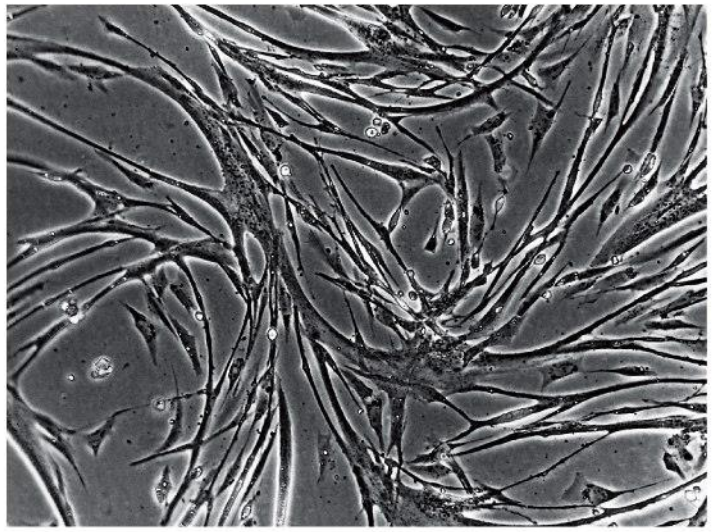
Lecture 3
Methods to study cell biology

Outline

1. How to obtain pure cell culture?
2. How are cells cultured?
3. What are cells composed of?
4. How to study the functions of these components in the cell?

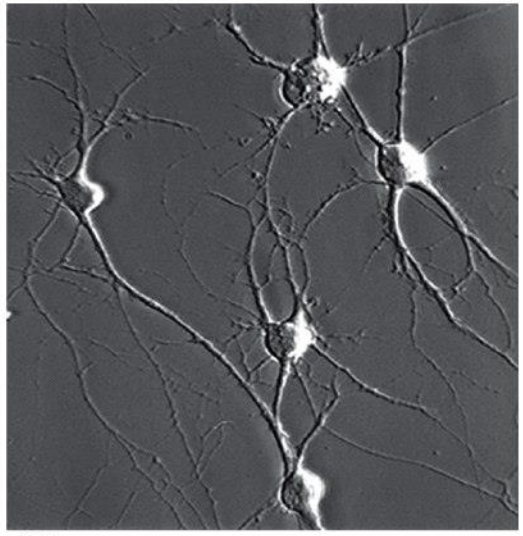
(A)

20 μm



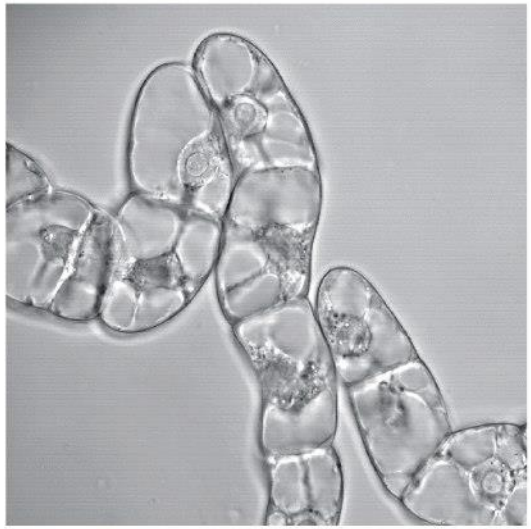
(B)

100 μm



(C)

50 μm



(D)

50 μm

1.1 An authorative resource to obtain pure cell line

www.atcc.org

♦ATCC serves by characterizing cell lines, bacteria, viruses, fungi and protozoa.

♦ More than 4,000 human and animal cell lines and an additional 1,200 hybridoma.

♦ More than 18,000 strains of bacteria from 900 genera, as well as 2,000 different types of animal viruses and 1,000 plant viruses.

♦ Over 49,000 yeast and fungi strains from 1,500 genera and 2,000 strains of protists.

Table 8–1 Some Commonly Used Cell Lines

CELL LINE*	CELL TYPE AND ORIGIN
3T3	fibroblast (mouse)
BHK21	fibroblast (Syrian hamster)
MDCK	epithelial cell (dog)
HeLa	epithelial cell (human)
PtK1	epithelial cell (rat kangaroo)
L6	myoblast (rat)
PC12	chromaffin cell (rat)
SP2	plasma cell (mouse)
COS	kidney (monkey)
293	kidney (human); transformed with adenovirus
CHO	ovary (Chinese hamster)
DT40	lymphoma cell for efficient targeted recombination (chick)
R1	embryonic stem cell (mouse)
E14.1	embryonic stem cell (mouse)
H1, H9	embryonic stem cell (human)
S2	macrophage-like cell *(Drosophila)*
BY2	undifferentiated meristematic cell (tobacco)

*Many of these cell lines were derived from tumors. All of them are capable of indefinite replication in culture and express at least some of the special characteristics of their cell's of origin.

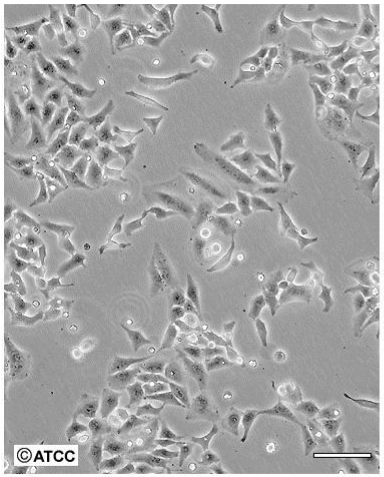
Table 8-1 *Molecular Biology of the Cell* (© Garland Science 2008)

HeLa cell --- The first immortalized human cell line

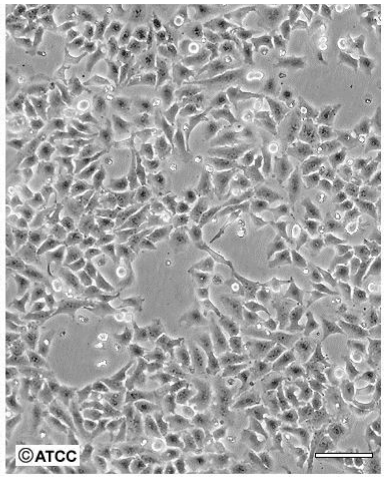
--- isolated in 1951

Henrietta Lacks circa 1920–1951

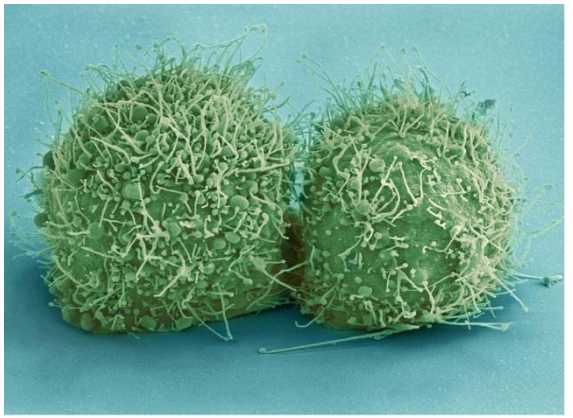
ATCC Number: **CCL-2**
Designation: **HeLa**



Low Density Scale Bar = 100µm

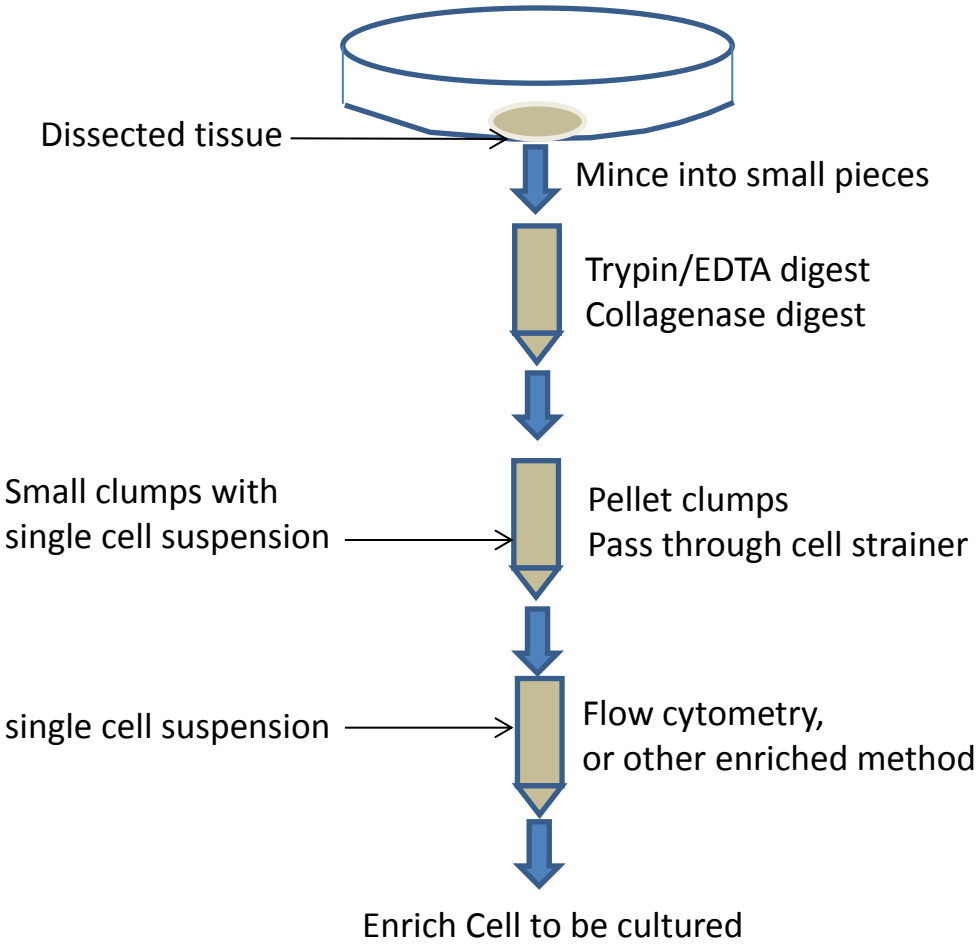


High Density Scale Bar = 100µm



TEM image for HeLa after dividing

1.2 How are cell generally isolated from tissue?



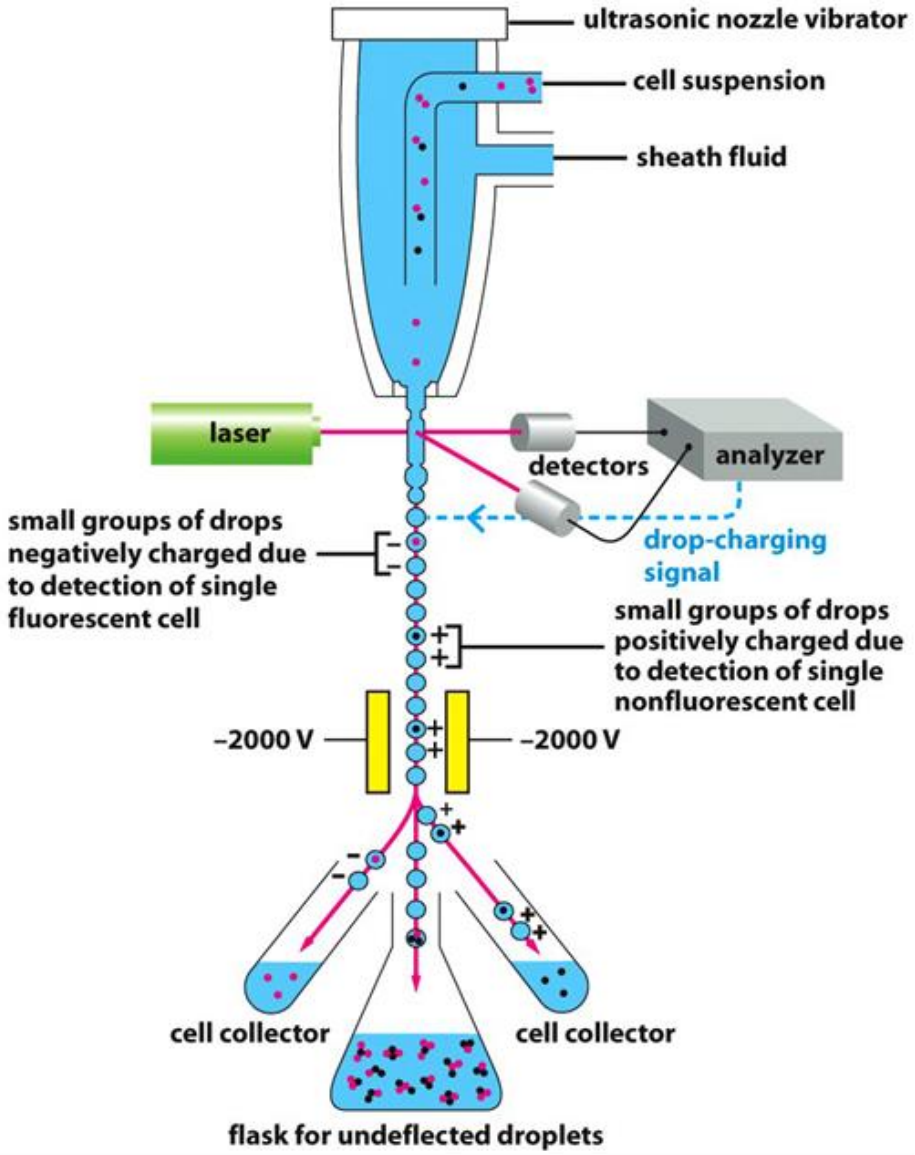
1.3 How to enrich homogenous cells?

1.3.1 Selectively grow single cell suspension in certain media which supports the outgrowth of single cell type over others

1.3.2 Fluorescence activated cell sorter (FACS)

1.3.3 Magnetic beads conjugated with cell-specific antibody

• 1.3.1 FACS (fluorescence-activated cell sorter)



1. Cells were labeled with antibody-conjugated fluorescence dye.
2. Sheath fluid flow (with no charge) focus the cells in the center where laser beam interacts with cells.
3. Optimal flow rate of cells allows them to pass on a single cell basis.
4. Based on its difference in charges, Cells will deflect in a certain angle in an electric field, and thus, be separated.

1.3.2 Magnetic beads-conjugated antibody

- Use magnetic force to enrich specific cells

  Example: isolation of T cells from thymus

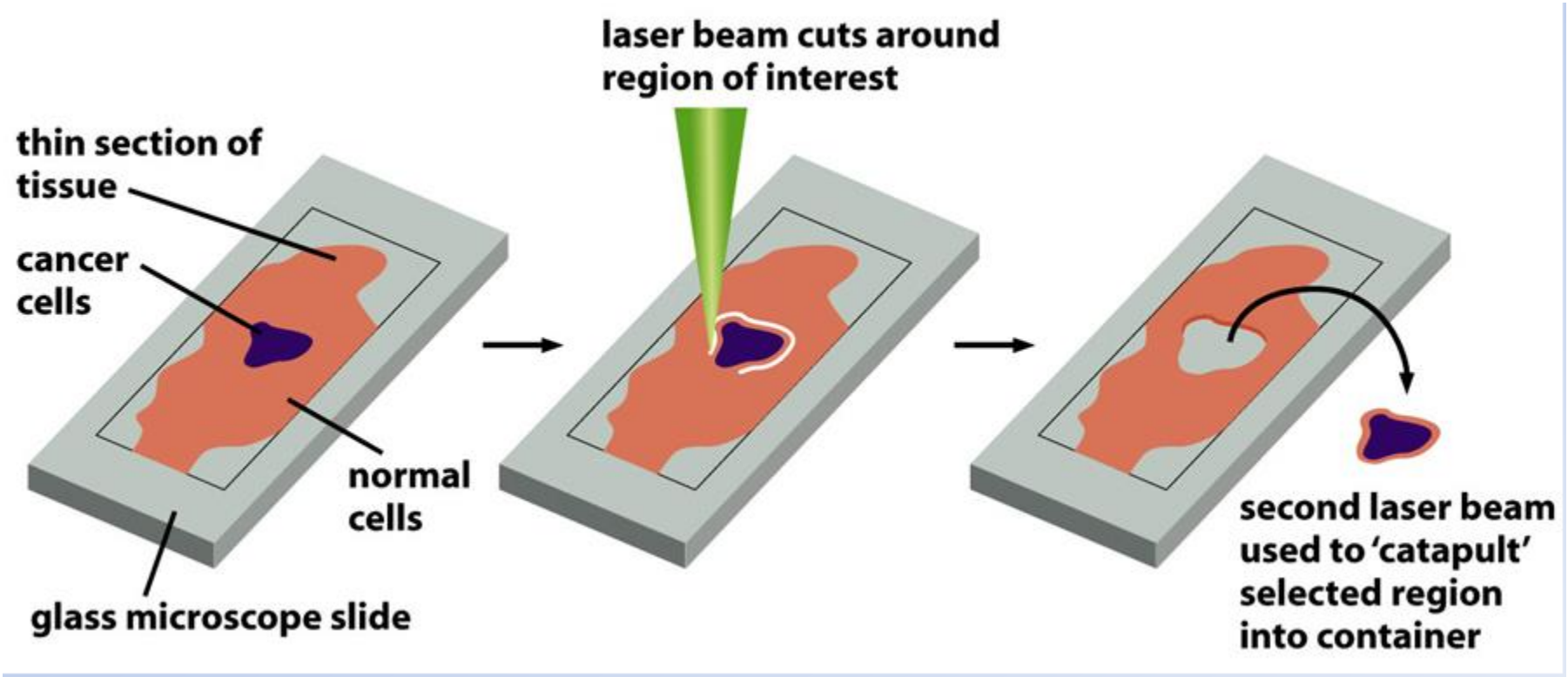
thymus tissue homogenous mixture(tissue grounder)

Add CD3-magnetic beads conjugate

Apply magentic force for this mixture, wash

T cell enrichment

1.3.3 Laser capture microdissection



Different types of cells can be fused together to display properties from the two sides

1.4 Hybridoma cell lines

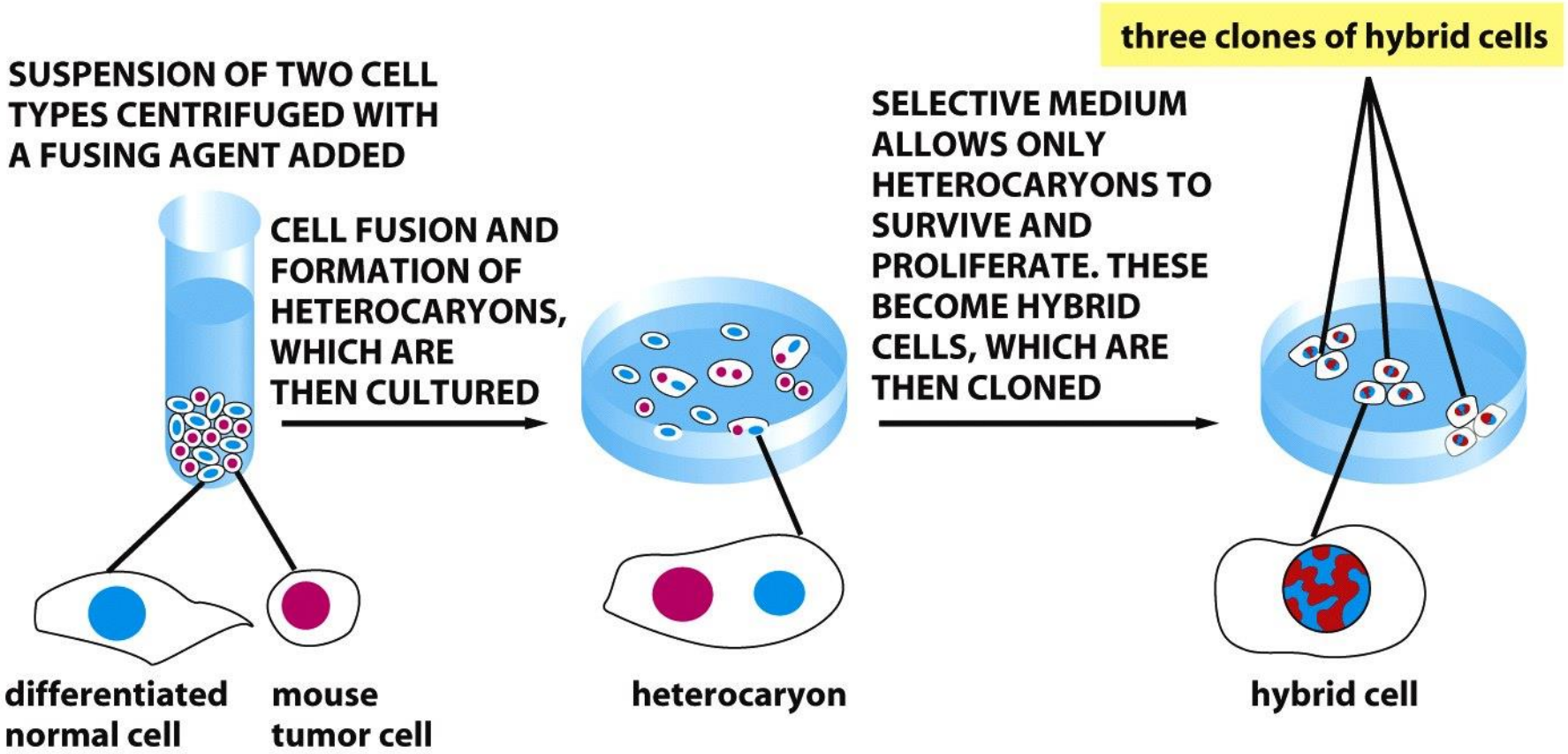


Figure 8-7 *Molecular Biology of the Cell* (© Garland Science 2008)

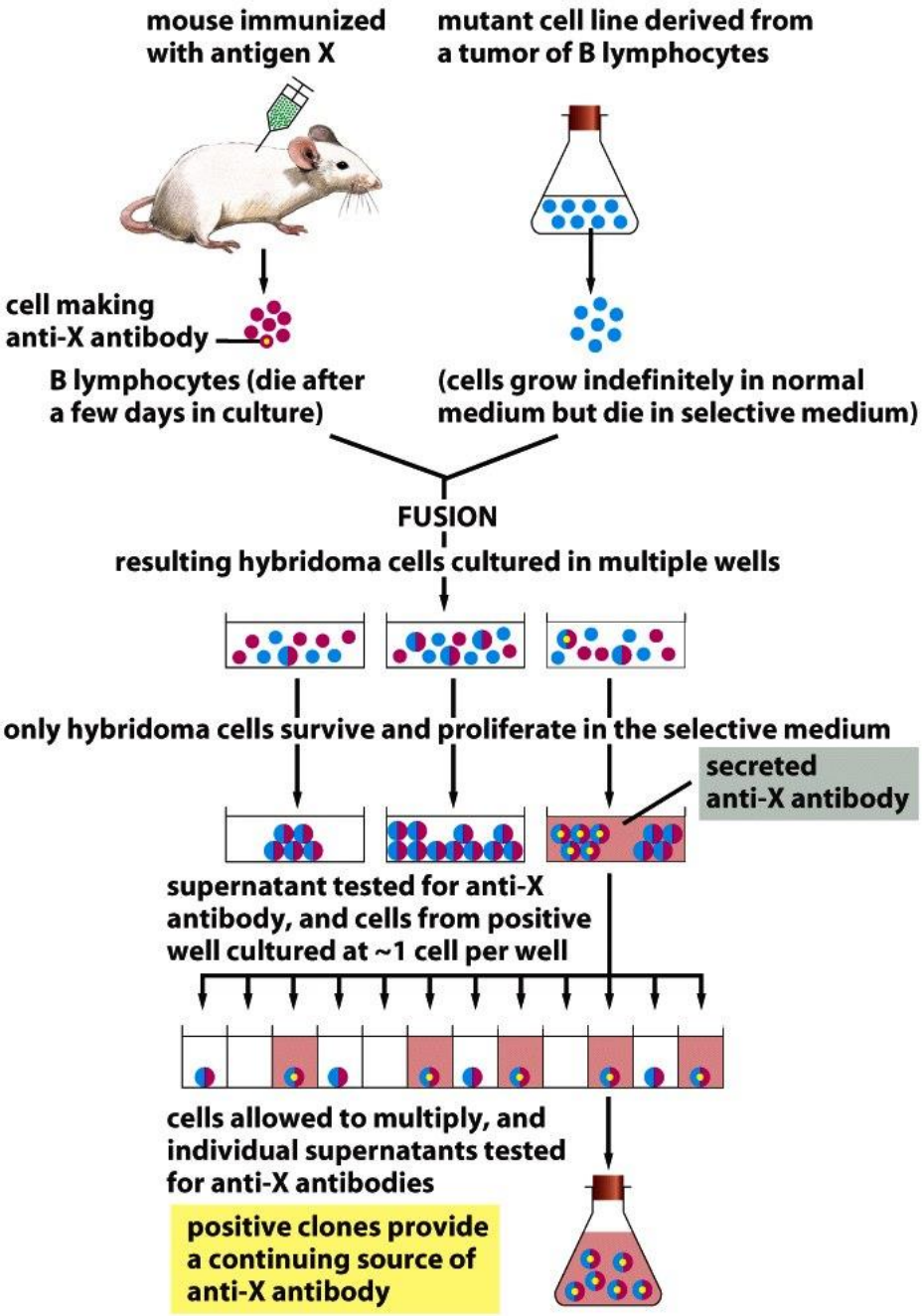


Figure 8-8 *Molecular Biology of the Cell* (© Garland Science 2008)

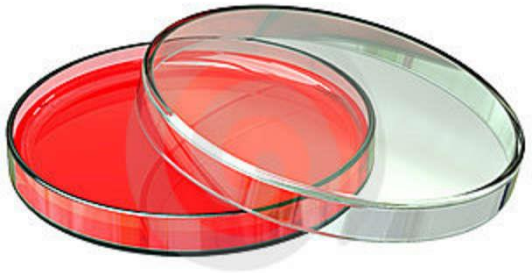
2. How to grow cells/maintain a cell line?

A basic cell culture media usually contains:

1. A buffer for pH level regulation,
2. a food source (e.g. Glucose, glutamine, amino acids, nucleotides, etc)
3. Serum / growth stimulant
4. minerals for metabolic functioning of the cells
5. Antibiotic additives and pH indicator

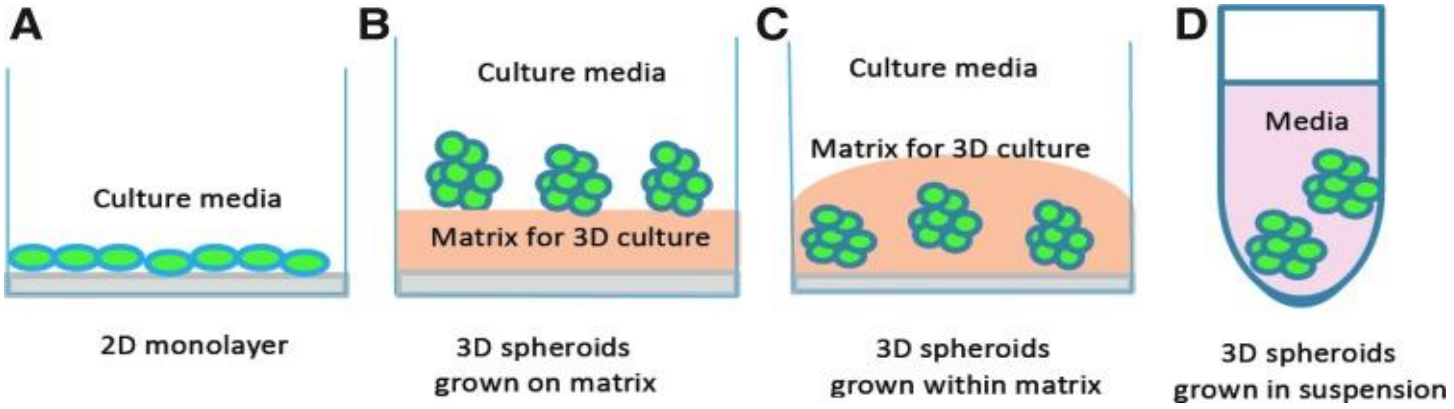
Some commonly used media:

Dulbecco's modified essential medium (DMEM)
RPMI-1640
McCoy's 5A
F12, etc



2D and 3D culture

- 3D more accurately reflects *in vivo* condition, in which cell-cell communication, extracellular matrix will have effects on cellular activity

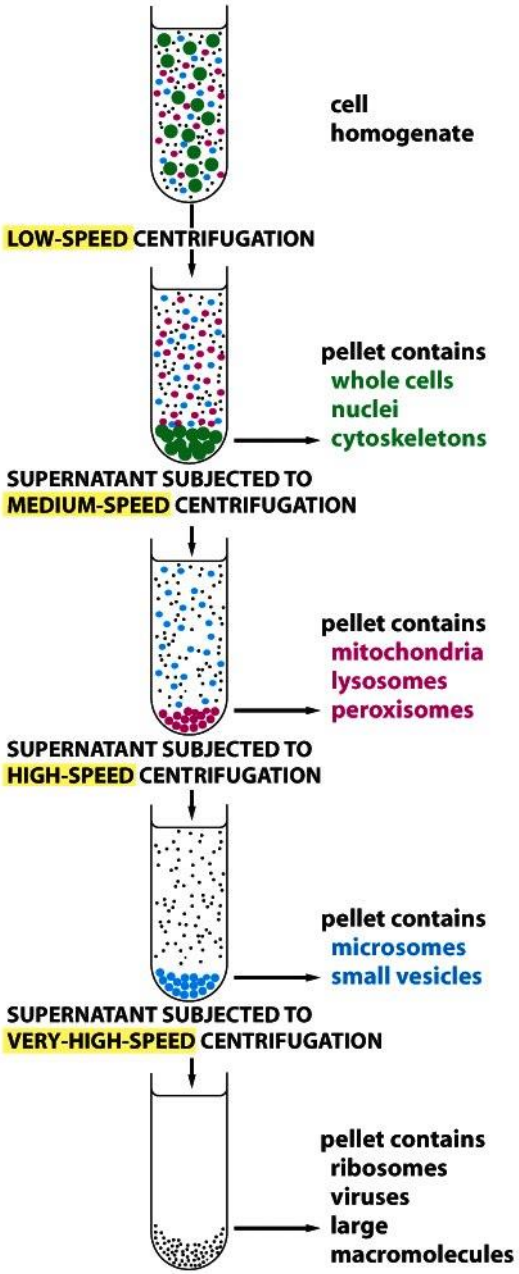


Comparison between 2D and 3D culture

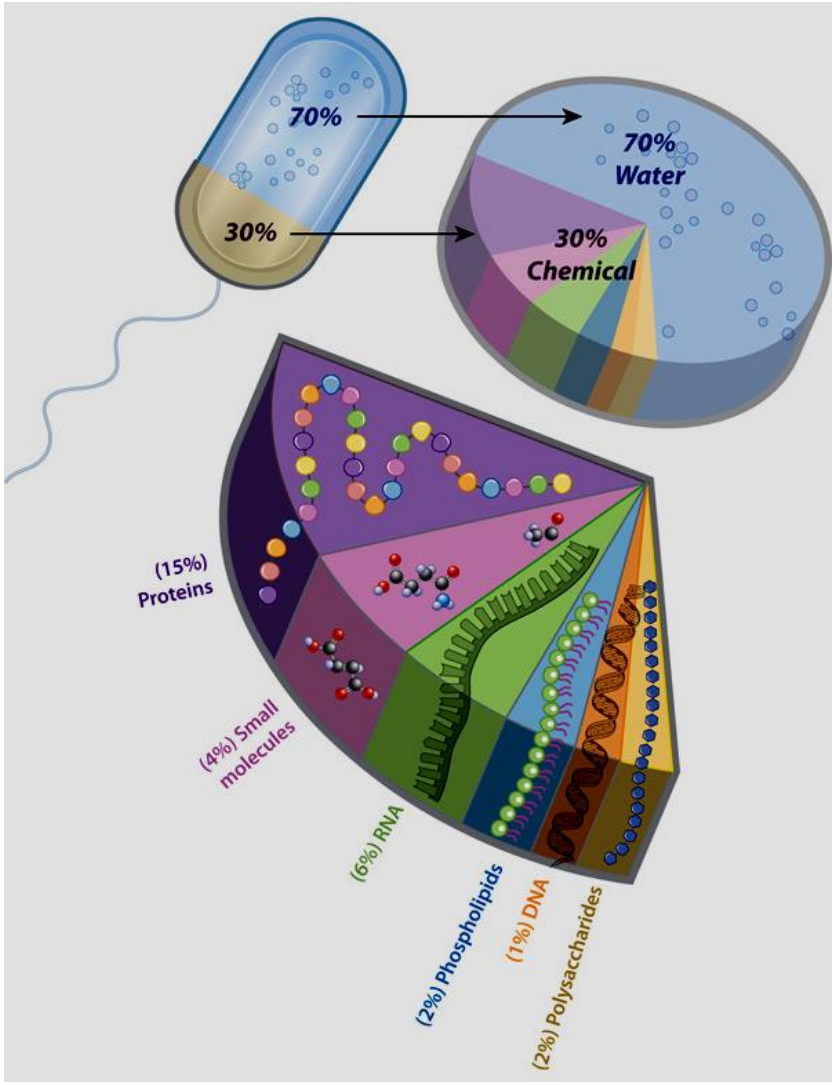
	2D	3D
Cell Shape	Flat and stretched	Natural shape (ellipsoid/polarized) is retained
Cell interface to medium	All cells are equally exposed to media components	As in physiological conditions, there is gradient availability of media components. Upper layer of cells are highly exposed over the lower layer (Heterogeneous exposure)
Cell junction	Cell junctions are less prevalent and does not resemble physiological conditions	Cell junctions are prevalent and enable cell to cell communication.
Cell Differentiation	Moderately and poorly differentiated	Well differentiated
Drug metabolism	Drug metabolism not well observed	Enhanced drug metabolism with increased expression of CYP enzymes
Drug Sensitivity	Cells are sensitive and drugs show high efficacy	Cells often show resistance and drugs show low potency
Cell Proliferation	Higher proliferation rate than in natural environment	Proliferation rate may be high or low, it is based on cell type and 3D-cell culture technique.
Response to stimuli	Poor response to mechanical stimuli of cells	well-established responses to mechanical stimuli of cells
Viability	Sensitive to cytotoxin	Greater viability and less susceptible to external factor
Apoptosis	Highly susceptible to drug-induced apoptosis	Enhanced resistance to drug-induced apoptotic stimuli

Adapted from Sigma/Aldrich

3. Cells can be separated into their functional fractions



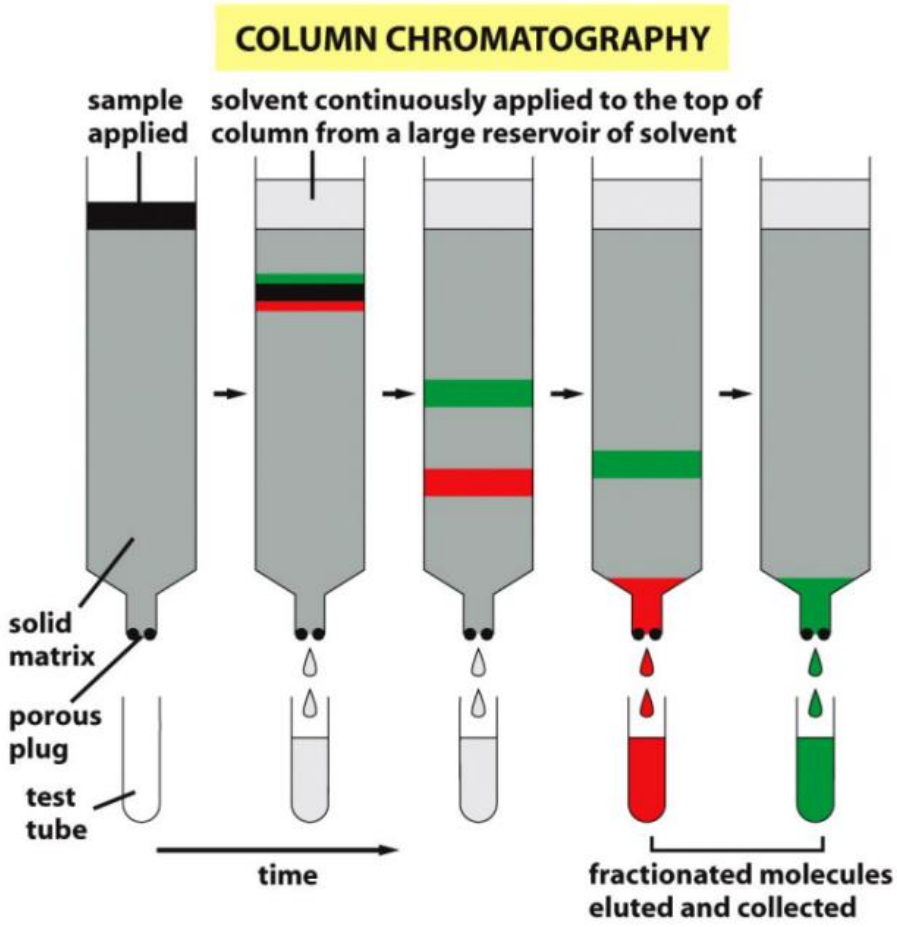
Chemical Components of a Cell



4. How to study cell biology?

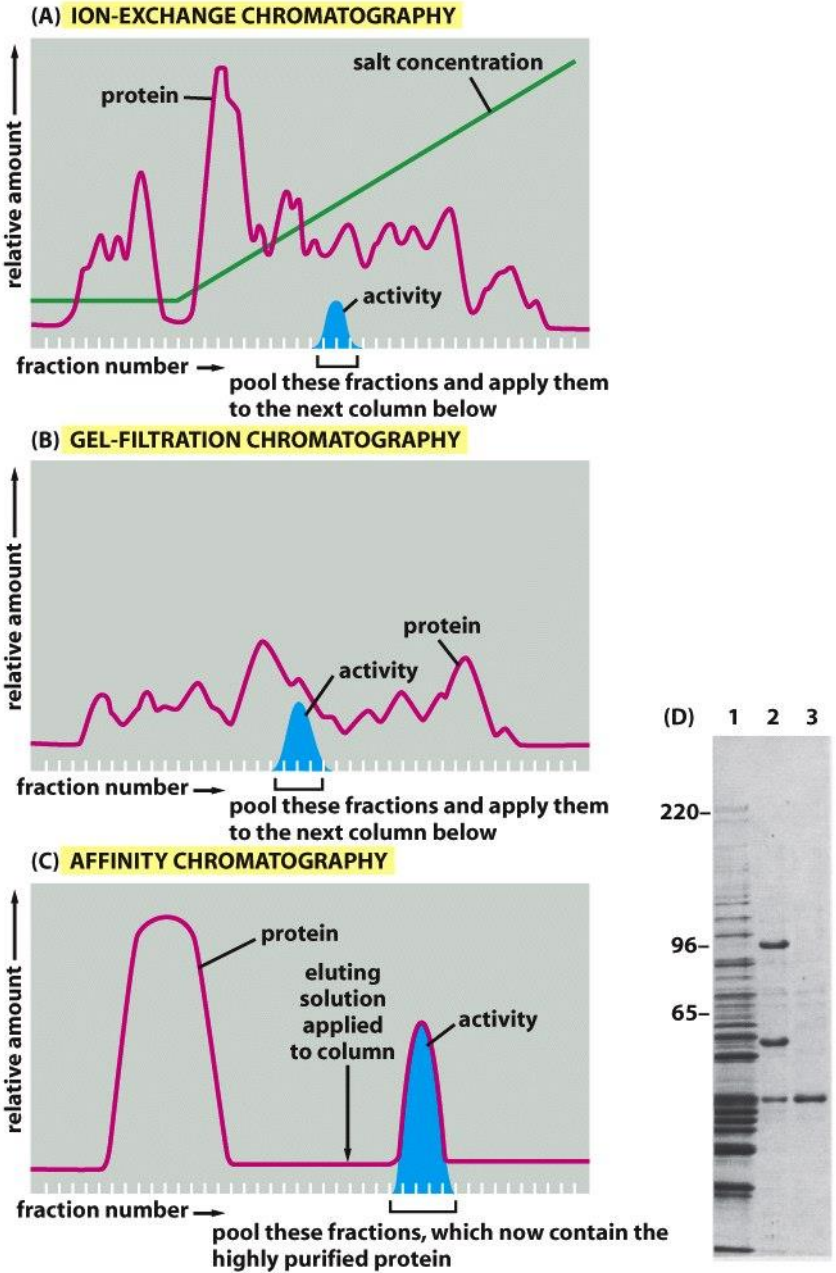
- Protein ?
- RNA?
- DNA?

4.1 Proteins can be isolated by chromatography

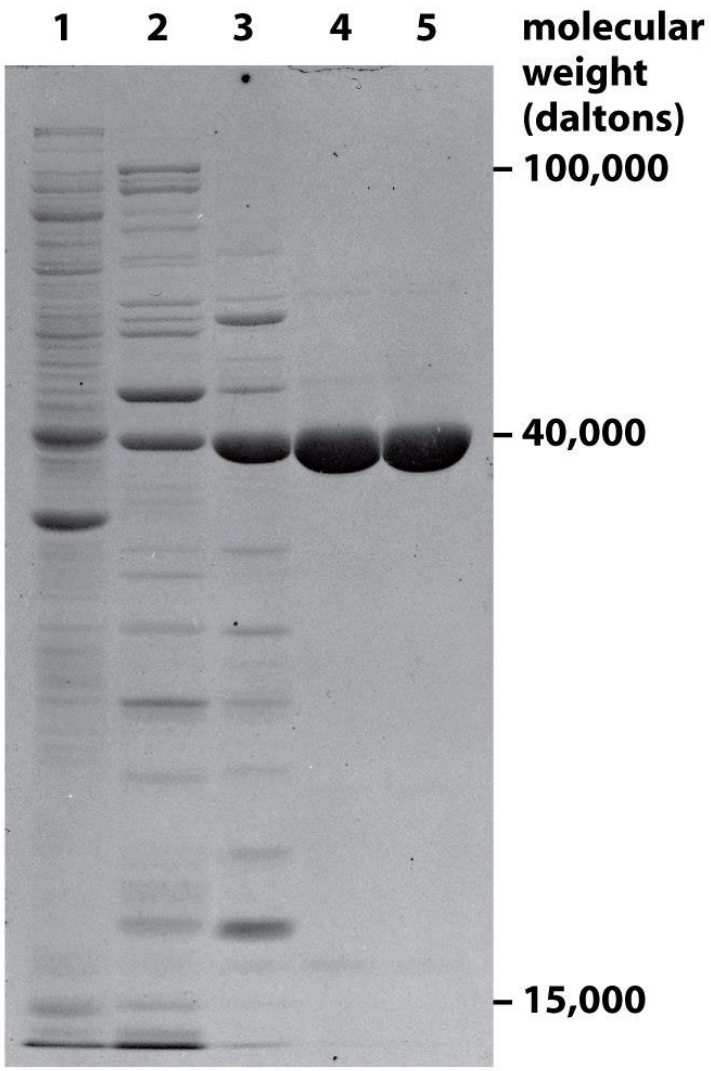


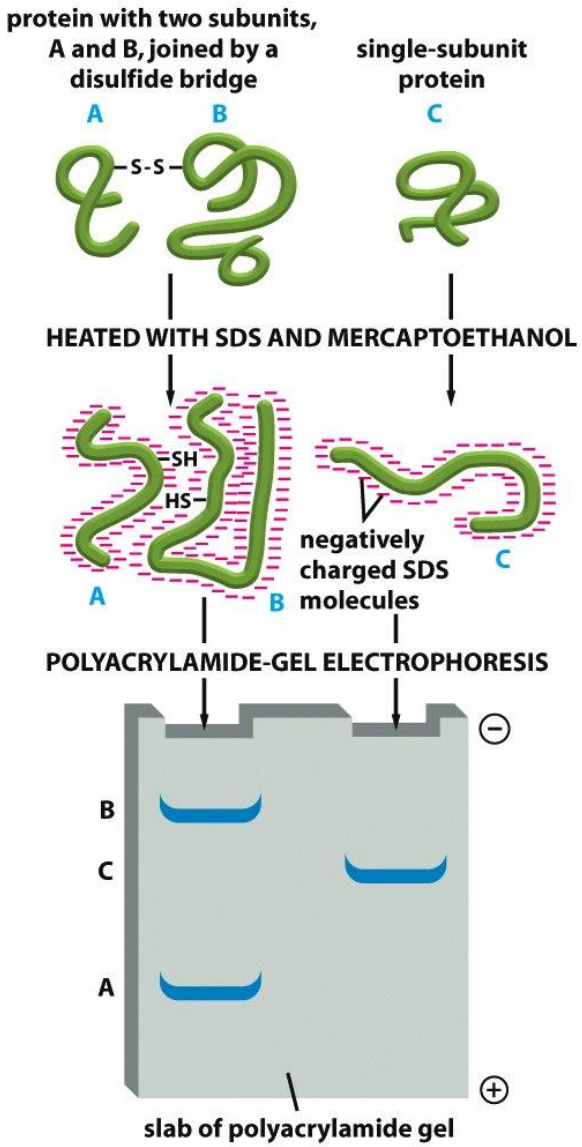
Gel filtration
Ion exchange
affinity

Protein chromatography



4.2 Protein analysis by SDS-PAGE





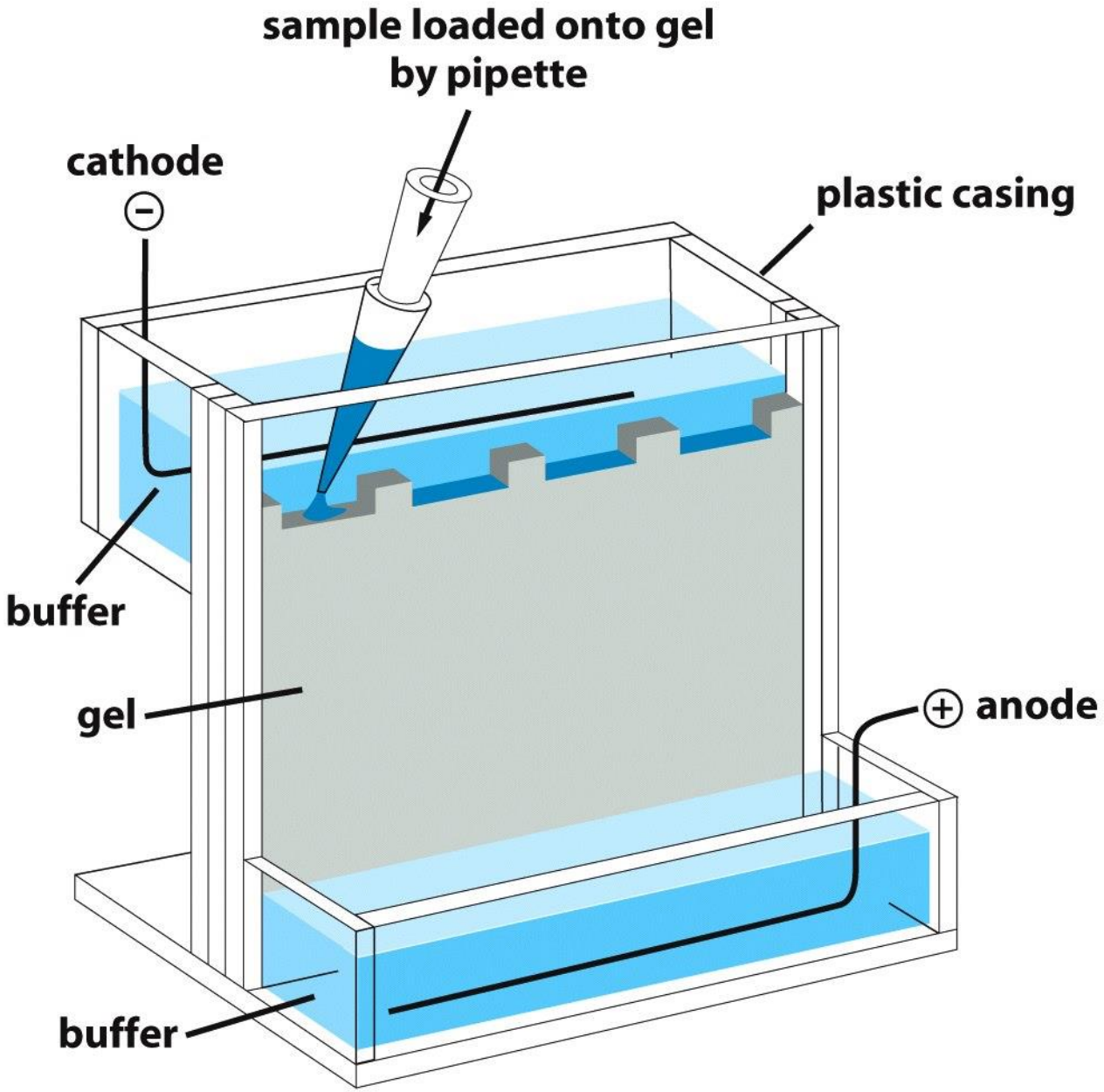
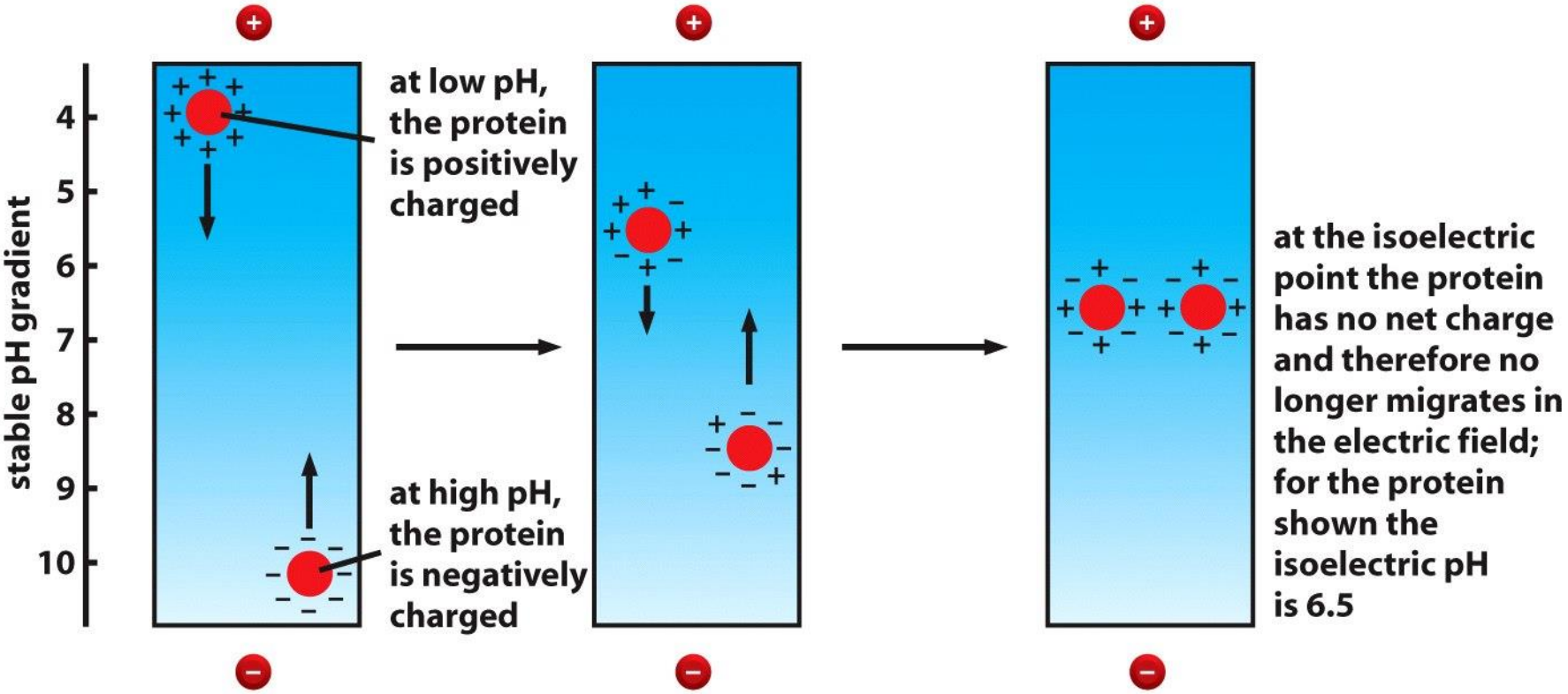
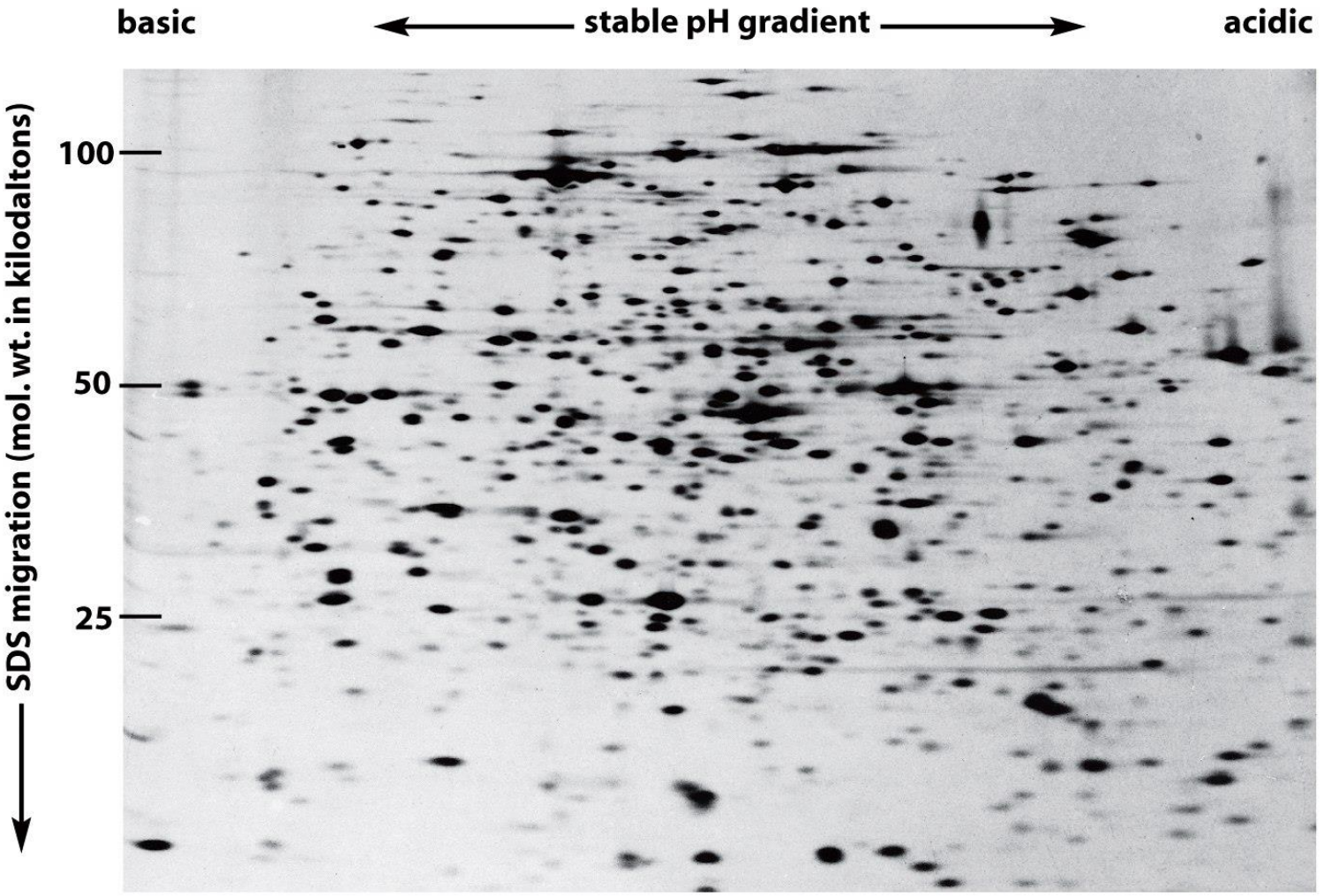


Figure 8-18a *Molecular Biology of the Cell* (© Garland Science 2008)

4.3 2-D protein analysis



2-D gel analysis



4.2 How to analyze DNA/RNA?

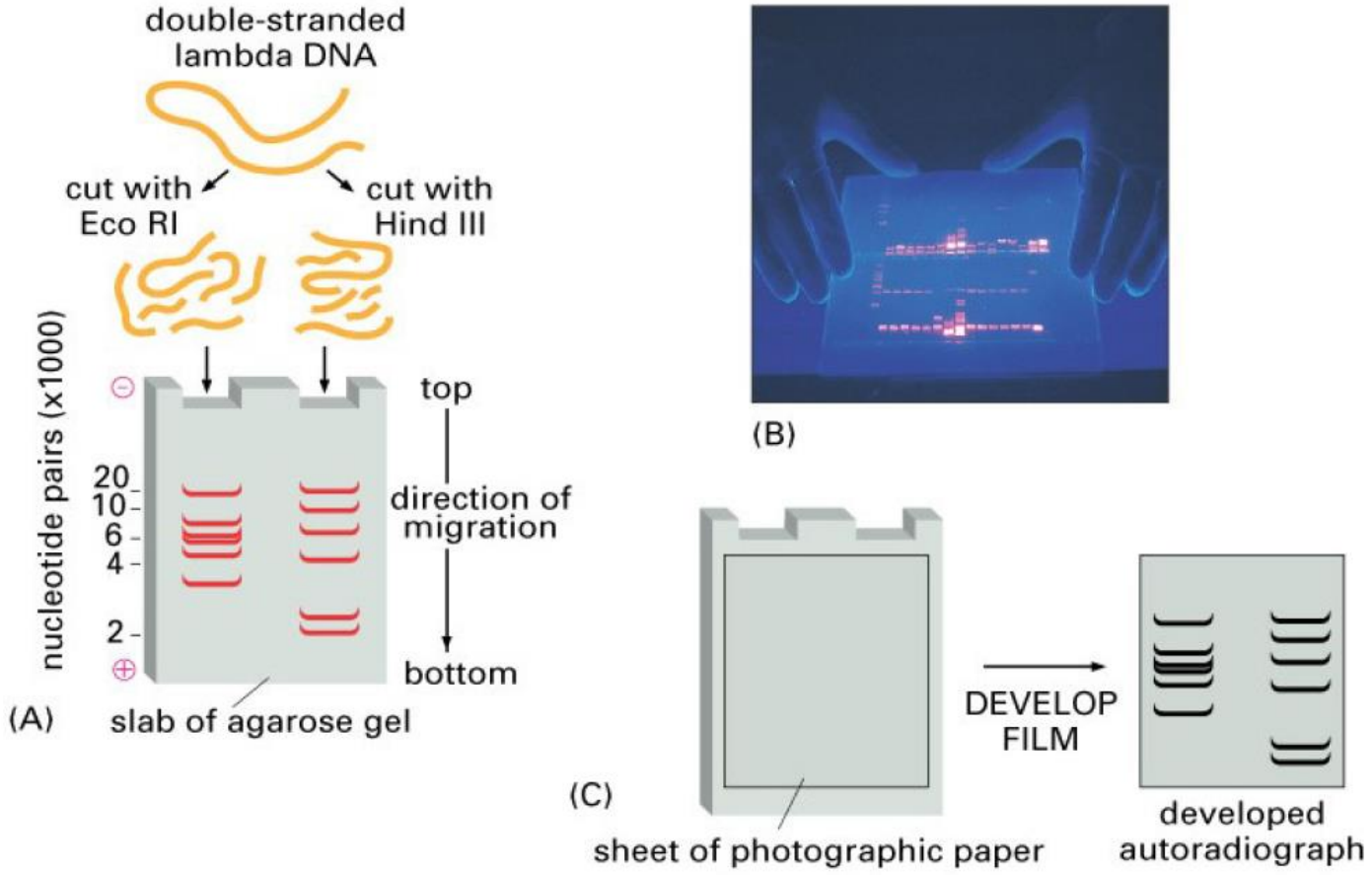
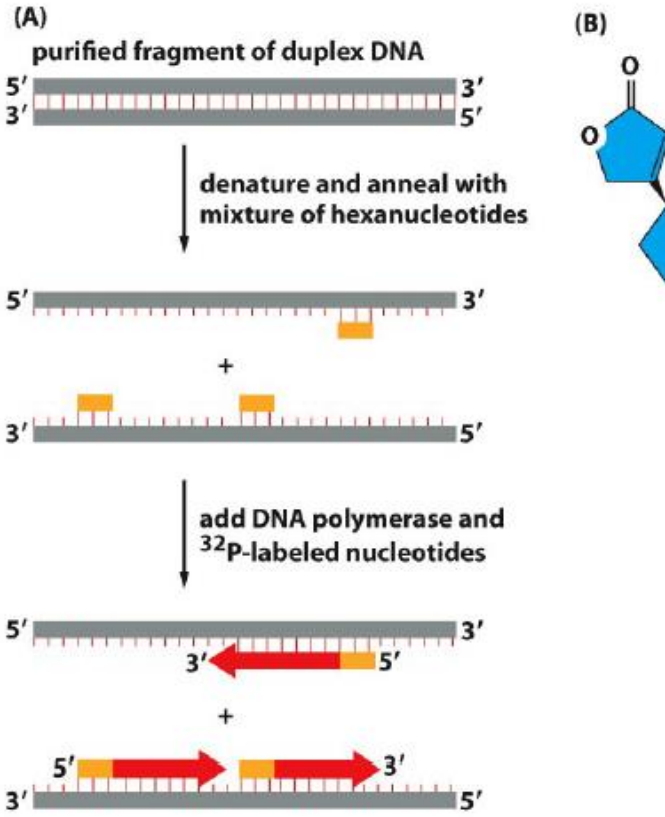


Figure 10-5 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA/RNA can be labeled



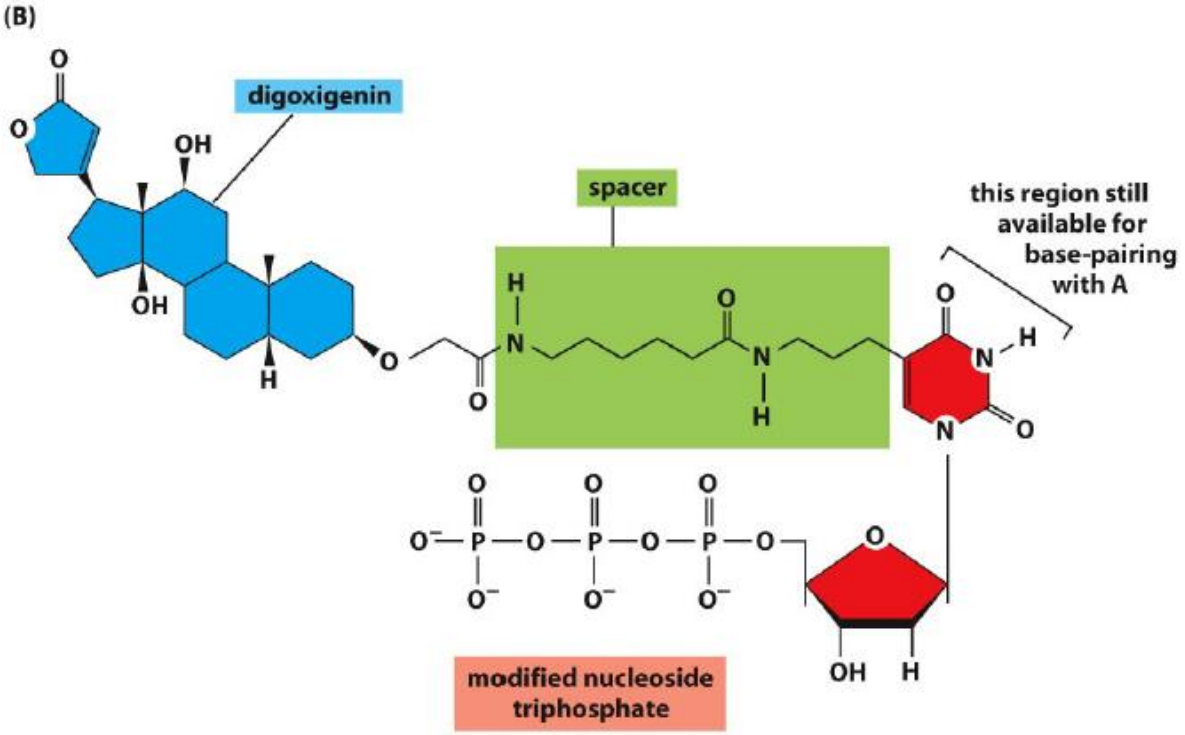
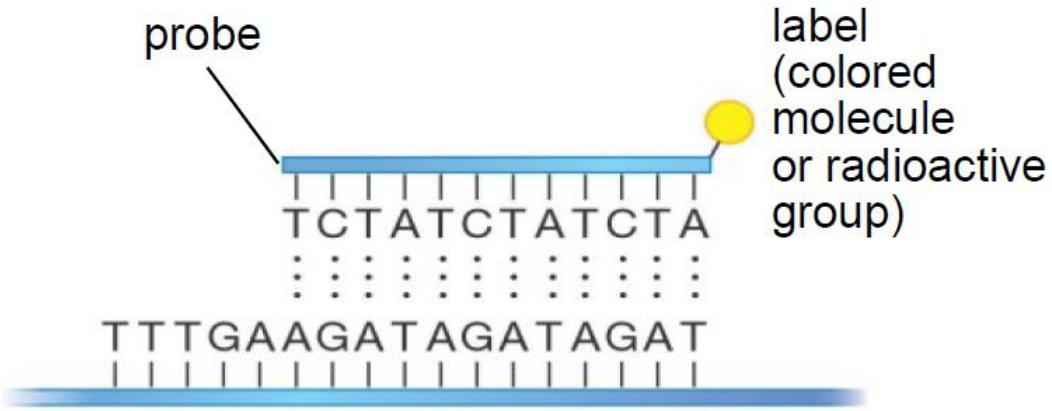
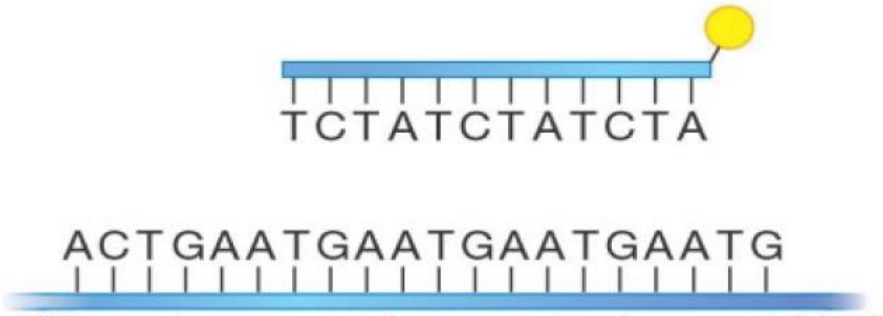


Figure 8-26 Molecular Biology of the Cell 6e (© Garland Science 2015)

Labeled RNA/DNA is called probe, which binds to RNA/DNA specifically



#1: probe base-pairs and binds

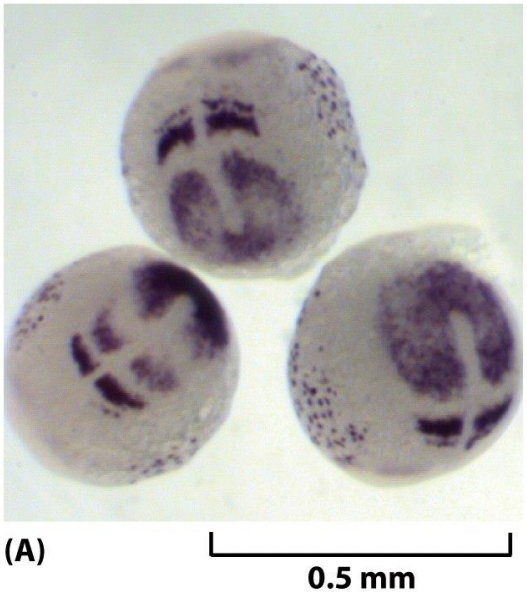


#2: probe cannot base-pair; does not bind

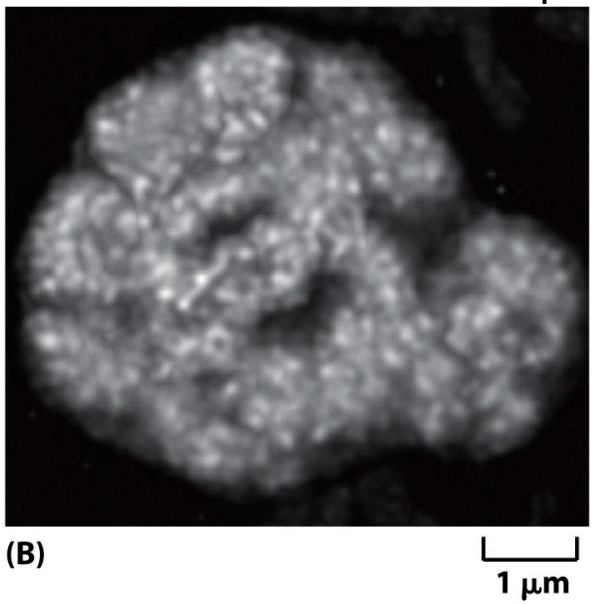
. RNA FISH

RNA Fluorescence in situ hybridization

deltaC-mRNA in zebrafish embryo



rRNA in the nucleolus of a pea



1. Make fluorescence labeled DNA/RNA probe
2. Cells were fixed and RNA can be accessible (permeabilized)
3. Apply the probe on fixed cells to allow hybridization
4. Visualization under fluorescence microscope

DNA FISH

How to clone specific gene or analyze gene expression levels?

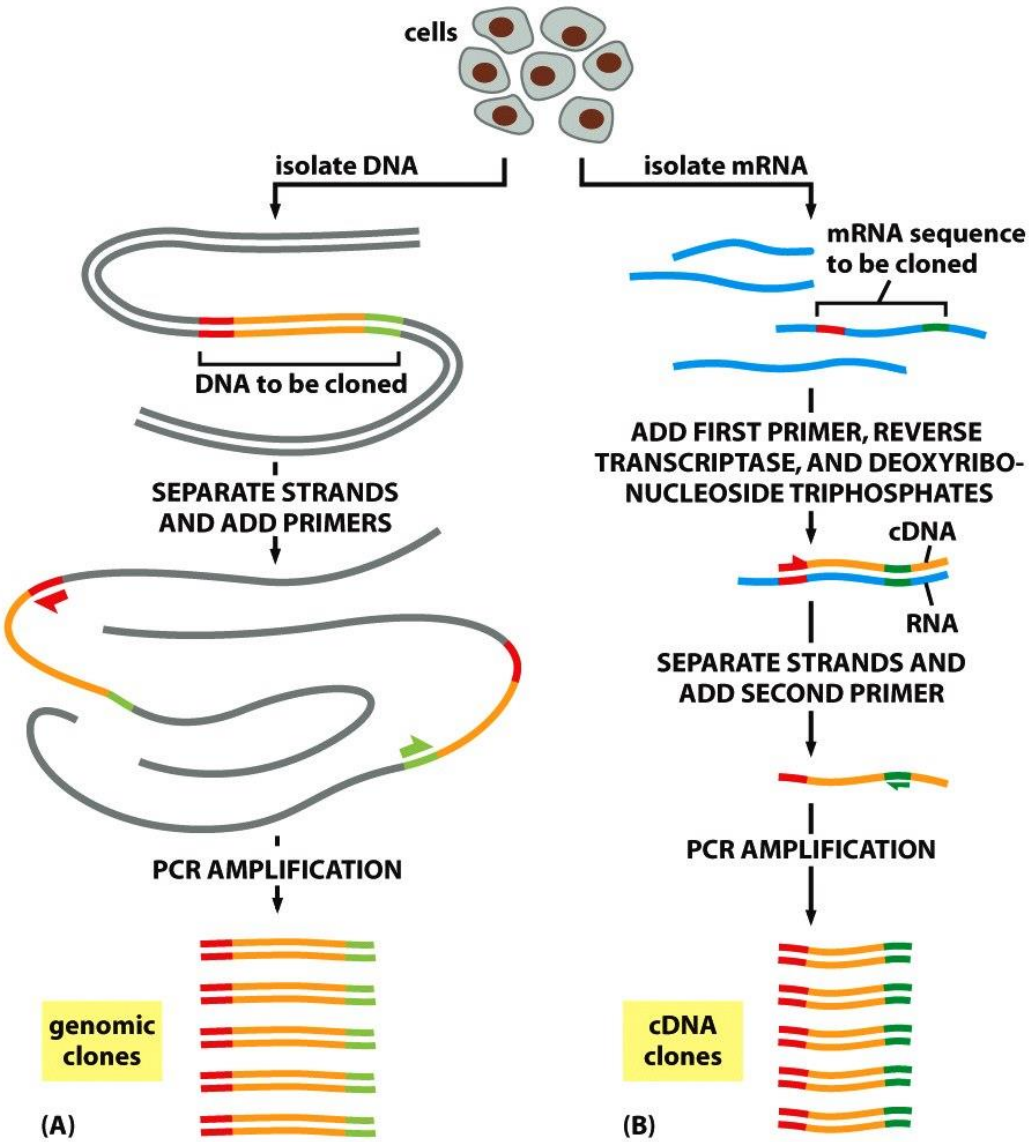
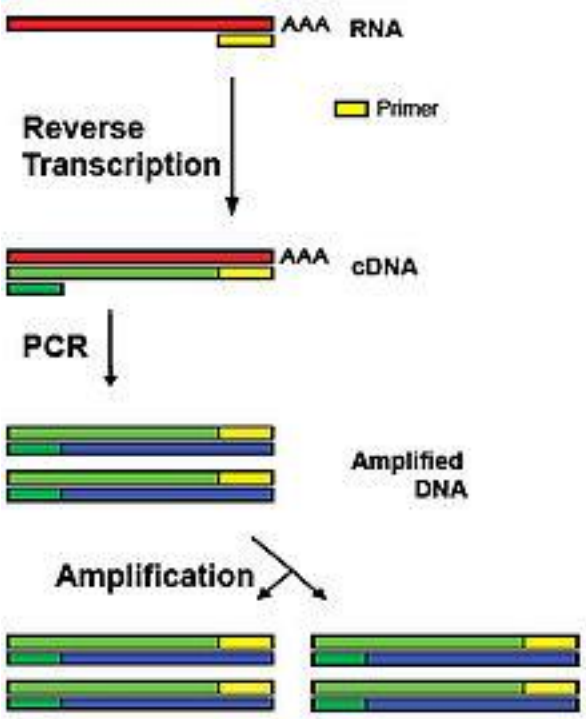


Figure 8-46 *Molecular Biology of the Cell* (© Garland Science 2008)

RT-PCR (reverse transcription-PCR)

Or other gene specific primer

1. Extract total RNA
2. Reverse transcribe RNA into its complementary DNA (cDNA) with oligo(dT) as primer
3. Use cDNA as template, use gene-specific primers to do PCR
4. Analyze PCR products by SYBR green incorporation or agarose gel analysis



Cell free system- a relatively pure system

- An *in vitro* system consists from pure biomolecules or cell homogenate needed to catalyze a biological process, such as:

  DNA replication,

  DNA transcription,

  Protein translation,

  RNA splicing, etc.

It provides direct evidence for the function of proteins while eliminating the influence from other cellular components in a complex *in vivo* system.