Molecular Techniques

- Disclaimer
- Nucleic Acids
- Proteins

3 Goals in Molecular Biology

Identify
All nucleic acids (and proteins) are chemically identical in aggregate - need to identify individual species

Amplify
The amount of an individual gene, mRNA species, or protein is vanishingly small

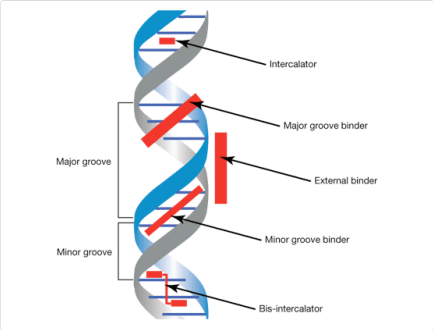
Visualize
Nucleic acids and proteins are invisible, so need to stain or label to detect and localize.

Nucleic Acids: DNA and RNA

- localization
  usually nucleus (DNA) or peri-nuclear (mRNA)
- amount
- length (size)
  in base pairs (bp)
- charge
  always negative
- sequence
  A,T(U) ,C,G
- epigenetic modifications
  proteins bound to DNA or RNA
  Modification of nucleotide bases, e.g. methylation of DNA

Localization of Nucleic Acids

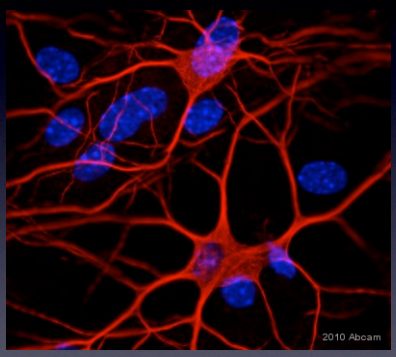
- **Generic Nucleic Acid Stains**
  DAPI for DNA in nucleus
  Ethidium Bromide, Syber Green in gels



http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook/Nucleic-Acid-Detection-and-Genomics-Technology/Nucleic-Acid-Stains.html

Example of DNA stain

DAPI stain of DNA in nucleus of cell



Blue = DAPI, DNA in nucleus
Red = MAP2, neuronal cytoplasm

http://www.abcam.com/index.html?pageconfig=reviews&intAbreviewID=21057&intAbID=5392

Localization of Nucleic Acids

- **In Situ Hybridization**
  RNA is single-stranded, but can form a double-helix with a complementary strand

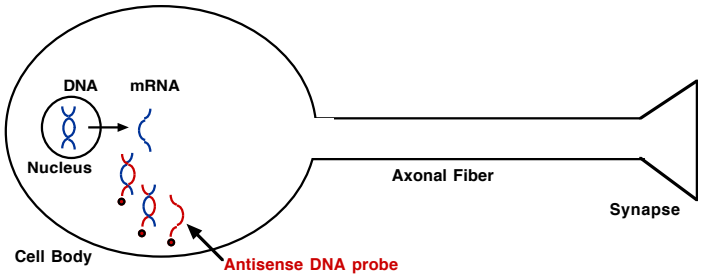
  Stick a labeled complementary stretch of DNA or RNA to the mRNA within a tissue section

For example:

DNA probe: ATCCGCATTAG
RNA in blot: TTAGCTTTAGGAGTAATCCGAATATGGC

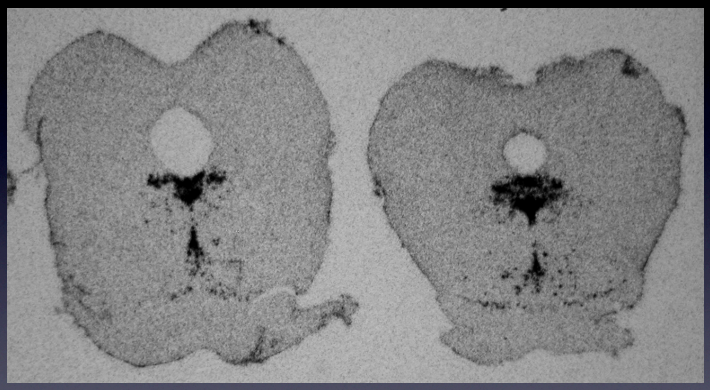
every T in probe is radioactive

use in situ hybridization to detect mRNA for enzymes, transporters, or neuropeptides



Label DNA or RNA probe with radioactive nucleotides, or fluorescent nucleotides or with a chemical that can be detected with antibodies
Specific to sequence of target mRNA
Doesn't distinguish different sizes of mRNA species, e.g. alternative splicings of same gene

Example of ISH

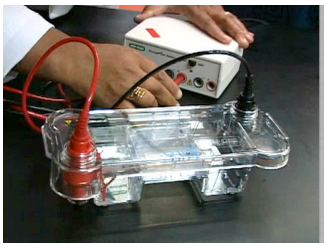


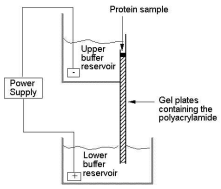
Cell bodies that express mRNA for serotonin transporter

Size Fractionation

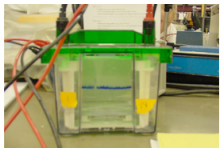
- Separation of macromolecules based on size, as measured by rate of travel through gels
- Charged macromolecules impelled to travel thru gels by applying an electrical field
- Identify macromolecules by **staining** (e.g. for generic nucleic acids or proteins) or by **probing** (e.g. with specific DNA or antibody probe).

Gel Electrophoresis



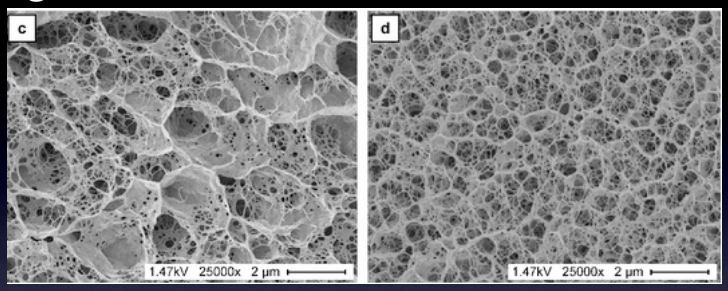


http://newarkbioweb.rutgers.edu/bio301s/lab 5- mol wt sds-page.htm



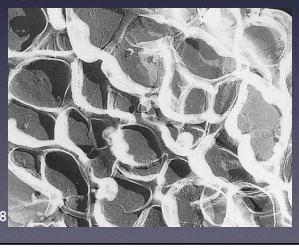
http://web.mit.edu/7.02/virtual_lab/PBC/PBC4Avirtuallab.html

Agarose Gel



Tuvikene et al., J. Appl. Phycol. 20 (2008)

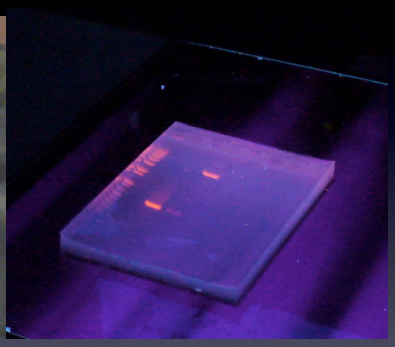
Polyacrylamide Gel



http://cellbiologyolm.stevegallik.org/node/78

Nucleic Acid Stain of Agarose Gel

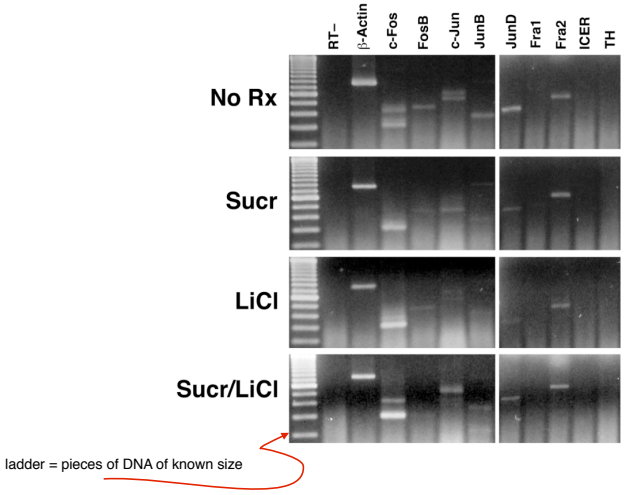
Wikimedia Commons: Agarosegel.jpg



Wikimedia Commons: AgarosegelUV.jpg

Example of DNA in agarose gel

PCR products



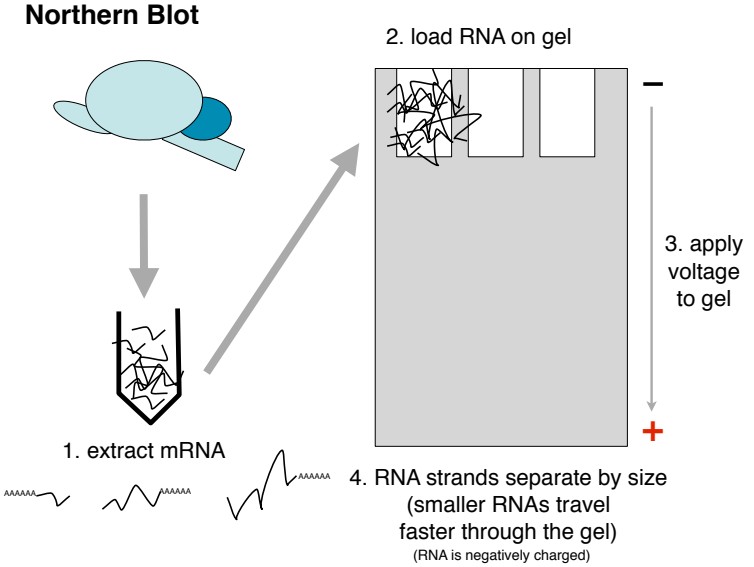
Blots

Southern Blot
Separate DNA fragments by size on a gel, then transfer to a nylon membrane (invented by Prof. Southern)

Northern Blot
Separate RNA species by size on a gel, then transfer to a nylon membrane

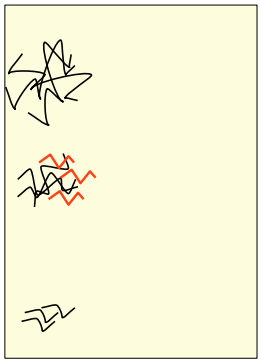
Western Blot
Separate proteins by size on a gel, then transfer to a nylon membrane

Northern Blot



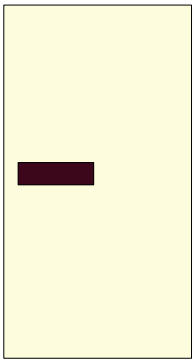
Northern Blot

5. Blot RNA onto nylon membrane



6. Incubate RNA blot with radiolabeled DNA probes

Expose Blot to Film



For example:

DNA probe: ATCCGCATTAG *(every T is radioactive)*

RNA in blot: TTAGCTTTAGGAGTAATCCGAATATGGC

Proteins

- localization
  - depending on function, proteins found throughout cell
- amount
- length (size)
  - molecular weight (kiloDaltons; kDa)
- hydrophobicity
  - hydrophilic (water soluble), so likely to be in cytoplasm
  - hydrophobic (lipid soluble) so likely to be in membrane
- charge
  - variable
- sequence
  - amino acid sequence
- epitopes
  - structural features that may be shared by multiple proteins
  - recognized by immune system, so can make antibodies
- postranslational modifications
  - phosphorylation

Protein Detection

Protein Stain

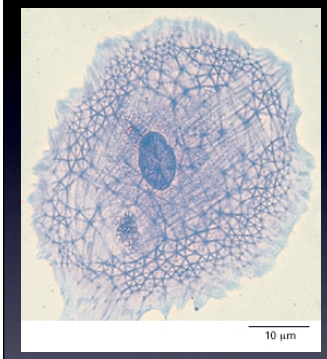
Immunohistochemistry

Western blot of protein

Western blot for phosphorylated protein

2-D Gel for detection of all proteins

Example of Protein Stain
Coomassie blue



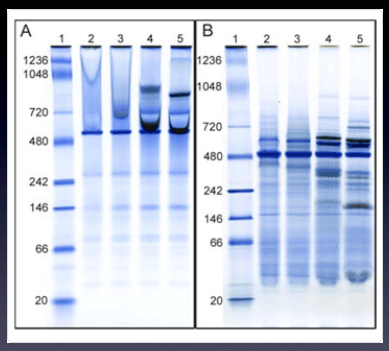


Figure 16-1 from Molecular Biology of the Cell

The cytoskeleton. A cell in culture has been fixed and stained with Coomassie blue, a general stain for proteins. Note the variety of filamentous structures that extend throughout the cell.

http://www.ncbi.nlm.nih.gov/books/NBK28304/

Gels were loaded with NativeMark™ standards (lane 1), or 18µg spinach chloroplast extract Staining was performed with Colloidal Blue Staining Kit.

Antibodies

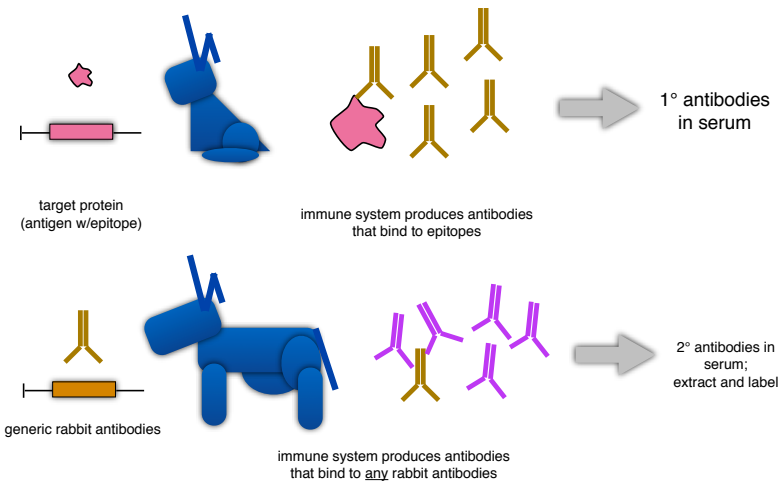
Protein stains do not discriminate different proteins

Protein sequences do not have complements

Immune system produces antibodies that recognizes specific structural features on the surface of proteins and other chemicals (epitopes)

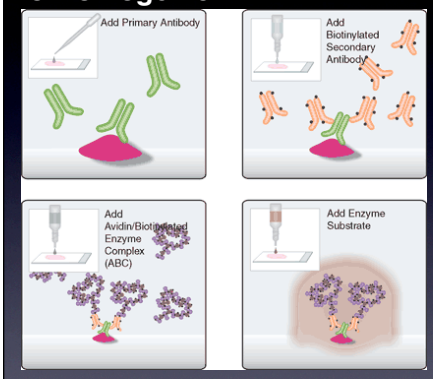
Label antibodies with fluoroscent, enzymatic, or radioactive tags

Antibody Production



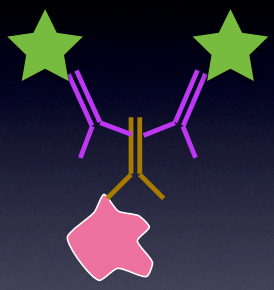
Antibody Detection

Chromogenic



epitope <- 1°Ab <- many 2° Ab <- enzyme comples <- colored reaction product

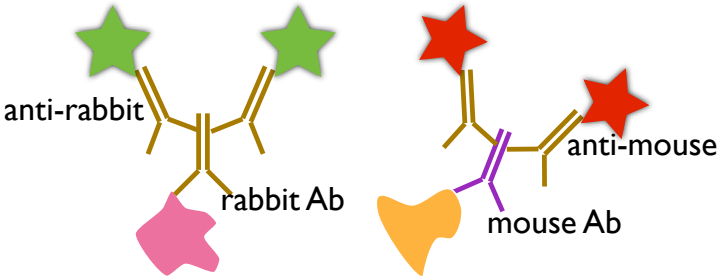
Fluorescent



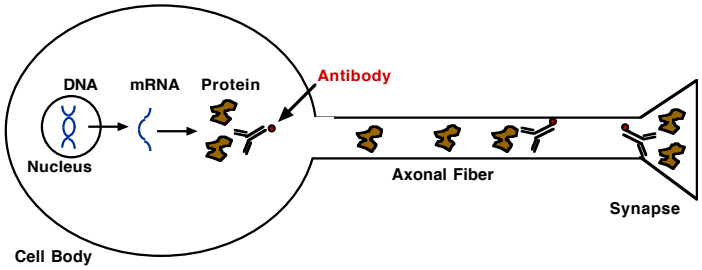
epitope <- 1°Ab <- many labeled 2° Ab <- fluorescence under UV light

www.vector labs

Double-labeling with 2-different species of antibodies



use immunohistochemistry to detect chemicals, proteins



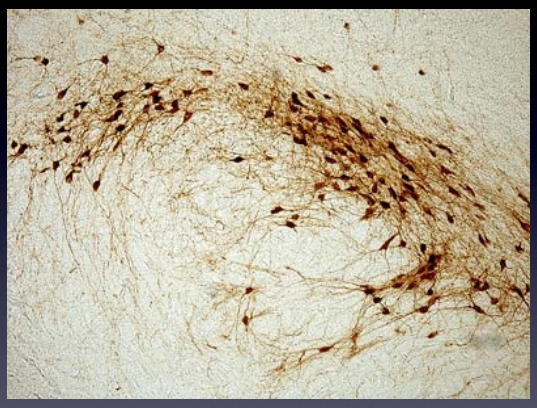
Pros:

Gives precise anatomical localization
detects specific molecular feature (epitope) of protein
can be used with non-proteins (e.g. 5HT, cAMP); anything that immune system can recognize

Cons:

Antibody may be non-specific; will stick to other proteins with same epitope
Cannot determine overall protein size or charge

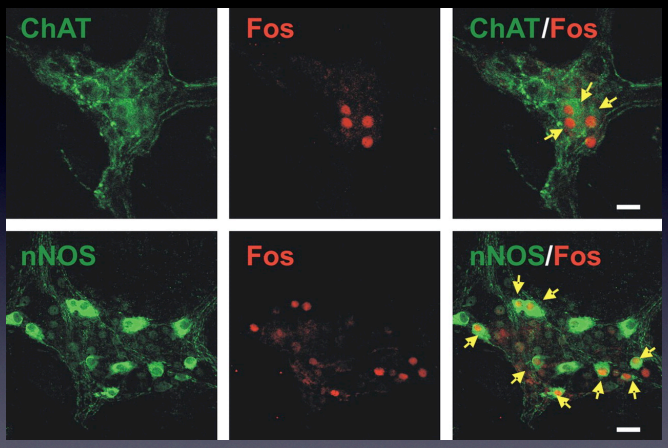
Chromogenic Immunohistochemistry



TH immunostaining of Dopaminergic cells in rat Substantia Nigra

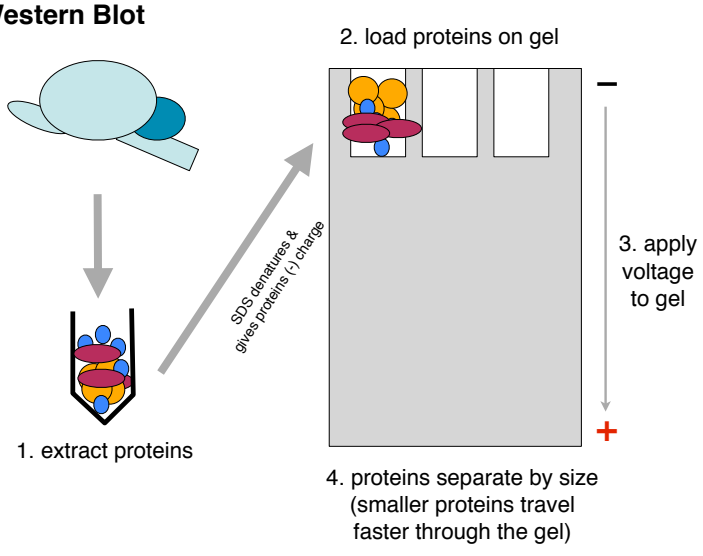
http://www.psy.herts.ac.uk/res/an-models.html

Immunofluorescence



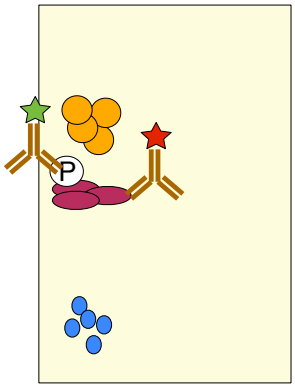
Zhou S et al. Am J Physiol Gastrointest Liver Physiol 2008;294:G1201-G1209

Western Blot



Western Blot

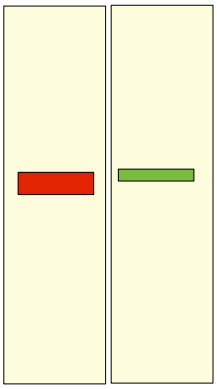
5. Blot proteins onto nylon membrane



6. Visualize proteins using labeled antibodies

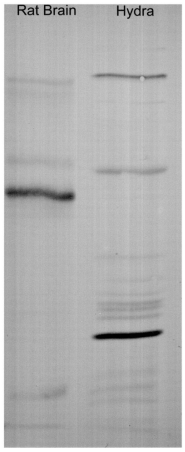
7. Visualize phosphoylated proteins using phospho-specific antibodies

Developed Blot



protein phospho protein

Example of western blot

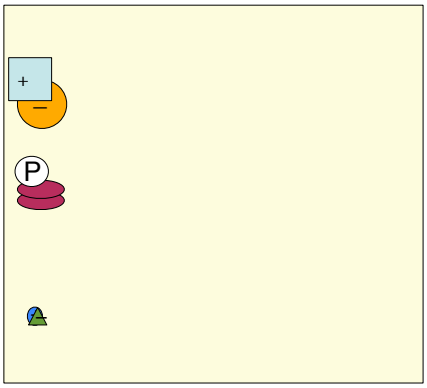


Western blot analysis of NMDA receptor proteins in rat brain (*left lane*) and whole hydra tissue (*right lane*). Numbers indicate the position of Kaleidoscope molecular weight standards. All minor bands were present in the absence of primary antibody. The most prominent band in each lane was only present when primary antibody was included

Immunocytochemical evidence for an NMDA1 receptor subunit in dissociated cells of Hydra vulgaris
Scappaticci et al, 2004

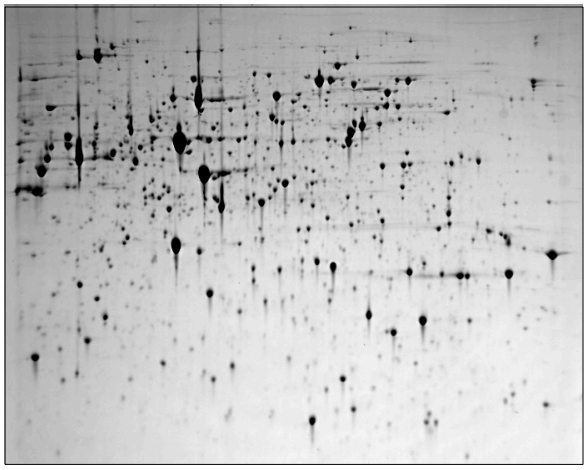
2-D Gel

1. Separate proteins by size on vertical axis



2. Separate proteins by charge on horizontal axis

2-D Gel stained with coomassie blue



http://abdn.ac.uk/proteome/ap-Saccharomyces-cerevisiae-2d-gel.hti