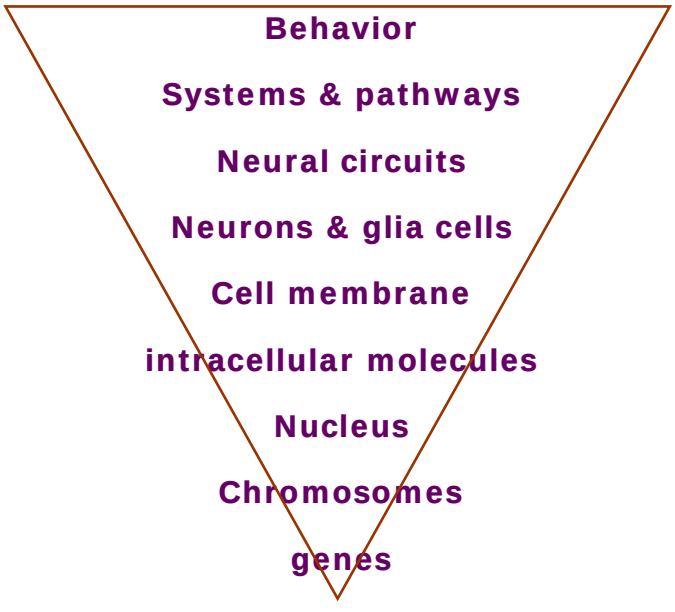
細胞分子學　vs　神經科學
Molecular biology vs Neuroscience

細胞分子神經科學
Molecular Neuroscience



Why not sequence everything?

- The cloning of the gene encoding proteins that are responsible for known neuronal activities can yield important information about the structure, function and regulation of these moieties.

Visualizing neuronal gene expression

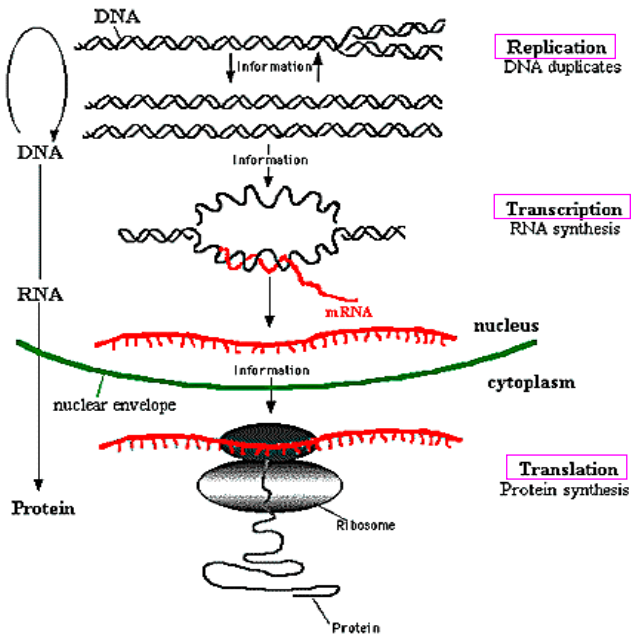
- Molecular neuroscience depends on our ability to monitor and measure the expression of genes.

The aim of the technique applied in molecular neuroscience

- To enable gene products to be assayed
- Qualitatively
- Quantitatively
- Any point of the expression pathway
- From transcription of the gene to the generation of the mature, functional peptide product

- What is the sensitivity of the technique?
- What is the anatomical resolution of the technique?
- Is the technique quantitative?
- Does the method tell us anything about the structure of the gene product?
- How can the results be interpreted in terms of gene functions?

- How the standard methods used in most molecular neuroscience laboratories to visualize gene expression measure?
- How these technologies will develop in the coming years?
- How might gene expression be monitored within the brain of a living organism?
- Could this ever be achieved non-invasively?

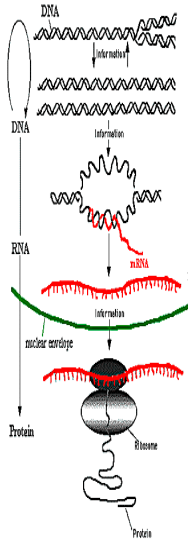


The Central Dogma of Molecular Biology

National Human Genome Research Institute (NHGRI)

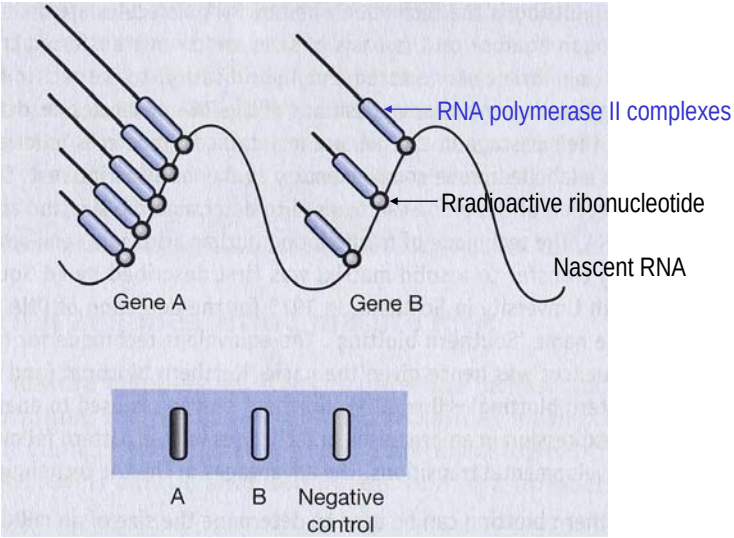
Monitoring and measuring transcription

Methods to Measure Gene Expression



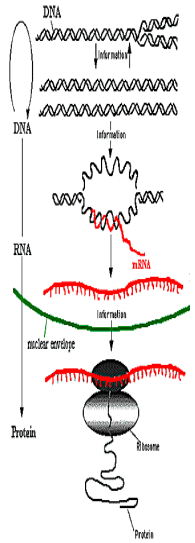
Gene Nuclear run-on assay +Southern blot

Nuclear run-on assay



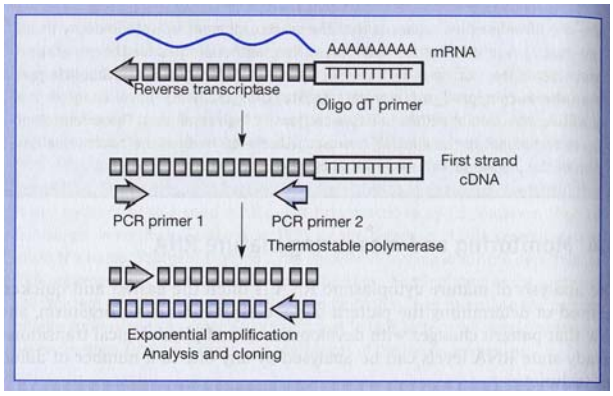
Monitoring and measuring RNA

Methods to Measure Gene Expression

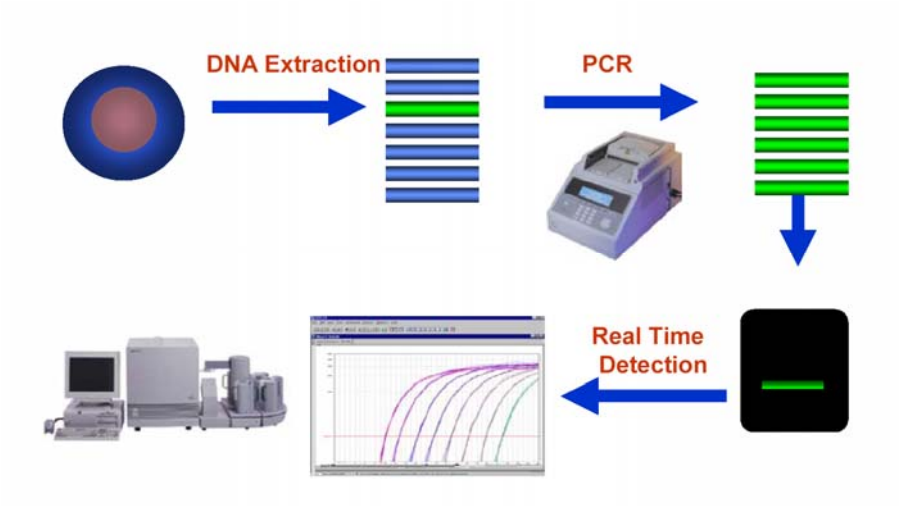


Gene	Nuclear run-on assay +Southern blot
RNA	Northern blot RT-PCR Real time RT-PCR In situ hybridization Microarray

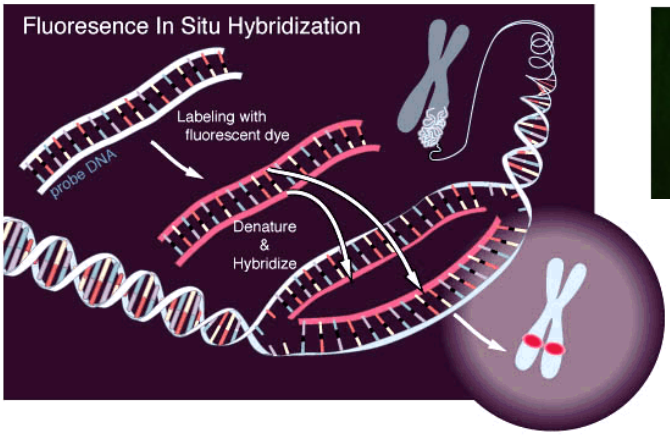
RT-PCR

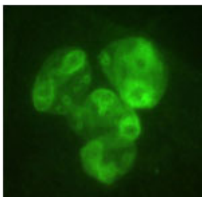


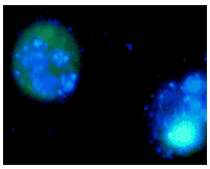
Real Time RT-PCR



In Situ Hybridization



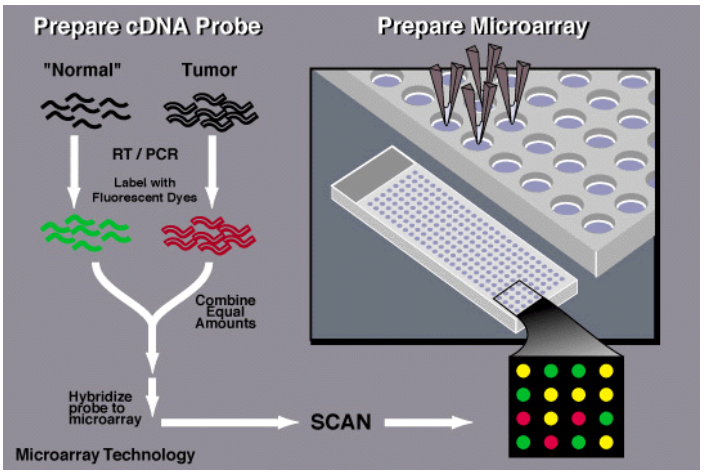




Detection of mRNA in tissue sections using labelled complementary cloned DNA.

National Human Genome Research Institute (NHGRI) by artist Darryl Leja

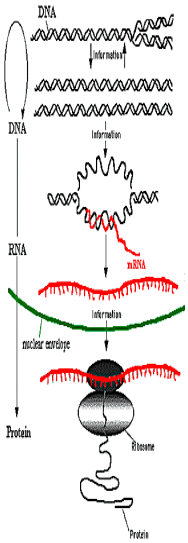
Microarray



National Human Genome Research Institute (NHGRI) by artist Darryl Leja

Monitoring and measuring protein

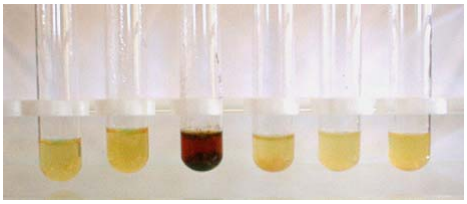
Methods to Measure Gene Expression



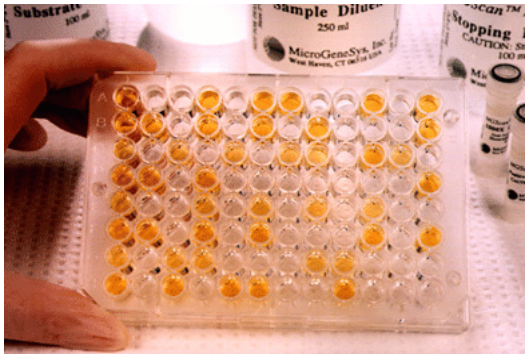
Gene	Nuclear run-on assay +Southern blot
RNA	Northern blot RT-PCR Real time RT-PCR In situ hybridization Microarray
Protein	Enzyme assay Electrophoresis (SDS-PAGE) Immunoassay (ELISA) Western blot Immunocytochemistry

Enzyme Assay

- Measurement of any biochemical reaction in which product appearance or substrate disappearance due to enzyme activity of interested protein can be measured.
  - colorimetric reactions using substrates which produce a colored product with the enzyme
  - radioactive substrates

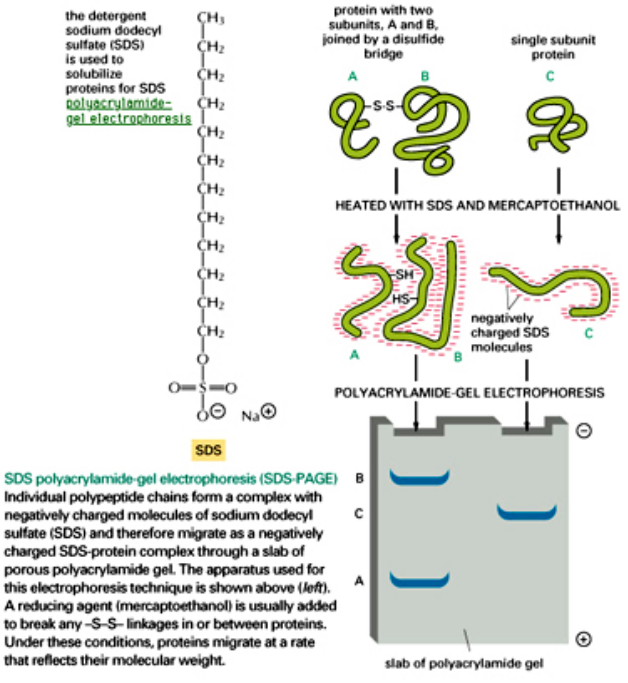


Immunoassay (ELISA)



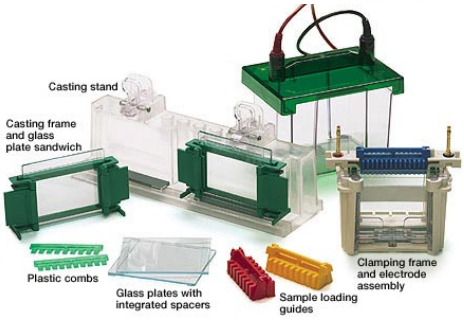
ELISA - (enzyme-linked immunosorbent assay) Quantification of protein amounts using antibodies conjugated to enzymes which can be assayed with a colorimetric or fluorimetric substrate and its color is proportional to amount of protein product

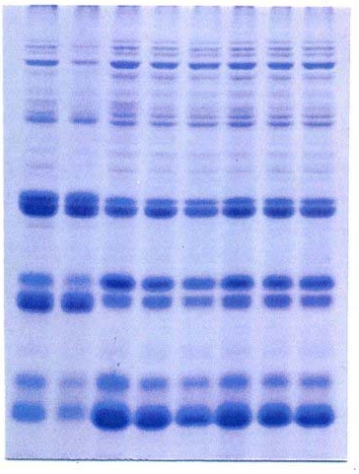
Electrophoresis (SDS-PAGE)



SDS polyacrylamide-gel electrophoresis (SDS-PAGE) Individual polypeptide chains form a complex with negatively charged molecules of sodium dodecyl sulfate (SDS) and therefore migrate as a negatively charged SDS-protein complex through a slab of porous polyacrylamide gel. The apparatus used for this electrophoresis technique is shown above (*left*). A reducing agent (mercaptoethanol) is usually added to break any –S–S– linkages in or between proteins. Under these conditions, proteins migrate at a rate that reflects their molecular weight.

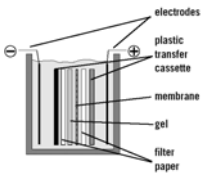
Electrophoresis (SDS-PAGE)

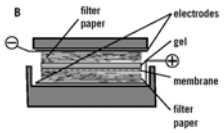




Western blot

1. PAGE to separate proteins

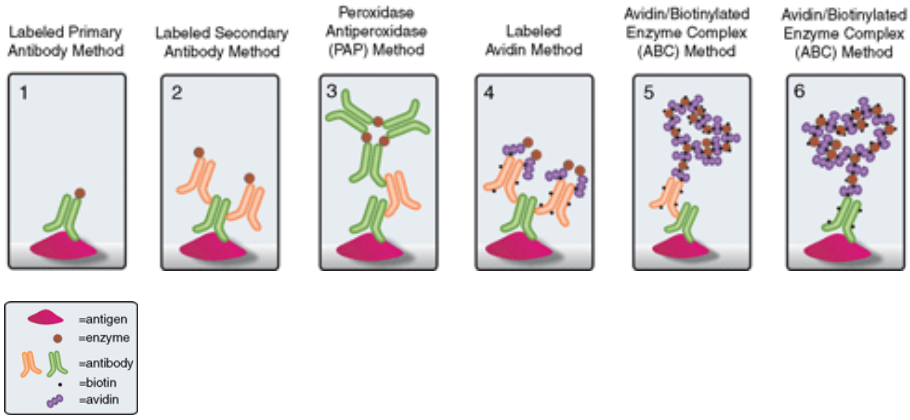




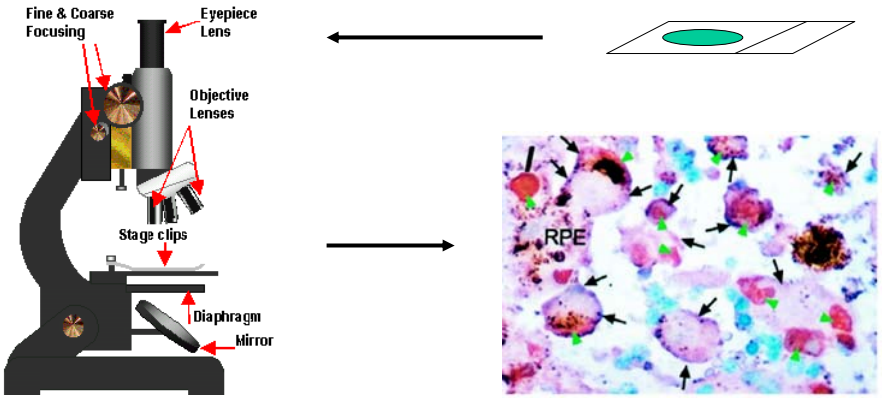
2. Transfer of proteins to membrane

3. Detection of proteins by specific antibody

Detection System for Western Blot



Immunocytochemistry



Detection of specific protein expression in tissue sections using labelled antibodies.

Monitoring and measuring protein

- The presence of an authentic RNA in a cell is not necessarily indicative of peptide synthesis or biologically active peptide
  - Poor translation
  - Posttranslational processes are absent
  - The peptide is rapidly degraded

Gene expression ➔ Protein expression ➔ Protein function

Monitoring and measuring protein

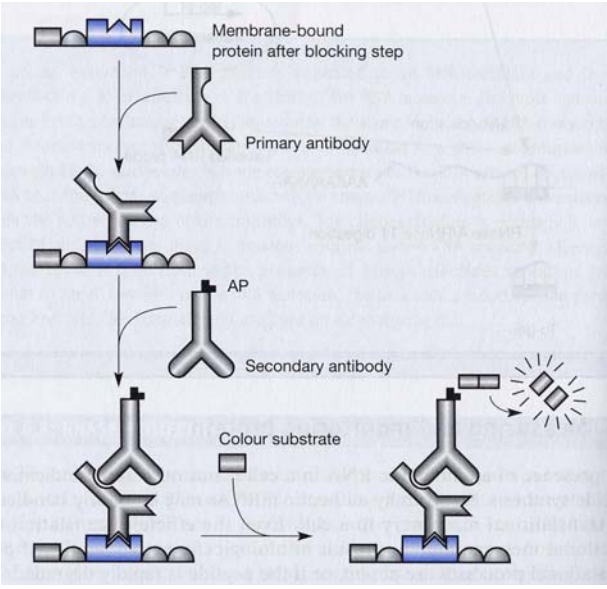
- Methods study the expression of protein in the brain
  - Radioimmunoassay
  - Western blotting
  - Immunocytochemistry

Radioimmunoassay (RIA)

- Samples can be fractionated by column chromatography → RIA
- The binding of a radioactively labeled antigen to a fixed amount of antibody can be inhibited by the addition of unlabeled antigen, and the extent of inhibition is a measure of the unlabeled material added

Western blotting

- Samples can be fractionated in SDS-polyacrylamide gel → electrophoretic separation → protein mass



Immunocytochemistry

- To localize the peptide product of a gene
- To identify particular cell types within tissue sections
- Detection
  - Enzyme
  - Fluorescent tag
  - Gold particle

Co-localization

- Whether the particular cells within the CNS express two or more genes of interest.?
- *In situ* hybridization
- Immunocytochemistry
- Combine *In situ* hybridization and Immunocytochemistry

- To correlate the expression of gene transcripts with functional properties of specific individual neurons in the brain
  - Combine the patch-clamp technique and RT-PCR

Neuroscience

Functions vs gene products