Expression-based Diagnostics

We have covered methods used to diagnose the presence or variation of genetic information. Here, we consider the methods used to answer whether the information is being used - expressed. Some of these methods are widely used clinically, others less so. Some are waiting for technological advances to become useful; for instance, a robust in situ PCR protocol will revolutionize the work of the pathologist.

Reading:

MBoC(6th) Ch8: ANALYSING PROTEINS, pgs. 452-455, *STUDYING GENE EXPRESSION AND FUNCTION*, *pgs. 502-506*. **Ch9:** 535-540.

Need to know and understand

Methods

- Northern, and Western blots
- Reverse Transcriptase (RT) PCR
- RNA in situ hybridization
- Immunohistochemistry/Immunofluorescence
- Expression Microarrays (chips)

Use the following to organize the methods.

Technique	Sample	Probe*	Use
Southern	DNA following electrophoresis	polynucleotide (denatured DNA or RNA)	Detection and/or quantitation of specific sequences in genomic DNA. Detection of viral genomes.
northern	RNA following electrophoresis	polynucleotide	Detection and/or quantitation of expression of specific sequences in tissue sample.
western	Protein following electrophoresis	Antibody	Detection of specific proteins in tissue sample.
RNA in situ	RNA in Tissue section	polynucleotide	Correlation of expression of specific sequences with cell-types/ morphologies.
immunohistochemistry	Protein in Tissue section	Antibody	Correlation of presence of specific proteins with cell-types/ morphologies.
DNA in situ, eg. FISH (fluorescent in situ hybridization)	DNA in Tissue section or metapahase spread	polynucleotide	Genome-wide arrangement of specific sequences in target cells.

*Note that some technique to detect the binding of the probe to its targets in the sample must be used. Polynucleotide probes may be either radioactively labelled with ^{32}P, ^{33}P, ^{3}H, or even ^{35}S, or labelled with a fluorochrome. Antibodies are sometimes radiolabelled with ^{125}I, but are more typically detected with a second antibody that specifically binds the constant regions of the IgG or IgM first antibody and that has been coupled with either a fluorochrome or an enzyme whose activity can be detected.