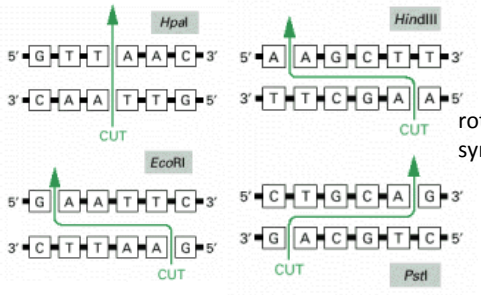

**Figure 3.22.** (Cooper, 2000)

- eukaryote : viruses
  - restriction sites
  - virus genes
  - terminal repeats



# *cut and paste* DNA : restriction

- restriction nucleases enzymes cut DNA
  - from unspecific (exonucleases) to highly specific (type II endonucleases)
  - leaves blunt or sticky ends




rotational symmetry !!

**Figure 8-21.** (Alberts *et al*, 2002)

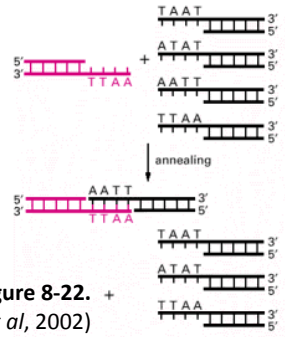



**Figure 8-22.** (Alberts *et al*, 2002)

# *cut and paste* DNA : restriction

- why sticky ends ?

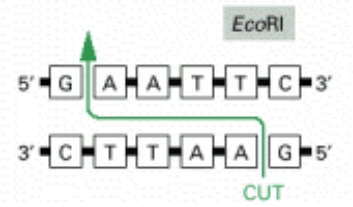



**Figure 8-21.** (Alberts *et al*, 2002)

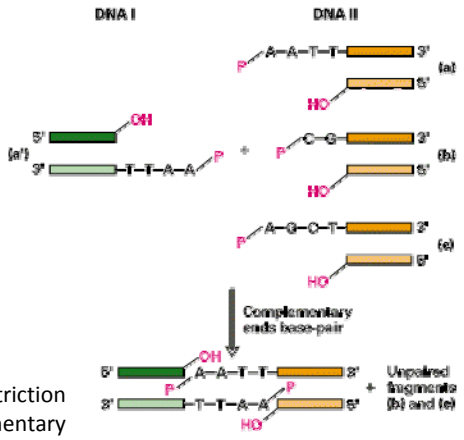



**Figure 7-7.** Ligation of restriction fragments with complementary sticky ends. (Lodish *et al*, 2000)

# *cut and paste* DNA : ligation

- DNA ligase

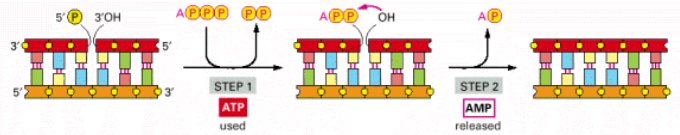



**Figure 5-14.** The reaction catalyzed by DNA ligase. (Alberts *et al*, 2002)

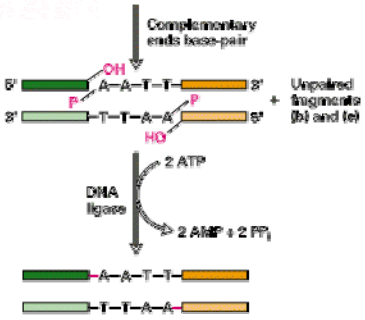



**Figure 7-7.** Ligation of restriction fragments with complementary sticky ends. (Lodish *et al*, 2000)

# bacterial transformation

- make cell membrane *permeable*
- introduce plasmid DNA into bacteria
- many different protocols

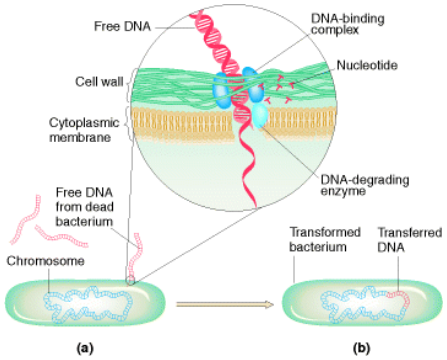
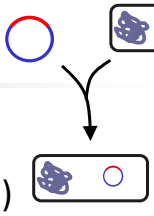



**Figure 7-16.** Bacterium undergoing transformation. (Grifftihs *et al*, 2000)

# transfection



- use of viruses to get DNA into cells
- in eukaryote and bacteria (transduction)

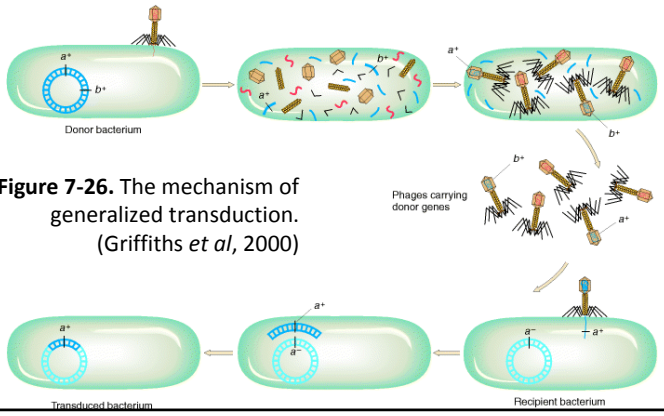
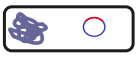



**Figure 7-26.** The mechanism of generalized transduction. (Griffiths *et al*, 2000)

# chromosome integration



- how to make stable mutants
- adds a gene to a chromosome
- homologous recombination
  - target DNA
- possible in bacteria
- usually necessary in eukaryote

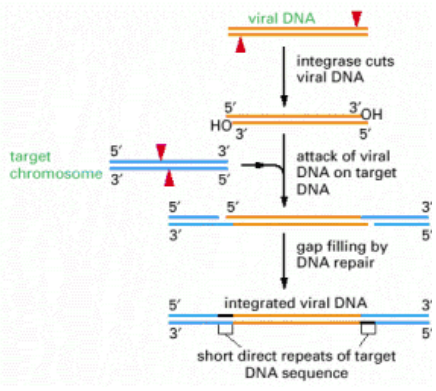



**Figure 5-75.** Transpositional site-specific recombination by a retrovirus or a retroviral-like retrotransposon. (Alberts *et al*, 2002)

# cellular screening

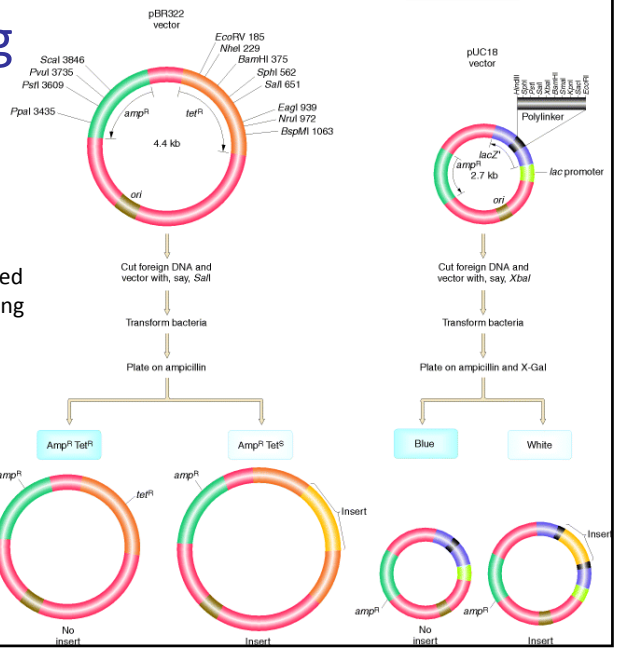
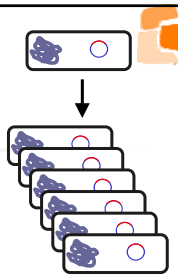
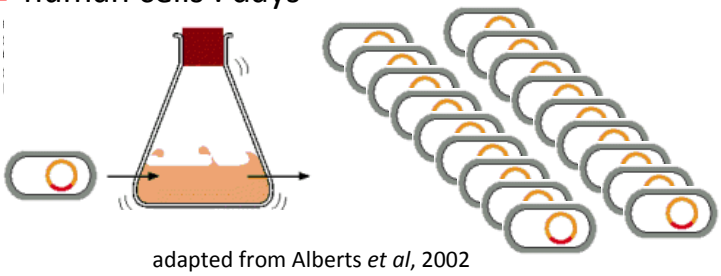



**Figure 12-6.** Two plasmids designed as vectors for DNA cloning, showing general structure and restriction sites. (Griffiths *et al*, 2000)

# cellular culture

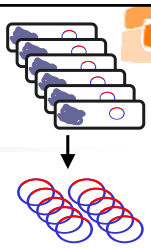


- get lots of cells
- solid or liquid media
- time varies
  - *E. coli* : overnight
  - human cells : days

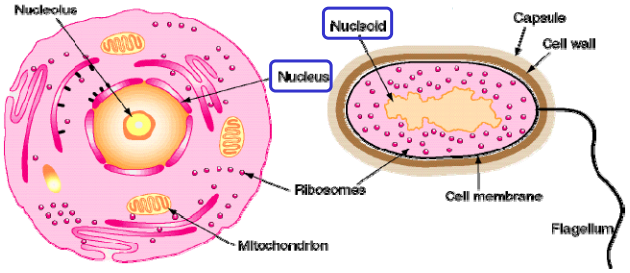


adapted from Alberts *et al*, 2002

# extraction of DNA



- take only DNA from the whole cell extract
  - brake cell membrane
  - precipitate proteins
- purify DNA from proteins bound, etc




# extraction of plasmids

- plasmids
  - small circular independent DNA molecules
- extract ***only*** plasmids
- take advantage
  - smaller, more compact
- a.k.a. maxi/midi/mini-prep

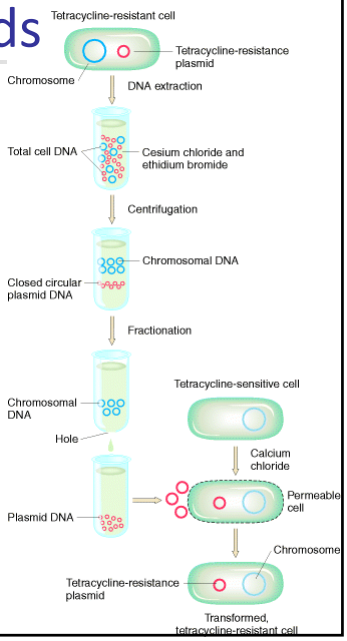
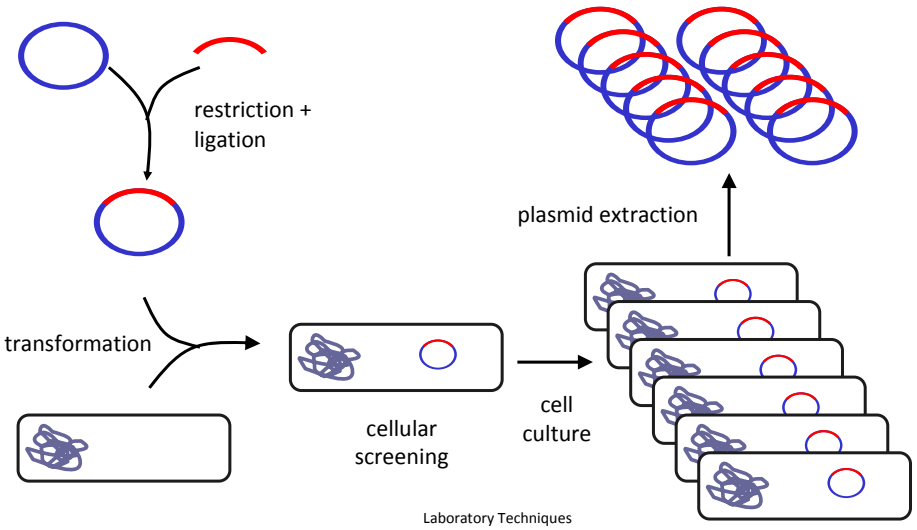


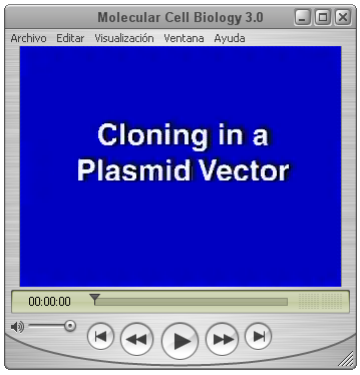

**Figure 12-2.** (Griffiths *et al*, 2000)

# DNA cloning overview, again






# movie : DNA cloning




Ch7anim1. Lodish *et al*, 2000

Polymerase Chain Reaction :
copying DNA

# DNA polymerase DNA dependent

- copies DNA into DNA ⟶ DNA replication
- adds dNTP to a 3'OH end of an existing strand

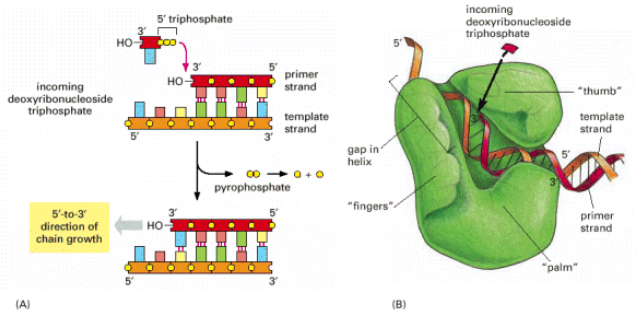



**Figure 5-4.** DNA synthesis catalyzed by DNA polymerase. (Alberts *et al*, 2002)

# Polymerase Chain Reaction

- double-stranded DNA
- primers
- heat tolerant DNA polymerase (*Taq* pol)
- dNTPs

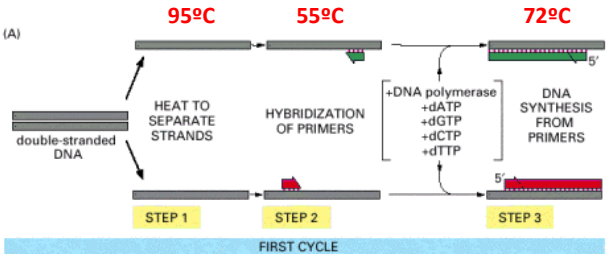
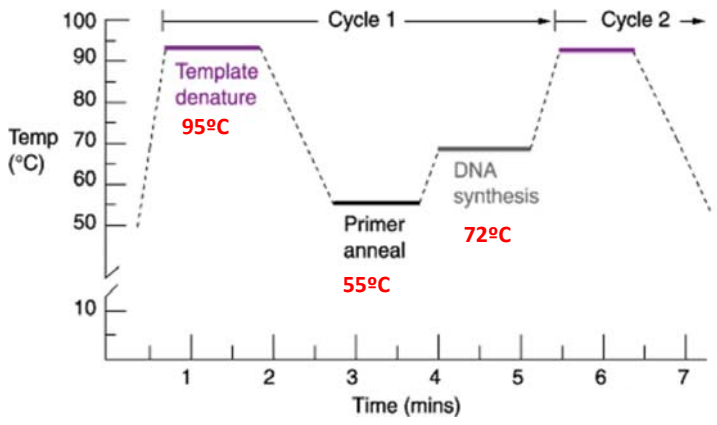



**Figure 8-39.** Amplification of DNA using the PCR technique (Alberts *et al*, 2002)



# PCR dynamics



# Polymerase Chain Reaction

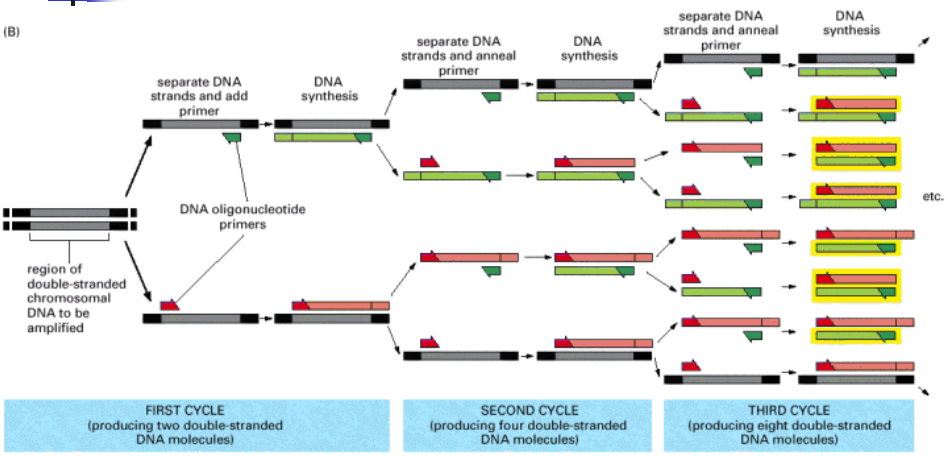
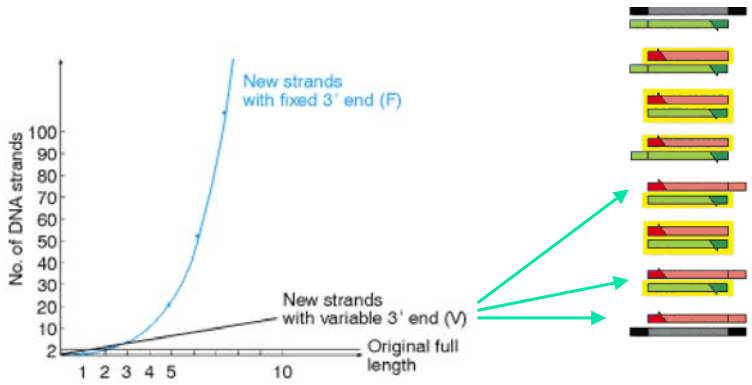
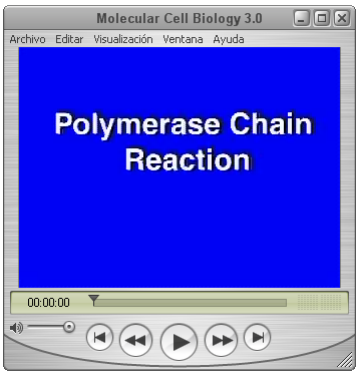



**Figure 8-39.** Amplification of DNA using the PCR technique. (Alberts *et al*, 2002)

# PCR amplifies DNA copies



# movie : PCR




Ch7anim4. Lodish *et al*, 2000



# PCR types

- Q-PCR
- Allele-specific PCR
- Assembly PCR
- Colony PCR
- Inverse PCR
- Ligation-mediated PCR
- Nested PCR and many more...

Gel electrophoresis :
separating molecules per length

# Gel electrophoresis

- nucleic acids in an agarose gel
- electric current through the gel

- nucleic acids
  - DNA or RNA
  - migrate to the **+** pole
    - P skeleton has **–** charge
  - separated per length
  - dyed (EtBr, SYBR green)

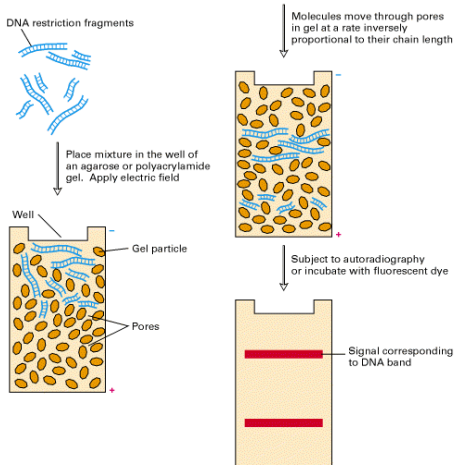
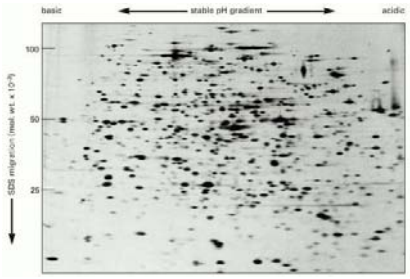
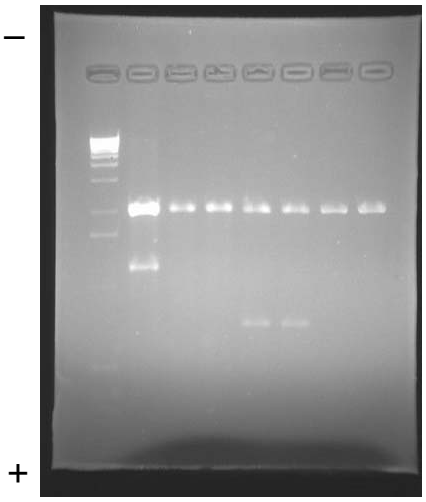



**Figure 7-22.** Separation of DNA fragments of different lengths by gel electrophoresis. (Lodish *et al*, 2000)

# Gel electrophoresis

- usually the length is inferred using a known sample
- proteins
  - SDS-PAGE gel
  - can be 2D: pI and weight




an EtBr gel electrophoresis

**Figure 8-17.** (Alberts *et al*, 2002)

reading and writing DNA

# DNA sequencing

- allows to know what is the exact sequence of A, T, C, G of a DNA molecule
- Sanger method (1977)
  - based on PCR reaction & gel electrophoresis
  - ddNTPs radioactively labelled



# DNA sequencing

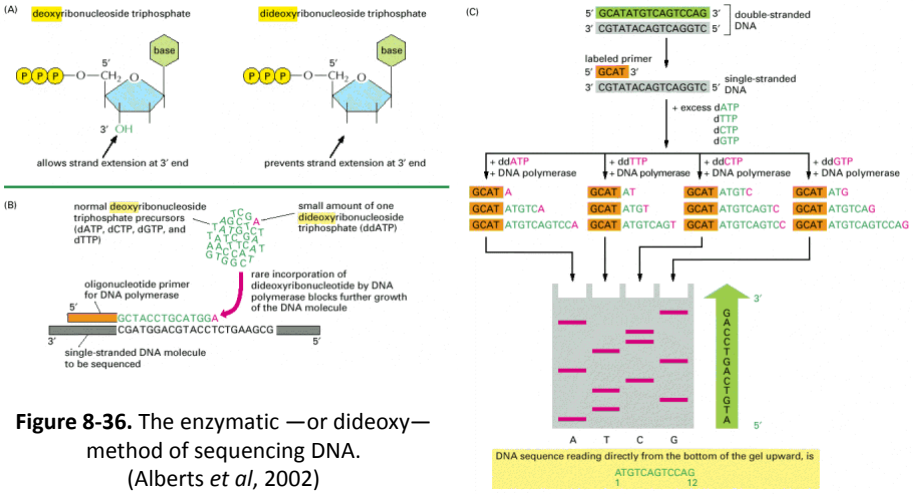
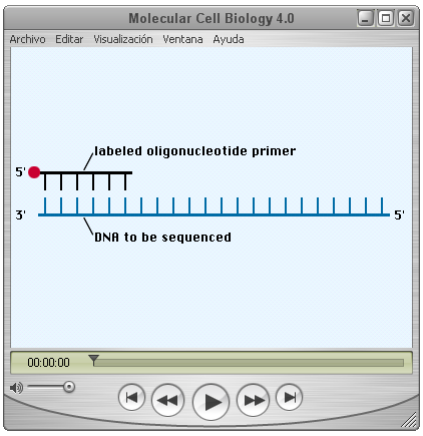



**Figure 8-36.** The enzymatic —or dideoxy— method of sequencing DNA. (Alberts *et al*, 2002)

# movie : Sanger sequencing

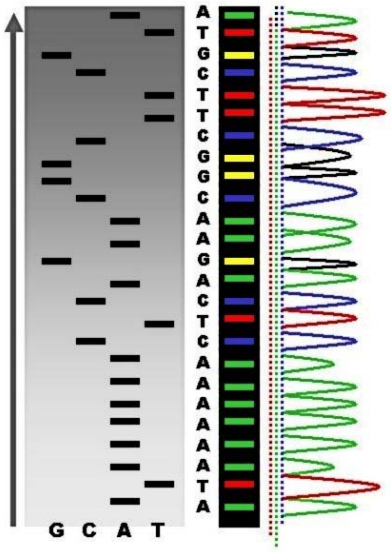



Ch7anim3. Lodish *et al*, 2000



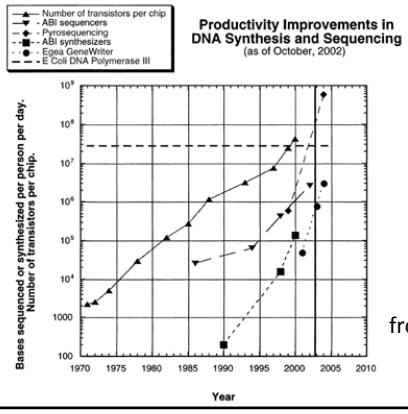
# DNA sequencing : present & future

- fluorescence labels
- capillar electrophoresis
- polonies
- nanopores
- pyrosequencing

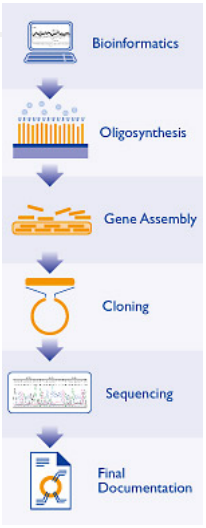


# DNA synthesis

- commercial synthesis
  - current price : from 0,80 €/bp
  - production time : from 2 weeks




from Carlson, 2003

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THE GENE OF YOUR CHOICE

molecular hybridization

# molecular hybridization

- nucleic acids specifically hybridize to nucleic acids
- using labelled n.a., specific detection is possible

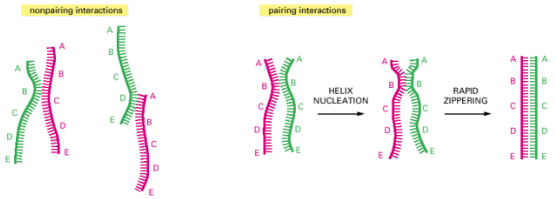



**Figure 5-57.** DNA hybridization. (Alberts *et al*, 2002)

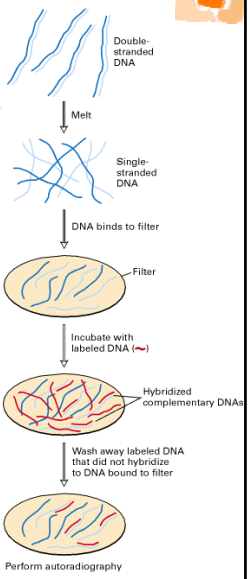
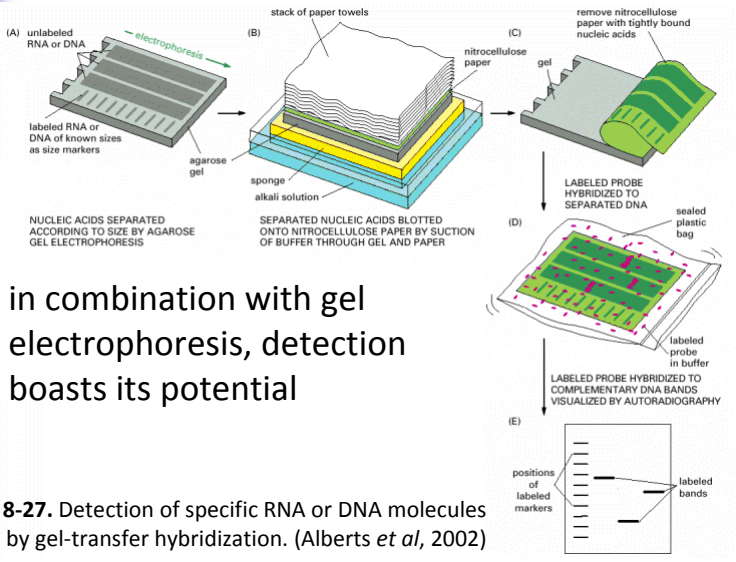



**Figure 7-17.** Membrane-hybridization assay for detecting nucleic acids. (Lodish *et al*, 2000)



# molecular hybridization




- in combination with gel electrophoresis, detection boasts its potential

**Figure 8-27.** Detection of specific RNA or DNA molecules by gel-transfer hybridization. (Alberts *et al*, 2002)

# molecular hybridization

- Southern blot (DNA)
  - DNA extraction
  - restriction
  - gel electrophoresis
  - denaturation
  - filter transfer
  - labelled probe hybridisation
    - DNA or RNA
  - detection
- Northern blot (RNA)
  - RNA extraction
  - denaturation
  - gel electrophoresis
  - filter transfer
  - labelled probe hybridisation
    - DNA
  - detection
- Western blot (protein)
  - polyacrilamide gel separation
  - filter transfer
  - probe reaction
    - antibody
  - detection

# Southern, Northern, Western

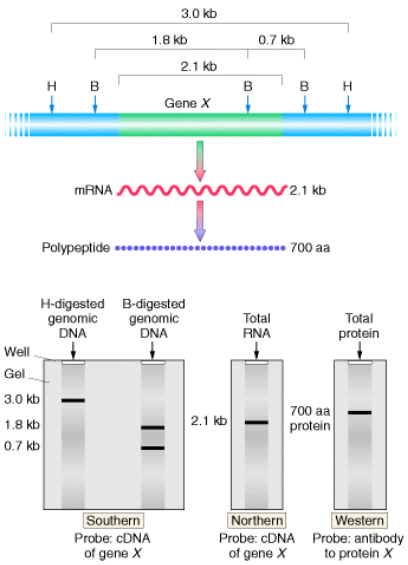



**Figure 10-20.** Comparison of Southern, Northern, and Western analyses of Gene *X*. (Griffiths *et al*, 2000)

rewriting DNA :
mutations

# mutations

- variations on a given DNA molecule
- basis of variability → evolution
- small-scale types
  - silent mutation : a.a. is not afected
  - missense mutation : different a.a.
  - nonsense mutation : a.a. → stop

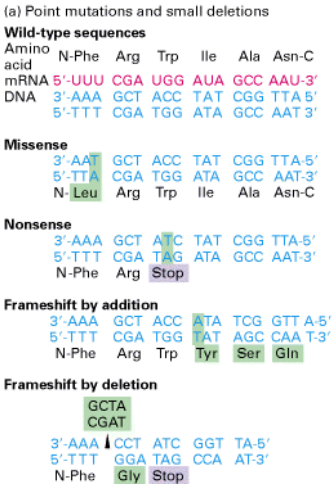



**Figure 8-4.** Different types of mutations. (Lodish *et al*, 2000)

# mutations

- large-scale types : chromosomes
  - inversion : changes order
  - insertion : adds genes
  - deletion
  - translocation : moves genes

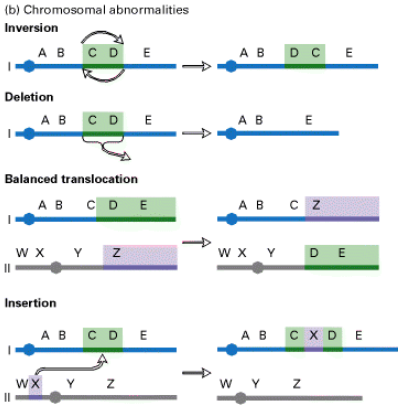



**Figure 8-4.** Different types of mutations. (Lodish *et al*, 2000)



# random mutagenesis

- use chemicals, UV or error-prone DNA replication
- fine screening needed !

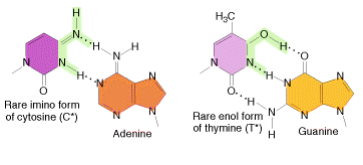



**Figure 16-2.** Mismatched bases. (Griffiths *et al*, 2000)

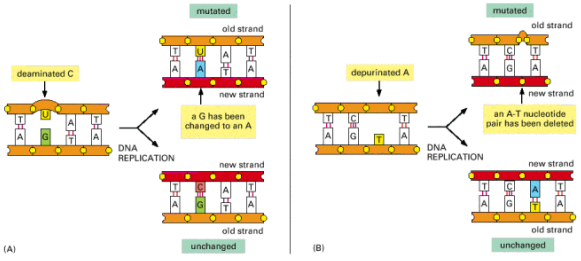



**Figure 5-49.** How chemical modifications of nucleotides produce mutations. (Alberts *et al*, 2002)

# point mutation

- point mutation on a given site
- PCR is widely used for this goal
  - site-directed
  - megaprimers
  - *in vitro* overlap-extension

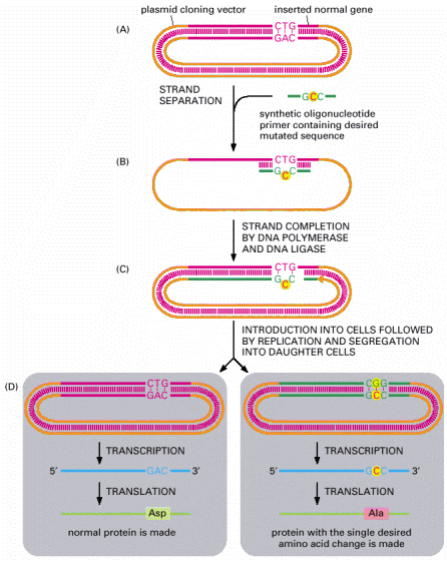
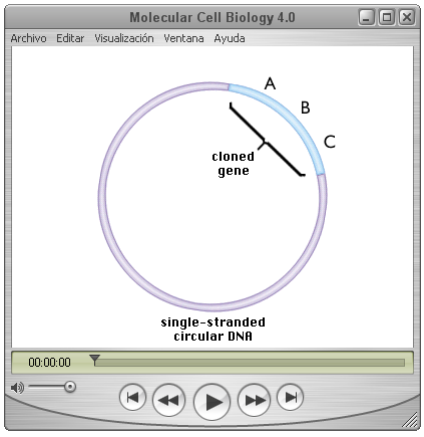



**Figure 8-69.** The use of a synthetic oligonucleotide to modify the protein-coding region of a gene by site-directed mutagenesis. (Alberts *et al*, 2002)

# movie : PCR mutagenesis




Ch8anim1. Lodish *et al*, 2000

# chromosome mutation

- mutate chromosomes
  - *knock out*
  - replace
  - insertion : add a gene
  - deletion
- homologous recombination
  - target DNA

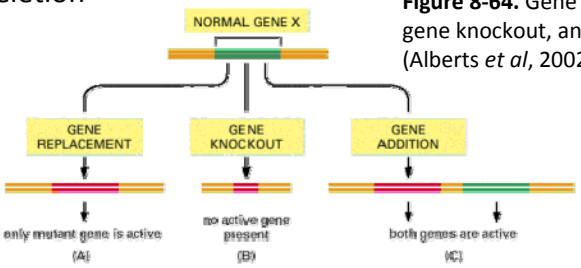



**Figure 8-64.** Gene replacement, gene knockout, and gene addition. (Alberts *et al*, 2002)

# chromosome mutation

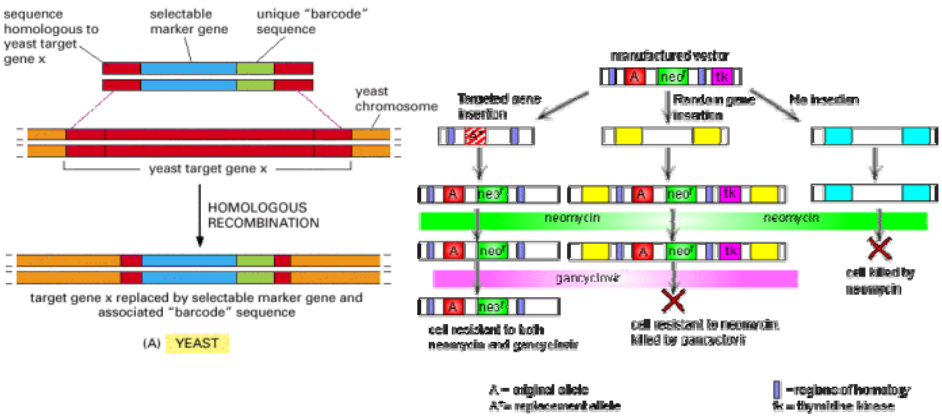



**Figure 8-73.** Making collections of mutant organisms. (Alberts *et al*, 2002)

arrays

# DNA array

- probe
  - DNA molecules of variable lenght on a solid support in a regular and fixed distribution
- sample
  - labelled DNA or RNA that will bind to the probes
- take advantage of nucleic acid's specific hybridization

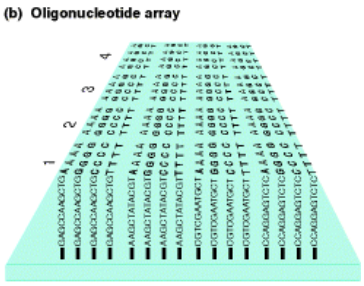
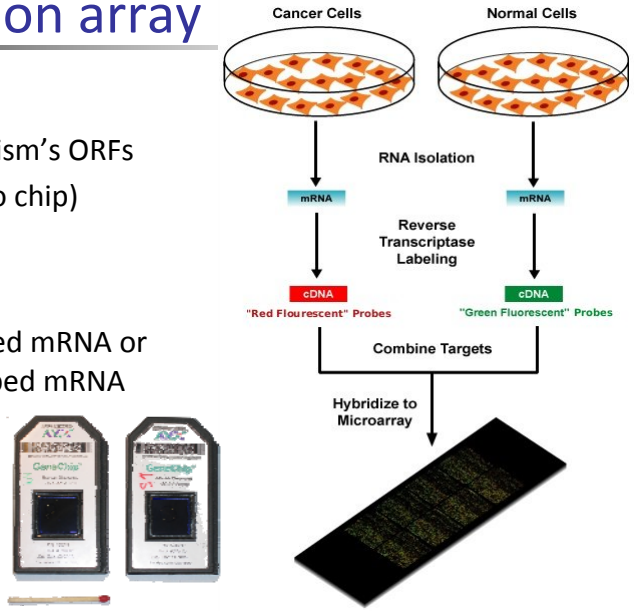



**Figure 14-27.** (Griffiths *et al*, 2000)

# expression array

- probe
  - usually organism's ORFs
  - ordered (oligo chip)
- sample
  - usually labelled mRNA or retrotranscribed mRNA (cDNA)






# expression array

- monitor mRNA quantities of the whole genome
- compare two states

**inputs**

- **sample 1**
- **sample 2**

**outputs**

- **sample 1 > sample 2**
- **sample 1 < sample 2**
- **sample 1 = sample 2**

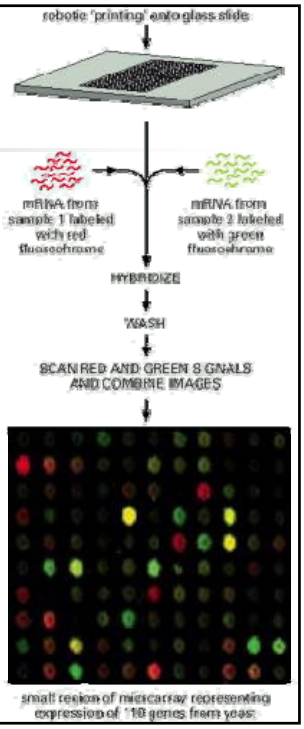
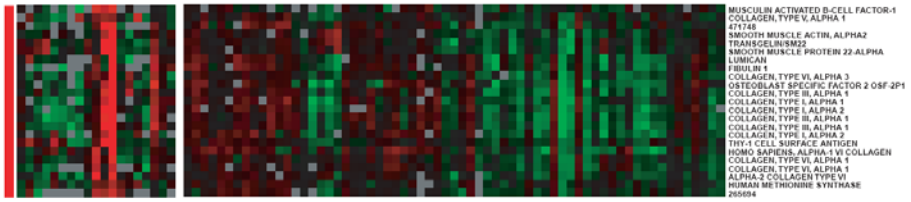



**Figure 8-62.** Using DNA microarrays to monitor the expression of thousands of genes simultaneously. (Alberts *et al*, 2002)

# expression array : breast cancer

84
number of experiments

1753
genes





below median

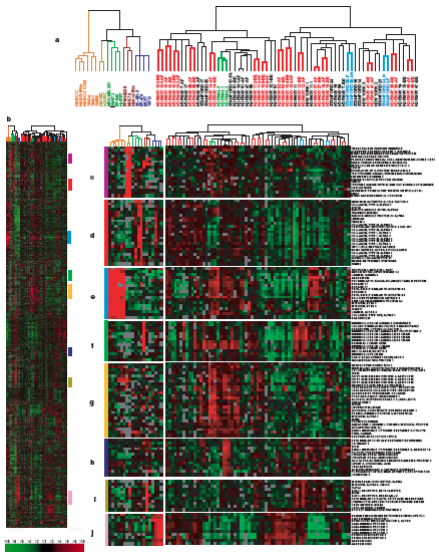
above median

median

Perou *et al* (2000)



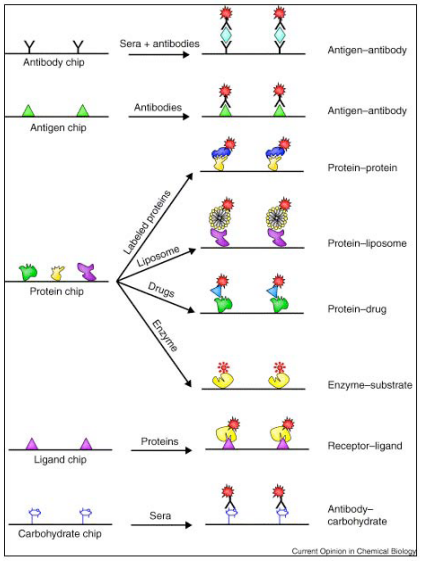
# expression array : breast cancer



Perou *et al* (2000)

# protein array

- probe
  - protein molecules on a solid support in a regular and fixed distribution
- sample
  - substrates that will bind to the probes
  - proteins that could interact with probes
  - antibodies that could recognize the probes




- take advantage of protein-substrate recognition



# sources


- Alberts *et al*, *Molecular Biology of the Cell*, **Garland Science**, 4th ed, 2002
- Lodish *et al*, *Molecular Cell Biology*, **Freeman & Co.**, 4th ed., 2000
- Cooper *et al*, *The Cell - A Molecular Approach*, **Sinauer Publishers**, 2nd ed., 2000
- Griffiths *et al*, *An Introduction to Genetic Analysis*, **Freeman & Co**. 2000
- Carlson. *The pace and proliferation of biological technologies*. **Biosecurity and Bioterrorism.** 2003
- Perou *et al*, *Molecular portraits of human breast tumours.* **Nature**. 2000
- www.ergito.com


InterTech

# farewell movie : the PCR song


http://www.youtube.com/watch?v=x5yPkxCLads

**YouTube**

Laboratory Techniques

# the PCR song

There was a time when to amplify DNA,
you had to grow tons and tons of tiny cells.
Then along came a guy named Dr. Kary Mullis,
said you can amplify *in vitro* just as well.
Just mix your template with a buffer and some primers,
nucleotides and polymerases, too.
Denaturing, annealing, and extending.
Well it's amazing what heating and cooling
and heating will do.

PCR, when you need to detect mutations.
PCR, when you need to recombine.
PCR, when you need to find out who the daddy is.
PCR, when you need to solve a crime.

# more !

Biotech Nation Feat. Notorious
GFP – Transformation

http://www.youtube.com/watch?v=cFeNIM1gJoo
**YouTube**

Biotech Nation –
Restriction enzymes


http://www.youtube.com/watch?v=Gy21KhSF3PM
**YouTube**