

Introduction to biotechnology

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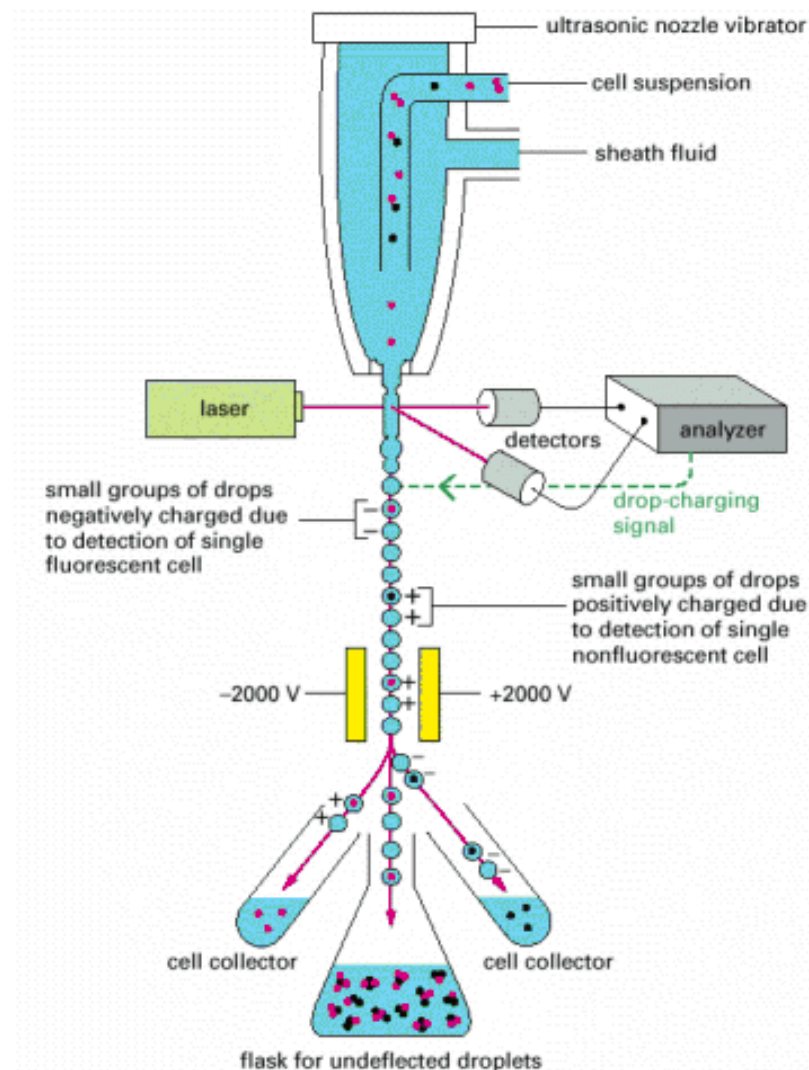
Biotechnology

- Cell technology
 - Isolating cells
 - Growing cells in culture
 - Fractionating cells
- Molecular technology
 - DNA cloning
 - DNA sequencing
 - Gene expression
 - Analyzing protein functions

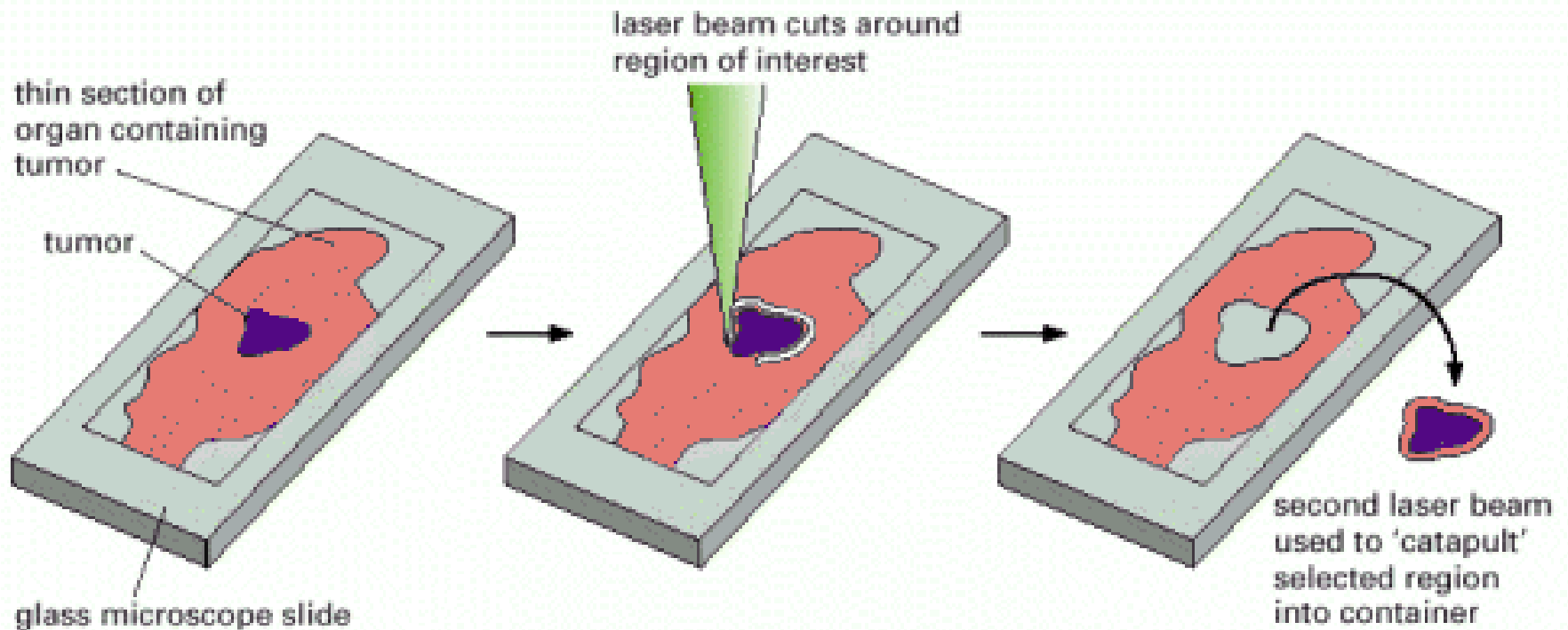
Isolating Cells

- Disrupting extracellular matrix: proteolytic enzymes or EDTA
- Separating different cell types
 - Antibodies coupled to a fluorescent dye (*fluorescence-activated cell sorter*)
 - Microscopic dissection
- Cell growth

Fluorescence-activated cell sorter



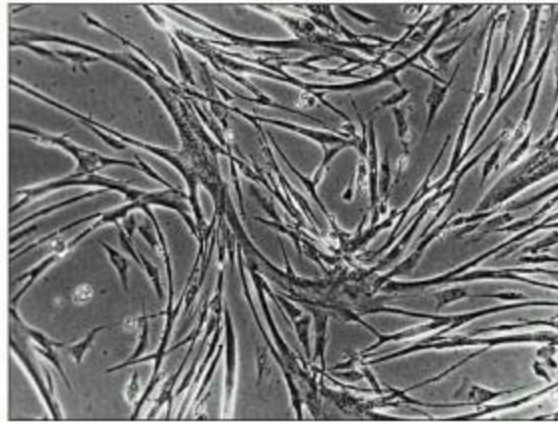
Microscopic dissection



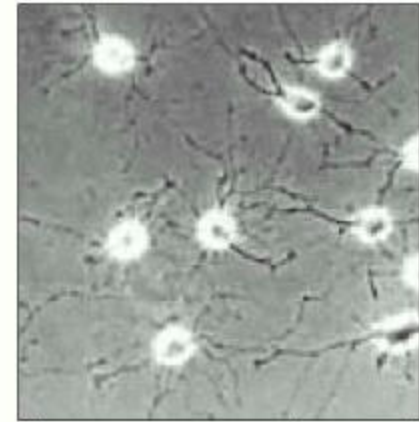
Growing cells in culture



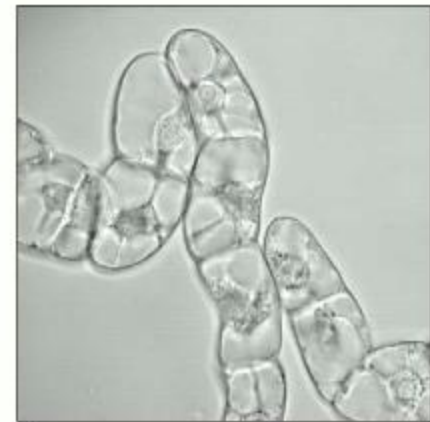
(A) 20 μm



(B) 100 μm



(C) 50 μm



(D) 50 μm

Composition of a Typical Medium Suitable for the Cultivation of Mammalian Cells

AMINO ACIDS

Arginine

Cystine

Glutamine

Histidine

Isoleucine

Leucine

Lysine

Methionine

Phenylalanine

Threonine

Tryptophan

Tyrosine

Valine

VITAMINS

biotin

choline

folate

nicotinamide

pantothenate

pyridoxal

thiamine

riboflavin

SALTS

NaCl

KCl

NaH_2PO_4

NaHCO_3

CaCl_2

MgCl_2

MISCELLANEOUS

glucose

penicillin

streptomycin

phenol red

whole serum

PROTEINS

insulin

transferrin

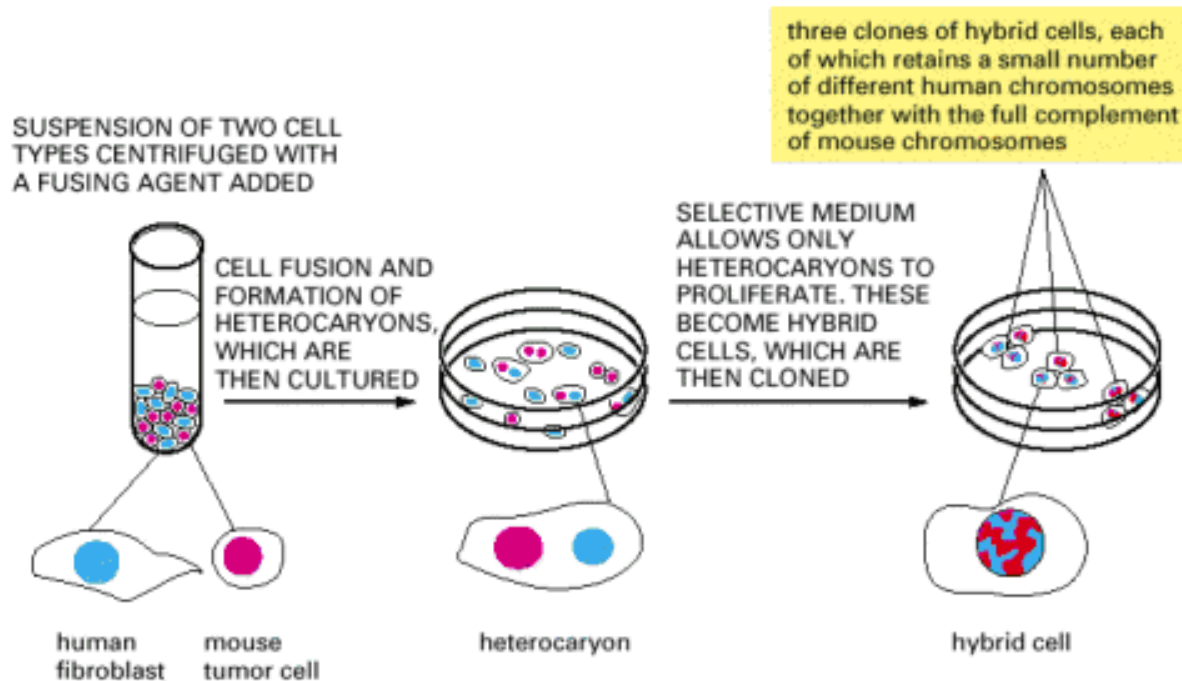
growth factors

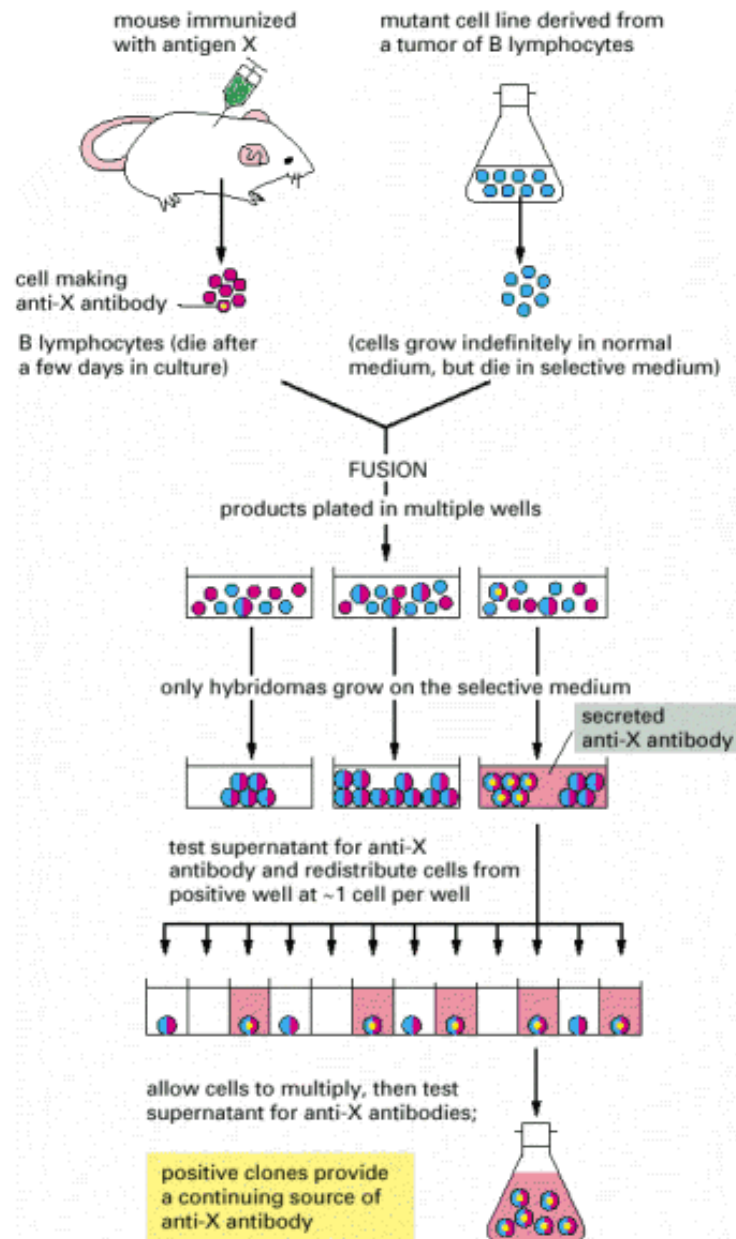
Cell line

- Most vertebrate cells stop dividing after a finite number of cell divisions in culture – senescence;
- "immortalized" cell line: telomerase
- Inactivate the checkpoint mechanisms
- Cell lines can often be most easily generated from cancer cells.

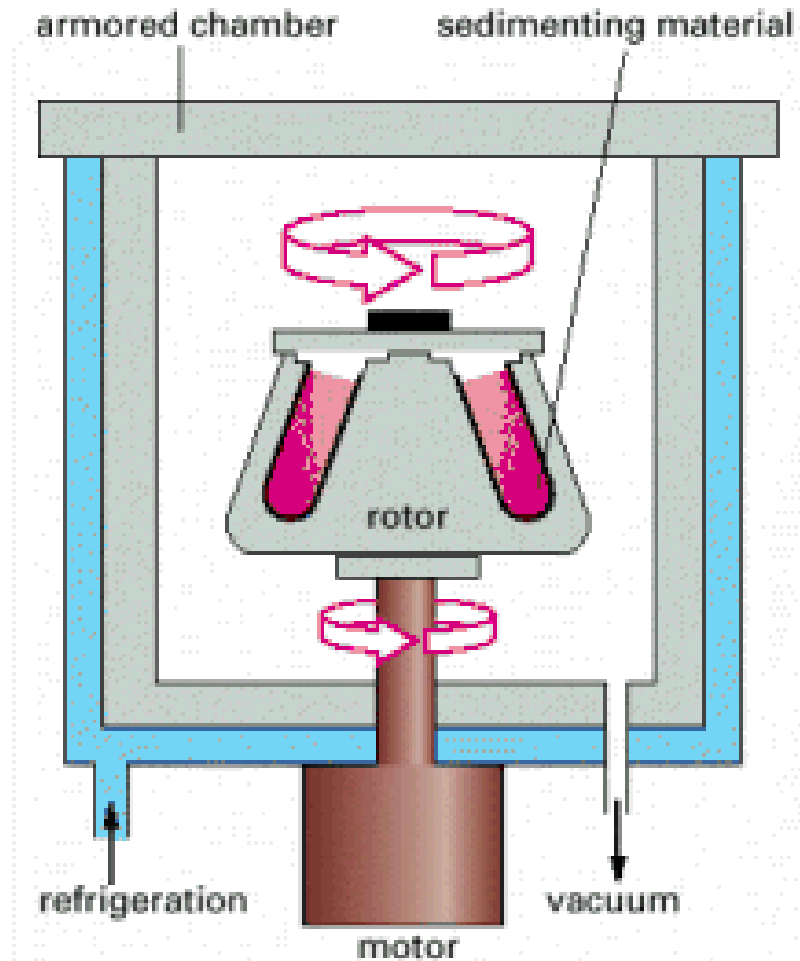
Hybrid cells

- a heterocaryon, a combined cell with two separate nuclei

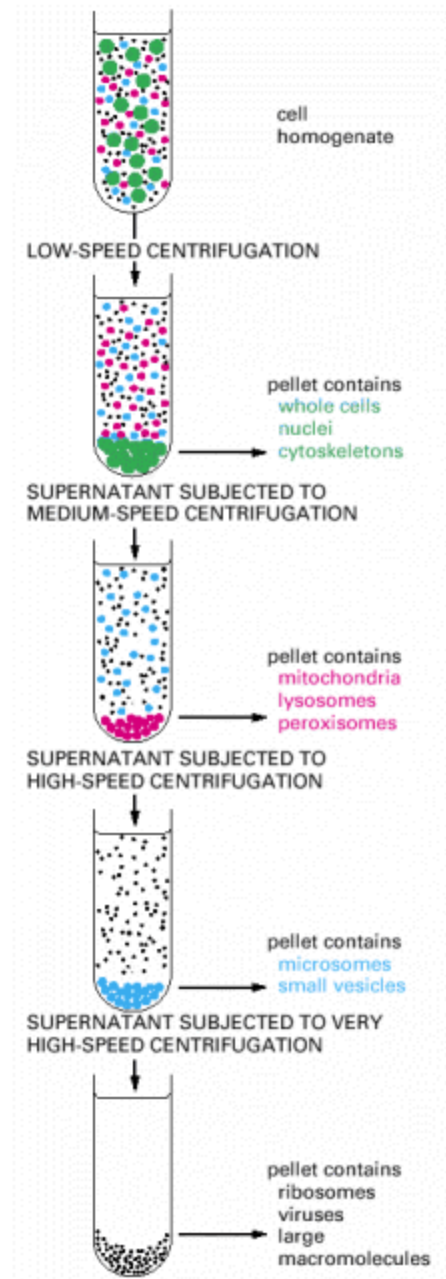




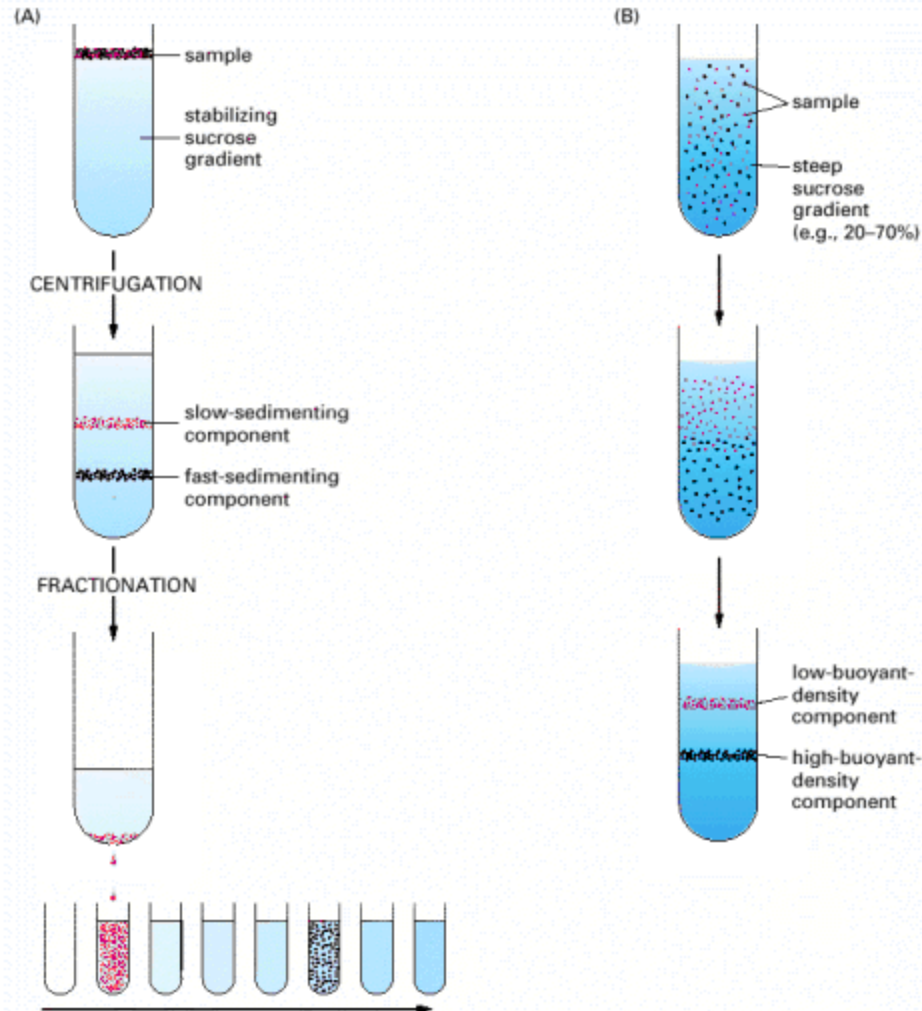
Ultracentrifuge



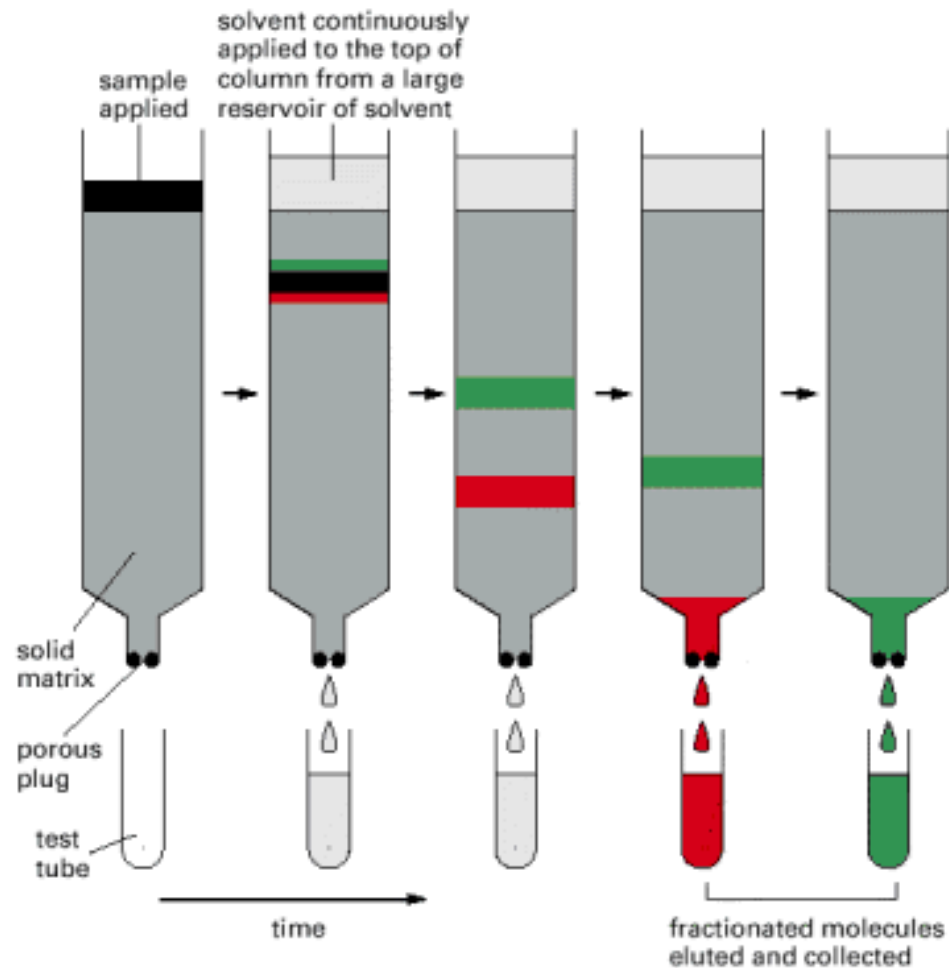
Cell fractionation by centrifugation



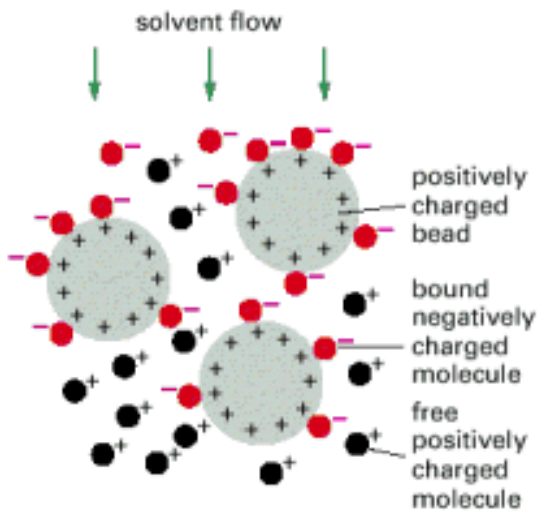
velocity sedimentation vs. equilibrium sedimentation



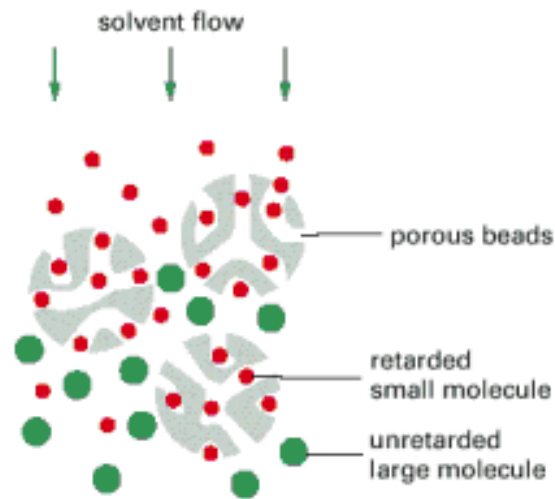
Column chromatography



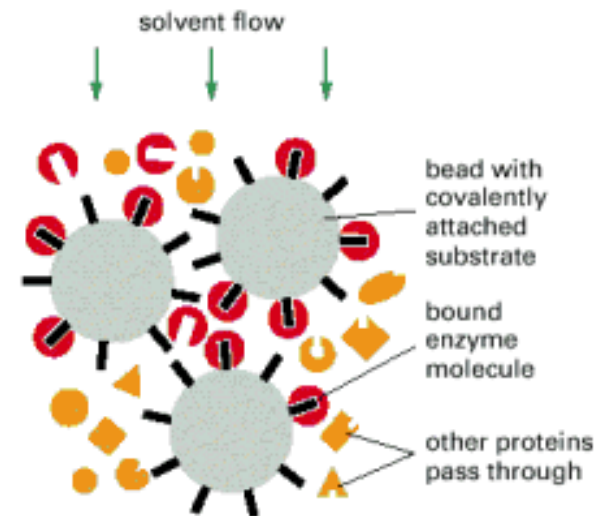
Matrices used for chromatography



(A) ION-EXCHANGE CHROMATOGRAPHY

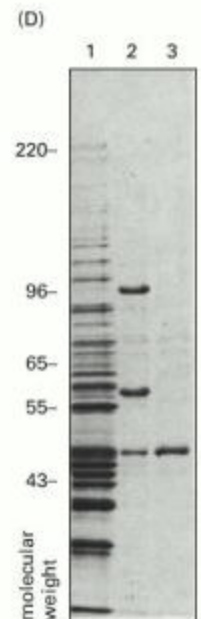
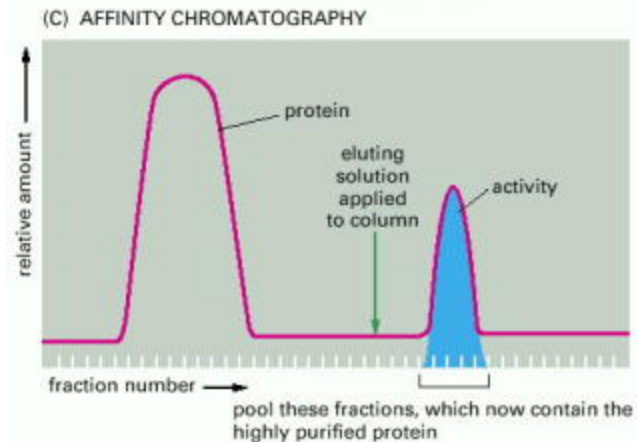
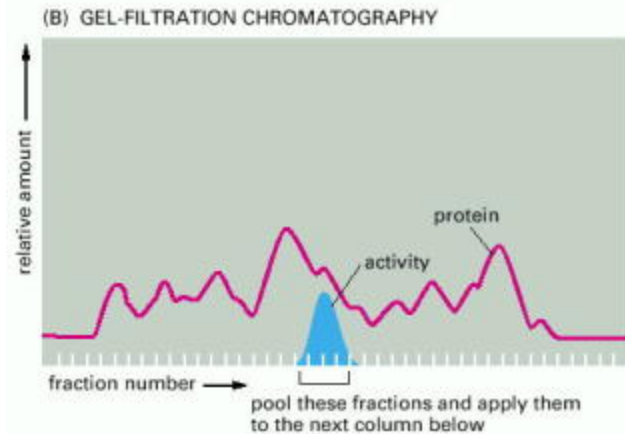
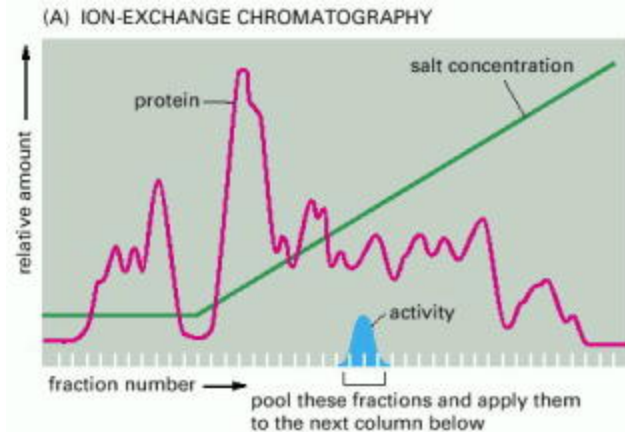


(B) GEL-FILTRATION CHROMATOGRAPHY

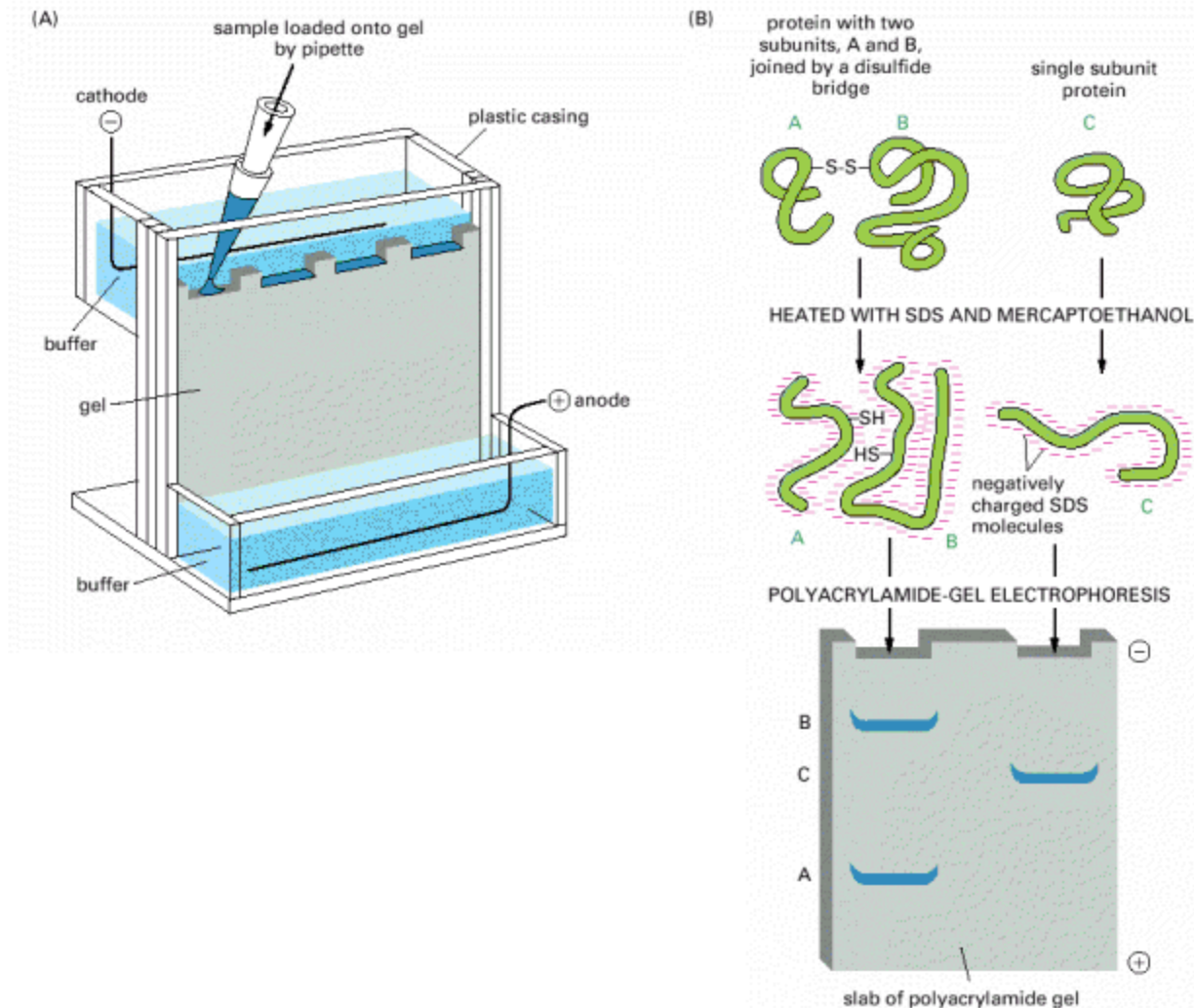


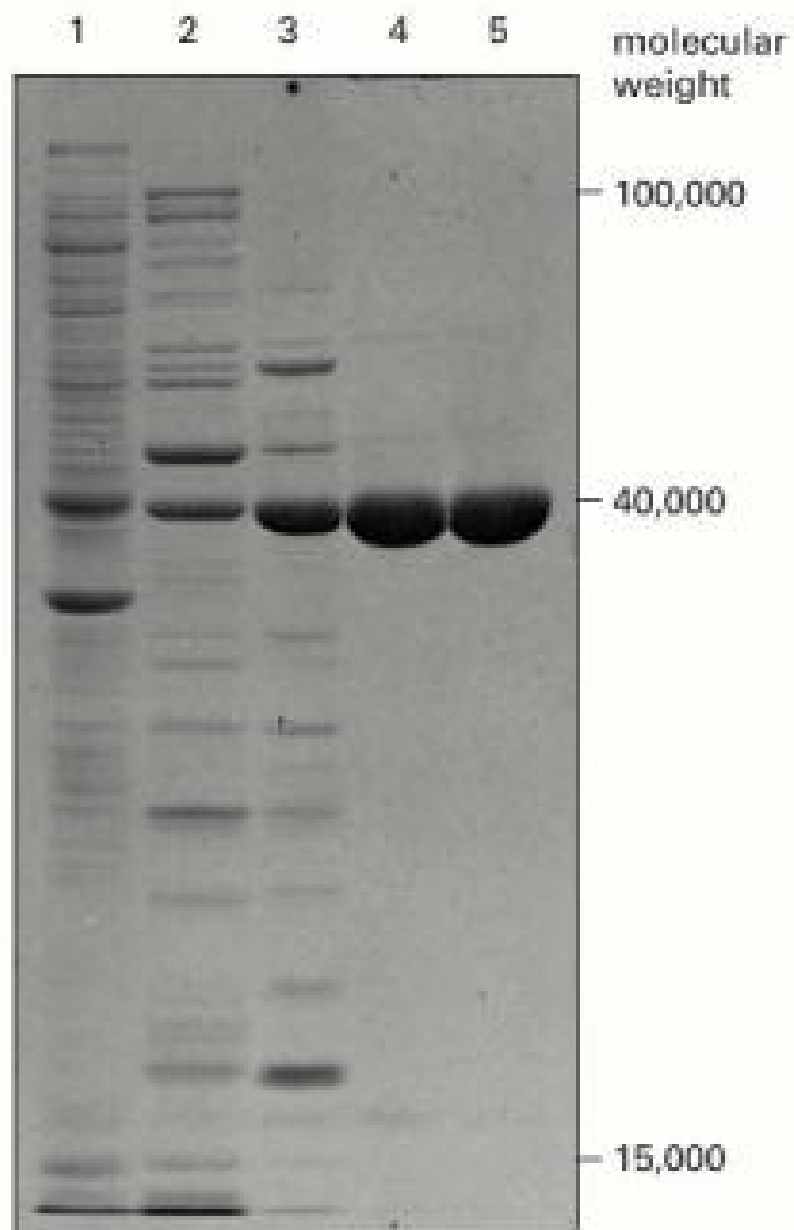
(C) AFFINITY CHROMATOGRAPHY

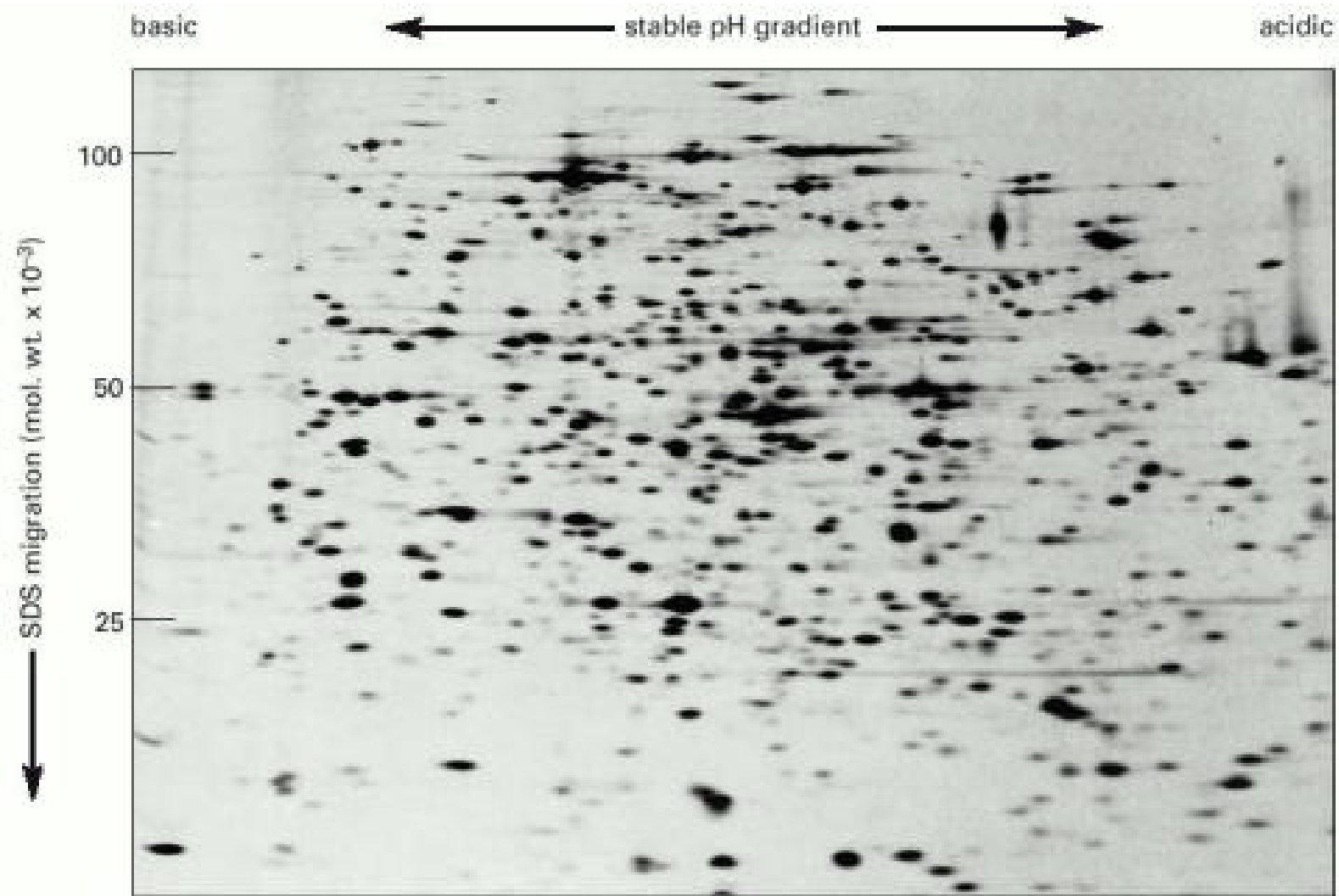
Protein purification by chromatography



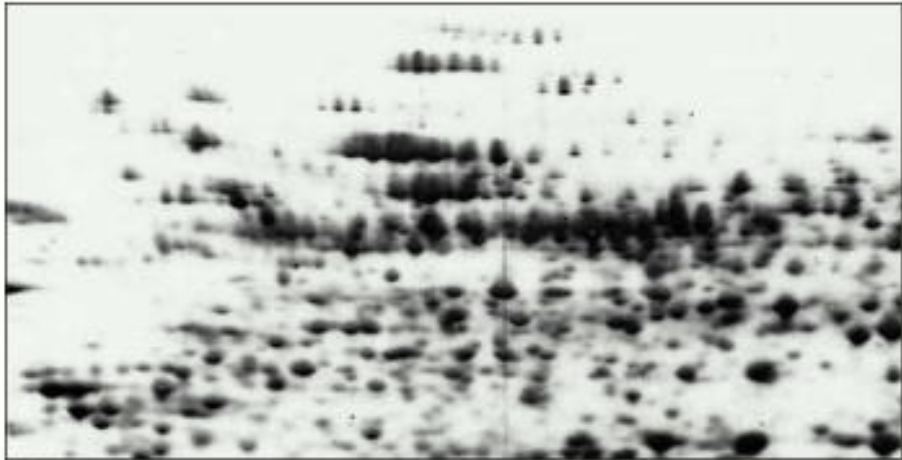
SDS polyacrylamide-gel electrophoresis



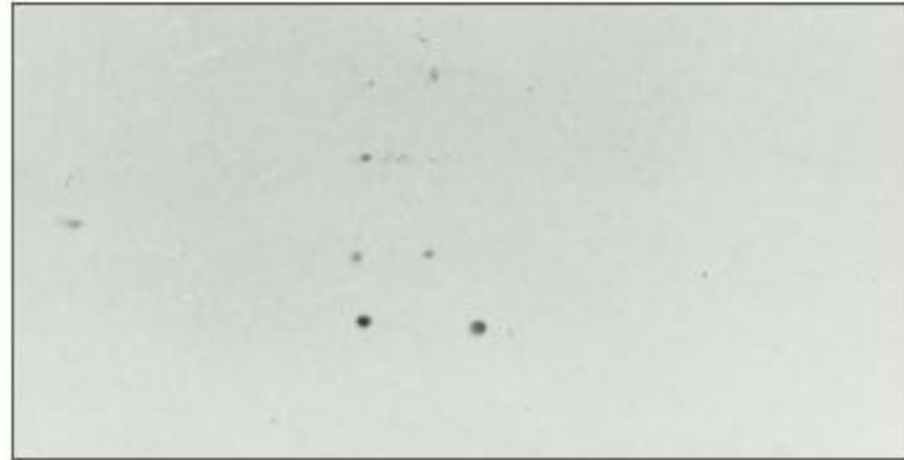




Western blotting

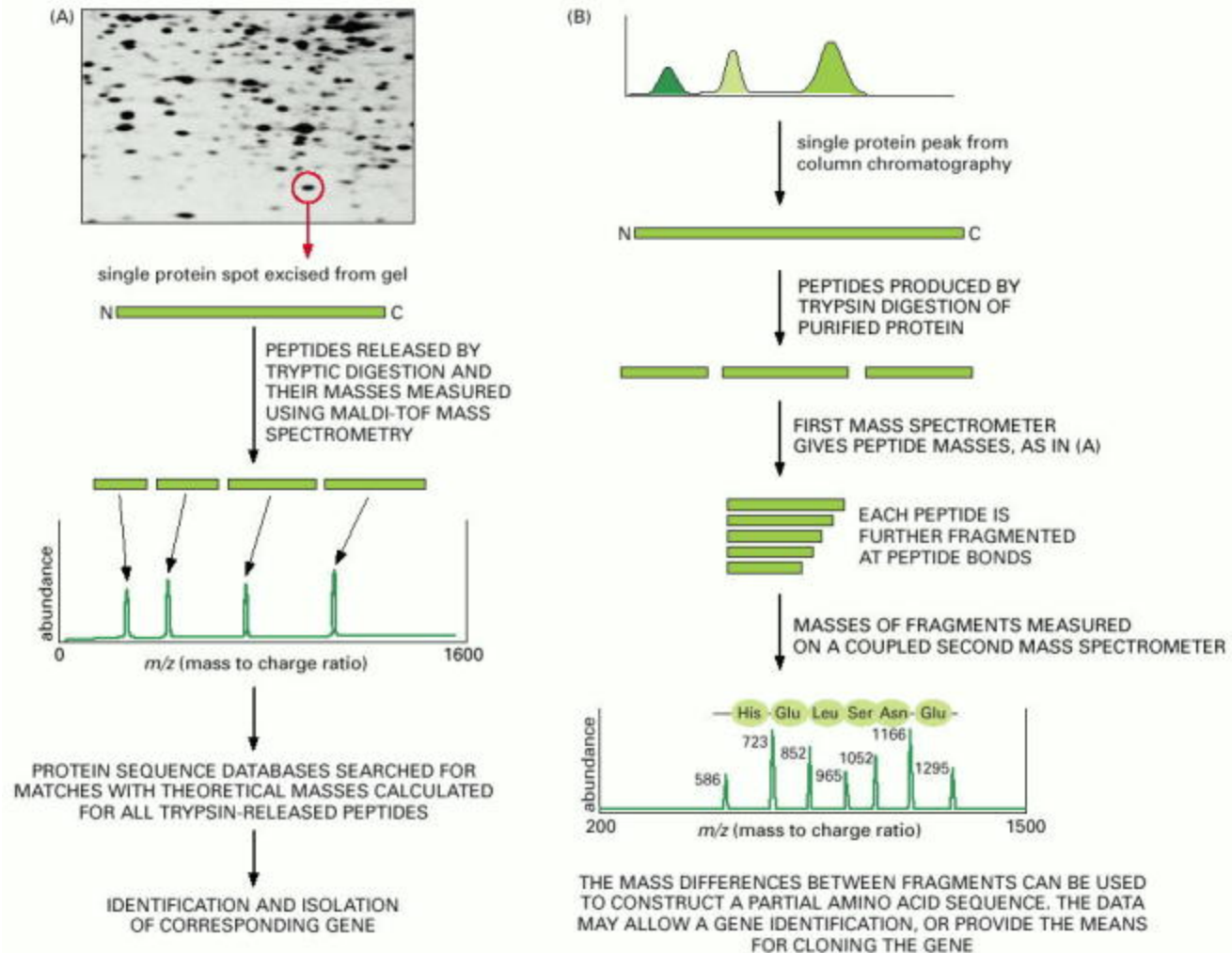


(A)



(B)

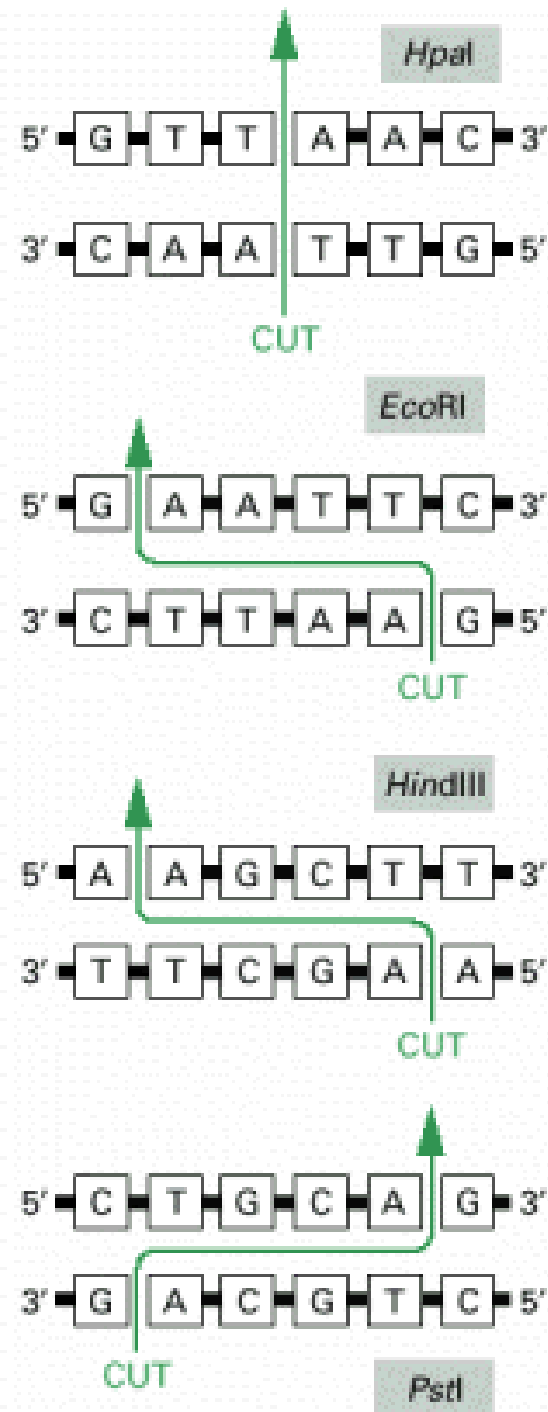
Protein identification via MS



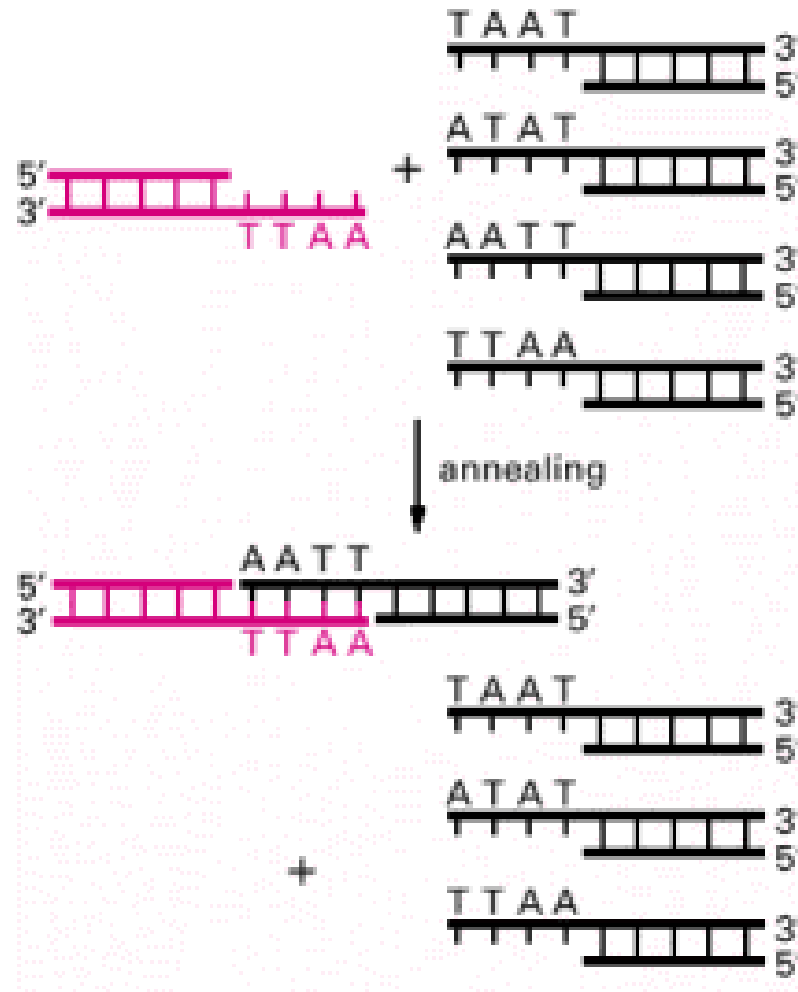
DNA recombination technology

- 1. Cleavage of DNA at specific sites by restriction nucleases, which greatly facilitates the isolation and manipulation of individual genes.
 2. DNA cloning either through the use of cloning vectors or the polymerase chain reaction, whereby a single DNA molecule can be copied to generate many billions of identical molecules.
 3. Nucleic acid hybridization, which makes it possible to find a specific sequence of DNA or RNA with great accuracy and sensitivity on the basis of its ability to bind a complementary nucleic acid sequence.
 4. Rapid sequencing of all the nucleotides in a purified DNA fragment, which makes it possible to identify genes and to deduce the amino acid sequence of the proteins they encode.
 5. Simultaneous monitoring of the expression level of each gene in a cell, using nucleic acid microarrays that allow tens of thousands of hybridization reactions to be performed simultaneously.

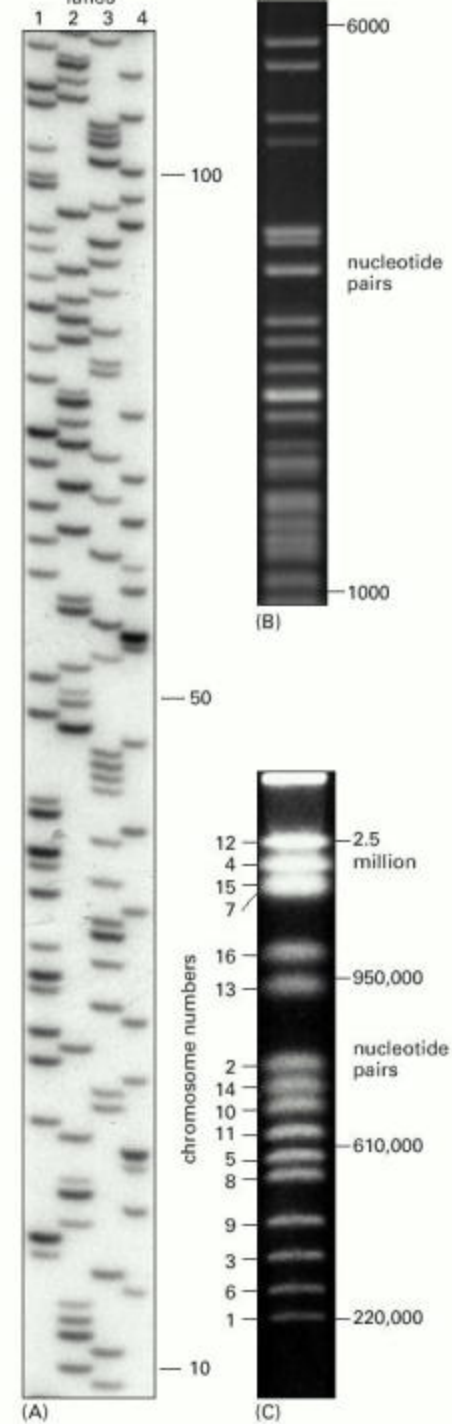
Restriction nucleases

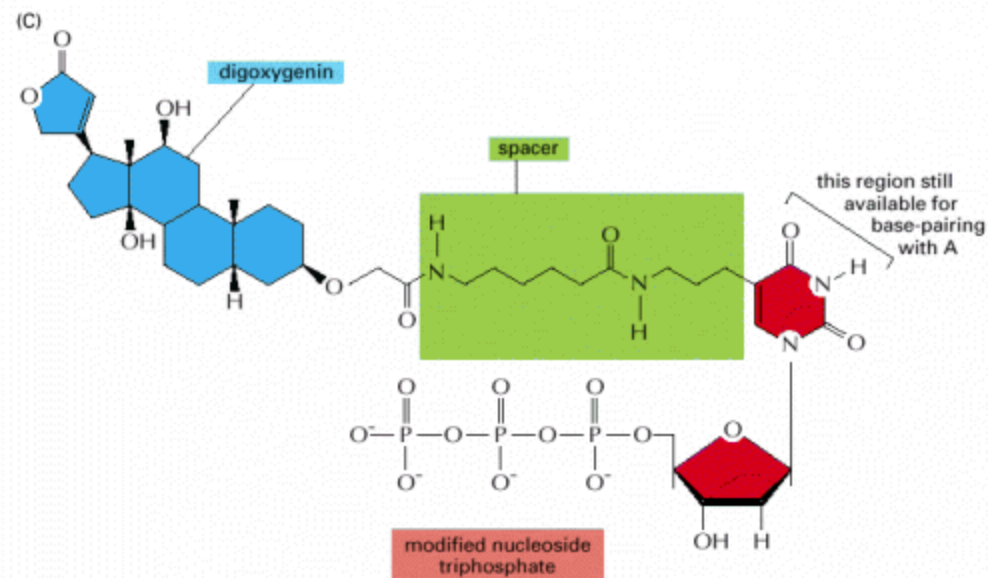
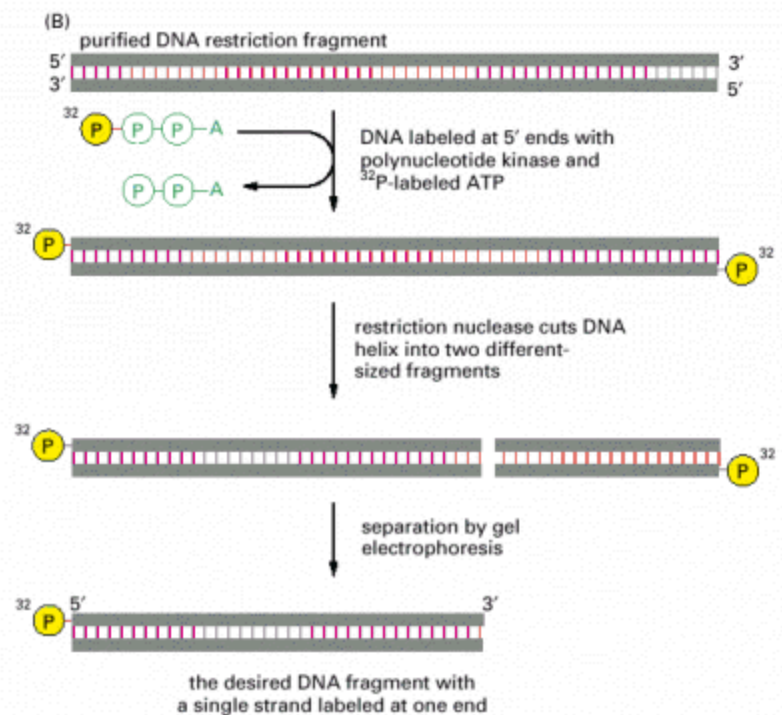
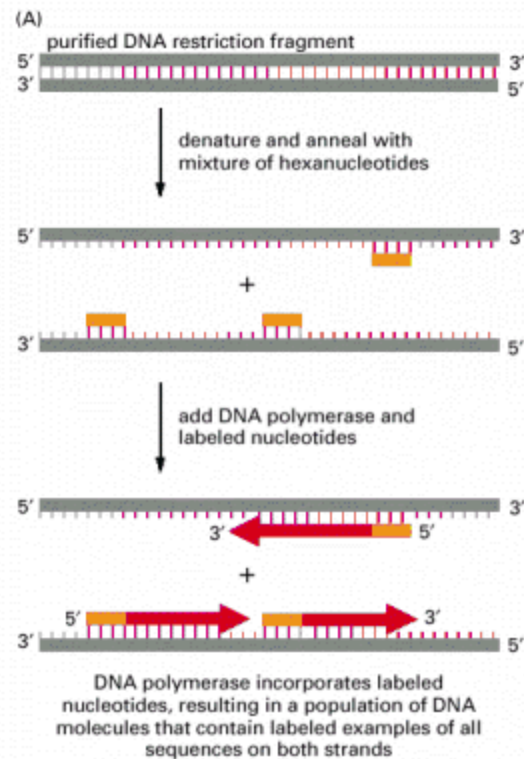


Restriction nucleases produce DNA fragments that can be easily joined together

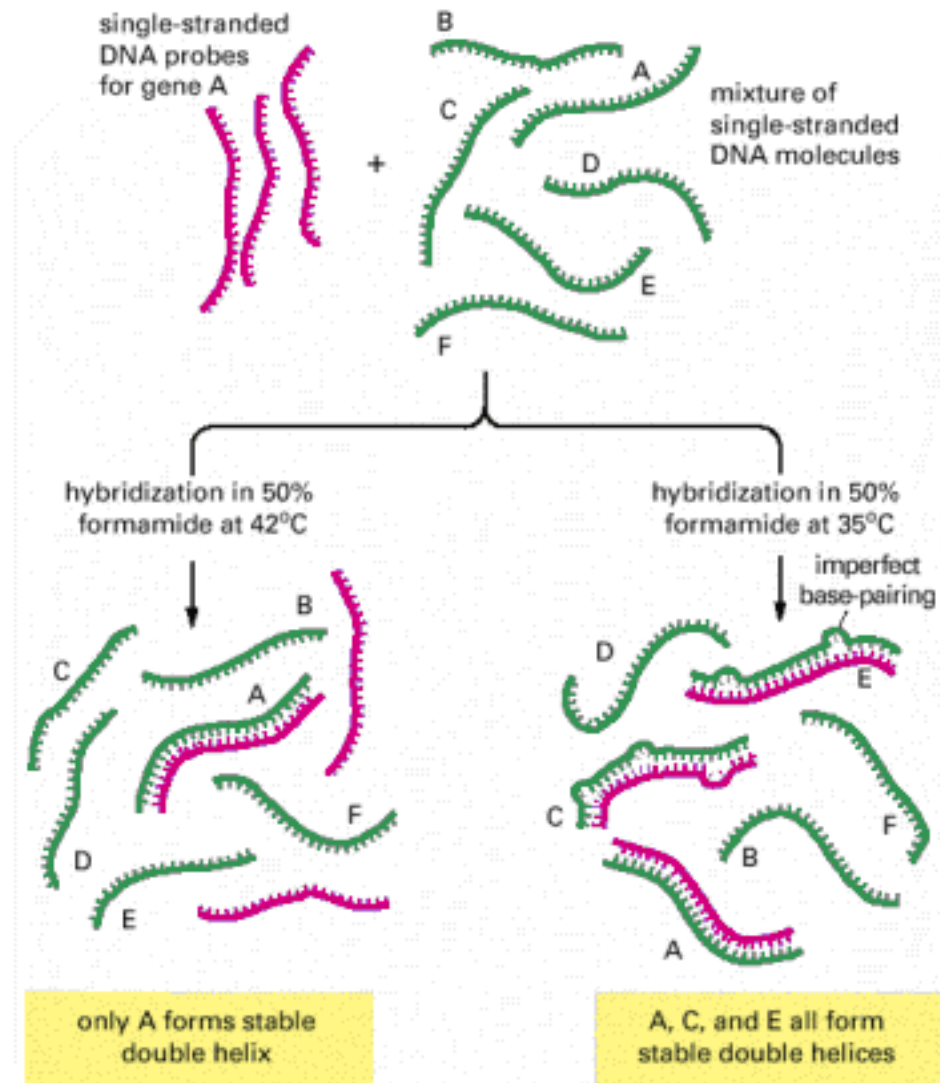


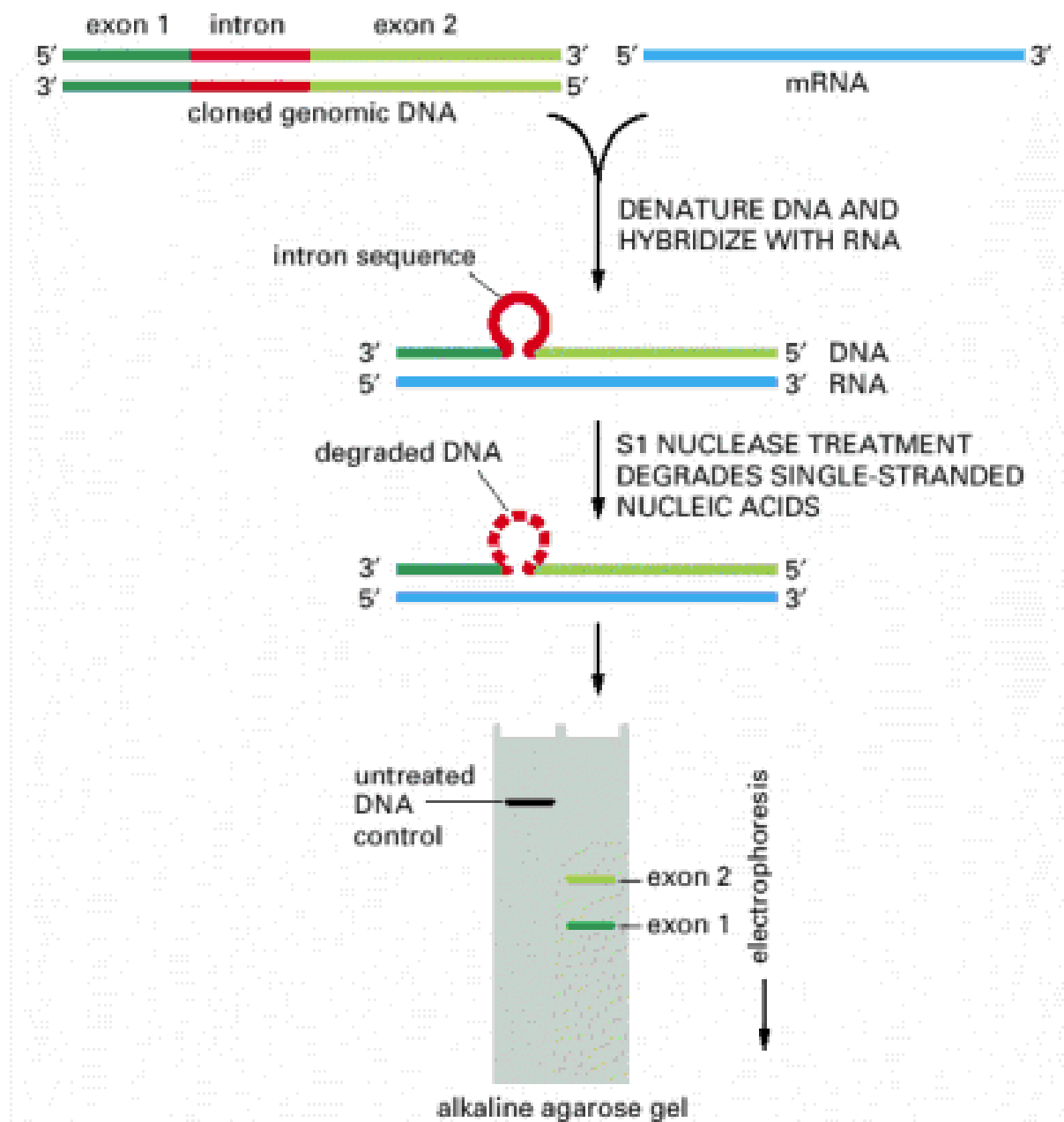
DNA Gel electrophoresis



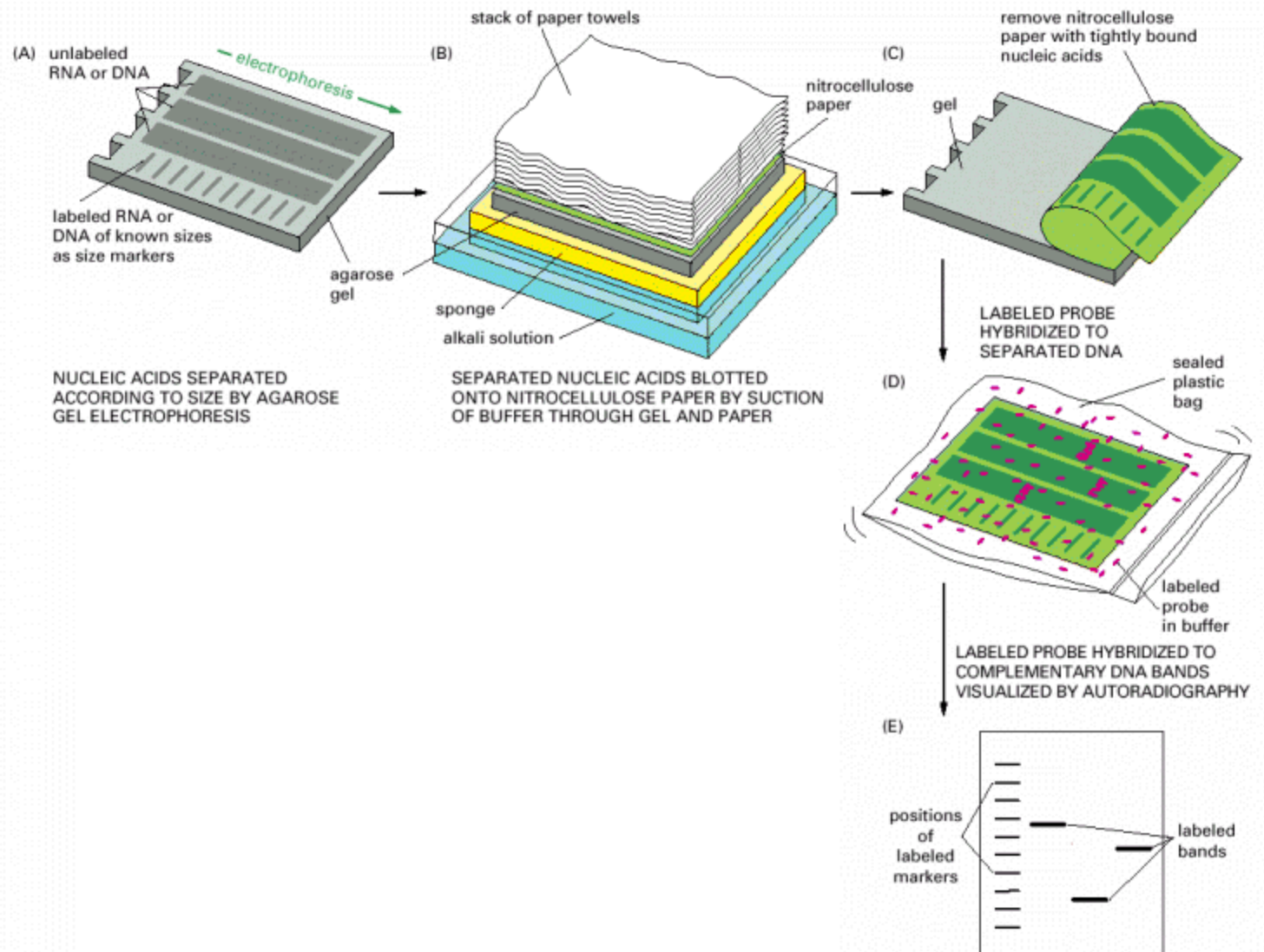


DNA hybridization

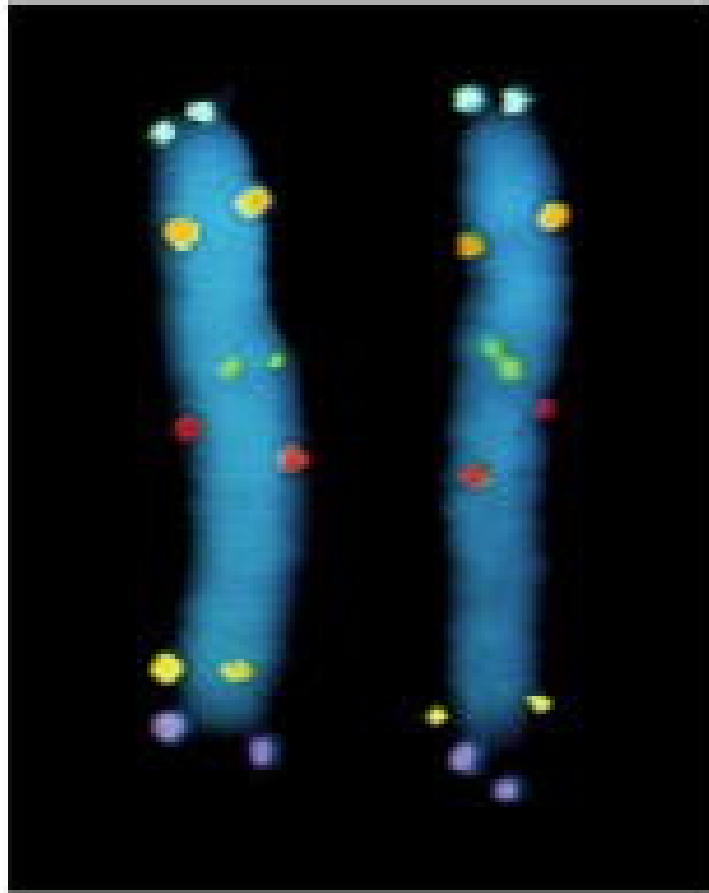




Gel transferred hybridization



In situ hybridization

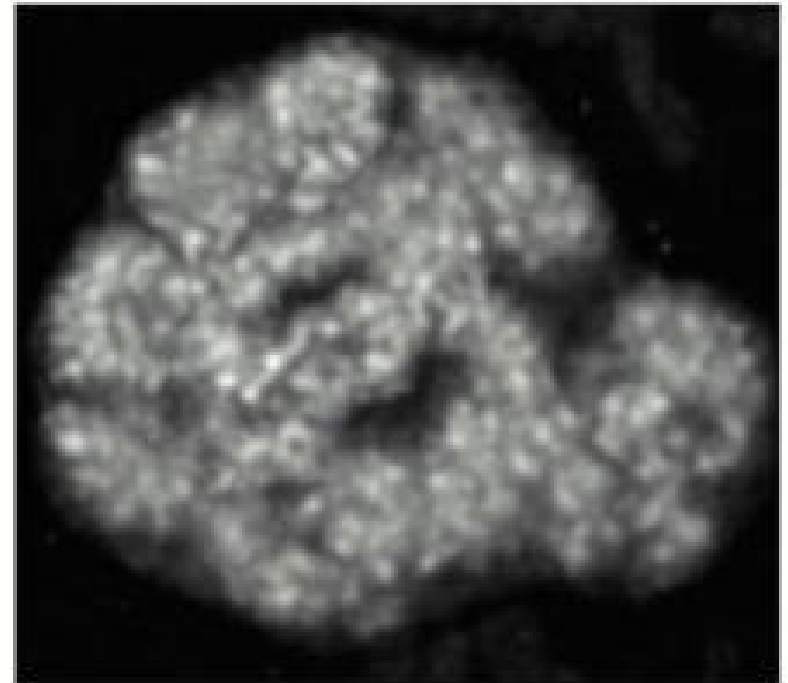


In situ hybridization for RNA localization



(A)

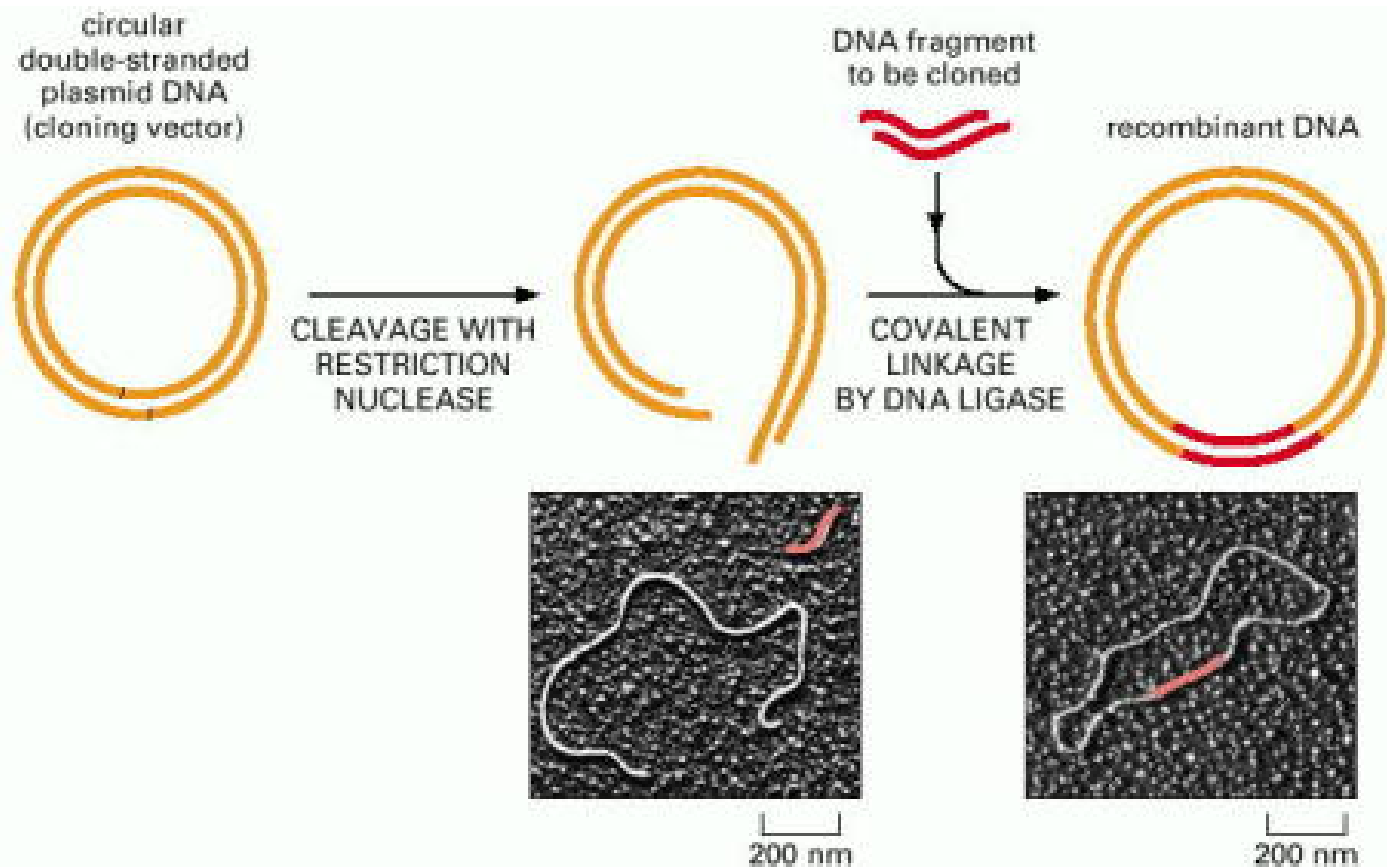
0.5 mm



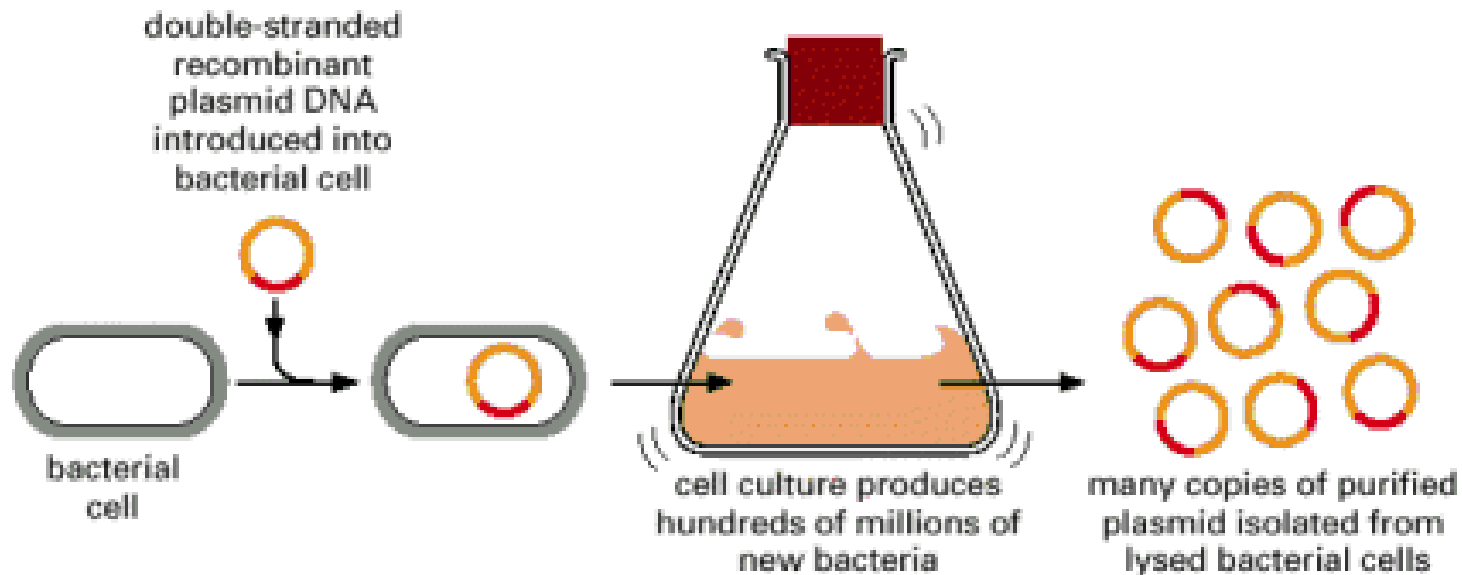
(B)

1 μm

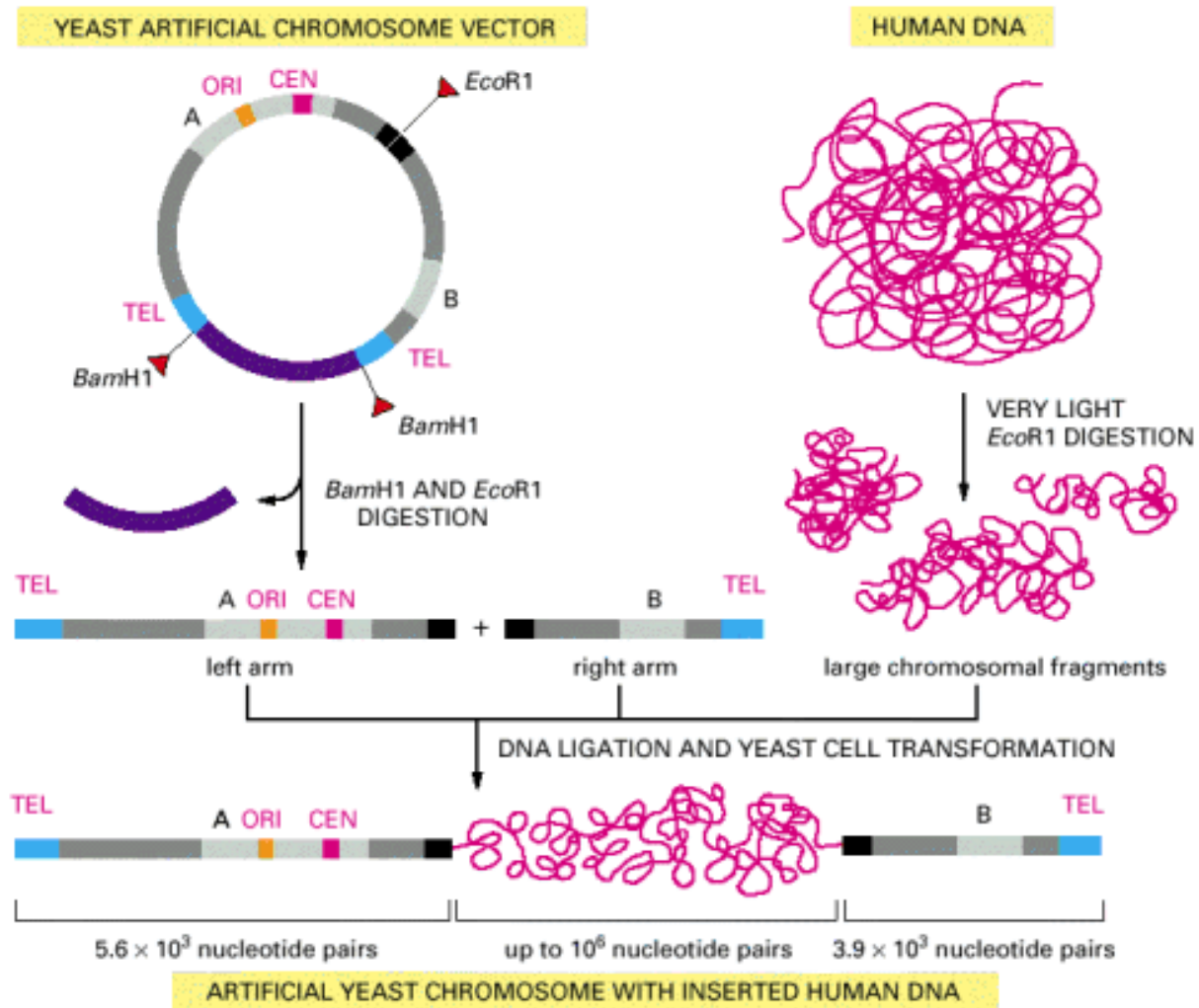
DNA recombination



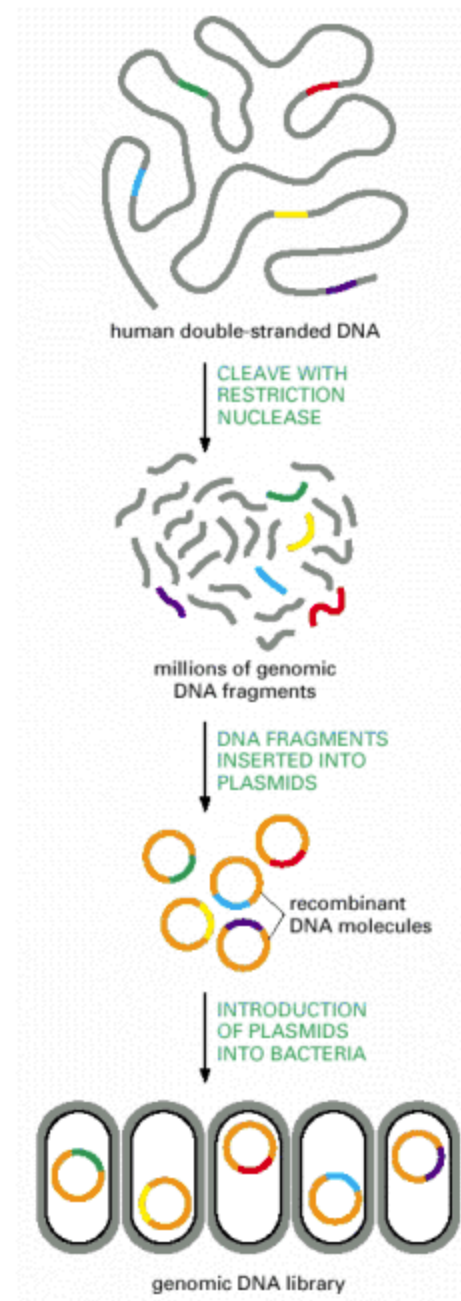
DNA cloning



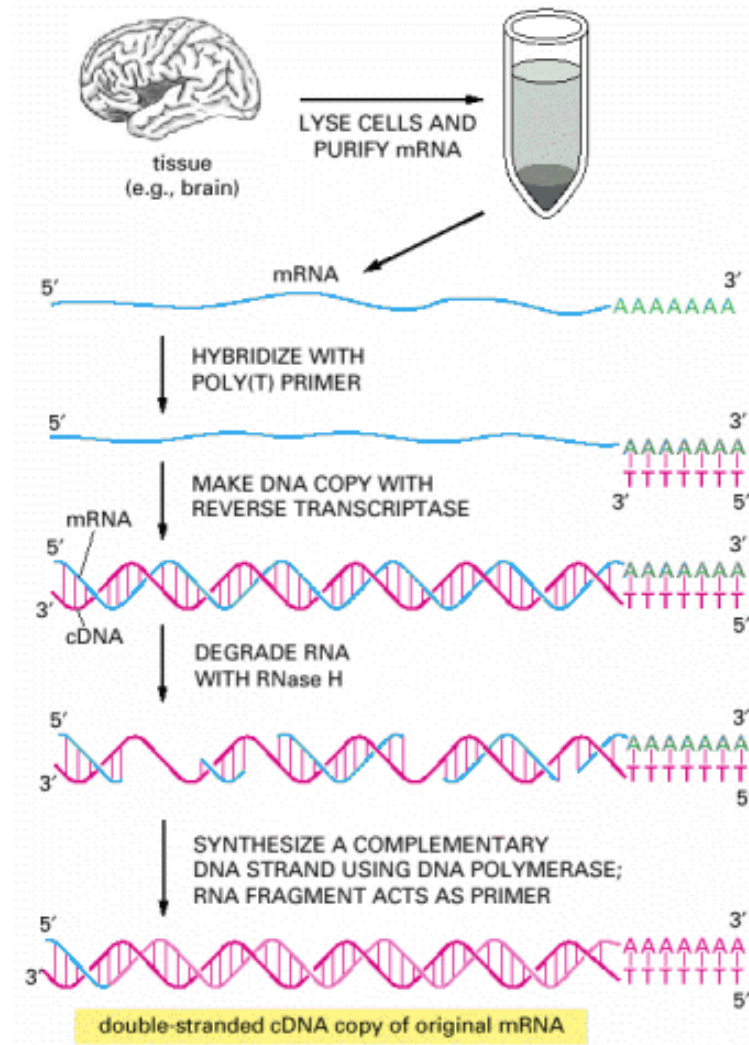
YAC



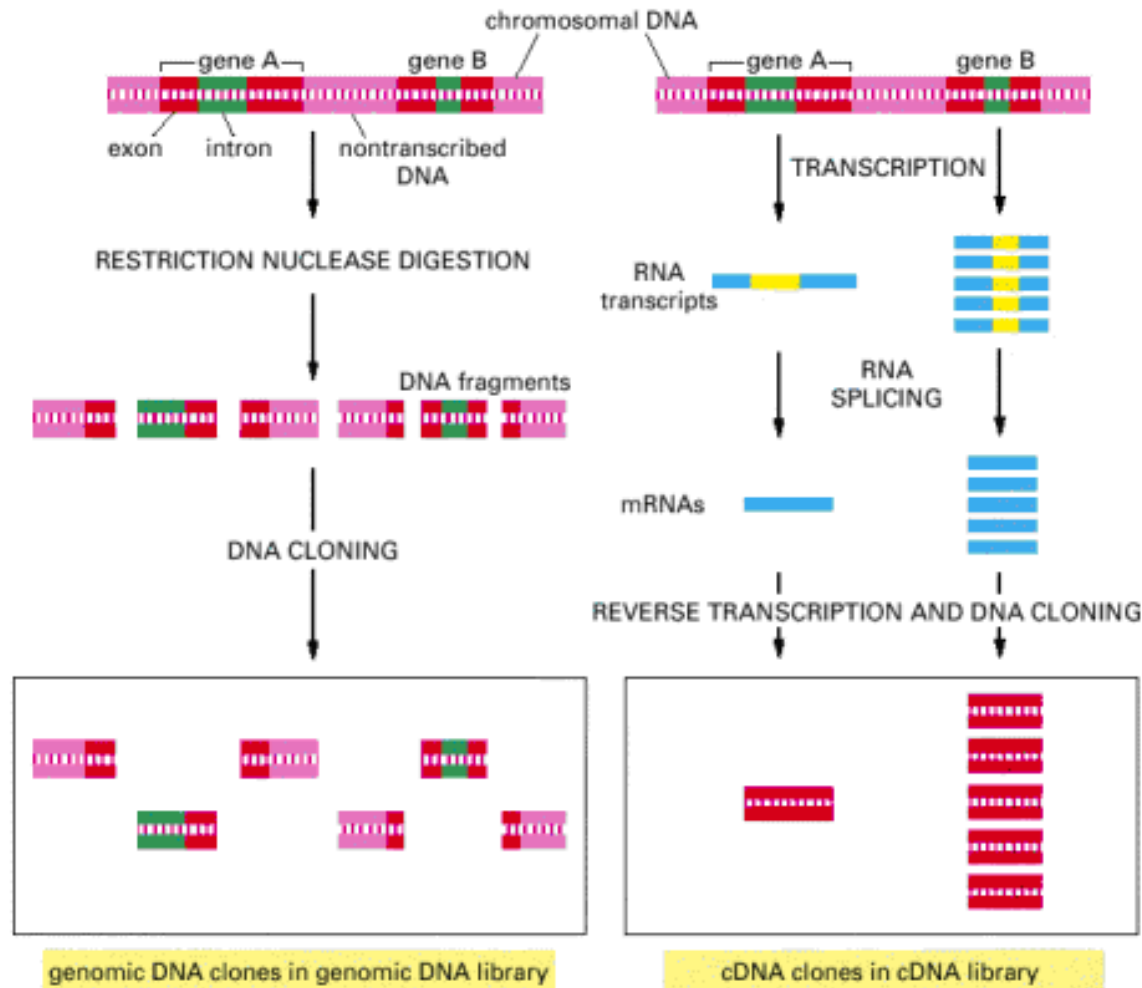
Human genome library



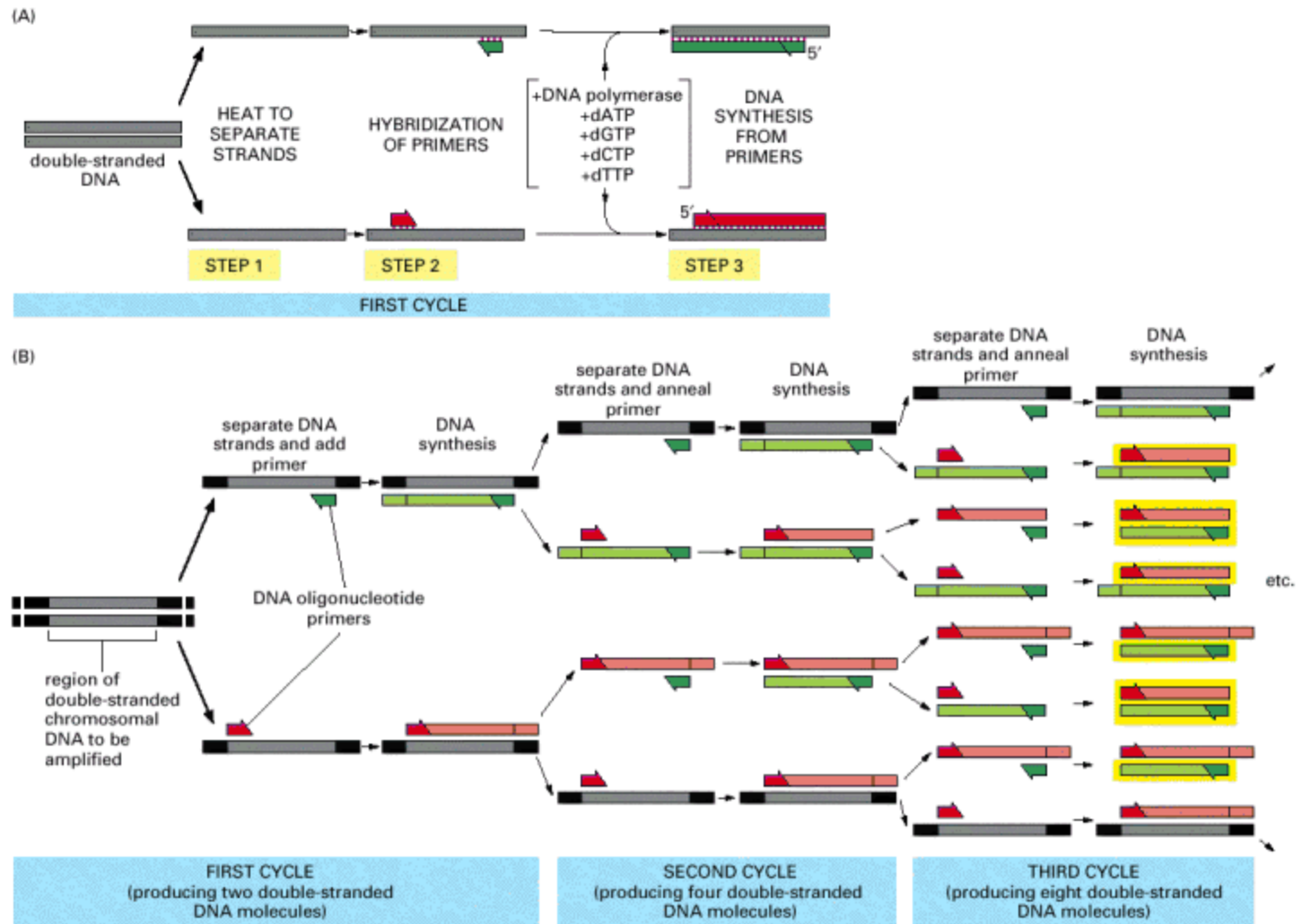
The synthesis of cDNA



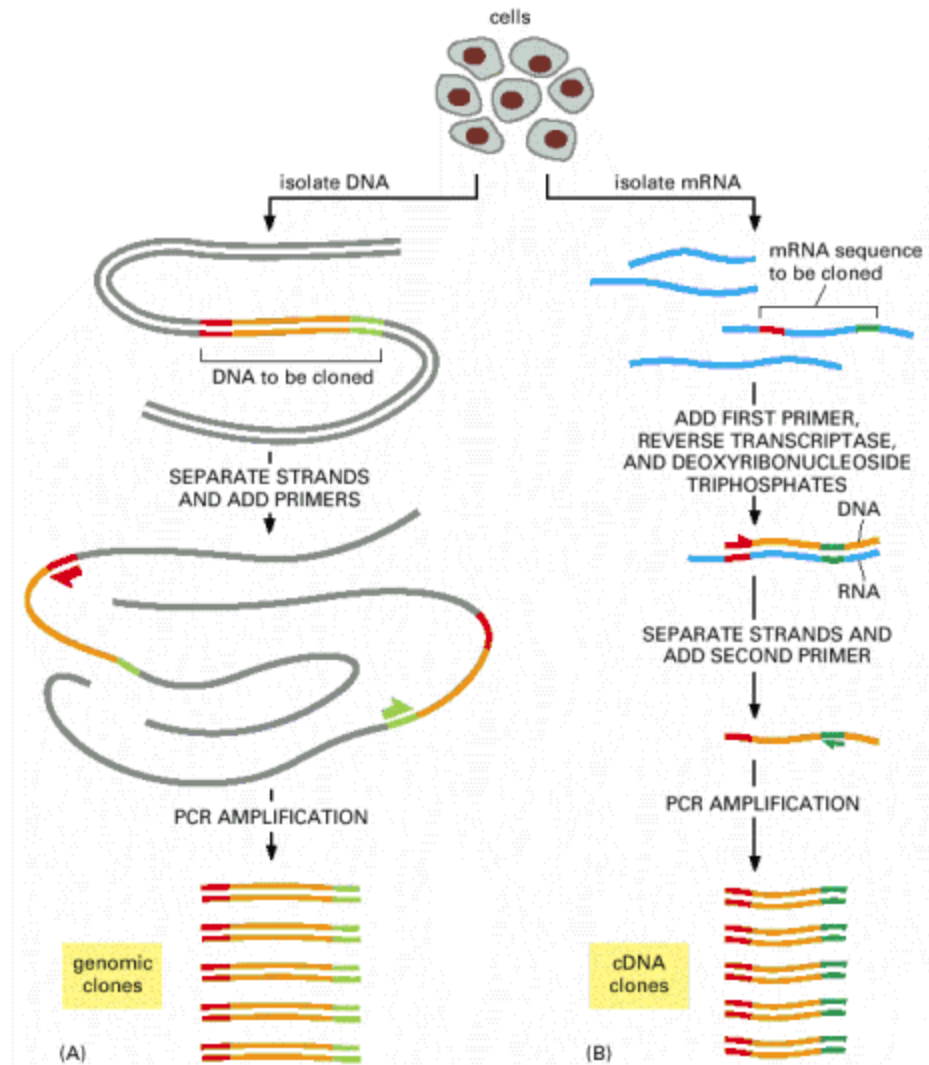
cDNA clones and genomic DNA clones



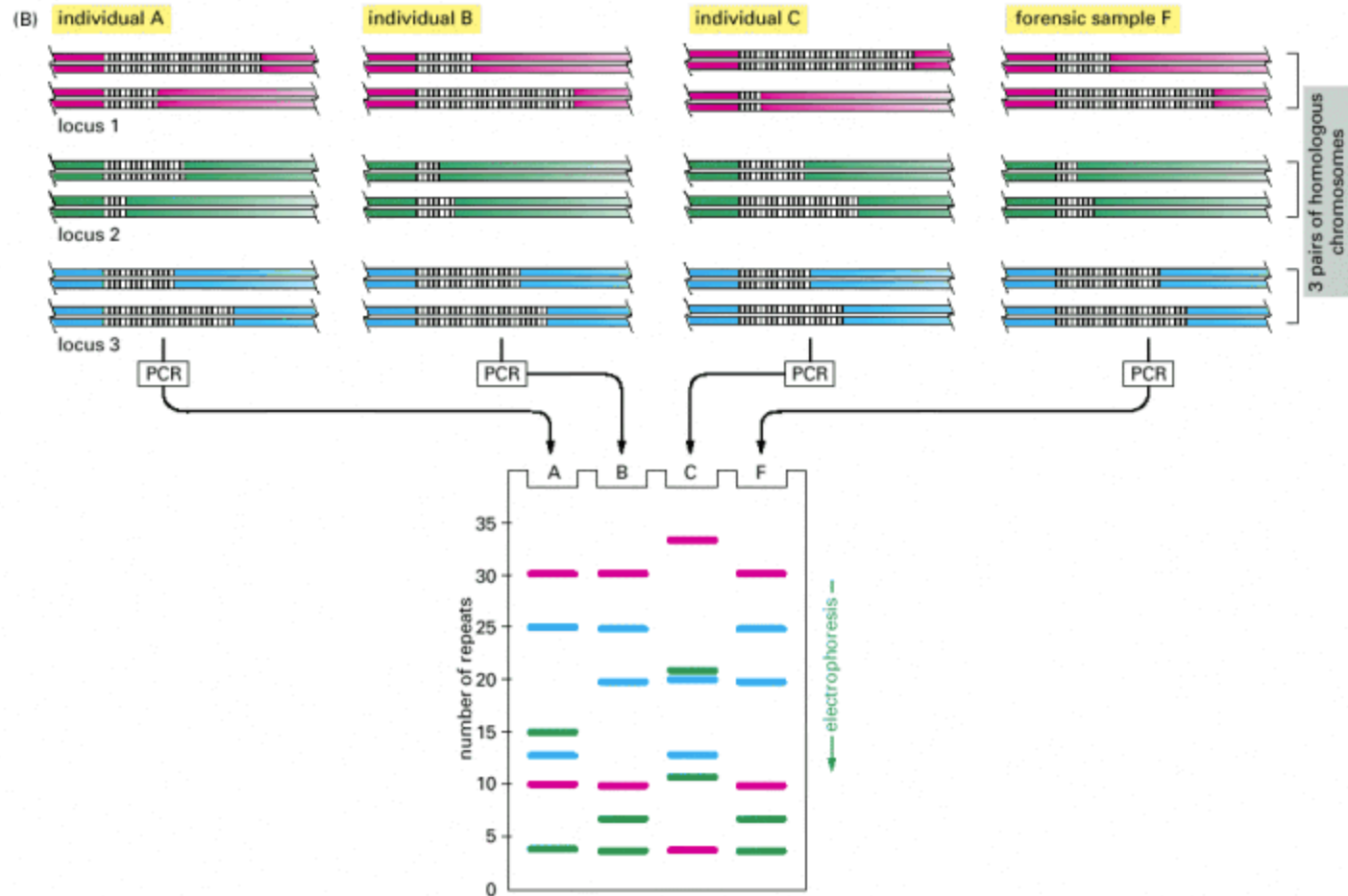
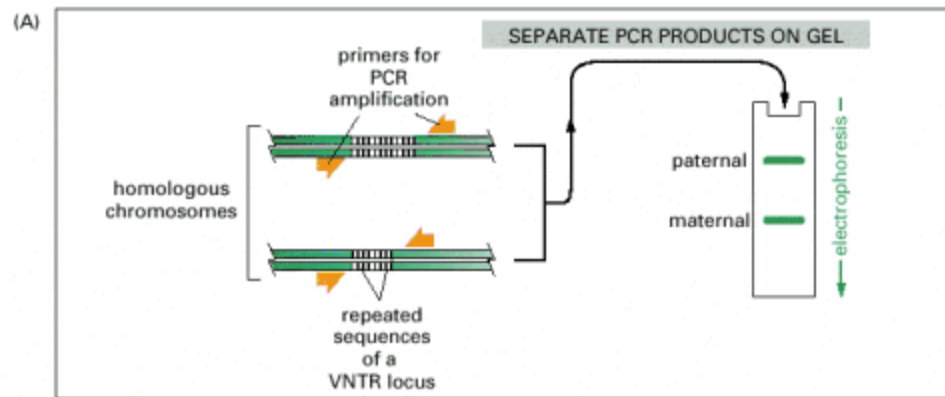
PCR



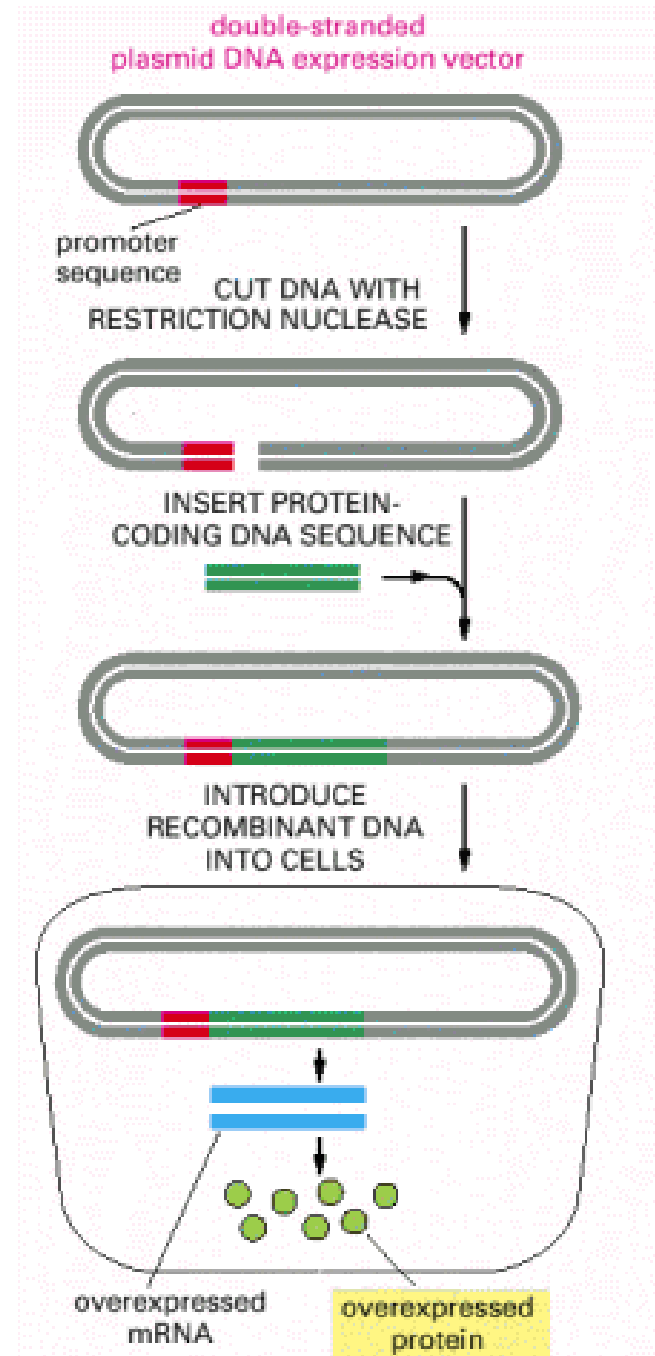
PCR based gene cloning



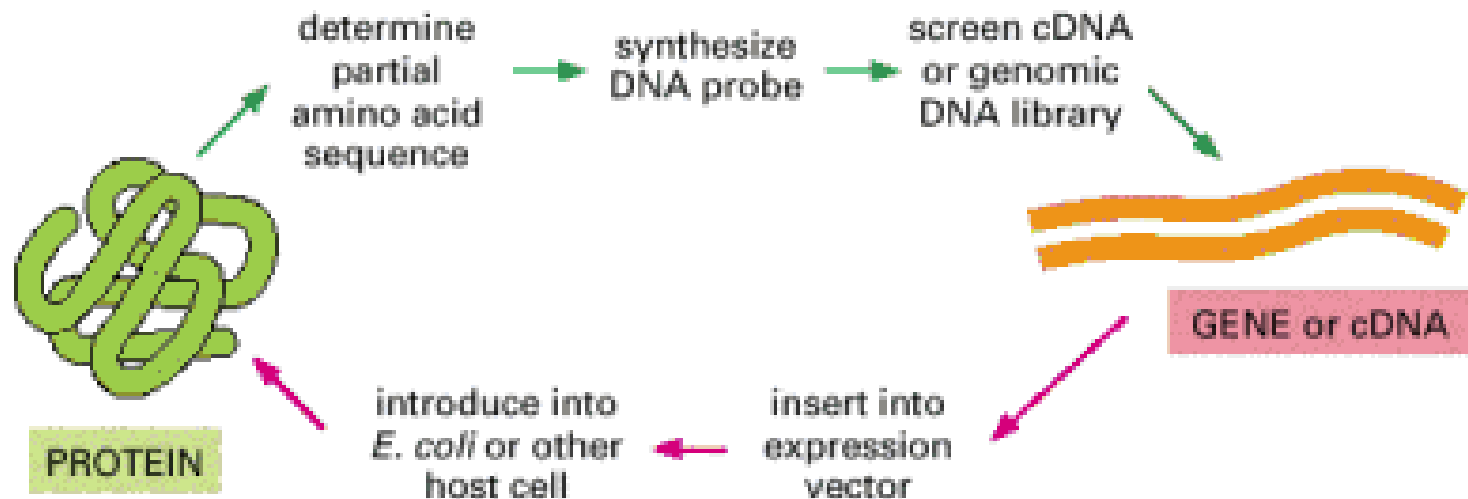
PCR used in forensic science



Protein expression system via a plasmid expression vector



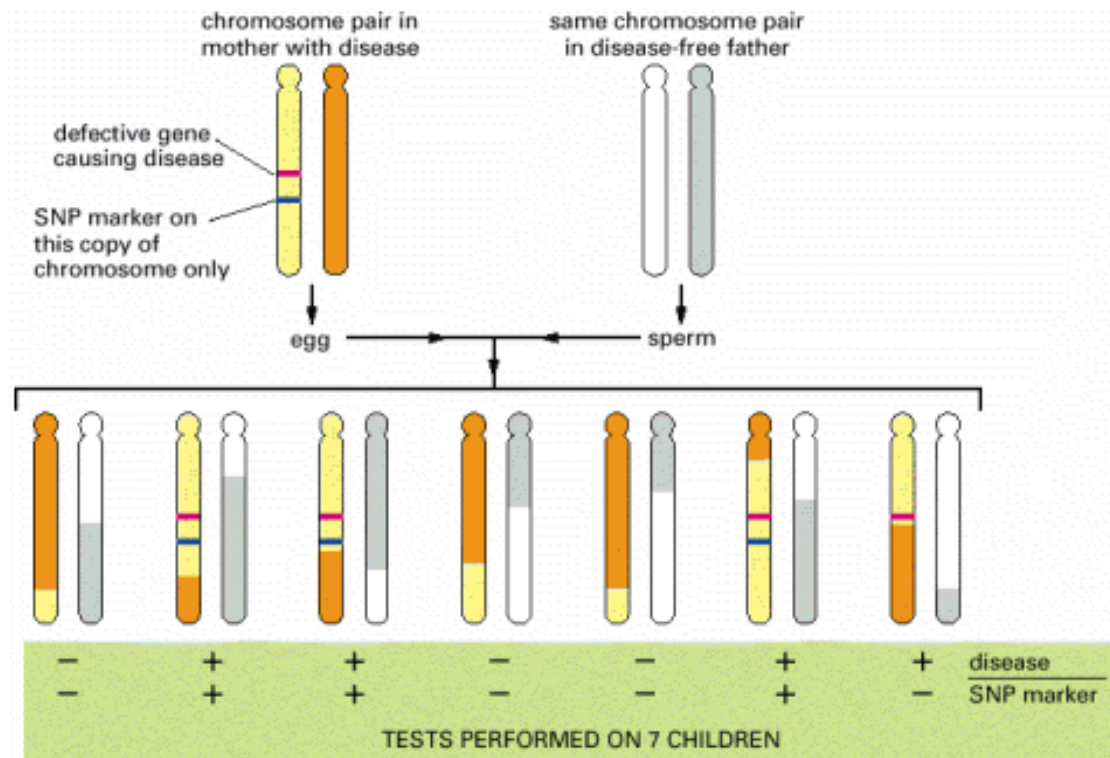
Molecular biology research



Studying gene functions

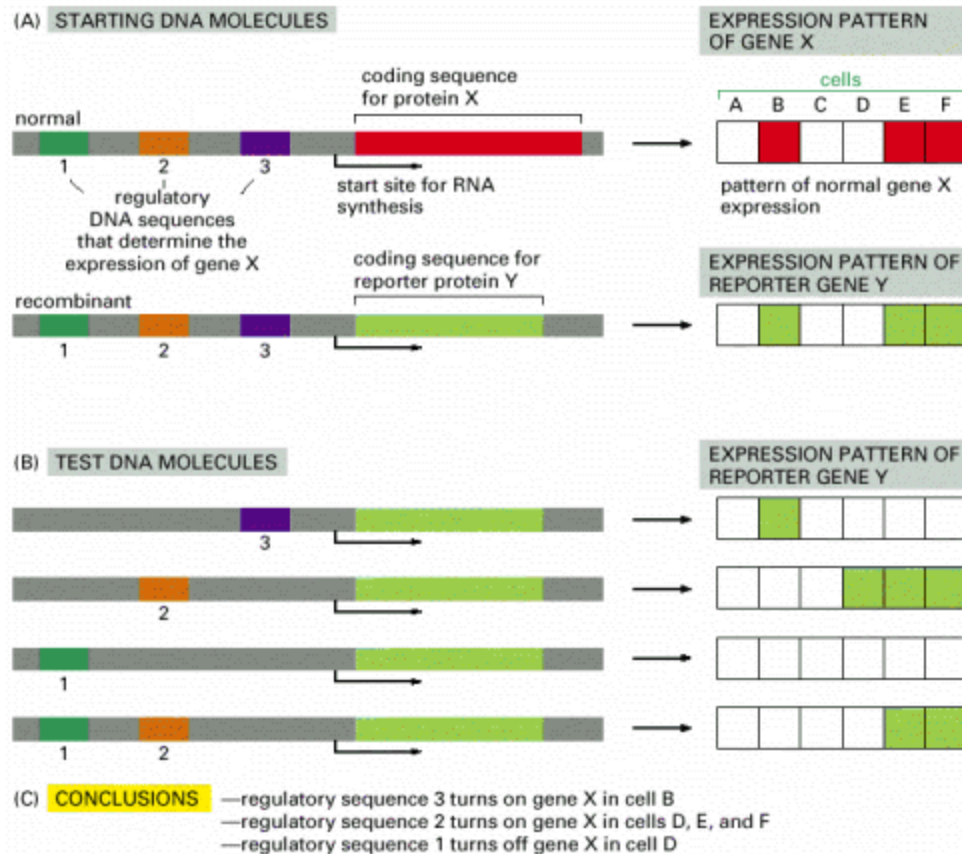
- Genetic screening
- Monitoring gene expression
- Site-directed mutagenesis
- Gene replacement or knockout

LDA

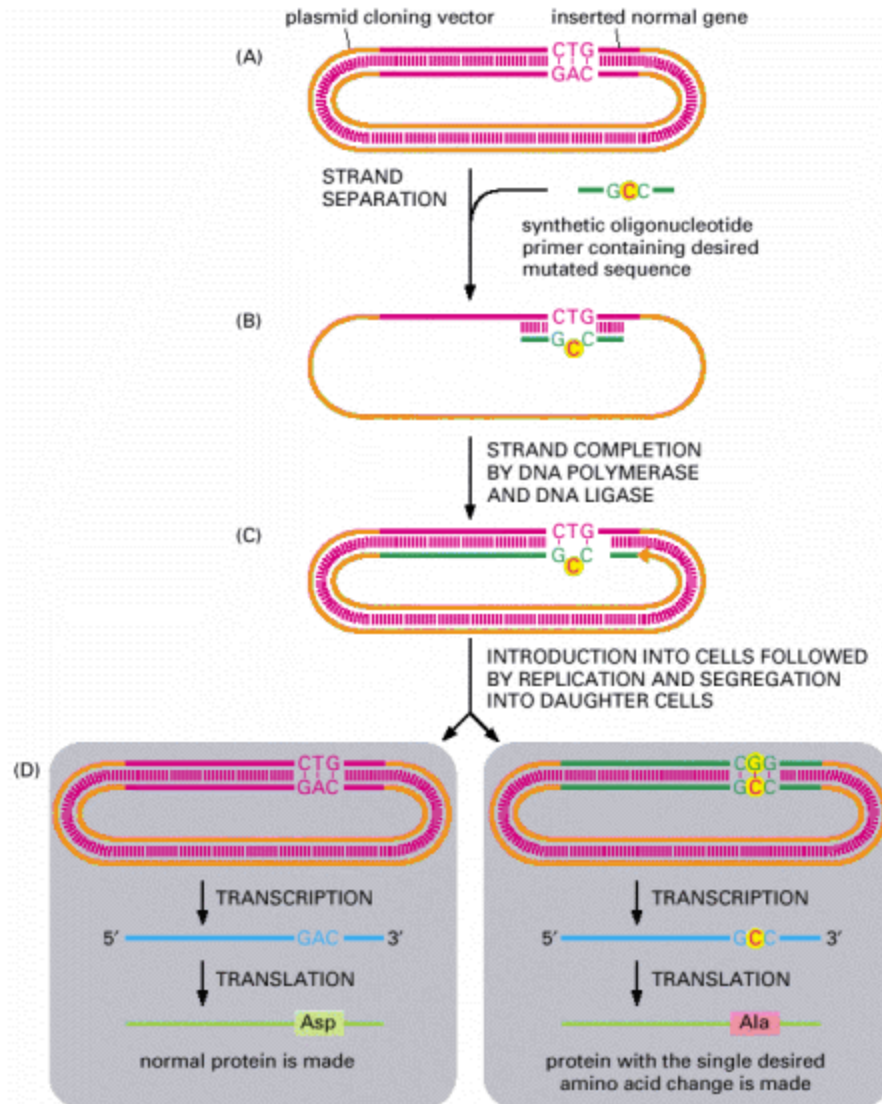


CONCLUSION: gene causing disease is coinherited with SNP marker from diseased mother in 75% of the diseased progeny. If this same correlation is observed in other families that have been examined, the gene causing disease is mapped to this chromosome close to the SNP. Note that a SNP that is either far away from the gene on the same chromosome, or located on a different chromosome than the gene of interest, will be coinherited only 50% of the time.

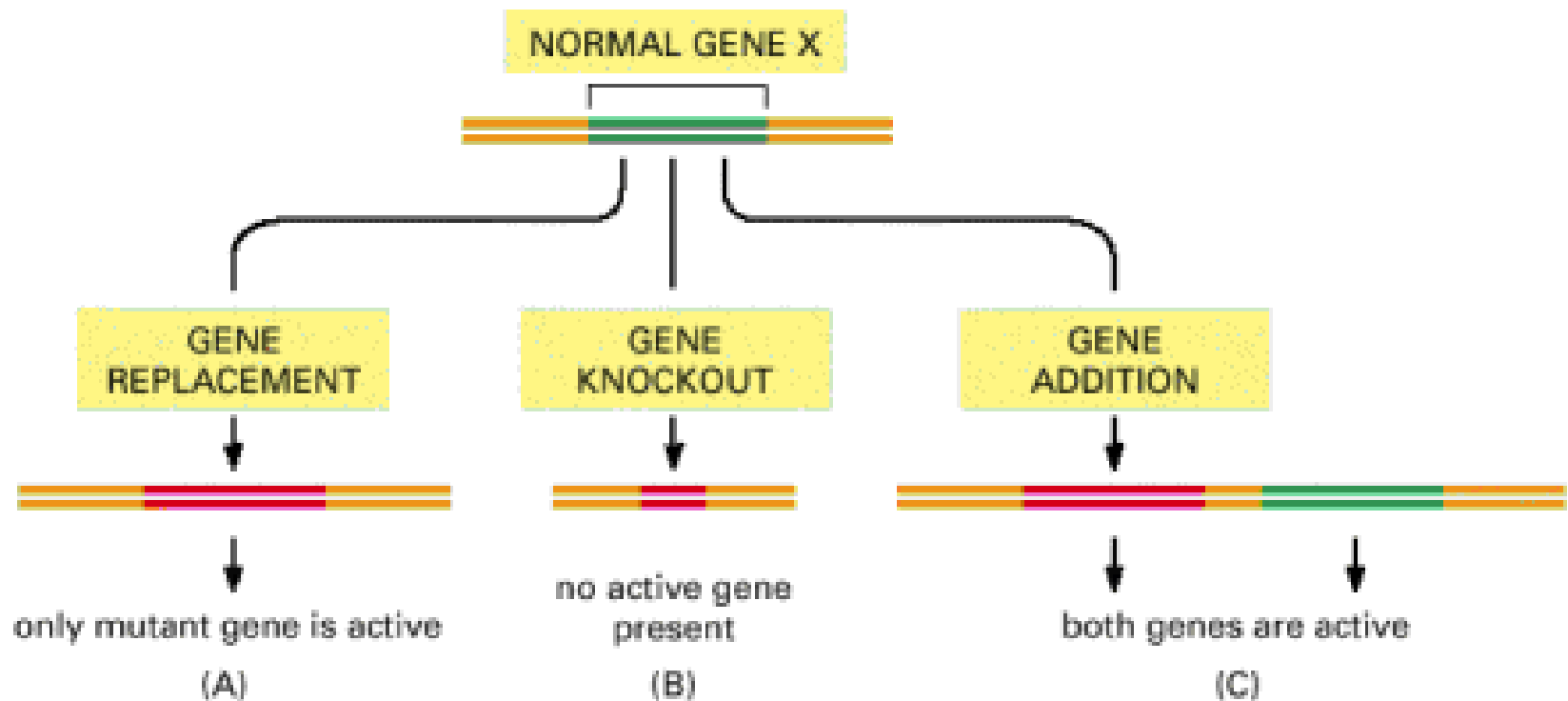
Reporter gene



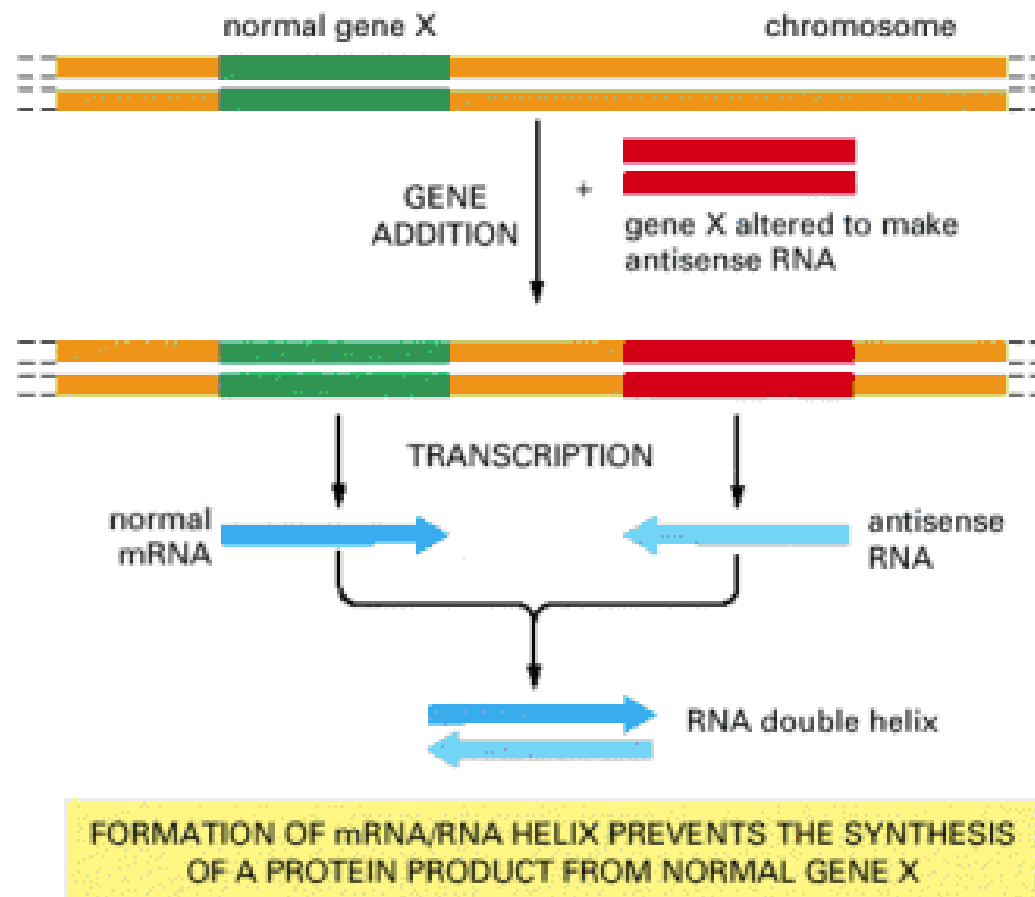
Site-directed Mutagenesis



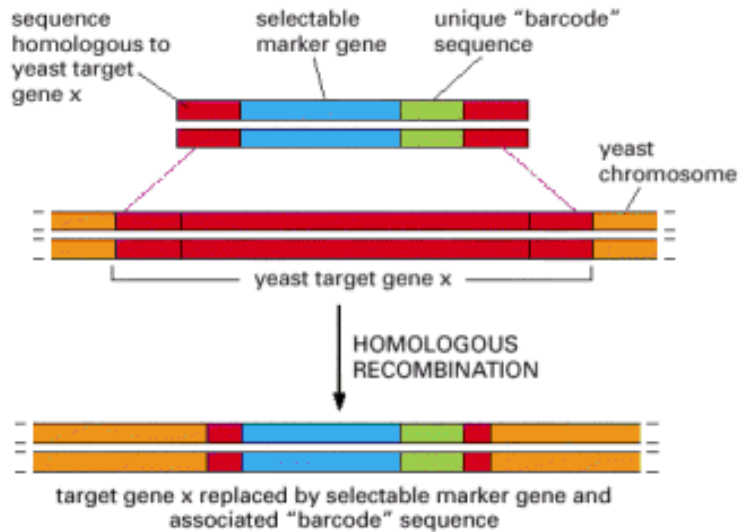
Genome manipulation



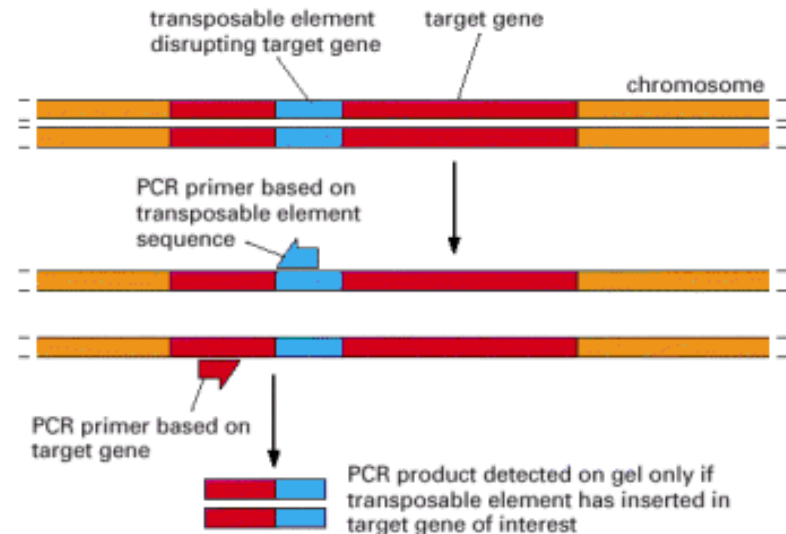
Anti-sense RNA strategy



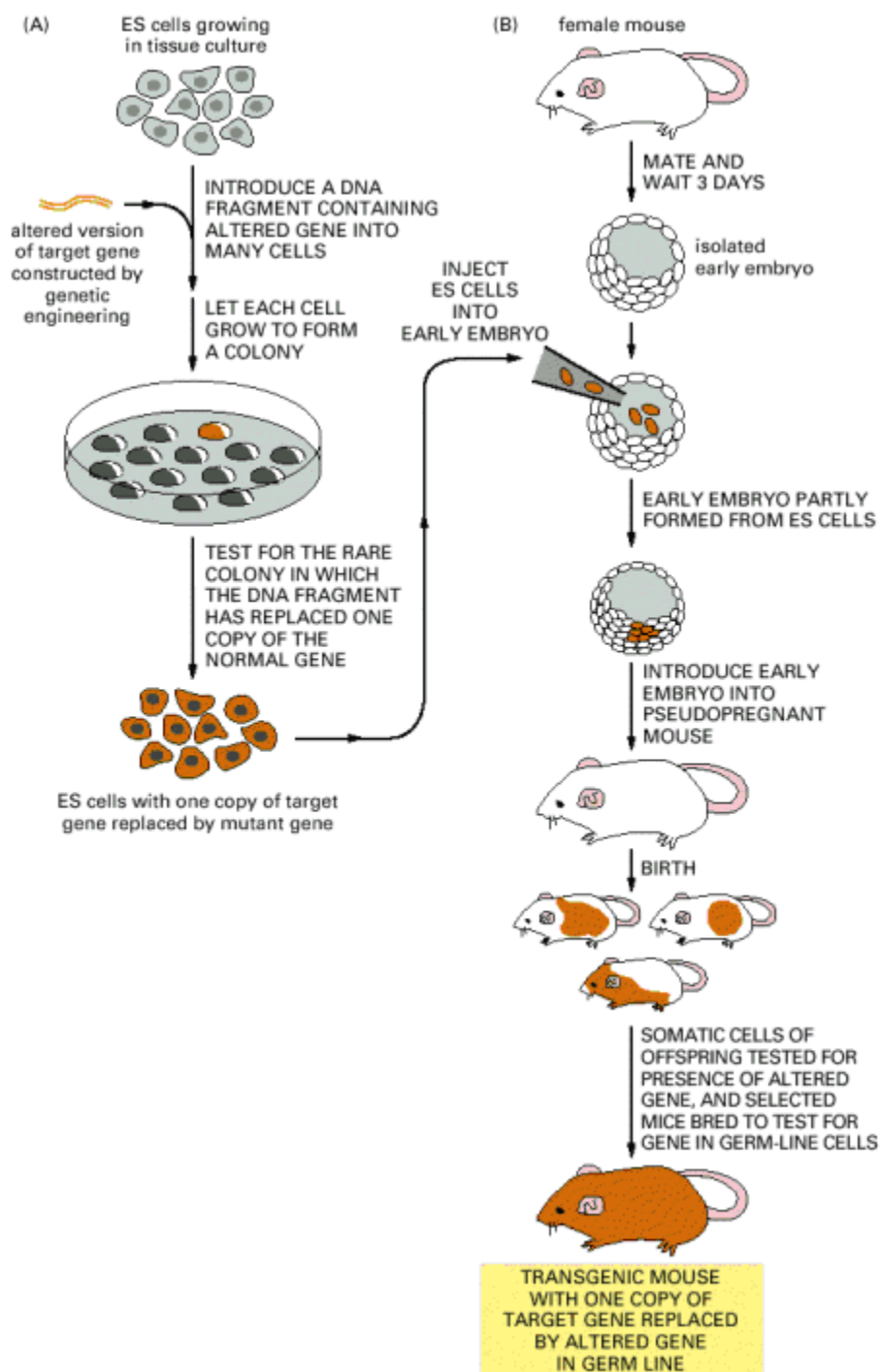
Making collections of mutant organisms



(A) YEAST



(B) ARABIDOPSIS AND DROSOPHILA



Mouse with an engineered defect in fibroblast growth factor 5 (FGF5)

