Transcriptomics & Proteomics

BIOL 426/626
Approaches to Molecular Biology


Class 18: Transcriptomics

- **Learning Goal**
  - To gain an appreciation for the tools available to study genome-wide the level of expression and structural diversity of mRNAs (transcriptomics) and the level and diversity of proteins expressed (proteomics)
- **Learning Objectives**
  - To identify and compare the many techniques for comparison of mRNA structure and expression level across genomes
  - To identify and compare these types of techniques operating at the level of proteins
- **Reading assignment:**
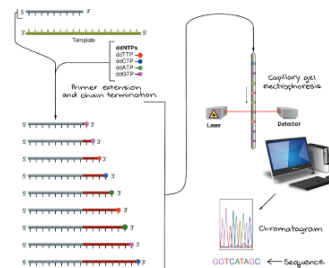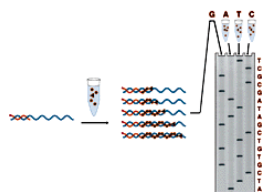 - Dale From Genes to Genomes: Chapt 10

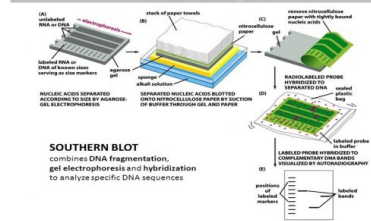## Shift to parallel technologies...

Enzyme assays
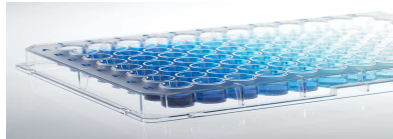
DNA sequencing

Shift to parallel technologies...

DNA analysis
Southern Blotting Technique
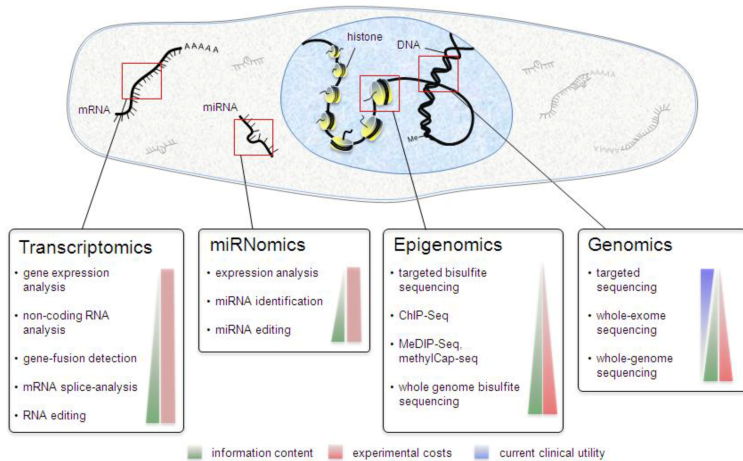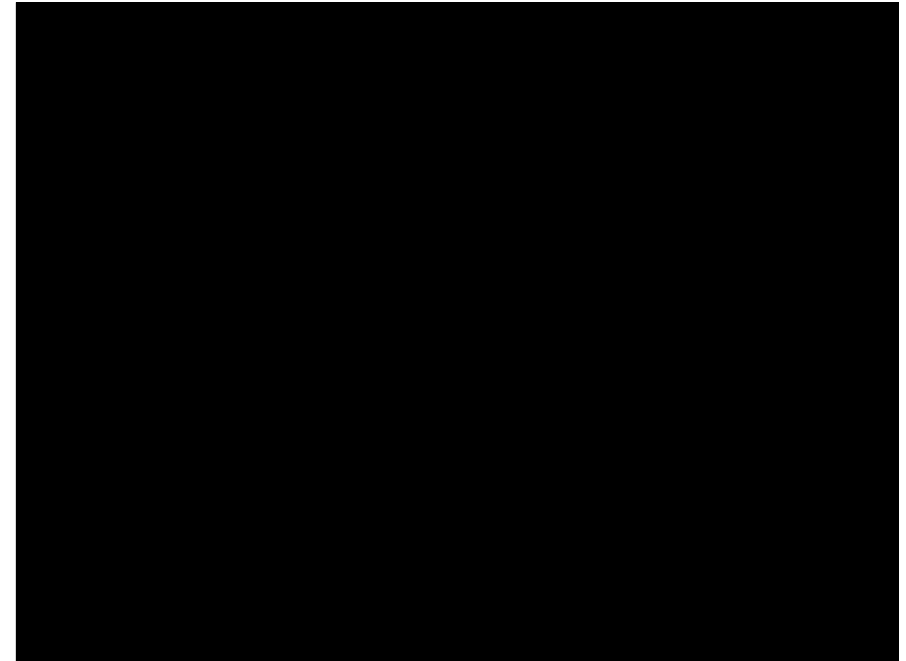
Protein analysis
Western Blotting Technique

http://www.onlinebiologynotes.com/

https://www.mybiosource.com/

Slide 5
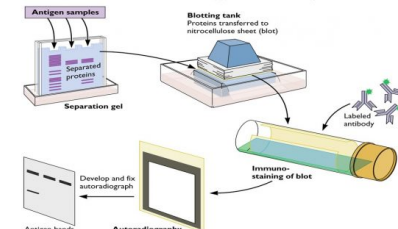
Applications of next-generation sequencing

Transcriptomics
- gene expression analysis
- non-coding RNA analysis
- gene-fusion detection
- mRNA splice-analysis
- RNA editing

miRNomics
- expression analysis
- miRNA identification
- miRNA editing

Epigenomics
- targeted bisulfite sequencing
- ChIP-Seq
- MeDIP-Seq, methylCap-seq
- whole genome bisulfite sequencing

Genomics
- targeted sequencing
- whole-exome sequencing
- whole-genome sequencing

■ information content ■ experimental costs ■ current clinical utility

Frese et al. Biology (Basel). 2013 2:378

Slide 6

Slide 7

Subtractive hybridization

- Prepare RNA from an experimental (sample A) and control (sample B) condition
- Convert to cDNA (not shown)
- Denature and mix excess sample B (driver) to sample A (tracer) cDNA
- Remove dsDNA, which should leave unannealed sample A DNA
- Convert remaining ssDNA to dsDNA and insert in a plasmid
- Result: a cDNA library enriched for sample A sequences
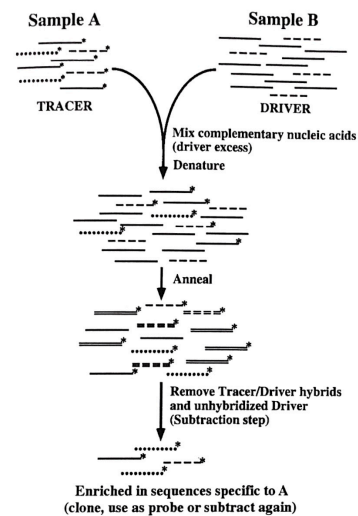
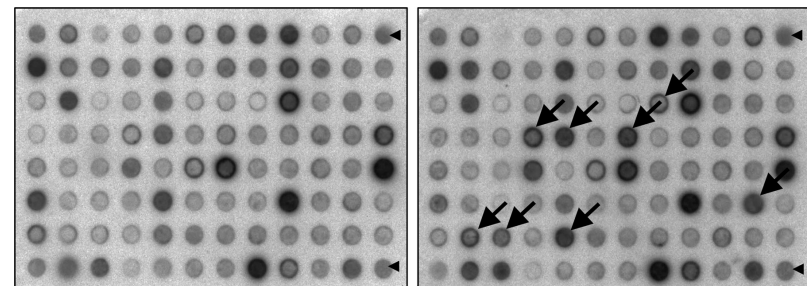Sagerström et al. Annu Rev Biochem. 1997 66:751

Slide 8

Differential Screening

Screen with labeled sample B cDNA

Screen with labeled sample A cDNA
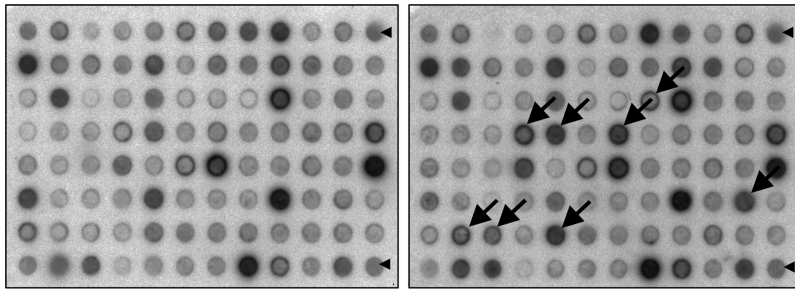

von Stein et al. Nucleic Acids Res. 1997 25:2598

Zenkel et al. Invest Ophthalmol Vis Sci (2005) 46:3742

Differential Screening–disadvantages

- Only good for identifying over-expressed genes
- High background for constitutively over-expressed genes
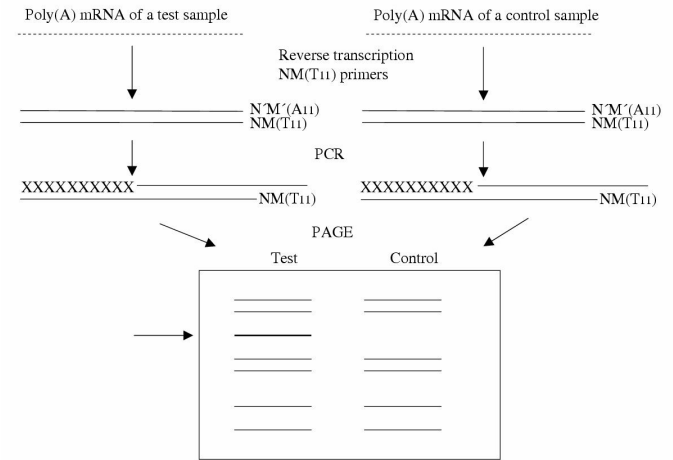- Less than full transcriptome coverage; time consuming

von Stein et al. Nucleic Acids Res. 1997 25:2598

Zenkel et al. Invest Ophthalmol Vis Sci (2005) 46:3742

Differential display

Chittka et al. BMC Devel Biol 2009 9:29

Differential display isn't always so clear...
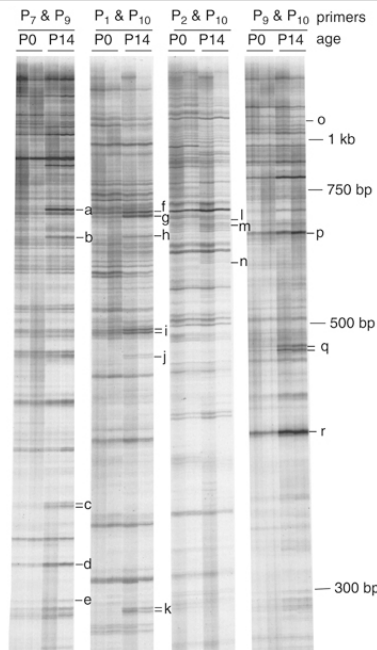
Expressed sequence tags

Craig Venter

https://nptel.ac.in/
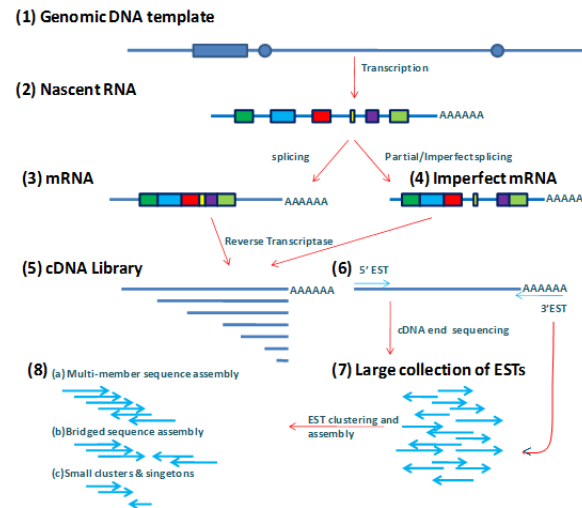
EST array screening
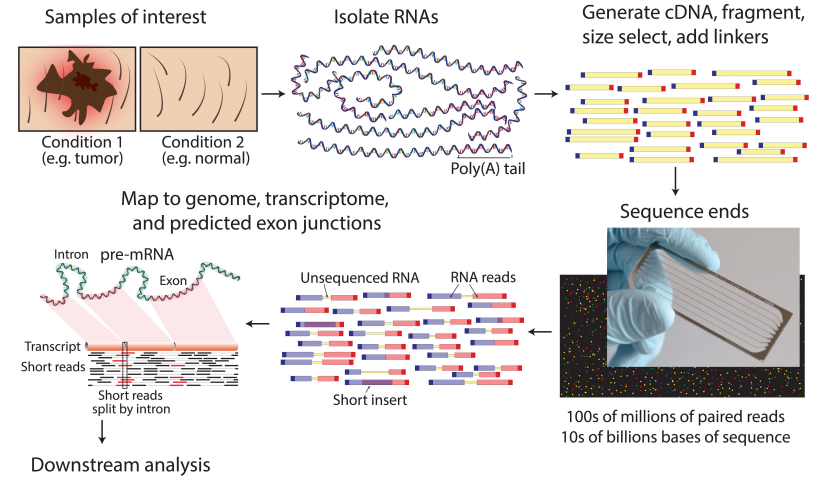
- Radioactive plant cDNAs from pathogen infected and control
- Transcription induced (red) or suppressed (blue) by infection

R. solani infected plants   uninfected control

RNAseq

Samples of interest   Isolate RNAs   Generate cDNA, fragment, size select, add linkers

Condition 1 (e.g. tumor)   Condition 2 (e.g. normal)   Poly(A) tail   Sequence ends

Map to genome, transcriptome, and predicted exon junctions

Intron pre-mRNA   Unsequenced RNA   RNA reads   Exon

Transcript
Short reads   Short insert   100s of millions of paired reads
Short reads split by intron   10s of billions bases of sequence

Downstream analysis
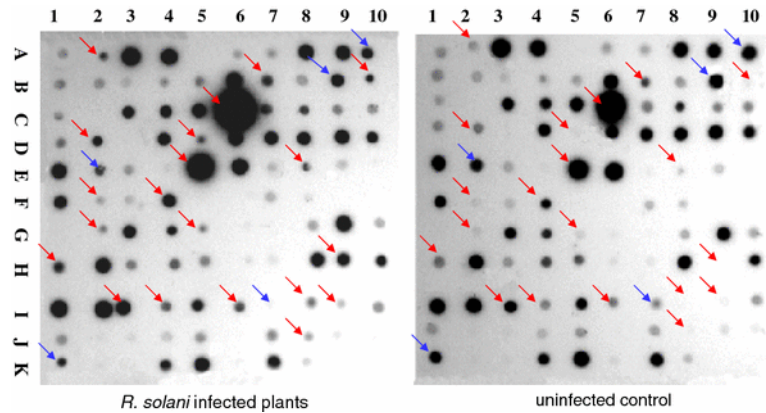
Ouellette et al. PLOS Com Biol 11:e1004293

RNAseq identifies splice junctions

Intron
pre-mRNA
Exon
mRNA
Short reads

Short read is split by intron when aligning to reference Genome

https://www.technologynetworks.com/
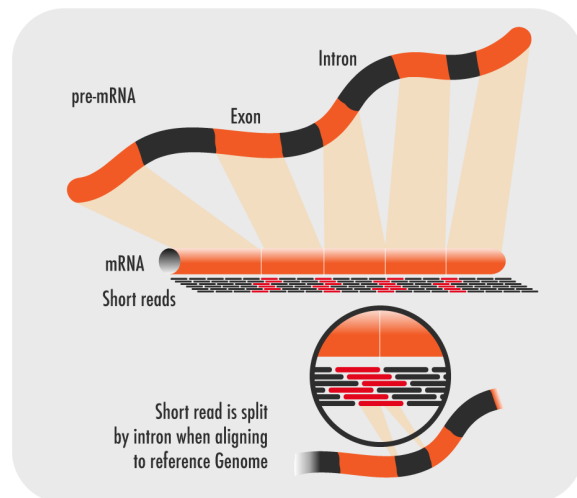
Matrix-assisted laser/disorption ionization (MALDI) mass spectrometry

analyte/matrix spot   laser beam   analyte ions

To mass spectrometer

matrix ions   University of BRISTOL

sample plate   cation   extraction grid   focussing lens

http://www.chm.bris.ac.uk/ms/maldi-ionisation.xhtml
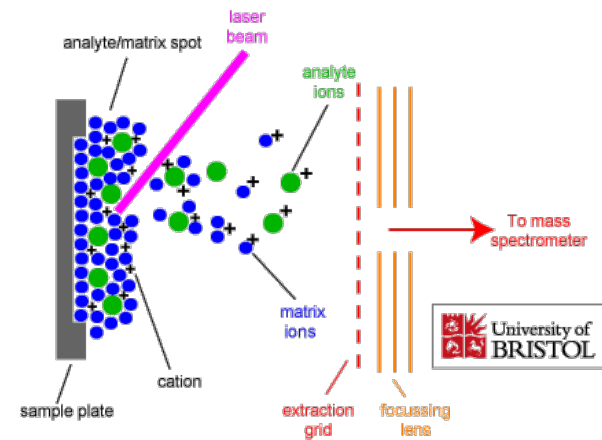
Electrospray ionization (ESI) mass spectrometry

Spray needle tip
Taylor cone
multiply charged droplet
solvent evaporation
the "Rayleigh" limit is reached
"Coulombic" explosion
multiply charged droplet
analyte molecule
Cone (counterelectrode)
analyte ions
+ve Power Supply -ve

University of BRISTOL

http://www.chm.bris.ac.uk/ms/fab-ionisation.xhtml
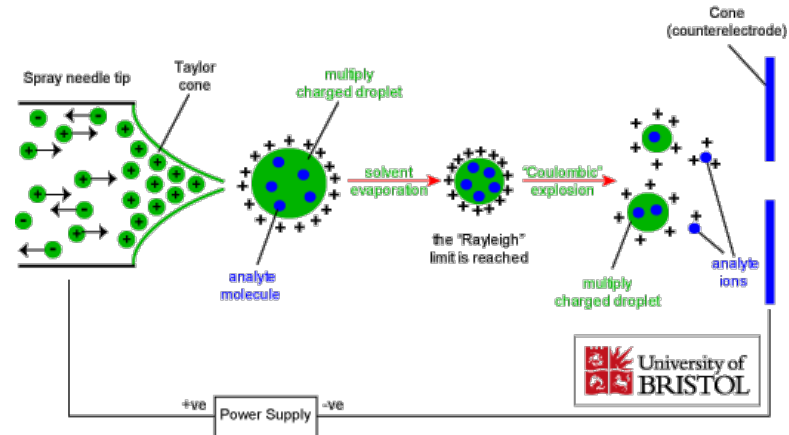
Tandem mass spectrometry

EI ESI MALDI
Collision Photon Surface
sample → ionization → m/z separation → fragment → m/z separation → detection

MS1
Precursor ion

MS2
Product ion

https://nationalmaglab.org/

Tandem MS of a car...

Mass selection (m/q isolation)
Detection of fragments
Ion source (ESI)
MS¹
Ion activation (CID, photons, electrons)
MS²
n+
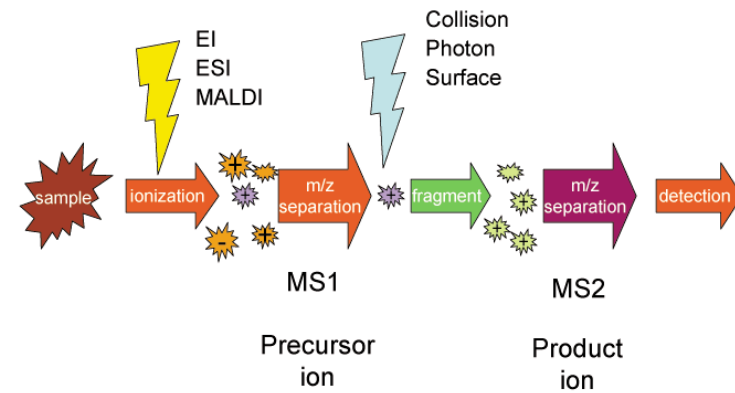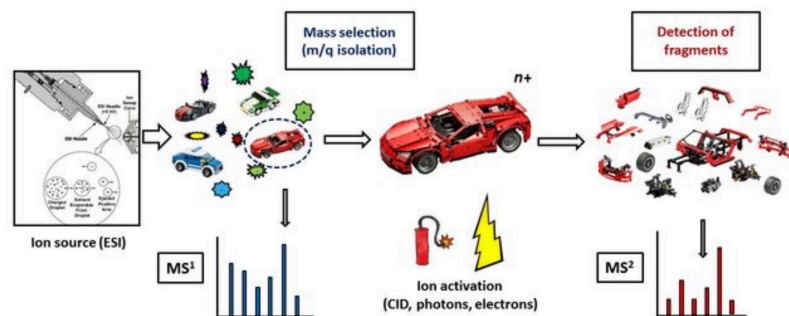
Miloš Lj Ranković, Czech Acad of Sci

Reading for next time:

- Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M. The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 2008 320:1344

- Final writing project due