Measuring Gene Expression

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Looking at Genes

- **Where? (where are genes located?)**
  - **Genes are located using gene finding programs (Glimmer, Genscan, GRPL)**
- **What? (what do these genes do?)**
  - **Genes are characterized using gene annotation tools (Pedant, Magpie, etc.)**
- **How Many? (how abundant are they?)**
  - **Gene expression is measured experimentally using SAGE or gene chips**

Different Kinds of "Omes"

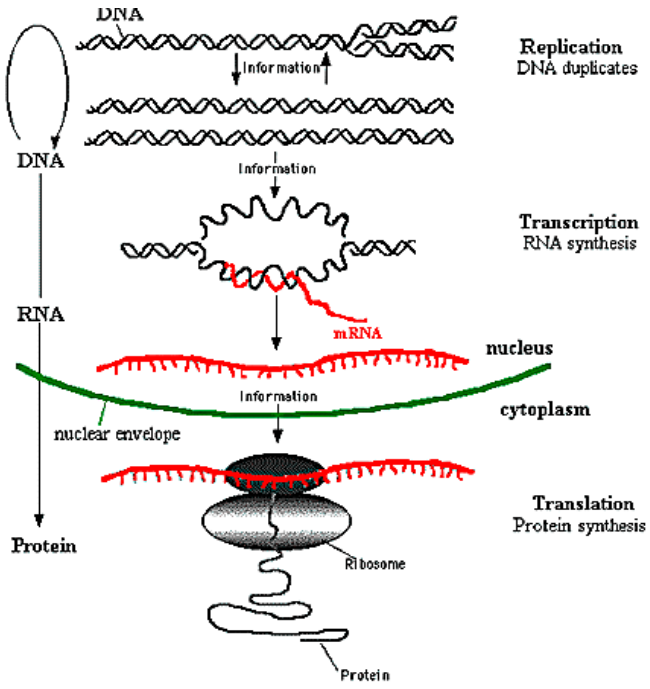
- Genome
  - Complement of all genes in a cell, tissue, organ or organism
- Transcriptome
  - Complement of all mRNA transcripts in a cell, tissue, organ or organism
- Proteome
  - Complement of all proteins in a cell, tissue, organ or organism

Different Kinds of "Omes"

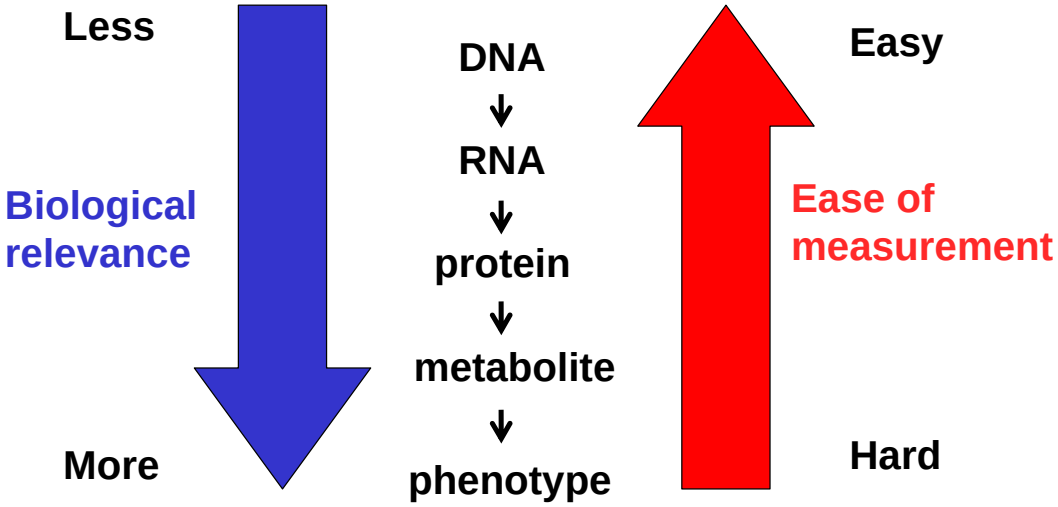
Genome

Transcriptome

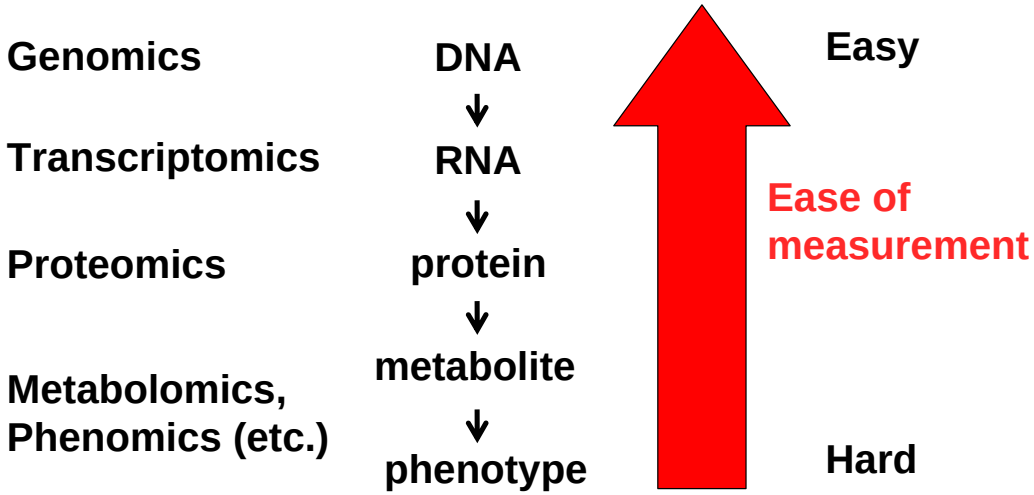
Proteome



The Measurement Dichotomy



High Throughput Measurement



-Omics Mania

biome, CHOmics, cellome, cellomics, chronomics, clinomics, complexome, crystallomics, cytomics, cytoskeleton, degradomics, diagnomics™, enzymome, epigenome, expressome, fluxome, foldome, secretome, functome, functomics, genomics, glycomics, immunome, transcriptomics, integromics, interactome, kinome, ligandomics, lipoproteomics, localizome, phenomics, metabolome, pharmacometabonomics, methylome, microbiome, morphome, neurogenomics, nucleome, secretome, oncogenomics, operome, transcriptomics, ORFeome, parasitome, pathome, peptidome, pharmacogenome, pharmacomethylomics, phenomics, phylome, physiogenomics, postgenomics, predictome, promoterome, proteomics, pseudogenome, secretome, regulome, resistome, ribonome, ribonomics, riboproteomics, saccharomics, secretome, somatonome, systeome, toxicomics, transcriptome, translatome, secretome, unknome, vaccinome, variomics...

http://www.genomicglossaries.com/content/omes.asp

Why Measure Gene Expression?

- **Assumption that more abundant genes/transcripts are more important**
- **Assumption that gene expression levels correspond to protein levels**
- **Assumption that a normal cell has a standard expression profile/signature**
- **Changes to that expression profile indicate something is happening**

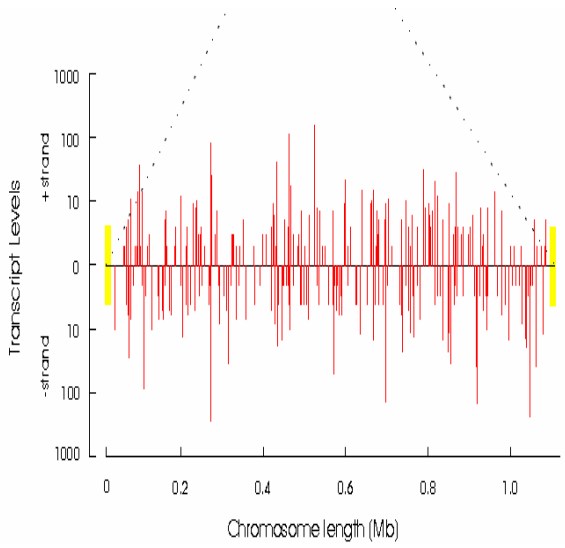
Why Measure Gene Expression?

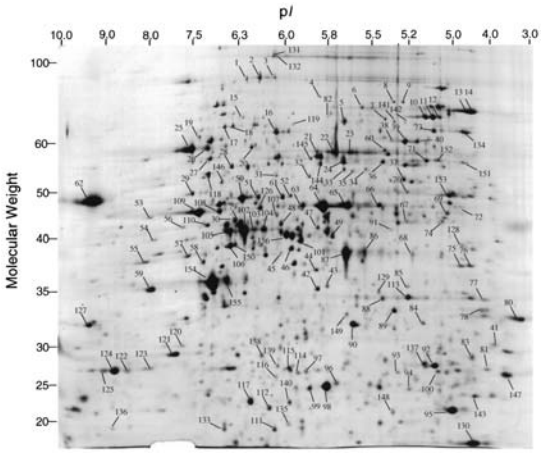
- Gene expression profiles represent a snapshot of cellular metabolism or activity at the molecular scale
- Gene expression profiles represent the cumulative interactions of many hard to detect events or phenomena
- Gene expression is a “proxy” measure for transcription/translation events

mRNA level = Protein level?

- Gygi et al. (1999) Mol. Cell. Biol. compared protein levels (MS, gels) and RNA levels (SAGE) for 156 genes in yeast
- In some genes, mRNA levels were essentially unchanged, but protein levels varied by up to 20X
- In other genes, protein levels were essentially unchanged, but mRNA levels varied by up to 30X

SAGE vs. 2D Gel

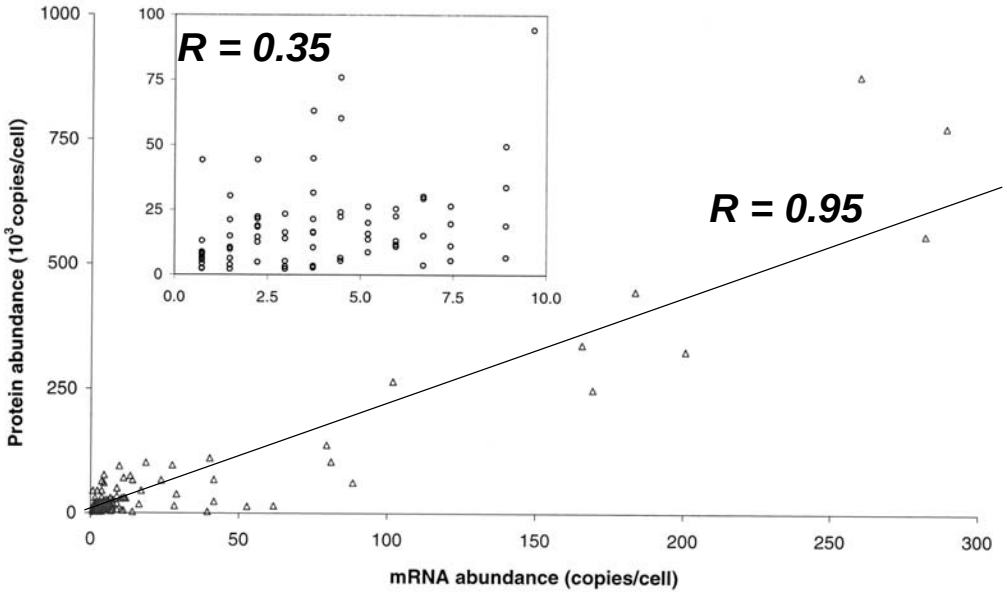




mRNA **Protein**

mRNA level = Protein level?

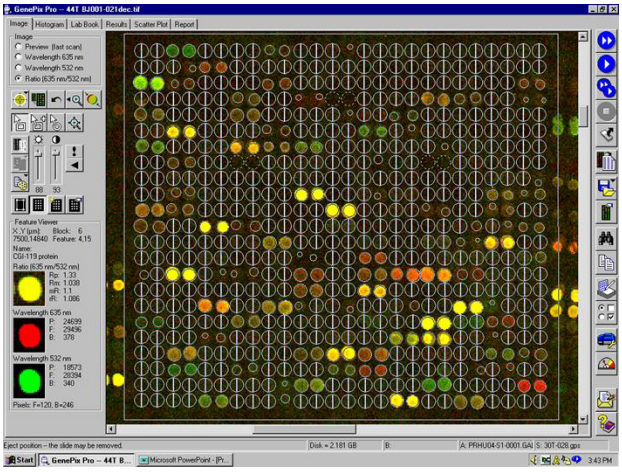
Gygi et al. (1999) Mol. Cell. Biol

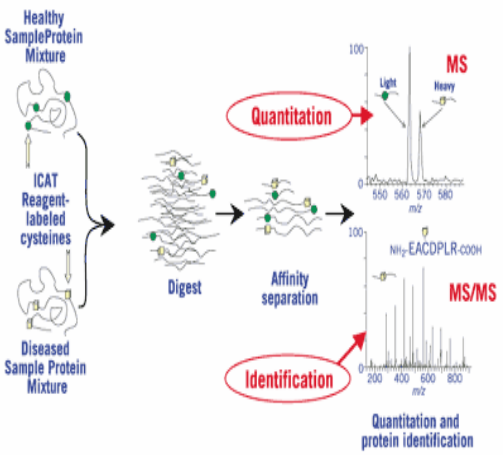


mRNA level = Protein level?

- Griffen TJ et al. (2002) *Mol. Cell. Proteomics* 1:323-333
- Compared protein levels (MS, ICAT) and RNA levels (microarray) for 245 genes in yeast on galactose/ethanol medium
- “Significant number of genes show large discrepancies between abundance ratios when measured at the levels of mRNA and protein expression”

Microarray vs. ICAT

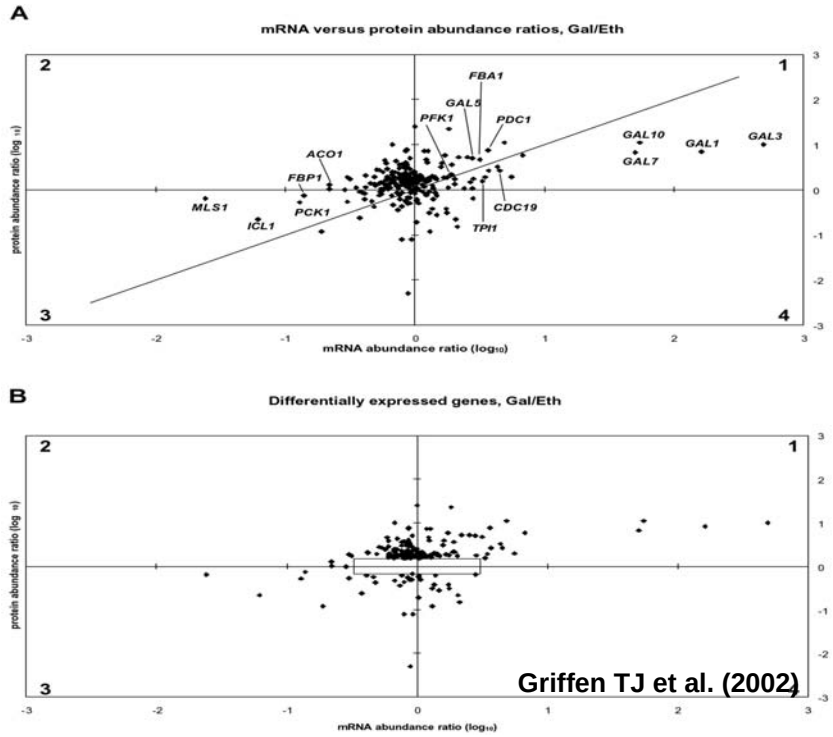




mRNA

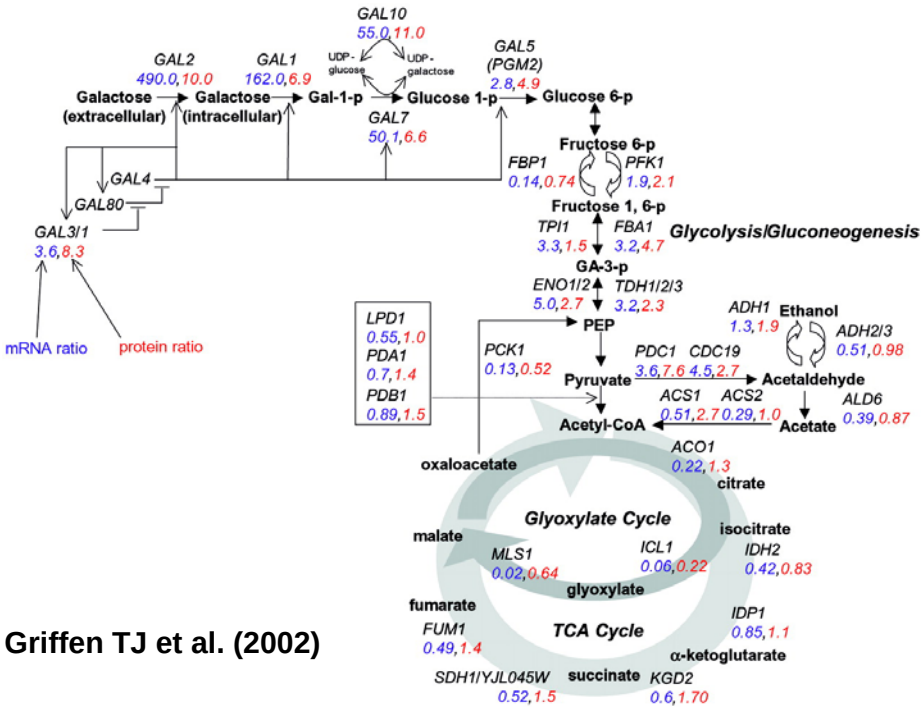
Protein

mRNA vs. Protein levels



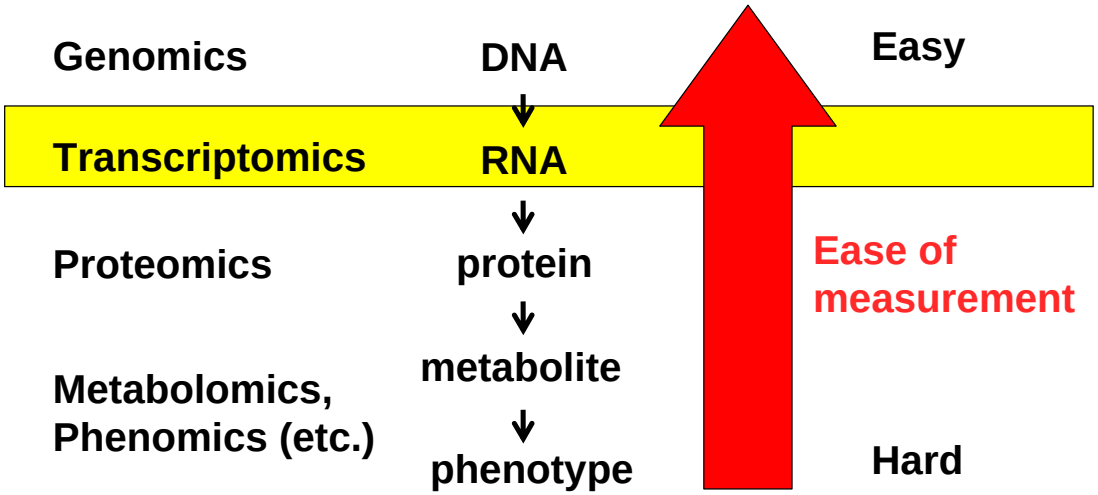
Griffen TJ et al. (2002)

mRNA vs. Protein levels



Griffen TJ et al. (2002)

Why Do It?



It's easier to do than the other measurements

How Relevant are RNA Levels to Protein Levels?

" [transcript abundance] doesn't tell us everything, but it tells us a lot more than we knew before "

--Pat Brown, Stanford
Microarray pioneer

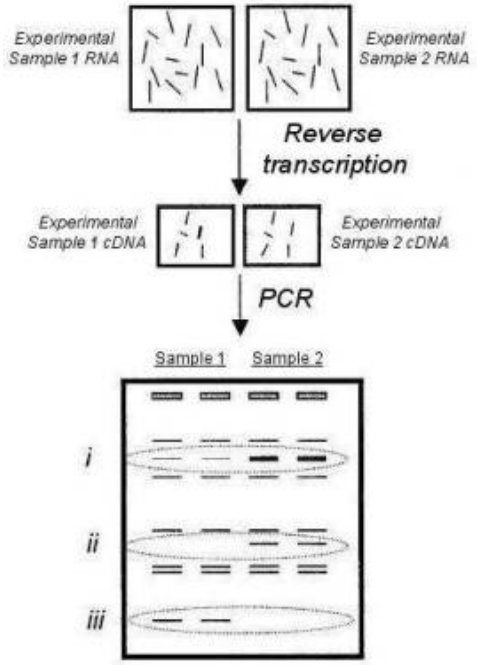
Measuring Gene Expression

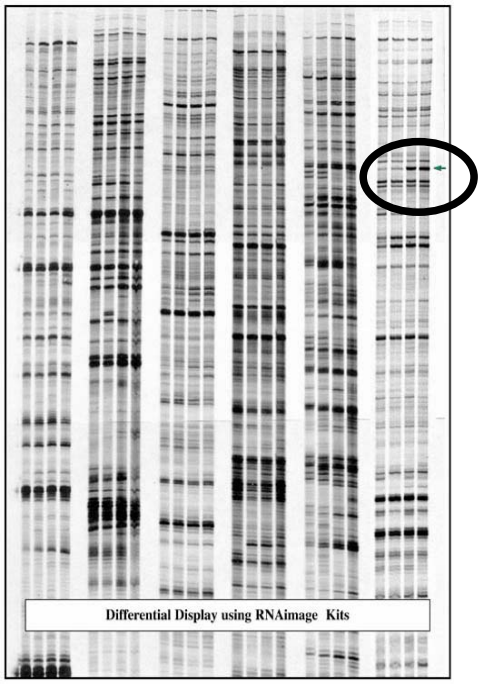
- Differential Display
- Serial Analysis of Gene Expression (SAGE)
- Rapid Analysis of Gene Expression (RAGE)
- RT-PCR (real-time PCR)
- Northern/Southern Blotting
- DNA Microarrays or Gene Chips

Differential Display (DD)

- **Basic idea:**
  - Run two RNA (cDNA) samples side by side on a gel
  - Excise and sequence bands present in one lane, but not the other
- **The clever trick:**
  - Reduce the complexity of the samples by making the cDNA with primers that will prime only a subset of all transcripts

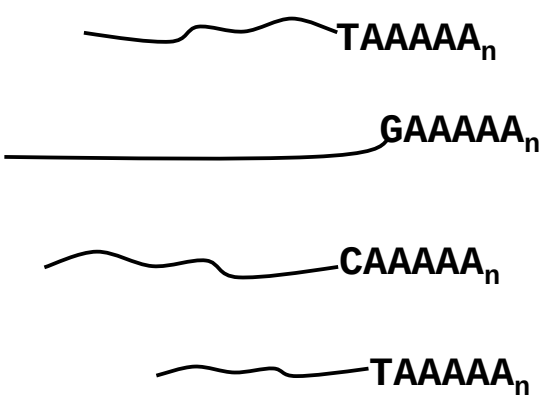
Differential Display



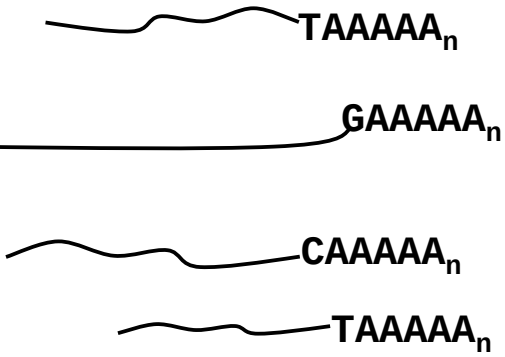


Differential Display (Detail)

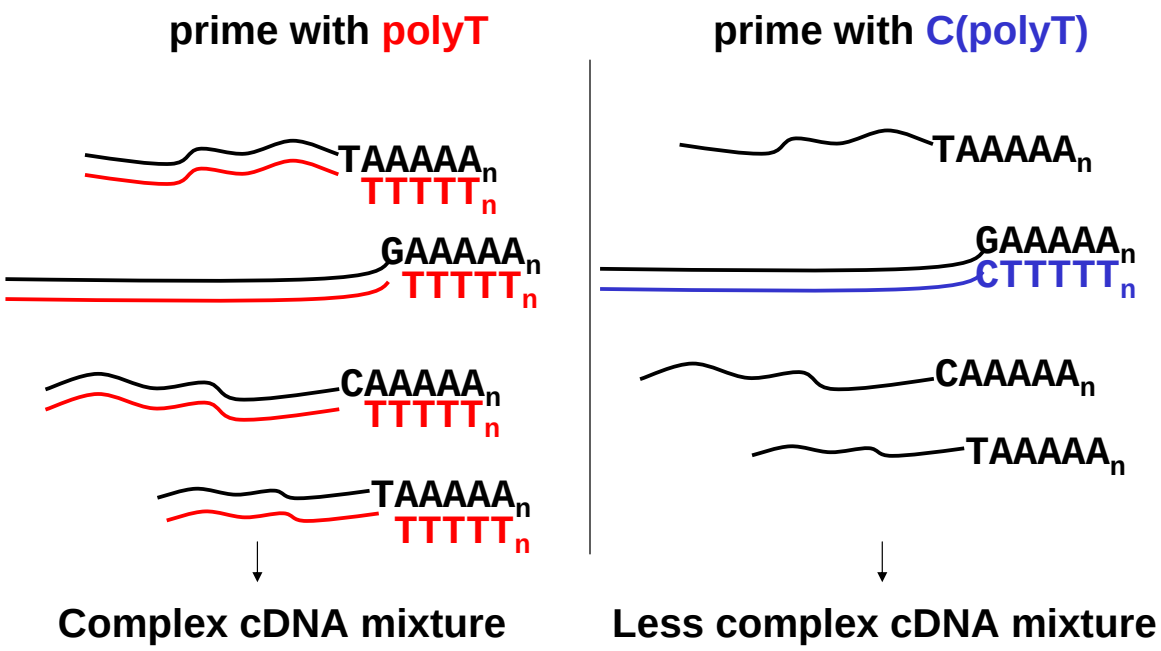
Prime with polyT



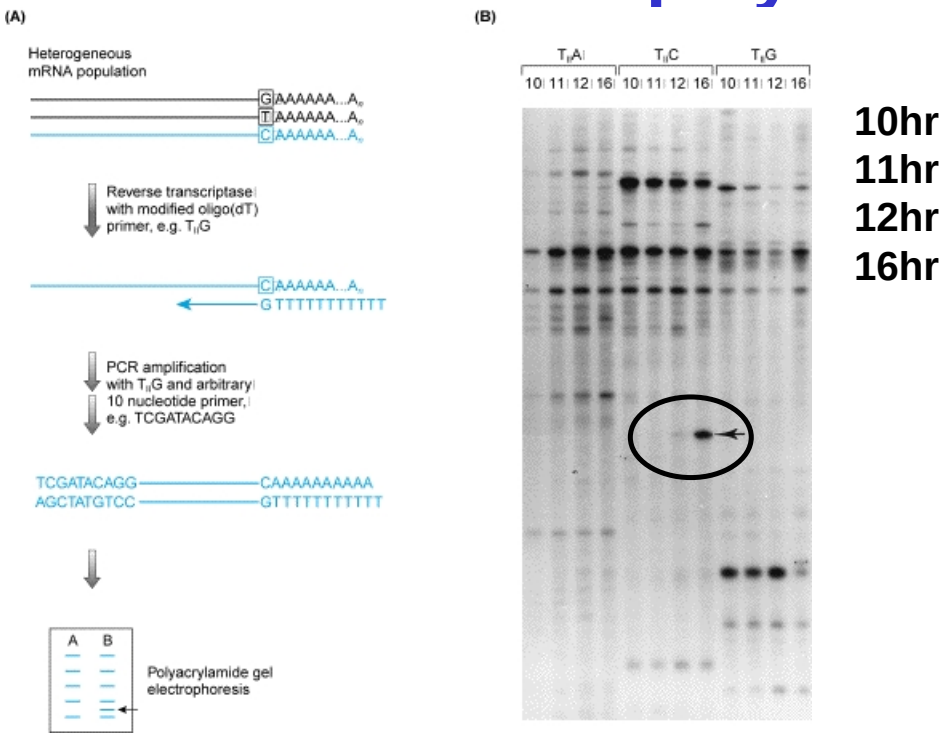
Prime with C(polyT)



Differential Display (Detail)



Differential Display



Advantages of DD

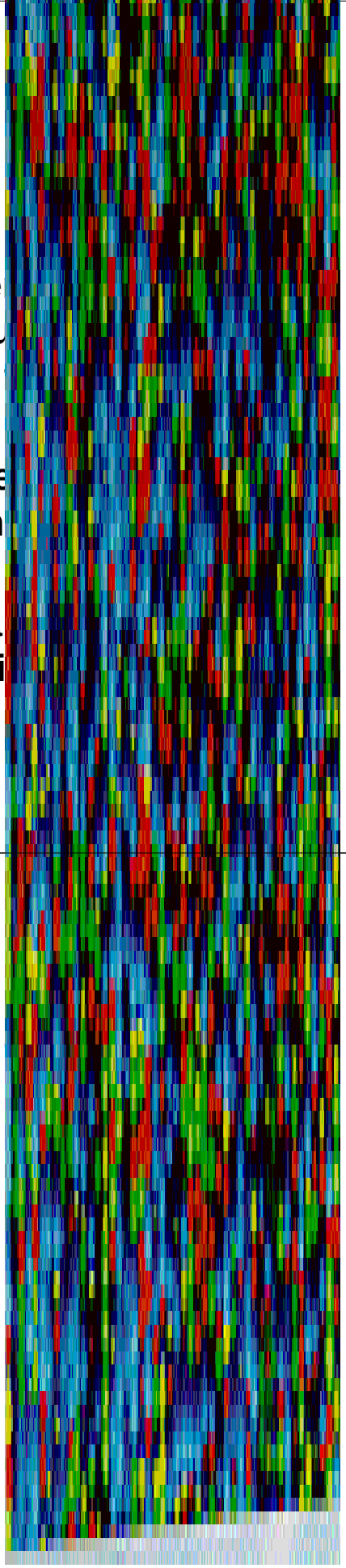
- Oldest of all transcript expression methods
- Technically and technologically simplest of all transcript methods
- Does not require ESTs, cDNA libraries, or any prior knowledge of the genome
- Open-ended technology

Disadvantages of DD

- Not very quantitative
- Sensitivity can be an issue
- Only a fraction of the transcripts can be analyzed in any single reaction
- Prone to false positives
- Not easily automated or scaled-up

SAGE

- Principle is to convert eve
  into a short (10-14 base), u
  Equivalent to reducing all
  city into a telephone book
- After creating the tags, the
  or concatenated into a lon
- The list can be read using
  and the list compared to a
  genes or proteins and thei



SAGE To

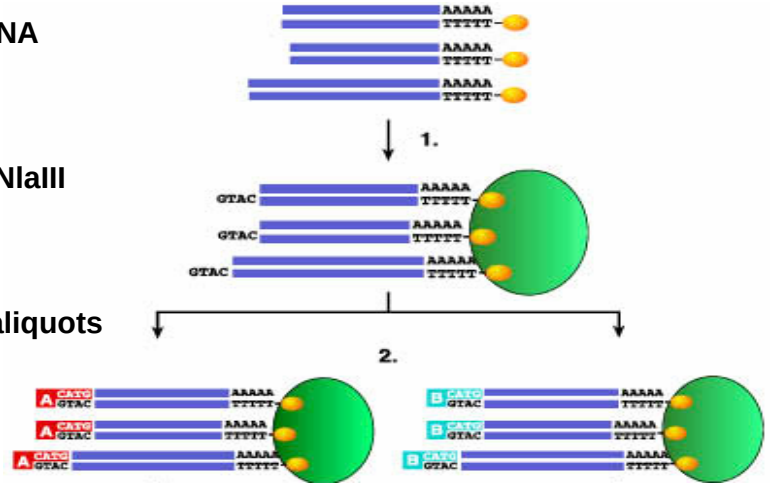
SAGE

Convert mRNA to dsDNA

Digest with NlaIII

Split into 2 aliquots

Attach Linkers



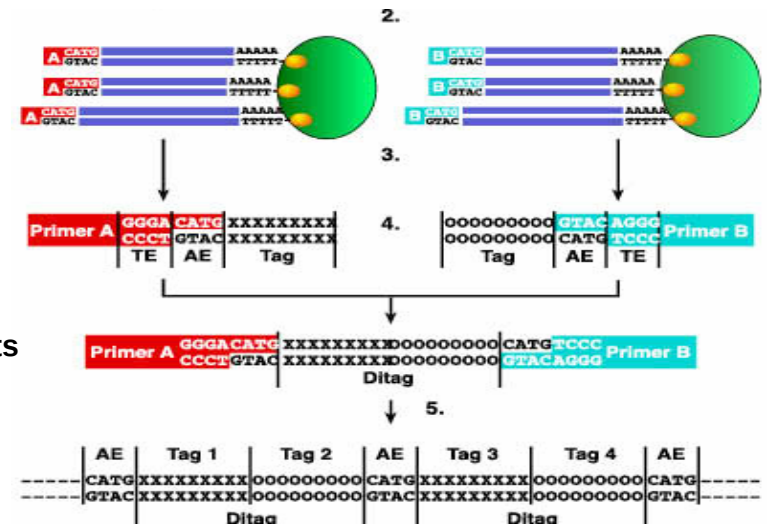
SAGE

Linkers have PCR & Tagging Endonuclease

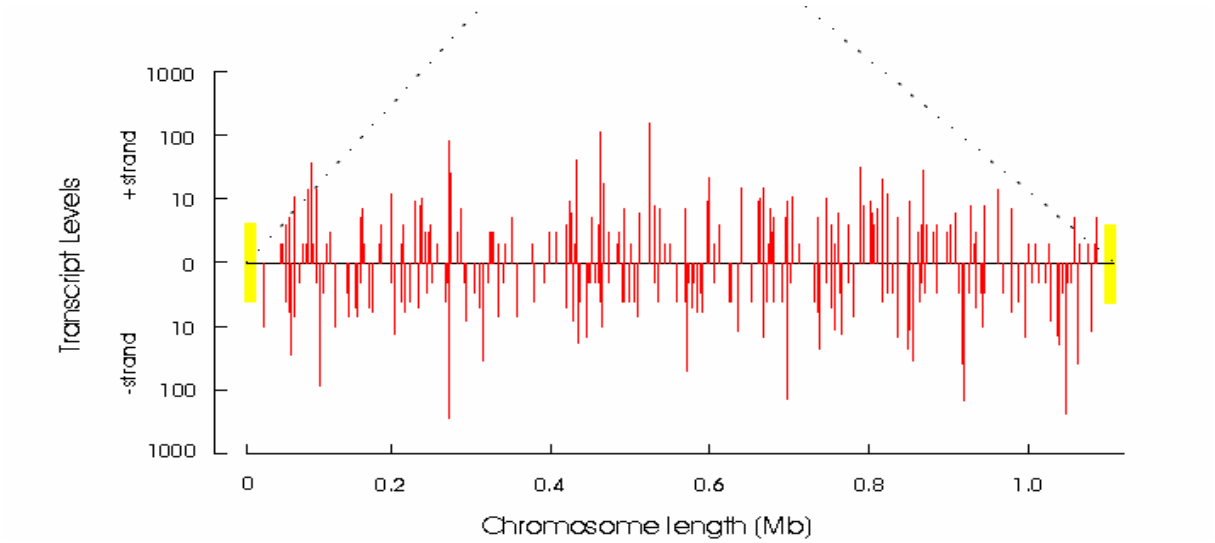
Cut with TE BsmF1

Mix both aliquots Blunt-end ligate to make “Ditag”

Concatenate & Sequence



SAGE of Yeast Chromosome



Advantages of SAGE

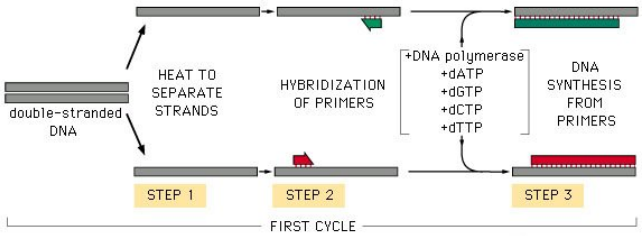
- **Very direct and quantitative method of measuring transcript abundance**
- **Open-ended technology**
- **Near infinite dynamic range**
- **Built-in quality control:**
  - **e.g. spacing of tags & 4-cutter restriction sites**

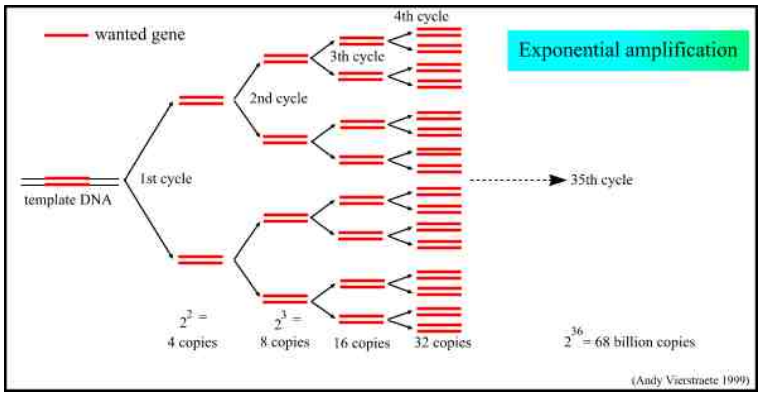
Disadvantages of SAGE

- Expensive, time consuming technology - must sequence >50,000 tags per sample (>$5,000 per sample)
- Most useful with fully sequenced genomes (otherwise difficult to associate 15 bp tags with their genes)
- 3’ ends of some genes can be very polymorphic

RT-PCR

Principles of PCR





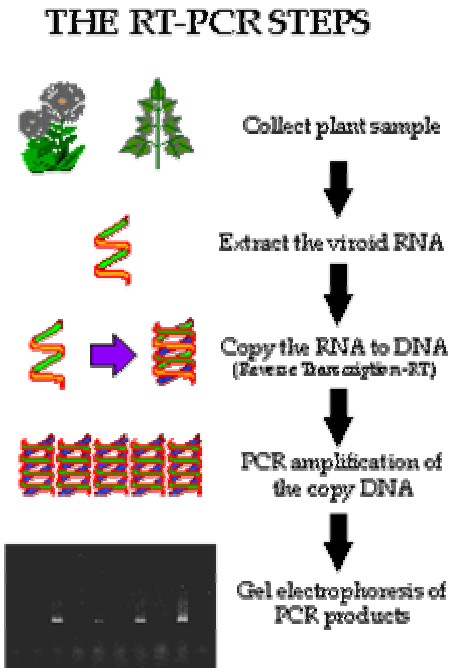
Polymerase Chain Reaction

PCR Tools

Thermocycler

Oligo Synthesizer

Reverse Transcriptase PCR

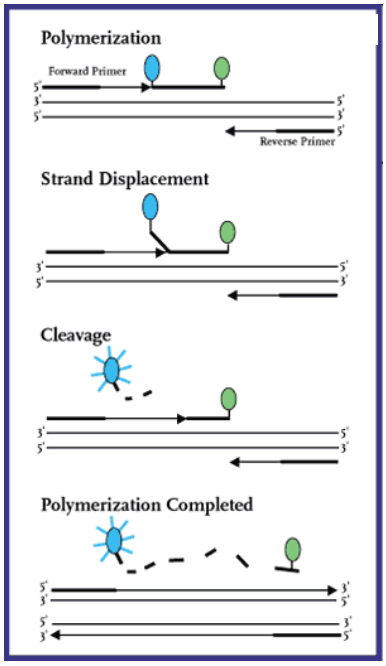


- Two kinds of "RT-PCR" - confusing
- One uses reverse transcriptase (RT) to help produce cDNA from mRNA
- Other uses real time (RT) methods to monitor PCR amplification

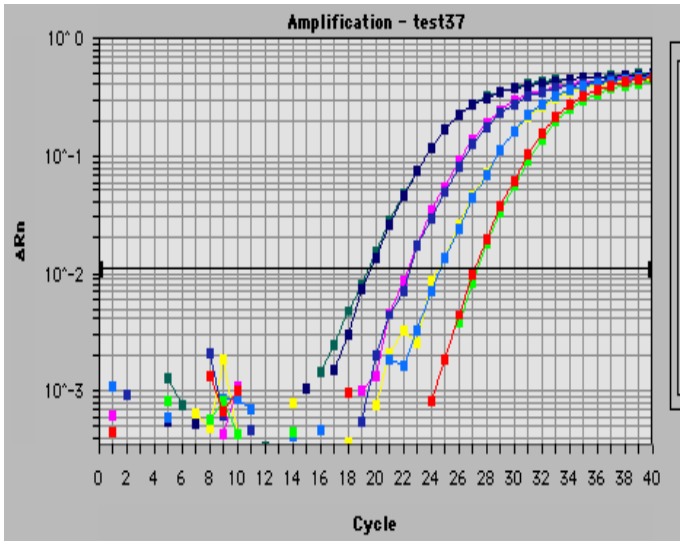
RT-PCR

- RT (Real Time) PCR is a method to quantify mRNA and cDNA in real time
- A ***quantitative PCR method***
- Measures the build up of fluorescence with each PCR cycle
- Generates quantitative fluorescence data at earliest phases of PCR cycle when replication fidelity is highest

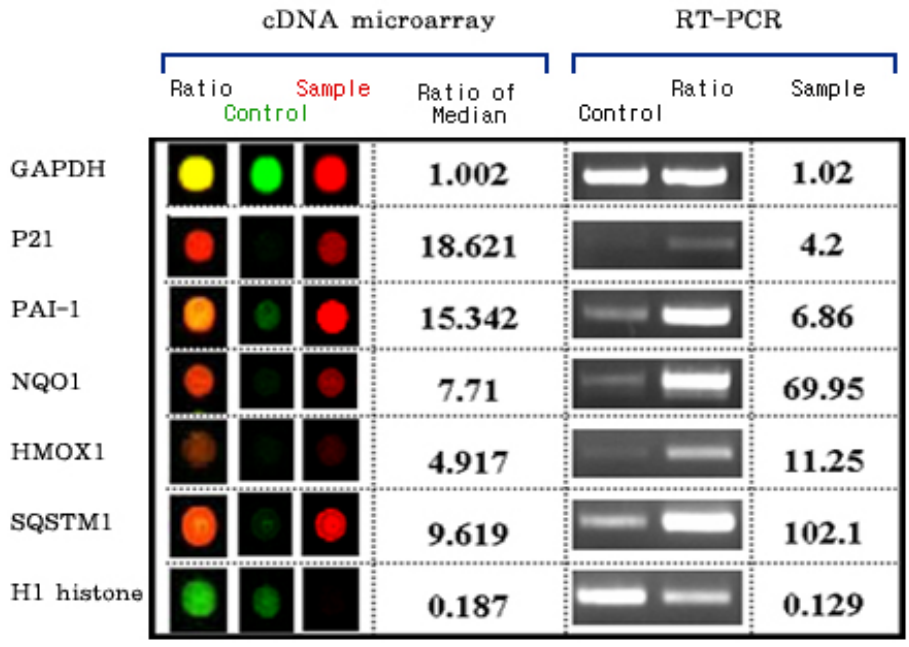
RT-PCR (Taqman)



An oligo probe with 2 flurophores is used (a quencher & reporter)



RT-PCR vs. Microarray



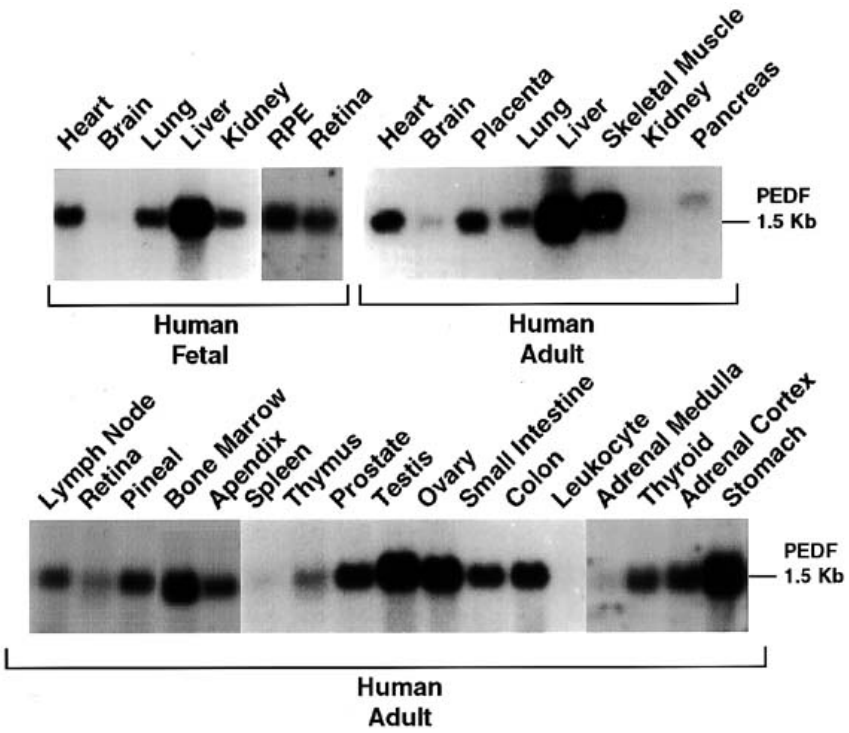
Advantages of RT-PCR

- Sensitive assay, highly quantitative, highly reproducible
- Considered “gold standard” for mRNA quantitation
- Can detect as few as 5 molecules
- Excellent dynamic range, linear over several orders of magnitude

Disadvantages of RT-PCR

- Expensive (instruments are >$150K, materials are also expensive)
- Not a high throughput system (10’s to 100’s of genes – not 1000’s)
- Can pick up RNA carryover or contaminating RNA leading to false positives

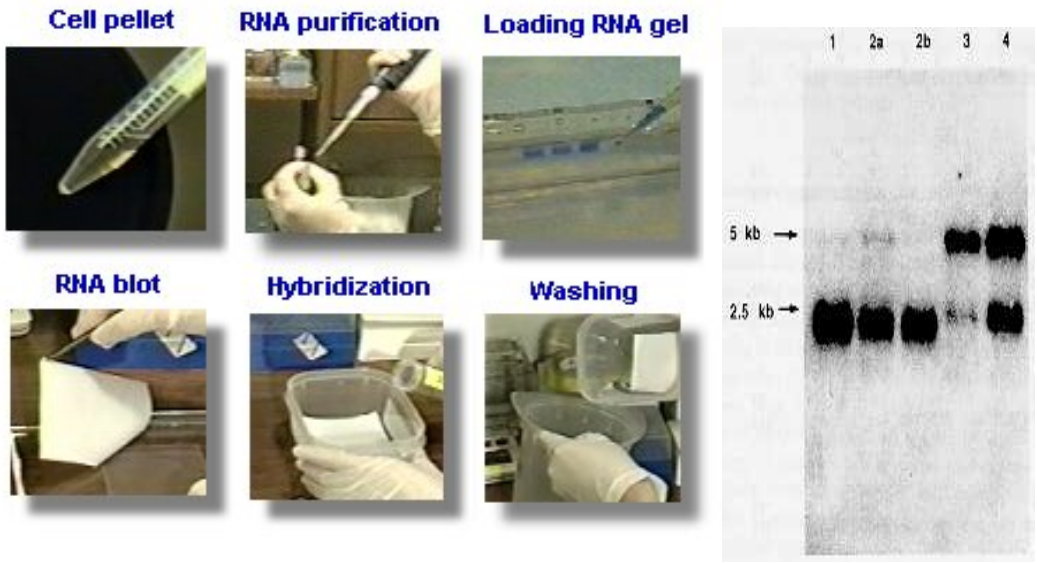
Northern Blots



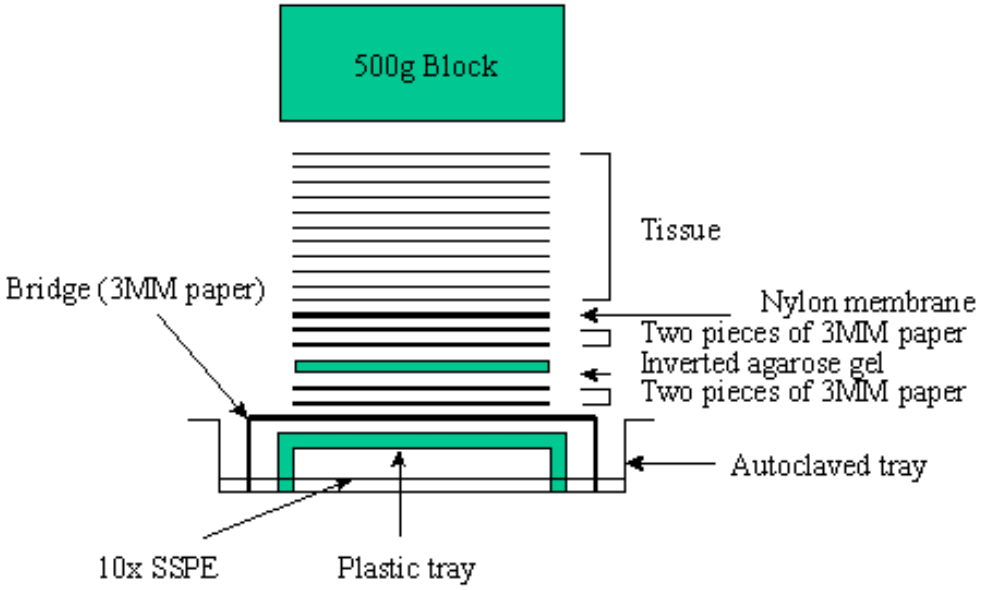
Northern Blots

- Method of measuring RNA abundance
- Name makes “fun” of Southern blots (which measure DNA abundance)
- mRNA is first separated on an agarose gel, then transferred to a nitrocellulose filter, then denatured and finally hybridized with ^{32}P labelled complementary DNA
- Intensity of band indicates abundance

Northern Blotting



The "Blot" Block



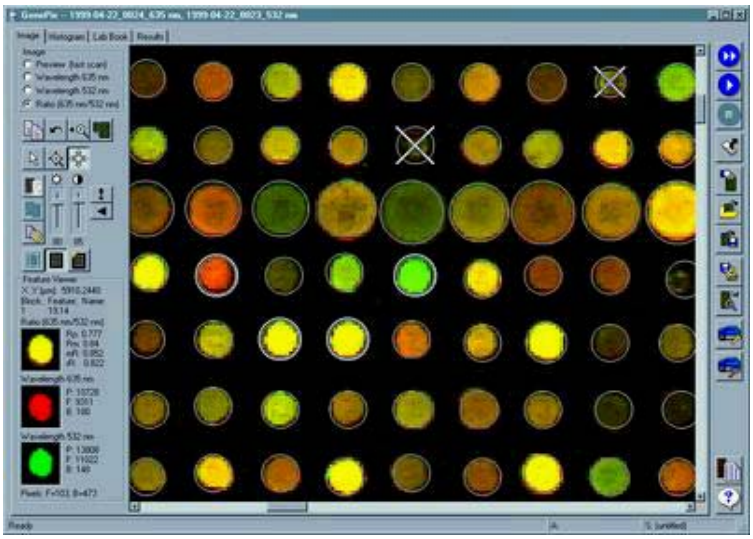
Advantages of Northerns

- Inexpensive, quantitative method of measuring transcript abundance
- Well used and well understood technology
- Use of radioactive probes makes it very sensitive
- Near infinite dynamic range

Disadvantages of Northerns

- Relies on radioactive labelling – “dirty” technology
- Quality control issues
- “Old fashioned” technology, now largely replaced by microarrays and other technologies

Microarrays



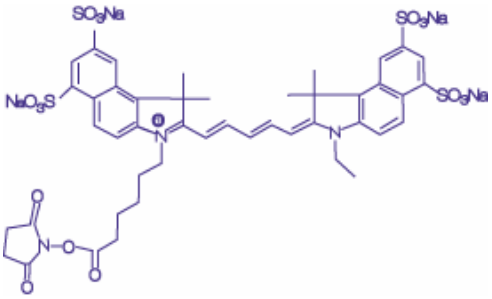
Microarrays

- **Basic idea:**
  - **Reverse Northern blot on a huge scale**
- **The clever trick:**
  - **Miniaturize the technique, so that many assay can be carried out in parallel**
  - **Hybridize control and experimental samples simultaneously; use distinct fluorescent dyes to distinguish them**

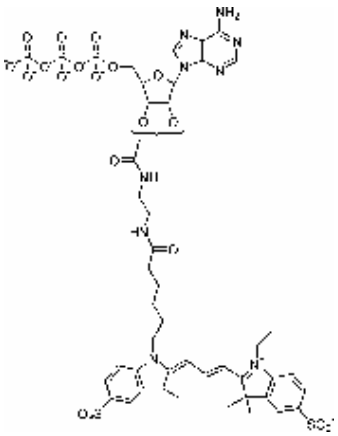
DNA Microarrays

- **Principle is to analyze gene (mRNA) or protein expression through large scale non-radioactive Northern (RNA) hybridization analysis**
- **Essentially high throughput Northern Blotting method that uses Cy3 and Cy5 fluorescence for detection**
- **Allows expressional analysis of up to 20,000 genes simultaneously**

Cy3 and Cy5 Dyes

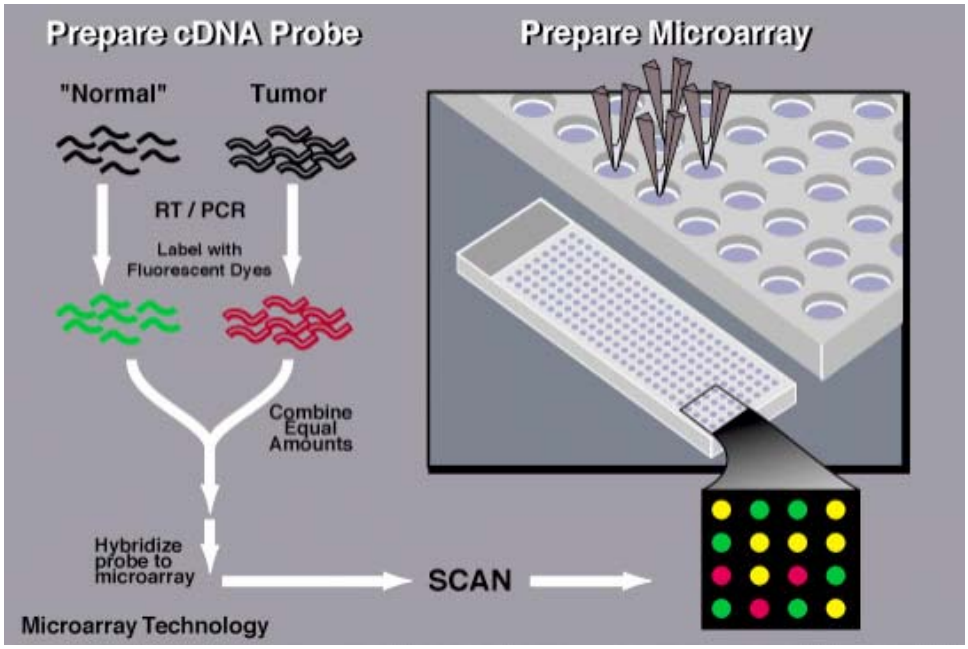


Cy5

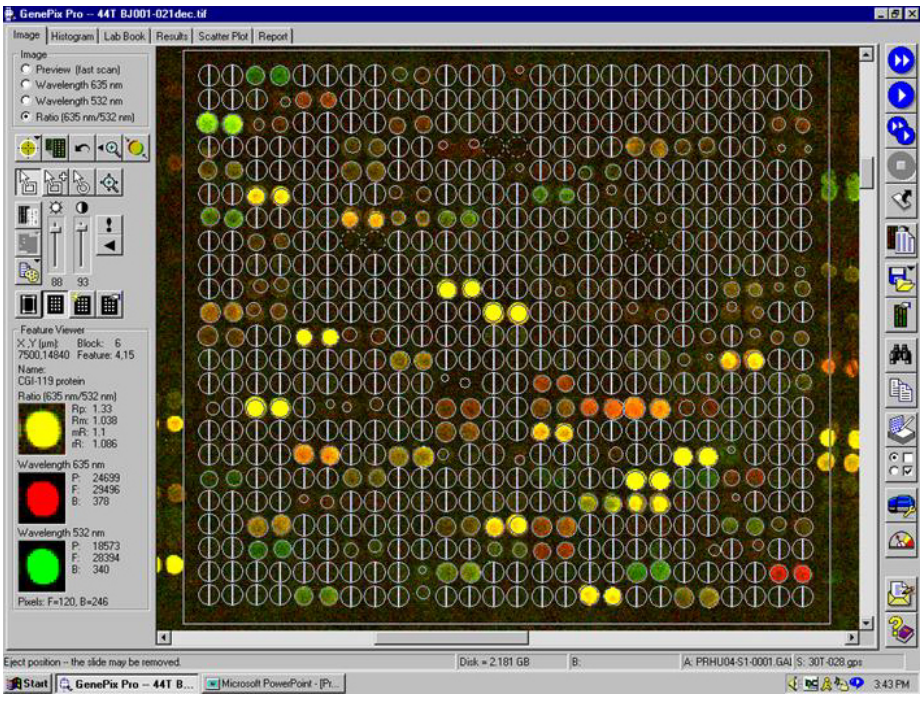


Cy3-ATP

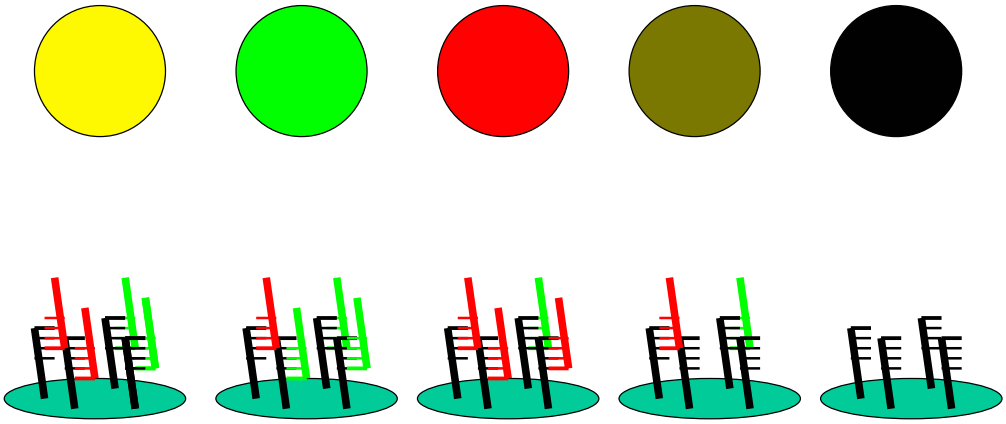
Principles of Microarrays



Typical Microarray Data



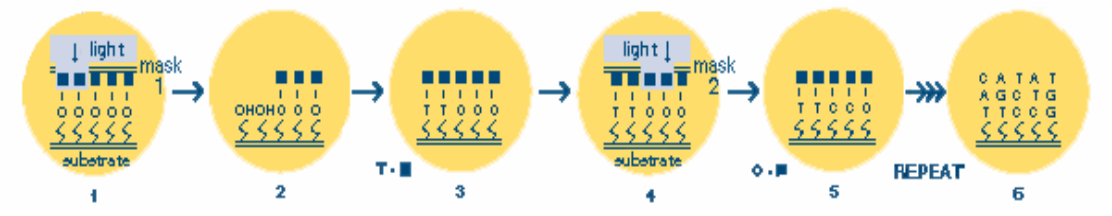
Microarrays & Spot Colour

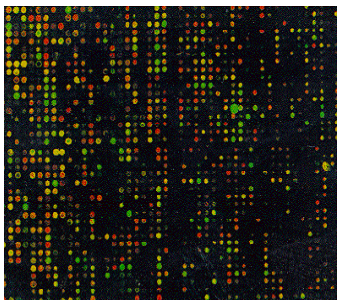


Four Types of Microarrays

- **Photolithographically prepared short oligo (20-25 bp) arrays**
- **Spotted glass slide cDNA (500-1000 bp) arrays**
- **Spotted nylon cDNA (500-1000 bp) arrays**
- **Spotted glass slide oligo (70 bp) arrays**

Affymetrix GeneChips





Glass Slide Microarrays

Advantages to Microarrays

- High throughput, quantitative method of measuring transcript abundance
- Avoids radioactivity (fluorescence)
- Kit systems and commercial suppliers make microarrays very easy to use
- Uses many “high-tech” techniques and devices – cutting edge
- Good dynamic range

Disadvantages to Microarrays

- Relatively expensive (>$1000 per array for Affy chips, $300 per array for “home made” systems)
- Quality and quality-control is highly variable
- Quantity of data often overwhelms most users
- Analysis and interpretation is difficult

Conclusions

- Multiple methods for measuring RNA or transcript abundance
  - Differential Display
  - Serial Analysis of Gene Expression (SAGE)
  - RT-PCR (real-time PCR)
  - Northern Blotting
  - DNA Microarrays or Gene Chips

Conclusions

- Some methods are better or, at least, more reliable than others
- Agreement between mRNA levels and protein levels is generally very poor – calls into question the utility of these measurements
- All mRNA measurement methods require a “second opinion”