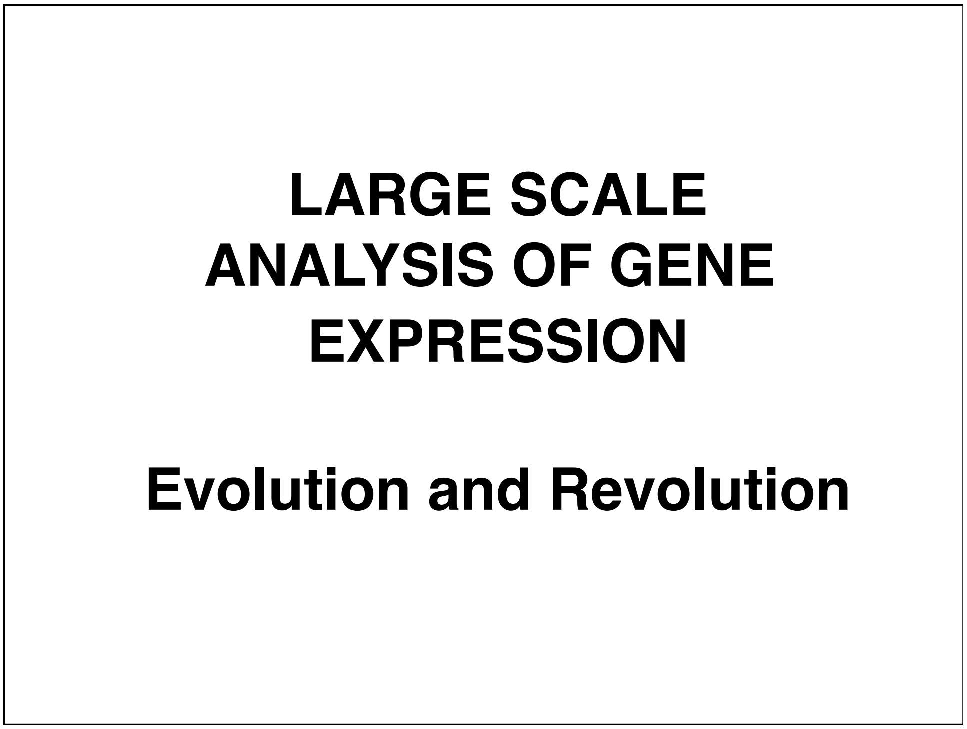
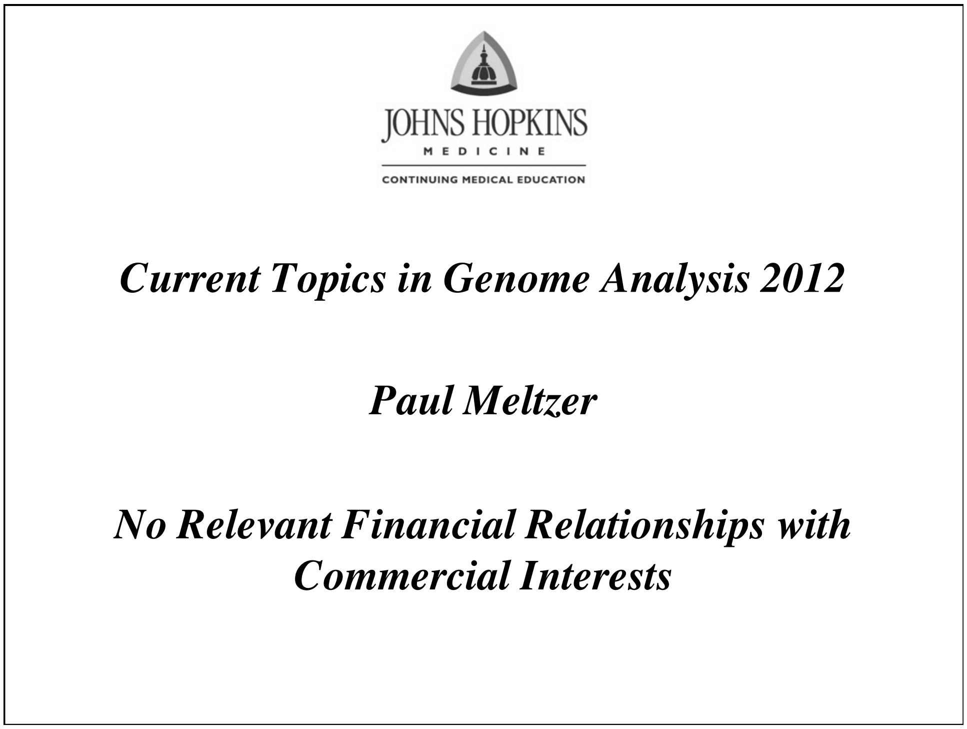


LARGE SCALE ANALYSIS OF GENE EXPRESSION

Evolution and Revolution

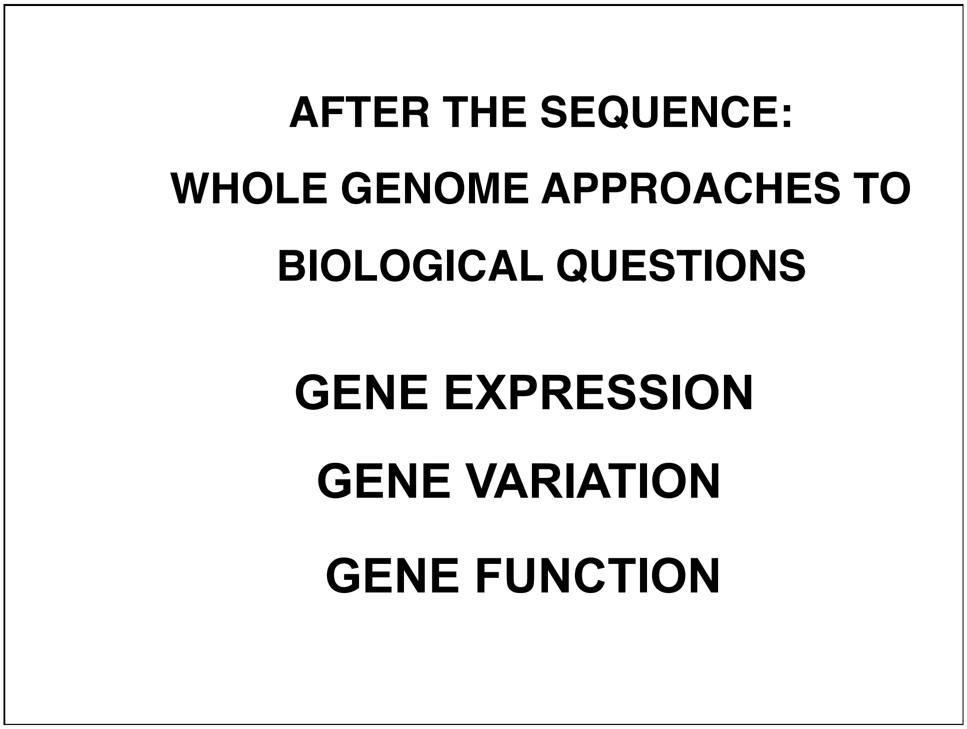


JOHNS HOPKINS
MEDICINE
CONTINUING MEDICAL EDUCATION

Current Topics in Genome Analysis 2012

Paul Meltzer

No Relevant Financial Relationships with Commercial Interests

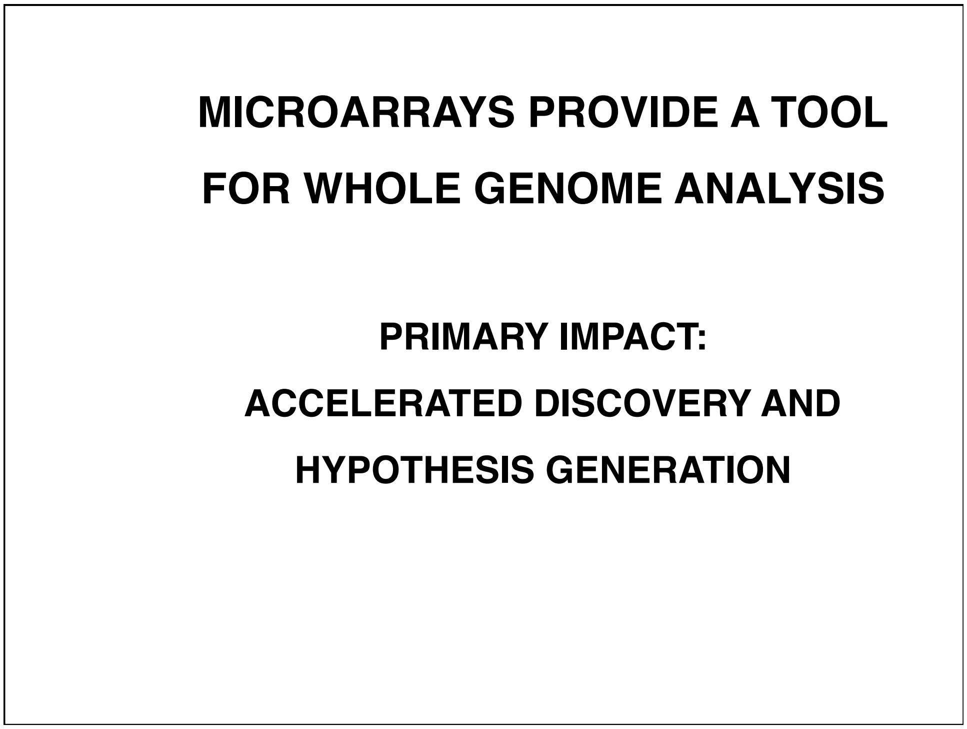


AFTER THE SEQUENCE:
WHOLE GENOME APPROACHES TO
BIOLOGICAL QUESTIONS

GENE EXPRESSION

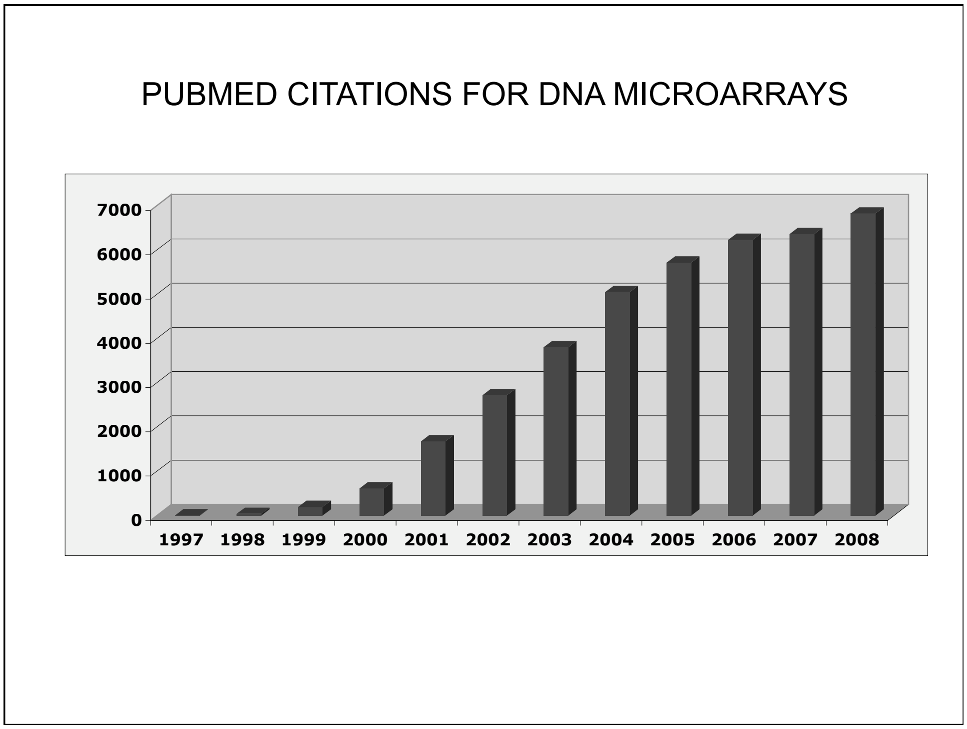
GENE VARIATION

GENE FUNCTION

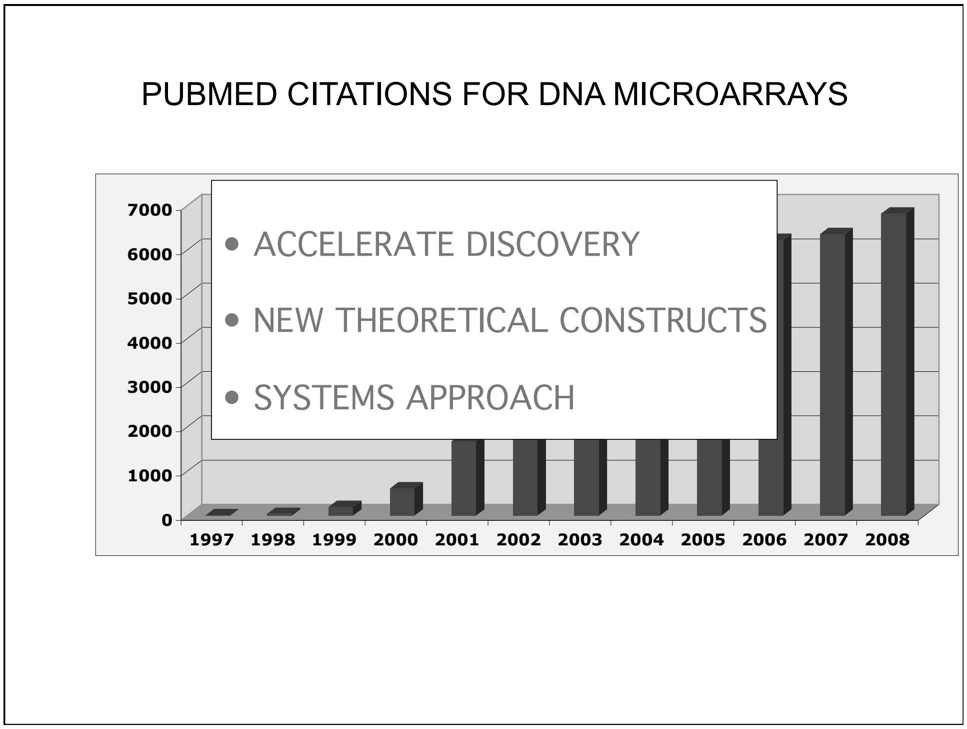


MICROARRAYS PROVIDE A TOOL
FOR WHOLE GENOME ANALYSIS

PRIMARY IMPACT:
ACCELERATED DISCOVERY AND
HYPOTHESIS GENERATION



PUBMED CITATIONS FOR DNA MICROARRAYS



PUBMED CITATIONS FOR DNA MICROARRAYS

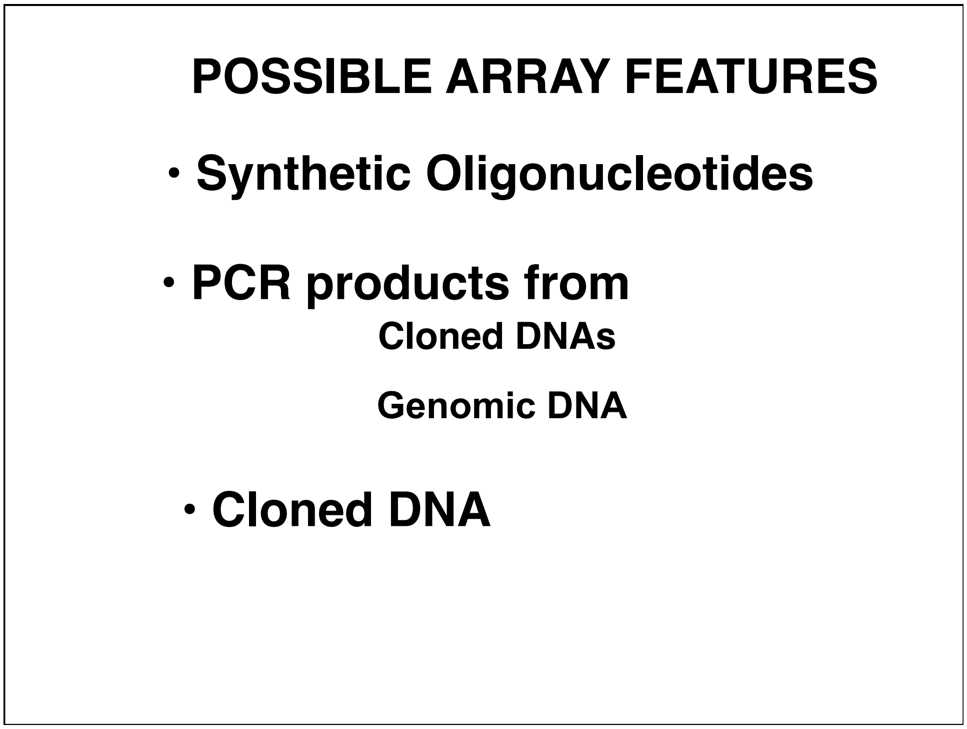
- ACCELERATE DISCOVERY
- NEW THEORETICAL CONSTRUCTS
- SYSTEMS APPROACH

INCREASE IN FEATURE DENSITY

SEQUENCING TECHNOLOGIES MAY SUPPLANT ARRAYS IN MANY APPLICATIONS

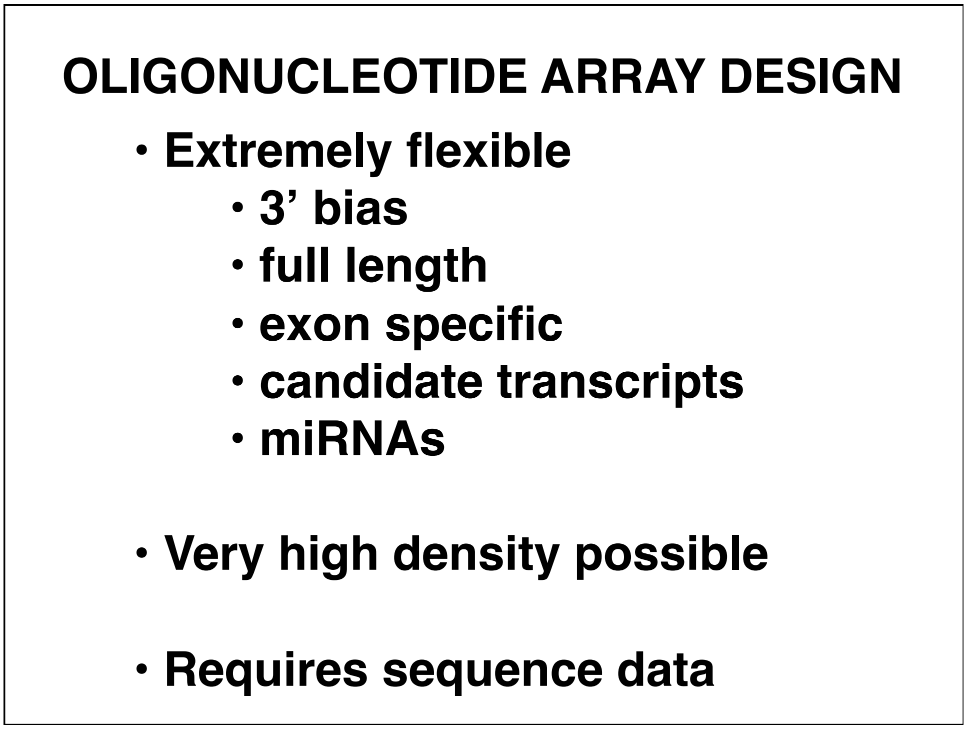
MICROARRAY TERMINOLOGY

- Feature--an array element
- Probe--a feature corresponding to a defined sequence
- Target--a pool of nucleic acids of unknown sequence



POSSIBLE ARRAY FEATURES

- Synthetic Oligonucleotides
- PCR products from
  - Cloned DNAs
  - Genomic DNA
- Cloned DNA

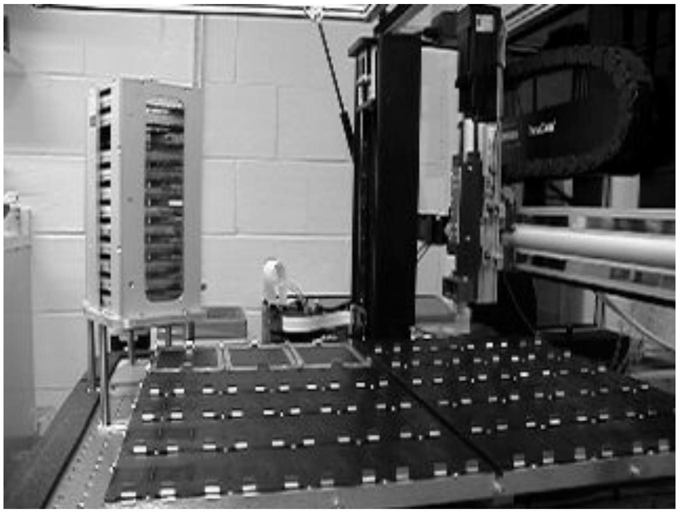


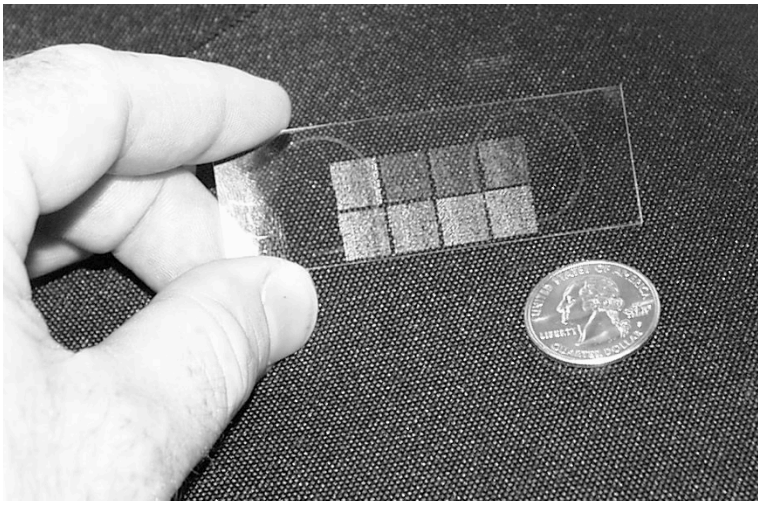
OLIGONUCLEOTIDE ARRAY DESIGN

- Extremely flexible
  - 3’ bias
  - full length
  - exon specific
  - candidate transcripts
  - miRNAs
- Very high density possible
- Requires sequence data

Microarray Manufacture

- **Printing**

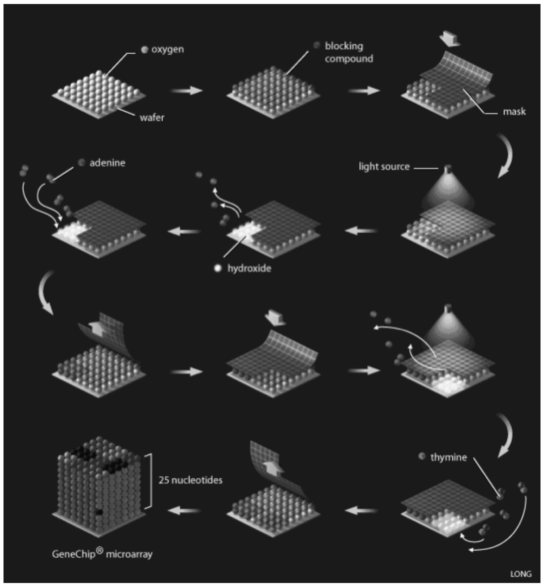




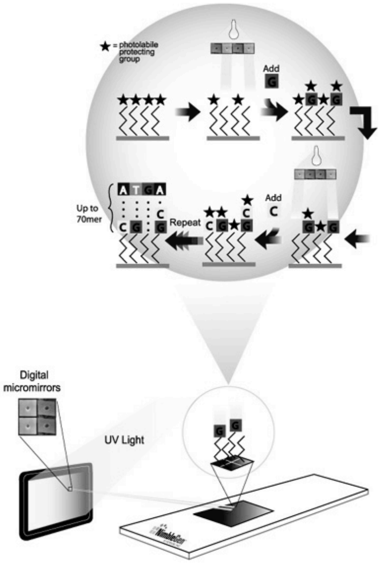
Microarray Manufacture

- Printing
- Synthesis *in situ*
  - light directed
  - mechanically directed

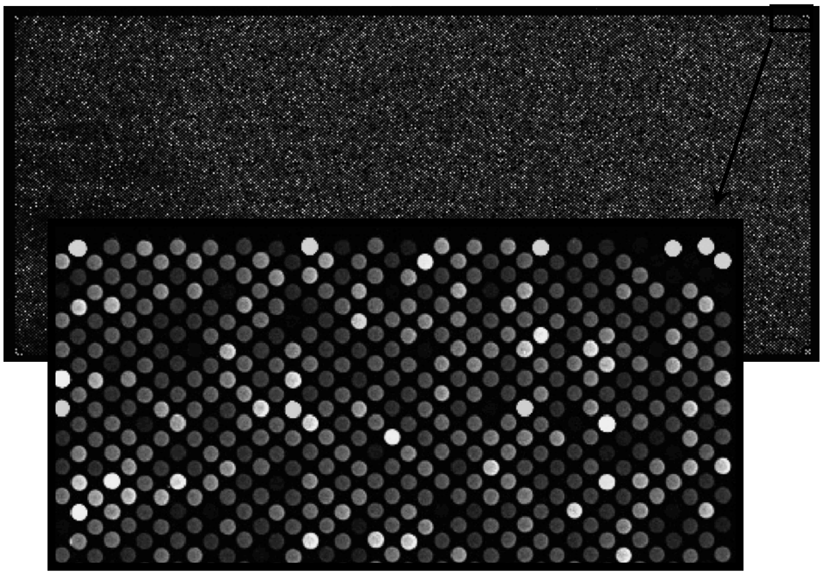
LIGHT DIRECTED OLIGONUCLEOTIDE SYNTHESIS

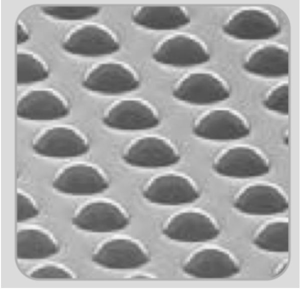


LIGHT DIRECTED OLIGONUCLEOTIDE SYNTHESIS

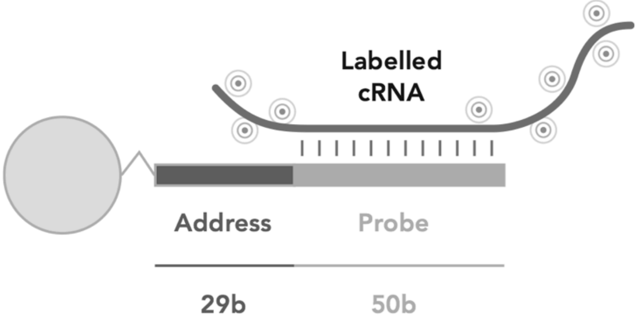


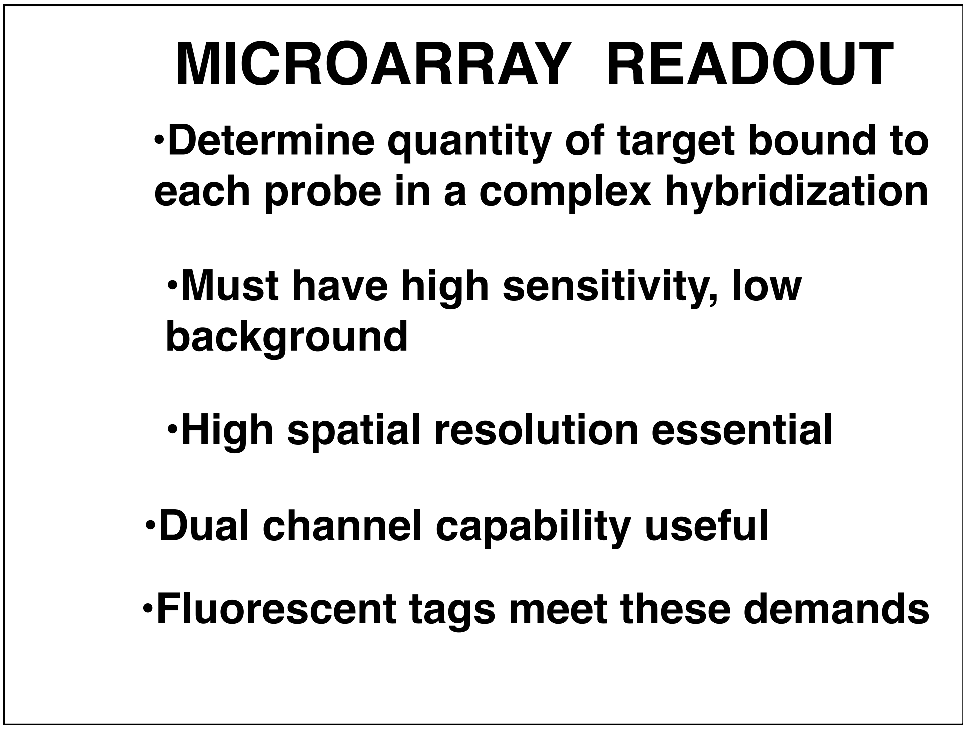
INK JET DIRECTED SYNTHESIS





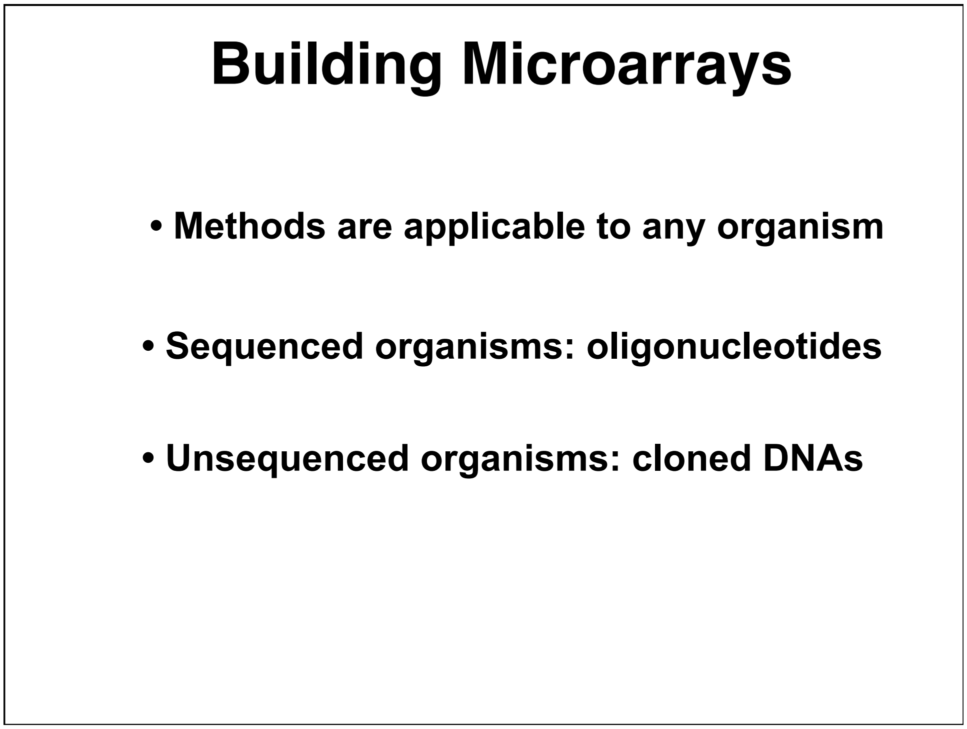
RANDOMLY POSITIONED HIGH DENSITY
ARRAYS OF ADDRESSABLE OLIGONUCLEOTIDES
COUPLED TO BEADS





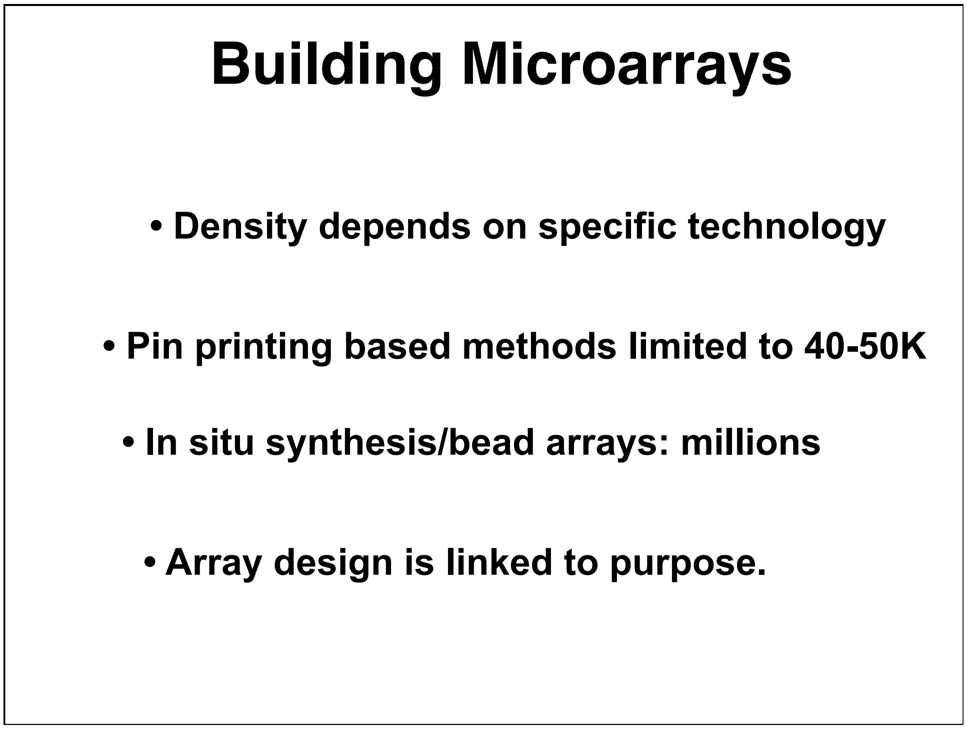
MICROARRAY READOUT

- Determine quantity of target bound to each probe in a complex hybridization
- Must have high sensitivity, low background
- High spatial resolution essential
- Dual channel capability useful
- Fluorescent tags meet these demands



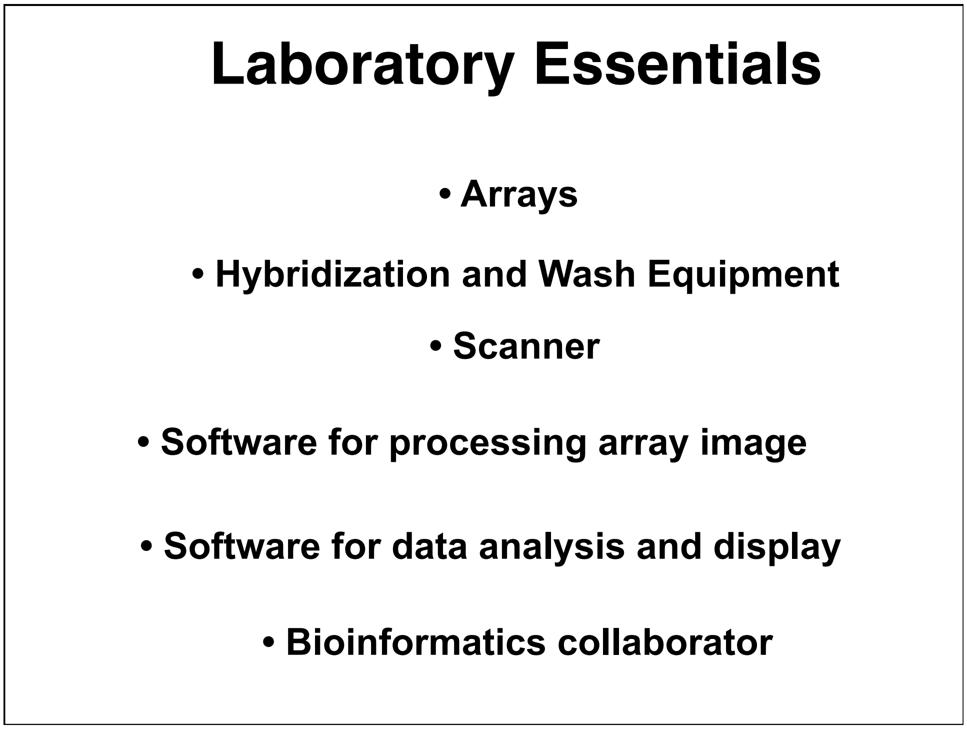
Building Microarrays

- Methods are applicable to any organism
- Sequenced organisms: oligonucleotides
- Unsequenced organisms: cloned DNAs



Building Microarrays

- Density depends on specific technology
- Pin printing based methods limited to 40-50K
- In situ synthesis/bead arrays: millions
- Array design is linked to purpose.



Laboratory Essentials

- Arrays
- Hybridization and Wash Equipment
- Scanner
- Software for processing array image
- Software for data analysis and display
- Bioinformatics collaborator

DNA Microarray Applications

- Gene Expression
- Comparative Genomic Hybridization
- Resequencing (SNPs)
- Transcription factor localization
- Chromatin/DNA modification

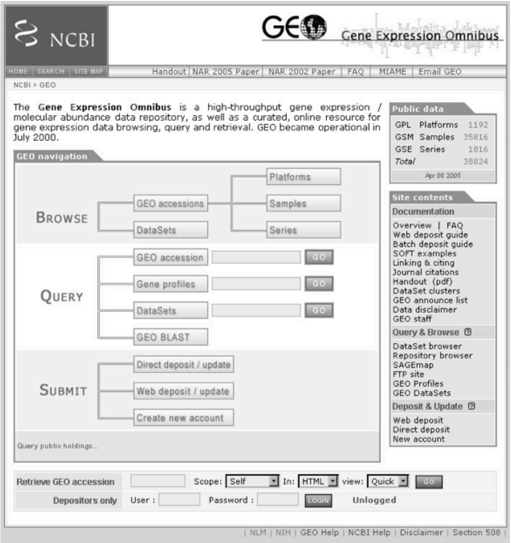
Reports on Microarray Data Quality

Nature Biotechnology

September 2006

Accessing Expression Data

- Individual Lab and Journal Sites; public databases



The **Gene Expression Omnibus** is a high-throughput gene expression / molecular abundance data repository, as well as a curated, online resource for gene expression data browsing, query and retrieval. GEO became operational in July 2000.

Public data

GPL	Platforms	1192
GSM	Samples	35816
GSE	Series	1816
Total		38824

Apr 08 2005

Retrieve GEO accession: Scope: Self In: HTML view: Quick GO

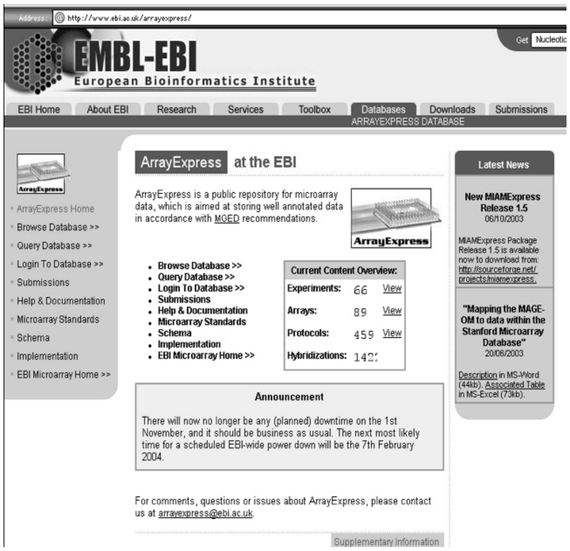
Depositors only: User : Password : LOGIN Unlogged

GEO

Currently contains expression data on 592,204 sample sets.

http://www.ncbi.nlm.nih.gov/geo/

Accessing Expression Data



ArrayExpress at the EBI

ArrayExpress is a public repository for microarray data, which is aimed at storing well annotated data in accordance with MGED recommendations.

- Browse Database >>
- Query Database >>
- Login To Database >>
- Submissions
- Help & Documentation
- Microarray Standards
- Schema
- Implementation
- EBI Microarray Home >>

Current Content Overview:		
Experiments:	66	View
Arrays:	89	View
Protocols:	459	View
Hybridizations:	142:	

Announcement

There will now no longer be any (planned) downtime on the 1st November, and it should be business as usual. The next most likely time for a scheduled EBI-wide power down will be the 7th February 2004.

For comments, questions or issues about ArrayExpress, please contact us at arrayexpress@ebi.ac.uk.

Latest News

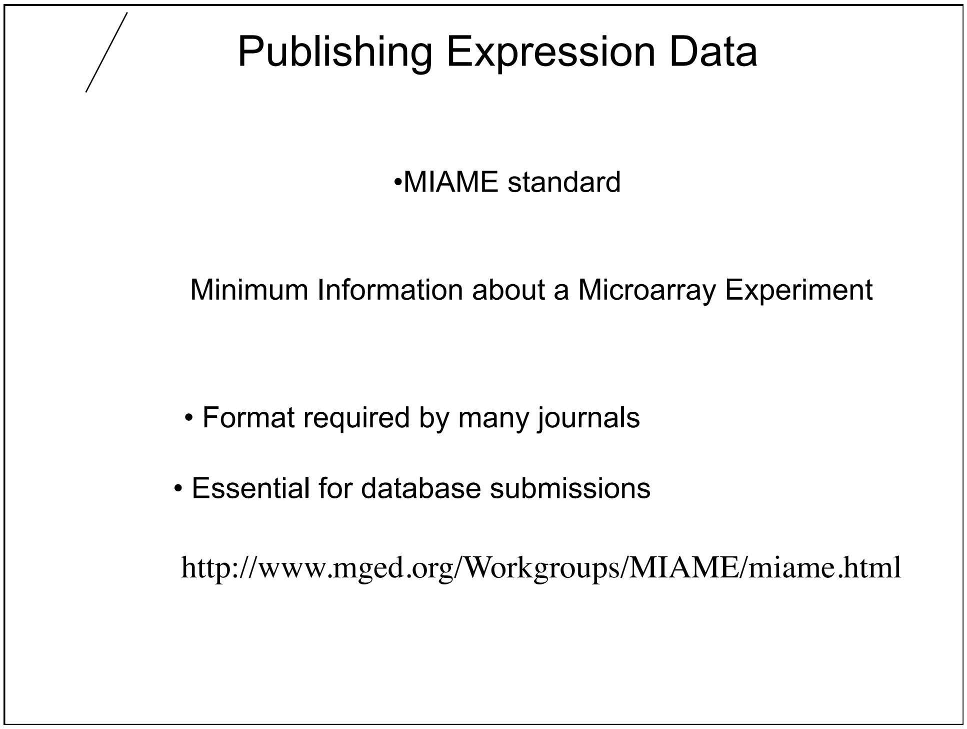
New MIAMExpress Release 1.5
06/10/2003

MIAMExpress Package Release 1.5 is available now to download from: http://sourceforge.net/projects/miamexpress.

"Mapping the MAGE-OM to data within the Stanford Microarray Database"
20/06/2003

Description in MS-Word (44kb). Associated Table in MS-Excel (73kb).

http://www.ebi.ac.uk/microarray-as/ae/



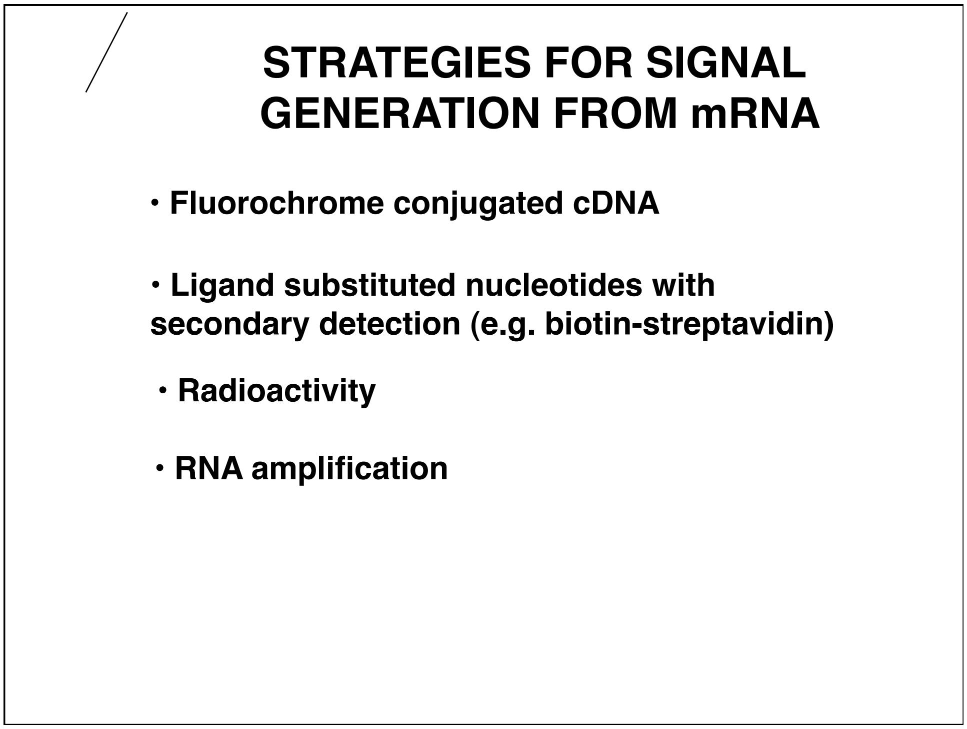
Publishing Expression Data

•MIAME standard

Minimum Information about a Microarray Experiment

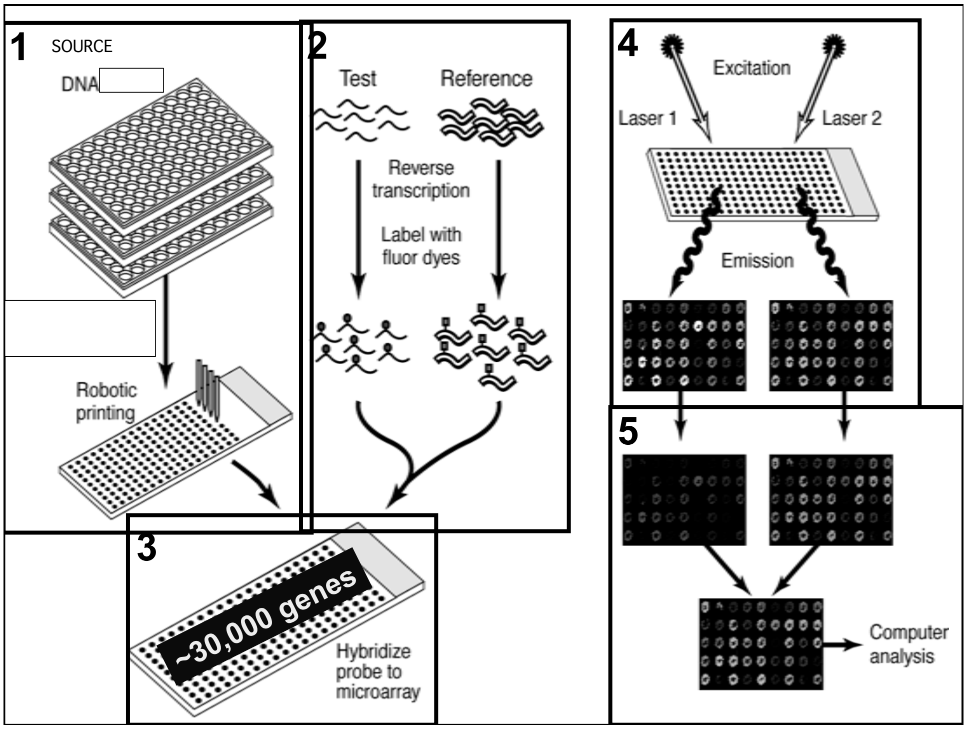
- Format required by many journals
- Essential for database submissions

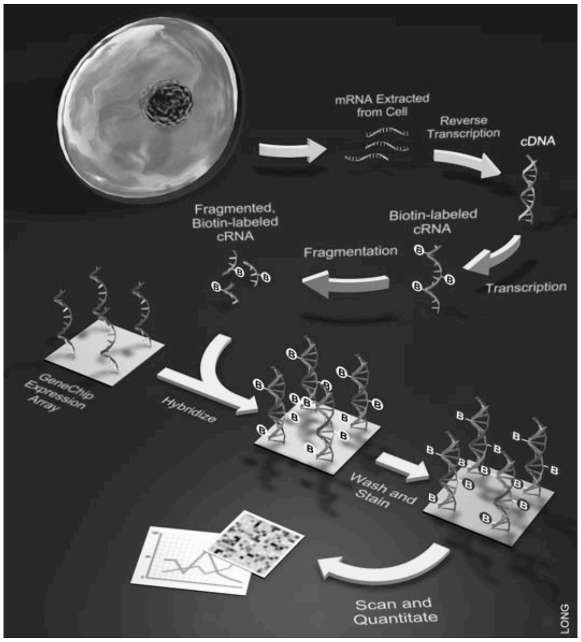
http://www.mged.org/Workgroups/MIAME/miame.html



STRATEGIES FOR SIGNAL GENERATION FROM mRNA

- **Fluorochrome conjugated cDNA**
- **Ligand substituted nucleotides with secondary detection (e.g. biotin-streptavidin)**
- **Radioactivity**
- **RNA amplification**



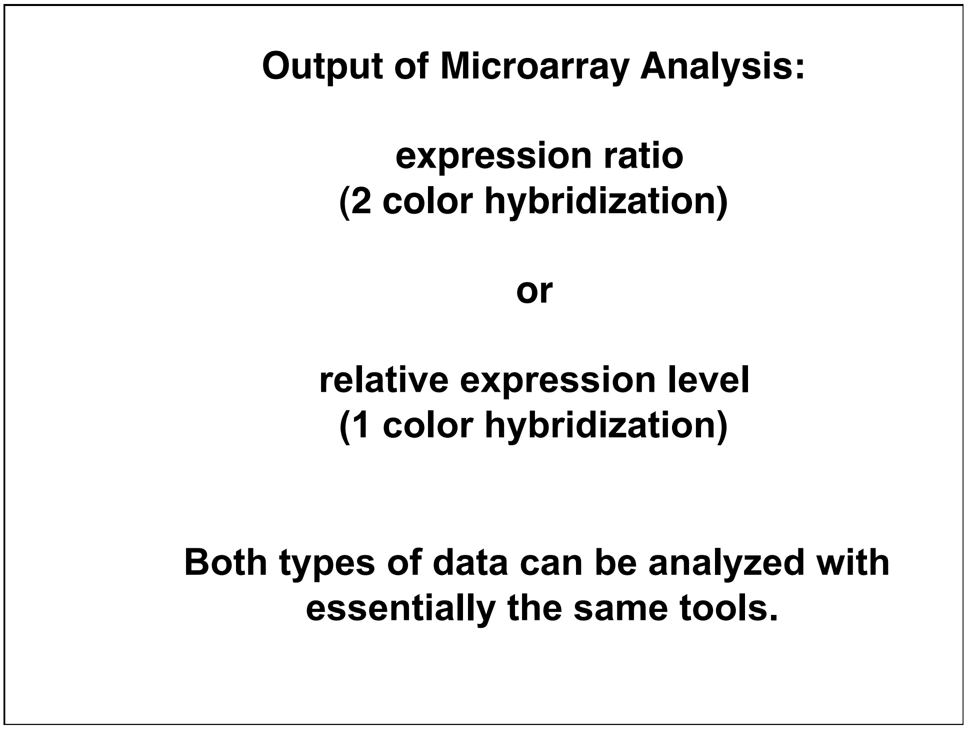


ONE COLOR

HYBRIDIZATION

ON AN OLIGO

ARRAY



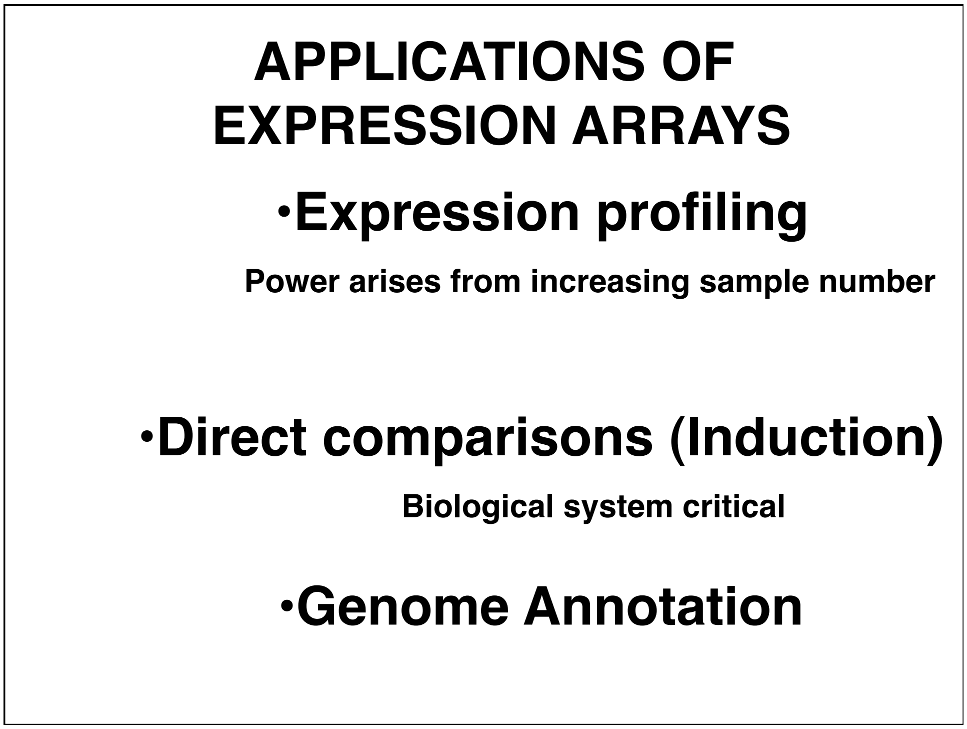
Output of Microarray Analysis:

expression ratio
(2 color hybridization)

or

relative expression level
(1 color hybridization)

Both types of data can be analyzed with essentially the same tools.



APPLICATIONS OF EXPRESSION ARRAYS

•Expression profiling

Power arises from increasing sample number

•Direct comparisons (Induction)

Biological system critical

•Genome Annotation

A RECURRING PROBLEM

Disease Genes

Transcription factors

Hormones/growth factors

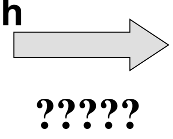
Drugs

Toxins

Infectious agents

Physical agents

siRNA's



<u>Downstream Genes</u>

•Direct targets

•Indirect targets

EXPRESSION DATA ANALYSIS

•Large amount of data

Examples: 200 samples x 25000 probes= 5,000,000 data points

•Requires analysis and visualization tools

Overview of microarray bioinformatics:
Simon R, Curr Opin Biotechnol. 2008 Feb;19(1):26-9.

EXPRESSION DATA ANALYSIS

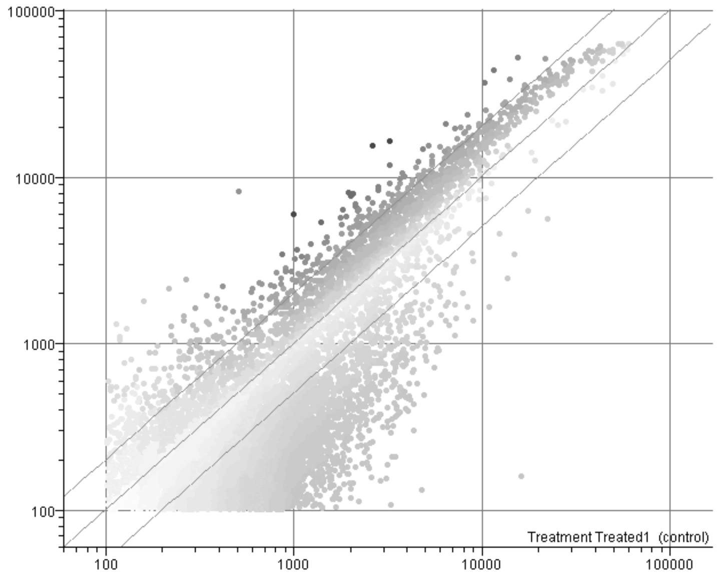
- Check quality of individual experiments
- **Preprocessing**

  Normalization

  Remove genes which are not accurately measured

  Remove genes which are similarly expressed in all samples
- **Unsupervised Clustering**
- **Supervised Clustering**

MICROARRAY SCATTER PLOT



Unsupervised Clustering

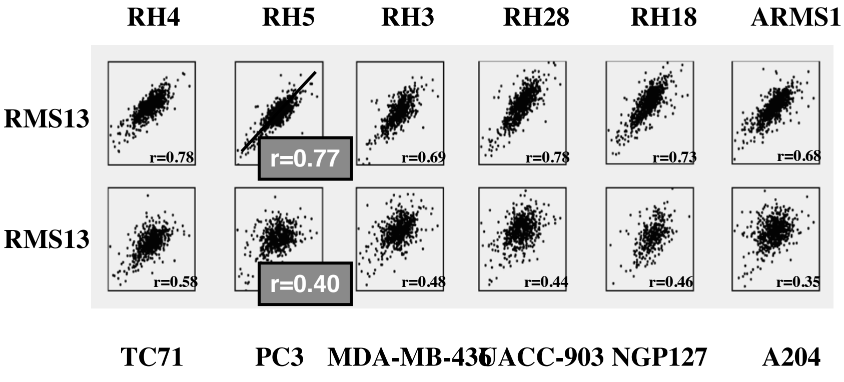
How do genes and samples organize into groups?

Powerful method of data display.

Does not prove the validity of groups.

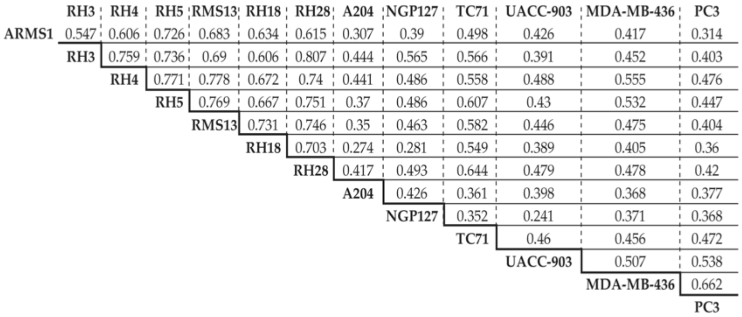
- **Clustered Samples Are Biologically Similar**
- **Clusters of Co-expressed genes**
  - **May be functionally related**
  - **May be enriched for pathways**

UNSUPERVISED CLUSTERING IS BASED ON A GLOBAL SIMILARITY METRIC



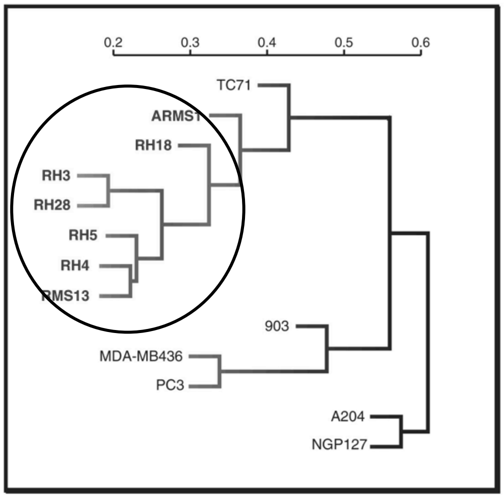
Khan et al. Can Res 58:5009

Matrix of Pearson Correlation Coefficients Distance Map

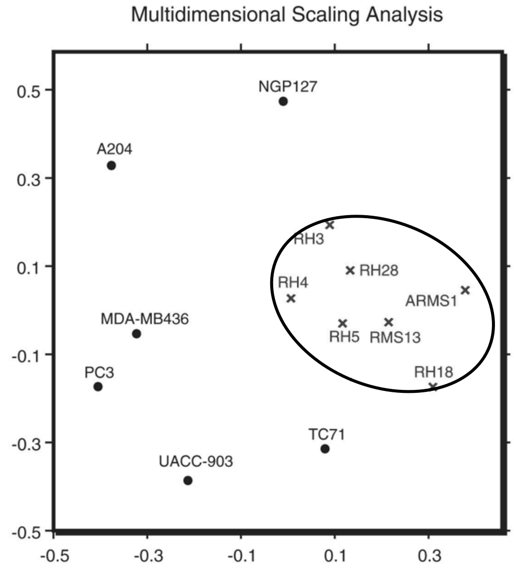


	RH3	RH4	RH5	RMS13	RH18	RH28	A204	NGP127	TC71	UACC-903	MDA-MB-436	PC3
ARMS1	0.547	0.606	0.726	0.683	0.634	0.615	0.307	0.39	0.498	0.426	0.417	0.314
RH3		0.759	0.736	0.69	0.606	0.807	0.444	0.565	0.566	0.391	0.452	0.403
RH4			0.771	0.778	0.672	0.74	0.441	0.486	0.558	0.488	0.555	0.476
RH5				0.769	0.667	0.751	0.37	0.486	0.607	0.43	0.532	0.447
RMS13					0.731	0.746	0.35	0.463	0.582	0.446	0.475	0.404
RH18						0.703	0.274	0.281	0.549	0.389	0.405	0.36
RH28							0.417	0.493	0.644	0.479	0.478	0.42
A204								0.426	0.361	0.398	0.368	0.377
NGP127									0.352	0.241	0.371	0.368
TC71										0.46	0.456	0.472
UACC-903											0.507	0.538
MDA-MB-436												0.662
PC3												

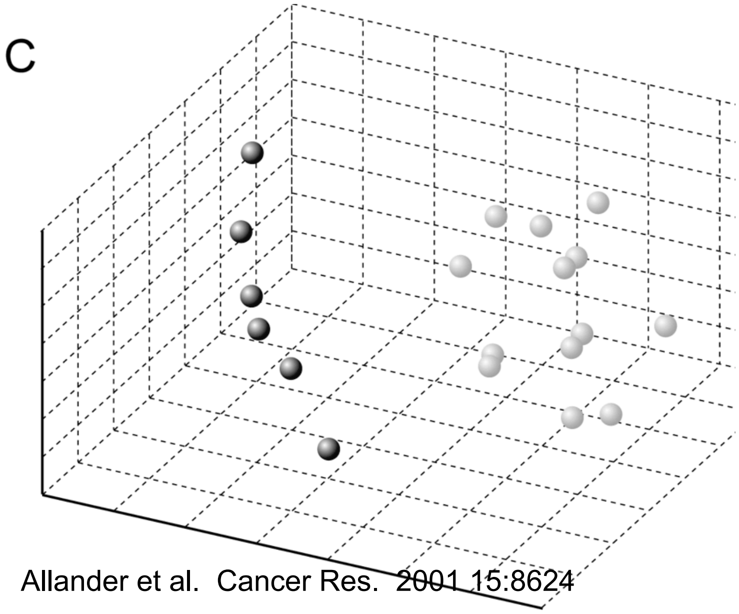
Hierarchical Clustering Dendrogram



DATA DISPLAY BY MULTIDIMENSIONAL SCALING

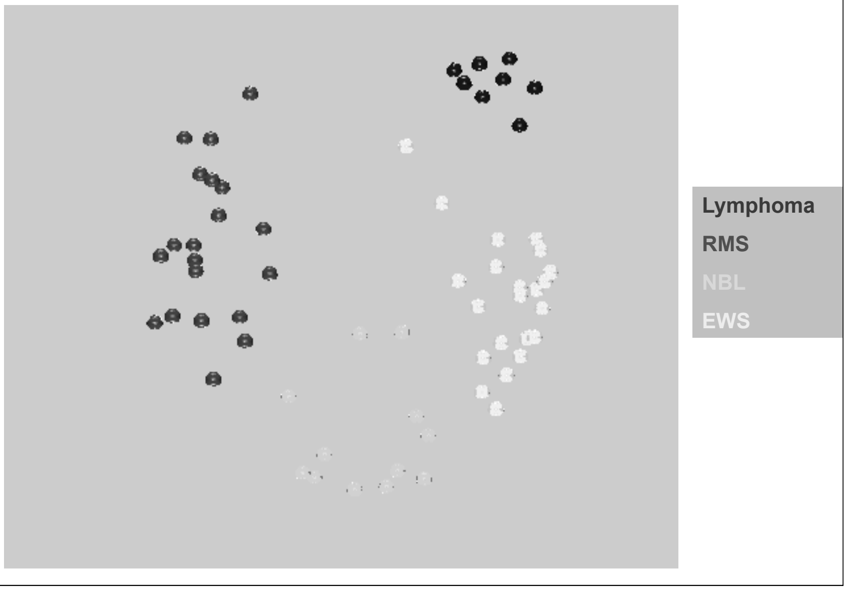


MDS PLOT



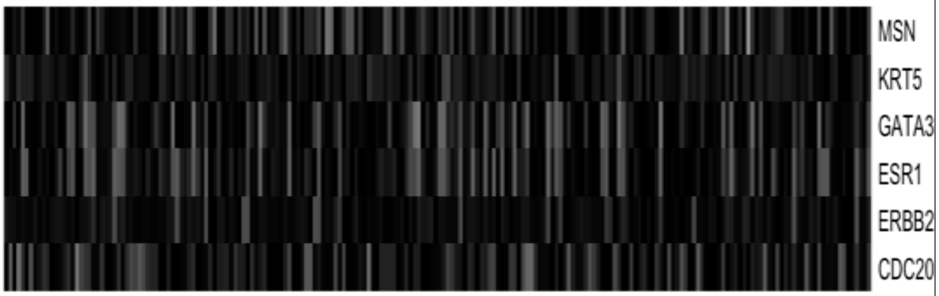
Allander et al. Cancer Res. 2001 15:8624

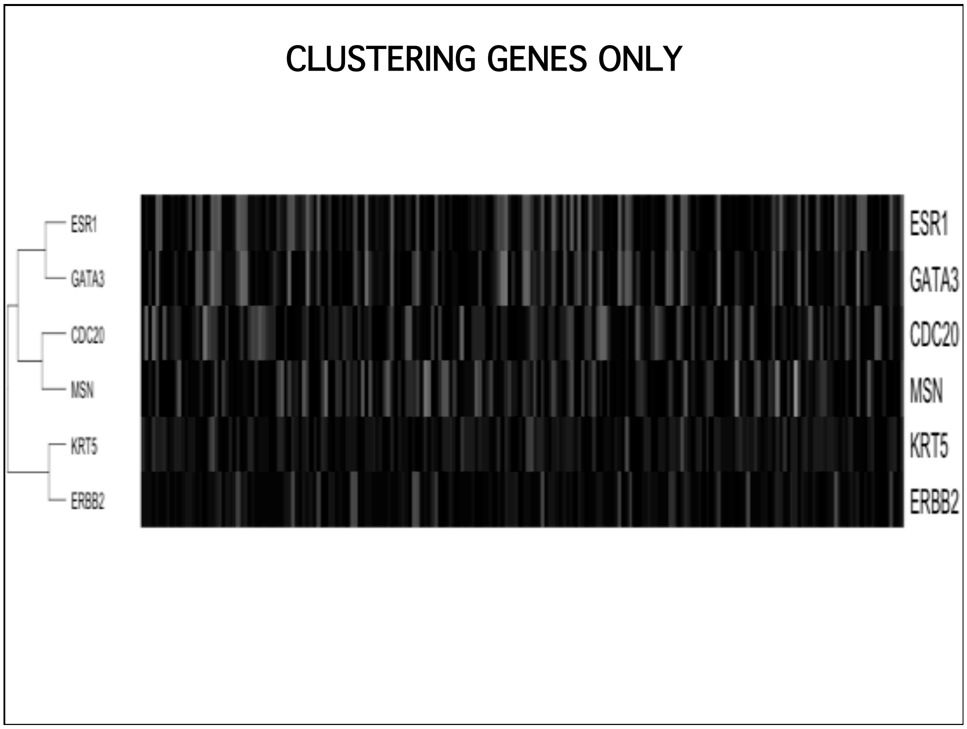
MULTIDIMENSIONAL SCALING OR PRINCIPLE COMPONENT ANALYSIS CAPTURE VARIATION IN SAMPLES AND ARE EXCELLENT VISUALIZATION TOOLS



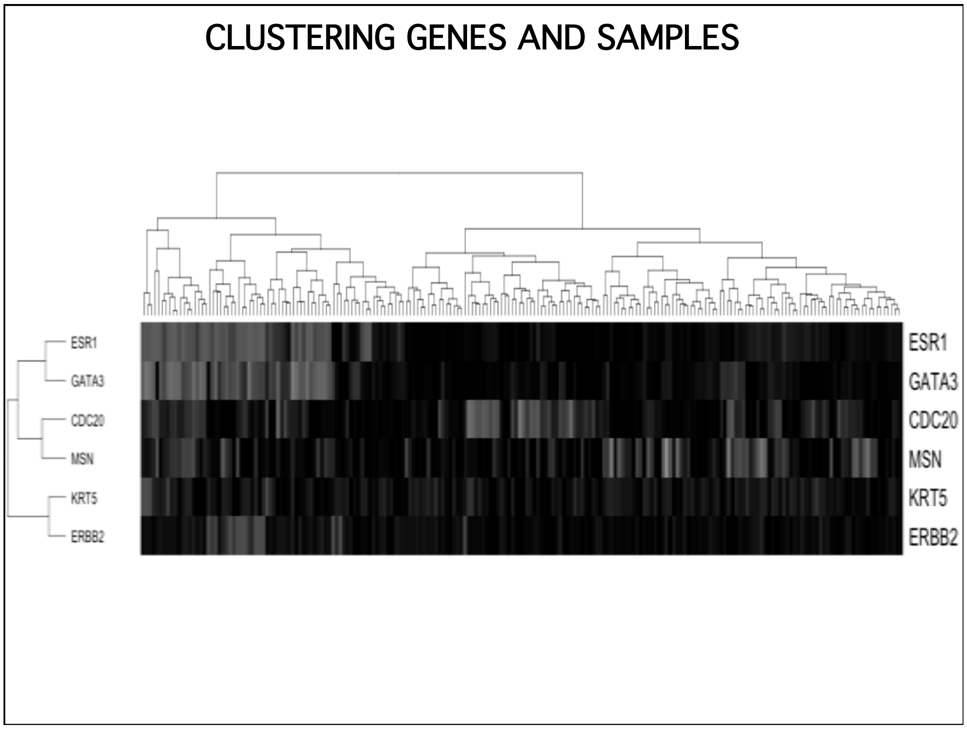
Khan et al
Nat Med
7:673

UNCLUSTERED DATA (6 GENES IN 200 SAMPLES)



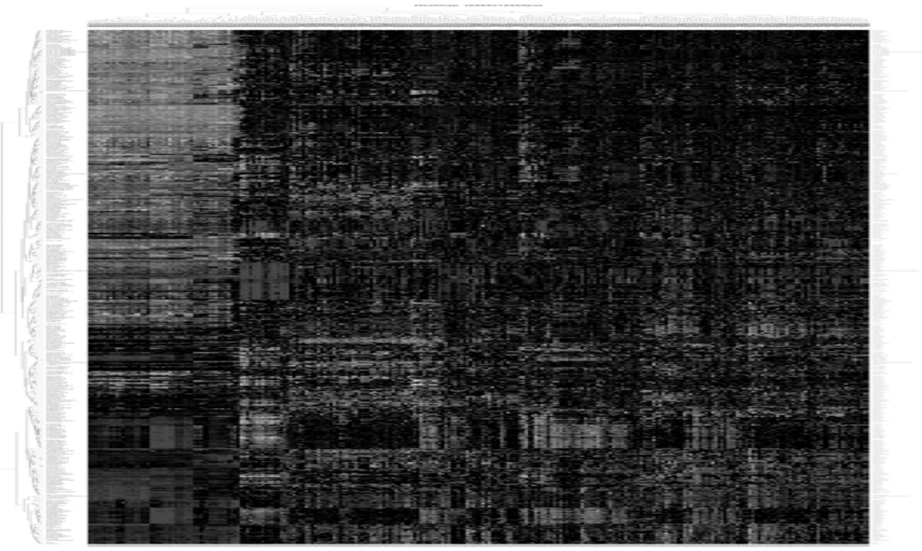


CLUSTERING GENES ONLY



CLUSTERING GENES AND SAMPLES

CLUSTERING GENES AND SAMPLES



DATA FROM GEO

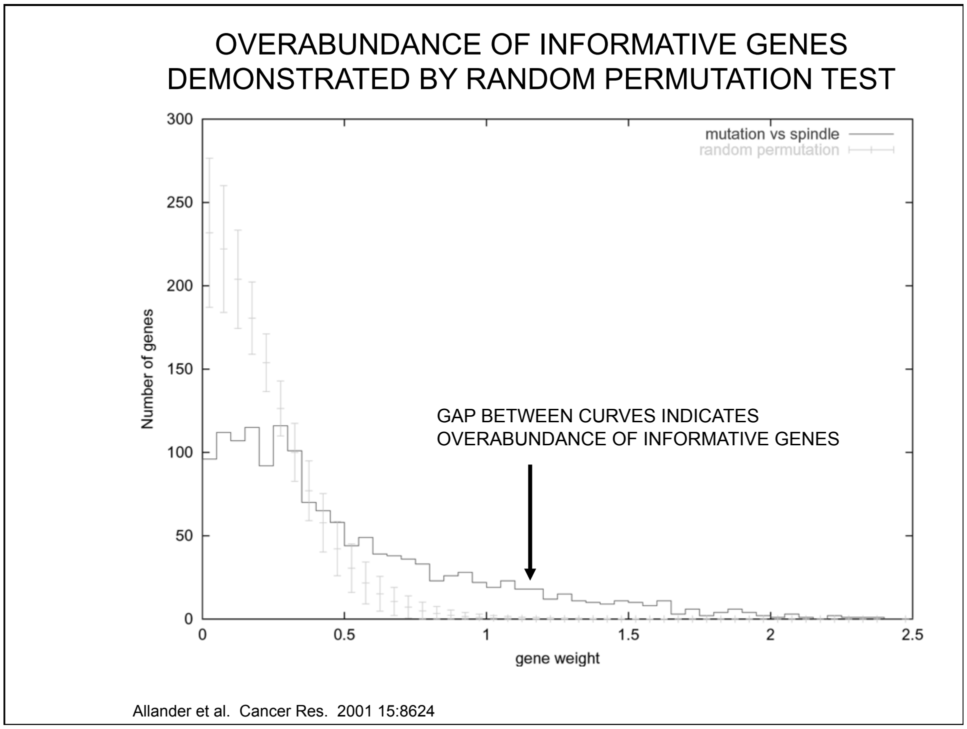
Supervised Clustering

What genes distinguish samples in selected groups from each other?

- **Choice of groups can be based on any known property of the samples.**
- **Many possible underlying methods: t-test or F-statistic frequently used.**
- **Output includes ranked gene list.**

- **Leads to the development of classifiers which can be applied to unknown samples.**

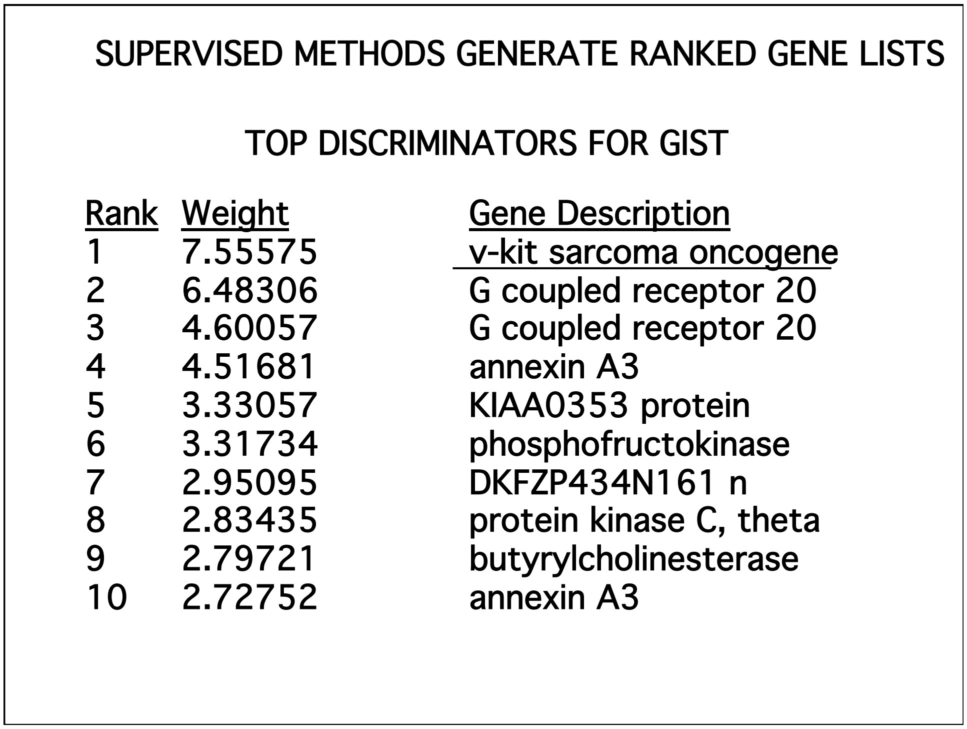
- **Must address the problem of false discovery due to multiple comparisons and discrepancy between sample/gene numbers.**



OVERABUNDANCE OF INFORMATIVE GENES
DEMONSTRATED BY RANDOM PERMUTATION TEST

GAP BETWEEN CURVES INDICATES
OVERABUNDANCE OF INFORMATIVE GENES

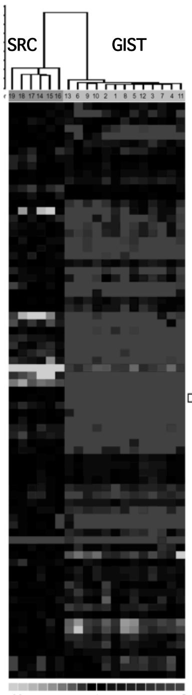
Allander et al. Cancer Res. 2001 15:8624



SUPERVISED METHODS GENERATE RANKED GENE LISTS

TOP DISCRIMINATORS FOR GIST

Rank	Weight	Gene Description
1	7.55575	v-kit sarcoma oncogene
2	6.48306	G coupled receptor 20
3	4.60057	G coupled receptor 20
4	4.51681	annexin A3
5	3.33057	KIAA0353 protein
6	3.31734	phosphofructokinase
7	2.95095	DKFZP434N161 n
8	2.83435	protein kinase C, theta
9	2.79721	butyrylcholinesterase
10	2.72752	annexin A3



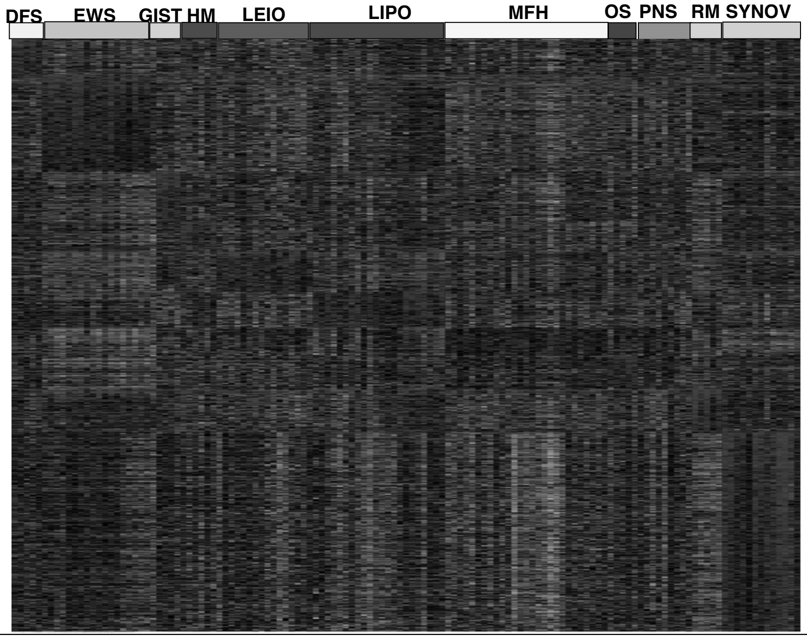
cloneID	Gene Symbol	Weight	α-value
378565	GHR	1	0
795309	SOD3	1.4	0
486641	MOCS2	1.5	0.00003
629896	MAP1B	1.8	0.00008
67769	KCNK3	1.7	0.00003
40304	SDHA	1.6	0.00006
2800976	CDX1	1.6	0
2556956	GOT2	1.1	0.0001
841370	GOT2	1.2	0.00003
42059	DLD	1.9	0.00001
41940	CXORF1	1.5	0.00006
878815	ARF3	1.4	0.00004
47202	ARF1	1.4	0
171912	EST	1.6	0.00008
347268	EST	1.5	0
1957165	RAB-R	2	0
2510105	PHF1	1.5	0.0001
450386	PHF1	1.6	0.00006
1461664	BCHE	2.8	0
2113077	KCNH2	1.9	0
50266	EST	1.7	0
824792	BM045	1.4	0.00009
292939	EST	1.3	0.00006
77533	INPP5A	1.9	0.00006
1049346	EST	1.5	0.00004
970684	MAP2	2.3	0.00008
789014	SLC12A4	2.3	0
789011	AAMP	1.7	0.00004
82225	SFRP1	1.9	0.00006
1435103	ATP2A3	2.1	0.00002
22493	RPL26	1.8	0.00004
52741	ABCA3	2.3	0
773421	EST	2.6	0
840575	EST	3	0.00008
2920924	ANXA3	2.7	0
489626	PFKM	3.3	0
1161564	DMN	3.3	0
2469213	ANXA3	4.5	0
2568905	GPR20	6.5	0
269806	KIT	7.6	0
375827	PTP4A3	2.6	0
174627	SCG2	2.6	0
205239	PRKCQ	2.8	0
2832322	TNFRSF6B	2	0
2164126	PRKCQ	2.6	0
303139	EST	2.4	0
2442397	ALDH2	1.6	0.00008
40026	SLC25A4	2.1	0
37196	EST	2.2	0.00008
39993	SOD1	1.6	0
950489	SOD1	1.4	0.00008
212165	PRDX2	1.6	0
2506776	PRDX2	1.3	0.00003
563444	FOXF1	1.6	0
753420	EST	1.9	0
2551468	GUCY1A3	1.6	0.00004
160485	GUCY1A3	1.5	0.00008
264951	NBS1	1.5	0.00008
839991	COL1A2	1.5	0.00001
1471829	DDXL	1.1	0.00004
2541394	EIF3S7	1.6	0
2576914	AKR1A1	1.5	0
44307	H2BFQ	1.5	0
745496	EST	1.3	0.00008
1594019	SUPT4H1	1.7	0
1492412	EST	1.6	0.00008
949940	RPL27A	1.5	0.00005
971367	RPS8	1.9	0
2467016	RPS27	1.3	0.00007
2548661	TIM44	1.7	0
2548854	RPL12	1.6	0.00008
2577093	MST1	1.7	0.0001
33632	GOT1	1.4	0
2578421	IGFBP4	1.3	0.00009
2545705	CNN3	1.6	0
486787	CNN3	1.5	0
755762	GRN	1.3	0.00003

HIERARCHICAL CLUSTERING OF SAMPLES/GENES DISCRIMINATING GASTROINTESTINAL STROMAL TUMOR USING THE GENES SELECTED BY SUPERVISED ANALYSIS

KIT, THE MUTATIONAL AND THERAPEUTIC TARGET IS HIGHLY SIGNIFICANT.

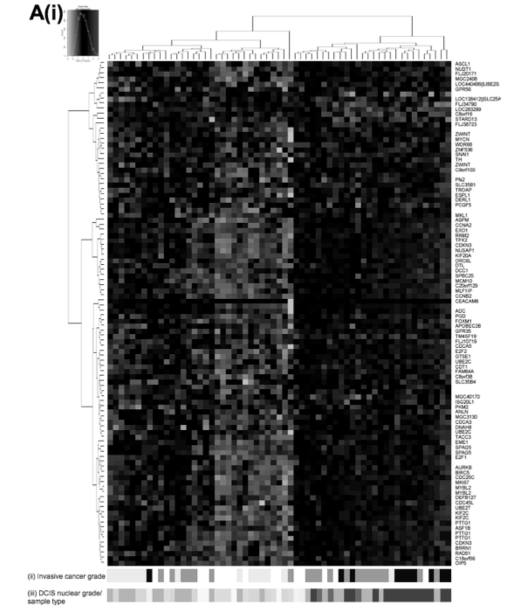
Allander et al. Cancer Res. 2001 15:8624

CHARACTERISTIC PATTERNS OF GENE EXPRESSION IN DIFFERENT SARCOMAS



BAIRD ET AL.
CANCER RES
2005

CLUSTERING GENES AND SAMPLES

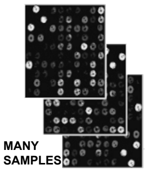


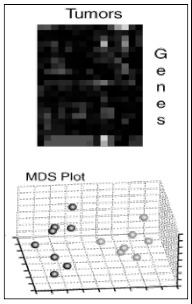
Separation
Of High and
Low Grade
Ductal Carcinoma
In Situ

Balleine et al. 14:8244 Clin Can Res 2008

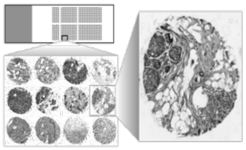
GENOMICS FROM BENCH TO BEDSIDE

WHOLE GENOME

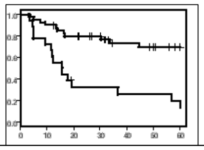




GENE SELECTION



GENE VALIDATION

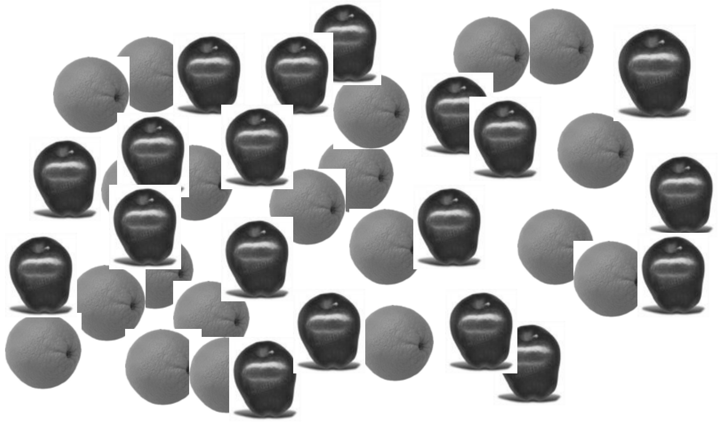


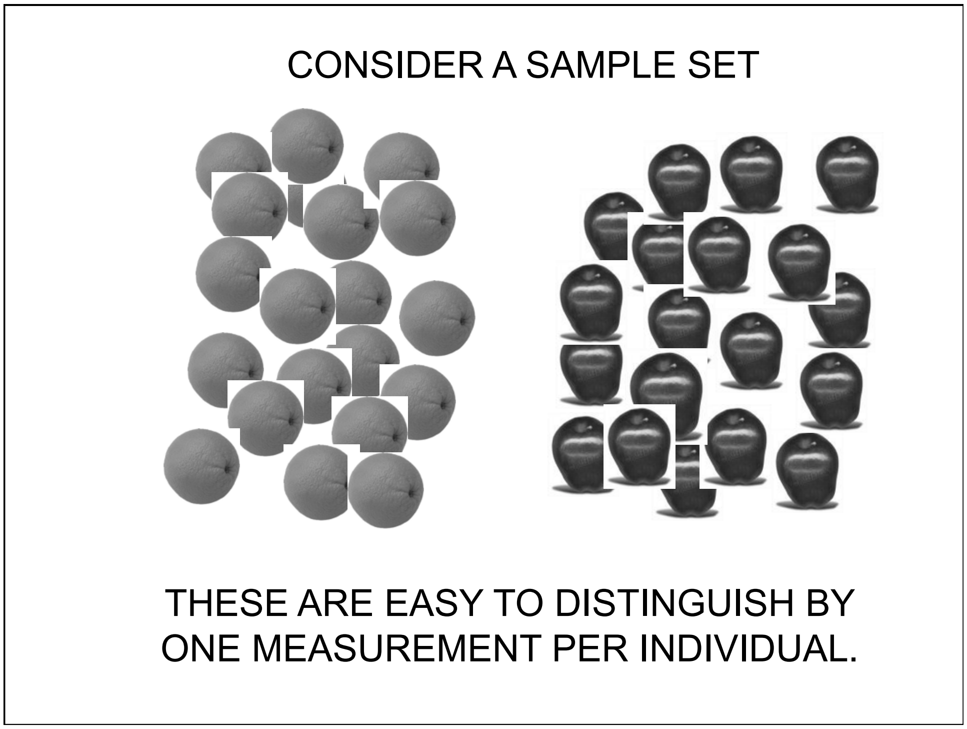
ASSAY DEVELOPMENT

SIGNAL STRENGTH VARIES IN TISSUE PROFILING EXPERIMENTS

THE MOST INTERESTING QUESTIONS TEND TO BE ASSOCIATED WITH WEAKER SIGNAL.

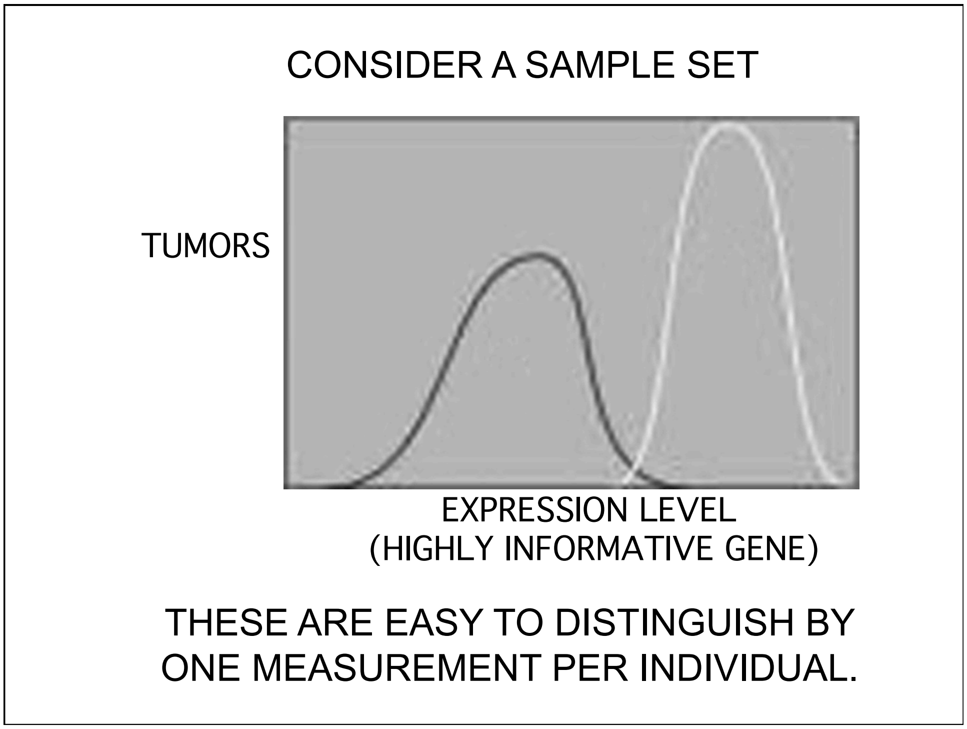
CONSIDER A SAMPLE SET





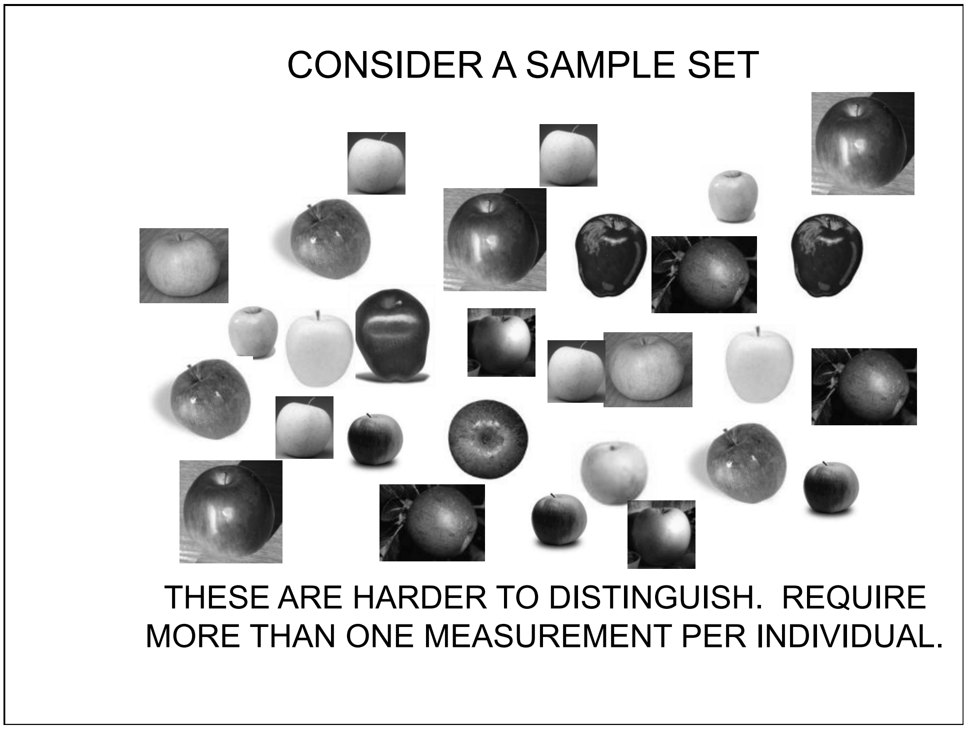
CONSIDER A SAMPLE SET

THESE ARE EASY TO DISTINGUISH BY ONE MEASUREMENT PER INDIVIDUAL.



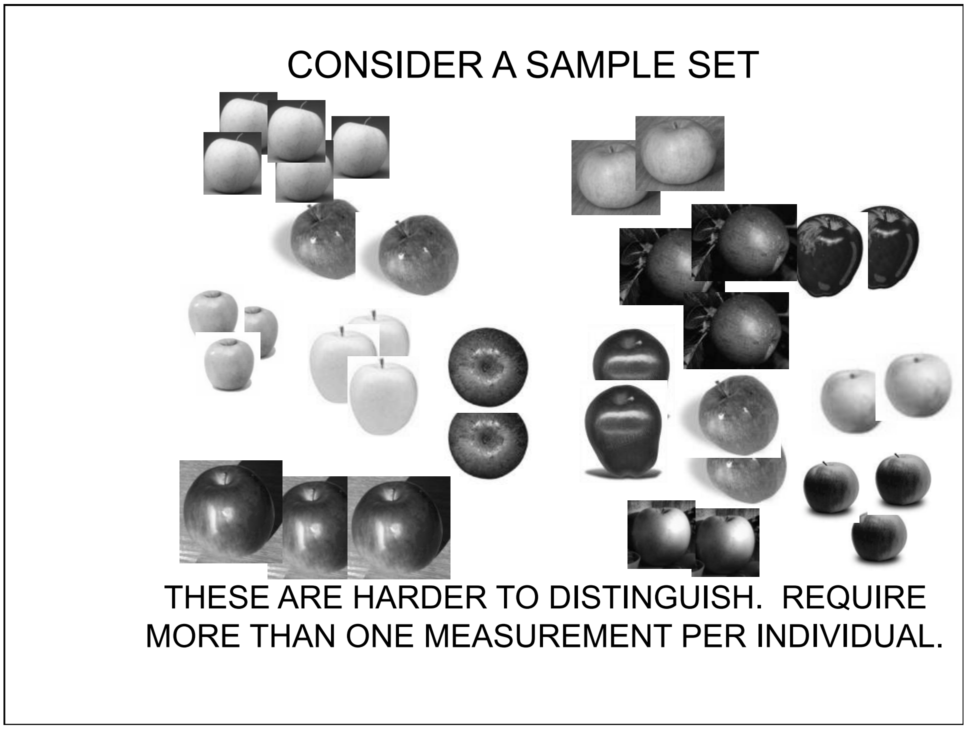
CONSIDER A SAMPLE SET

THESE ARE EASY TO DISTINGUISH BY ONE MEASUREMENT PER INDIVIDUAL.



CONSIDER A SAMPLE SET

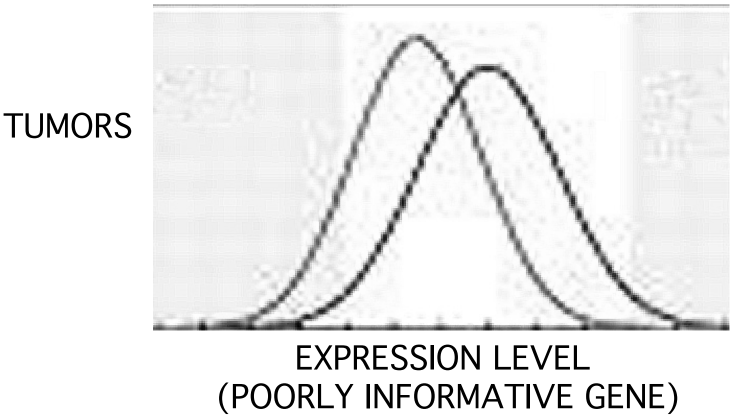
THESE ARE HARDER TO DISTINGUISH. REQUIRE MORE THAN ONE MEASUREMENT PER INDIVIDUAL.



CONSIDER A SAMPLE SET

THESE ARE HARDER TO DISTINGUISH. REQUIRE MORE THAN ONE MEASUREMENT PER INDIVIDUAL.

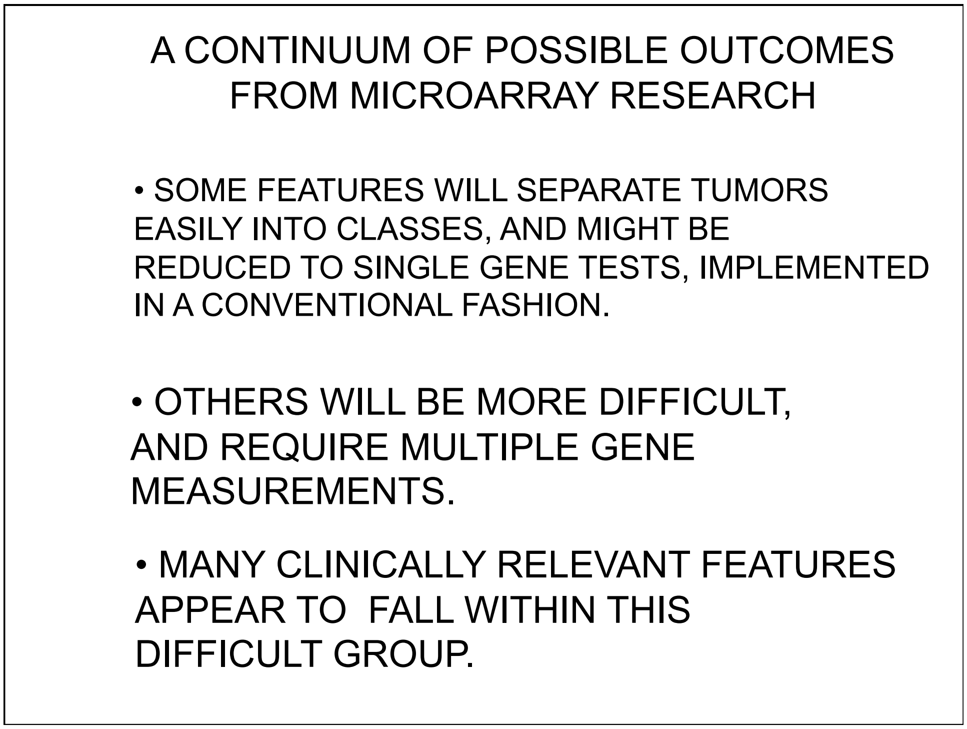
CONSIDER A SAMPLE SET



THESE ARE HARDER TO DISTINGUISH. REQUIRE MORE THAN ONE MEASUREMENT PER INDIVIDUAL.

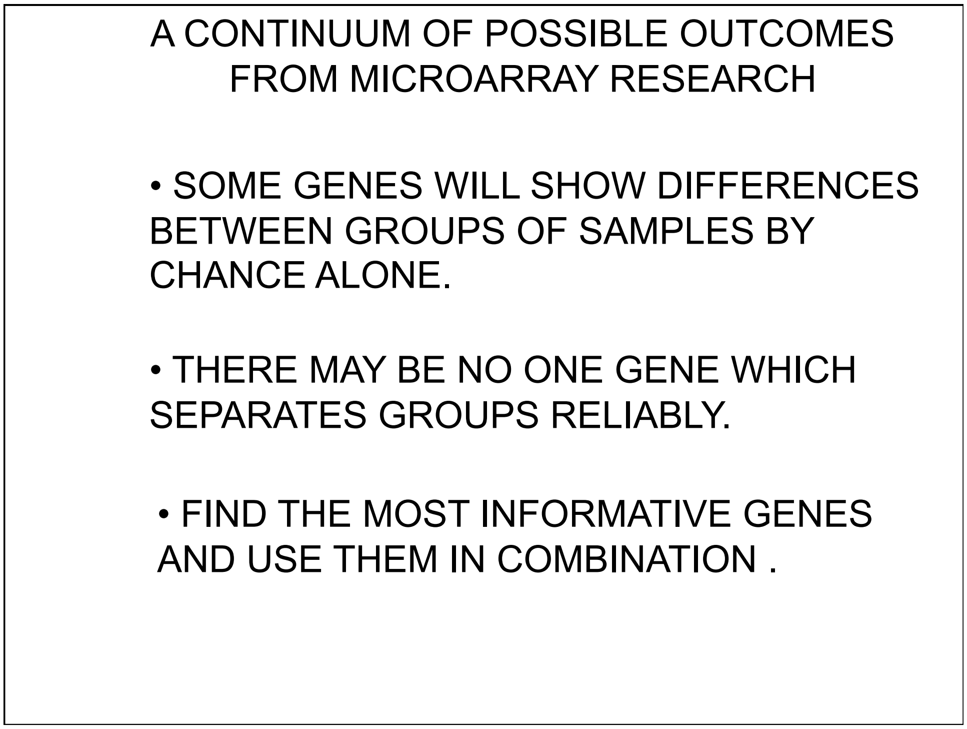
WE CAN TELL APPLES FROM ORANGES.

CAN WE DISTINGUISH DIFFERENT KINDS OF APPLES?



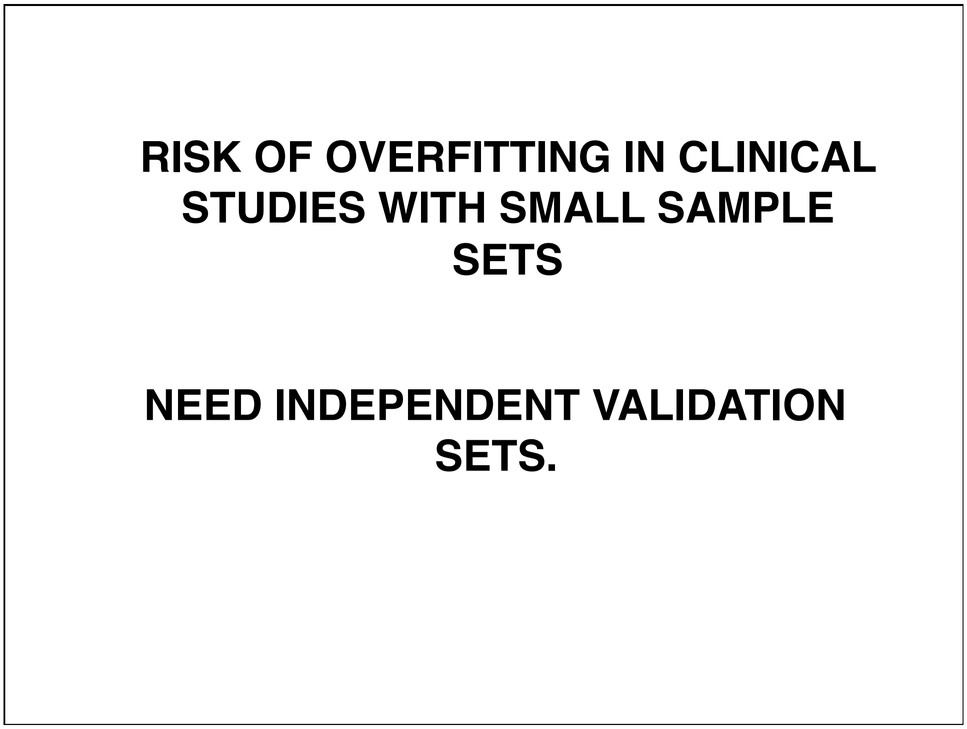
A CONTINUUM OF POSSIBLE OUTCOMES FROM MICROARRAY RESEARCH

- SOME FEATURES WILL SEPARATE TUMORS EASILY INTO CLASSES, AND MIGHT BE REDUCED TO SINGLE GENE TESTS, IMPLEMENTED IN A CONVENTIONAL FASHION.
- OTHERS WILL BE MORE DIFFICULT, AND REQUIRE MULTIPLE GENE MEASUREMENTS.
- MANY CLINICALLY RELEVANT FEATURES APPEAR TO FALL WITHIN THIS DIFFICULT GROUP.



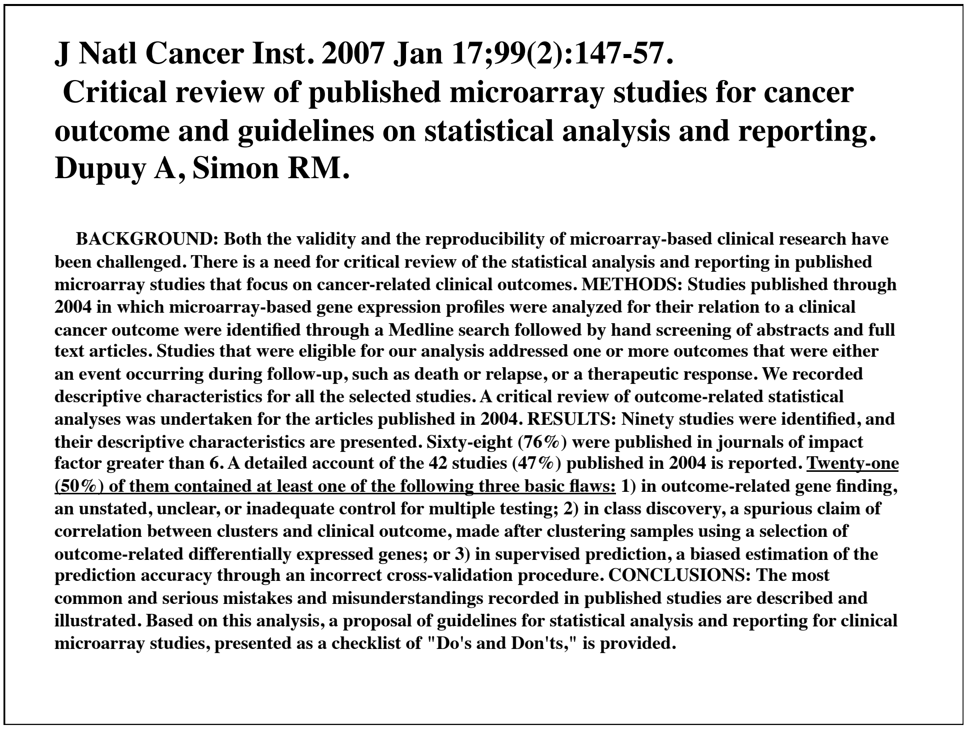
A CONTINUUM OF POSSIBLE OUTCOMES FROM MICROARRAY RESEARCH

- SOME GENES WILL SHOW DIFFERENCES BETWEEN GROUPS OF SAMPLES BY CHANCE ALONE.
- THERE MAY BE NO ONE GENE WHICH SEPARATES GROUPS RELIABLY.
- FIND THE MOST INFORMATIVE GENES AND USE THEM IN COMBINATION .



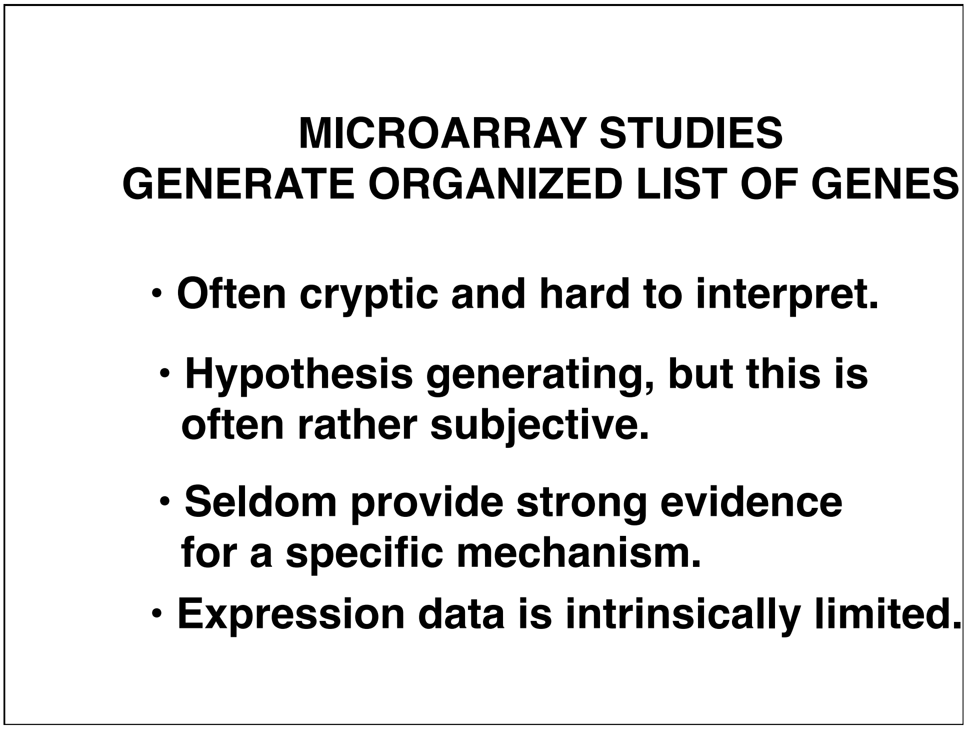
RISK OF OVERFITTING IN CLINICAL STUDIES WITH SMALL SAMPLE SETS

NEED INDEPENDENT VALIDATION SETS.



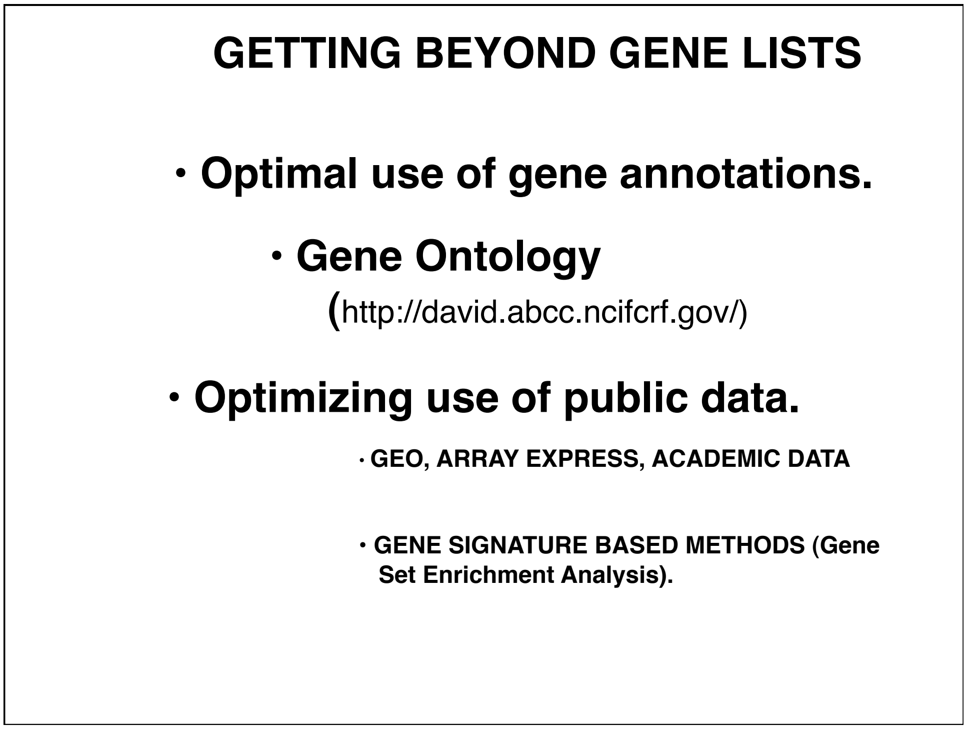
J Natl Cancer Inst. 2007 Jan 17;99(2):147-57.
Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting.
Dupuy A, Simon RM.

BACKGROUND: Both the validity and the reproducibility of microarray-based clinical research have been challenged. There is a need for critical review of the statistical analysis and reporting in published microarray studies that focus on cancer-related clinical outcomes. METHODS: Studies published through 2004 in which microarray-based gene expression profiles were analyzed for their relation to a clinical cancer outcome were identified through a Medline search followed by hand screening of abstracts and full text articles. Studies that were eligible for our analysis addressed one or more outcomes that were either an event occurring during follow-up, such as death or relapse, or a therapeutic response. We recorded descriptive characteristics for all the selected studies. A critical review of outcome-related statistical analyses was undertaken for the articles published in 2004. RESULTS: Ninety studies were identified, and their descriptive characteristics are presented. Sixty-eight (76%) were published in journals of impact factor greater than 6. A detailed account of the 42 studies (47%) published in 2004 is reported. Twenty-one (50%) of them contained at least one of the following three basic flaws: 1) in outcome-related gene finding, an unstated, unclear, or inadequate control for multiple testing; 2) in class discovery, a spurious claim of correlation between clusters and clinical outcome, made after clustering samples using a selection of outcome-related differentially expressed genes; or 3) in supervised prediction, a biased estimation of the prediction accuracy through an incorrect cross-validation procedure. CONCLUSIONS: The most common and serious mistakes and misunderstandings recorded in published studies are described and illustrated. Based on this analysis, a proposal of guidelines for statistical analysis and reporting for clinical microarray studies, presented as a checklist of "Do's and Don'ts," is provided.



MICROARRAY STUDIES GENERATE ORGANIZED LIST OF GENES

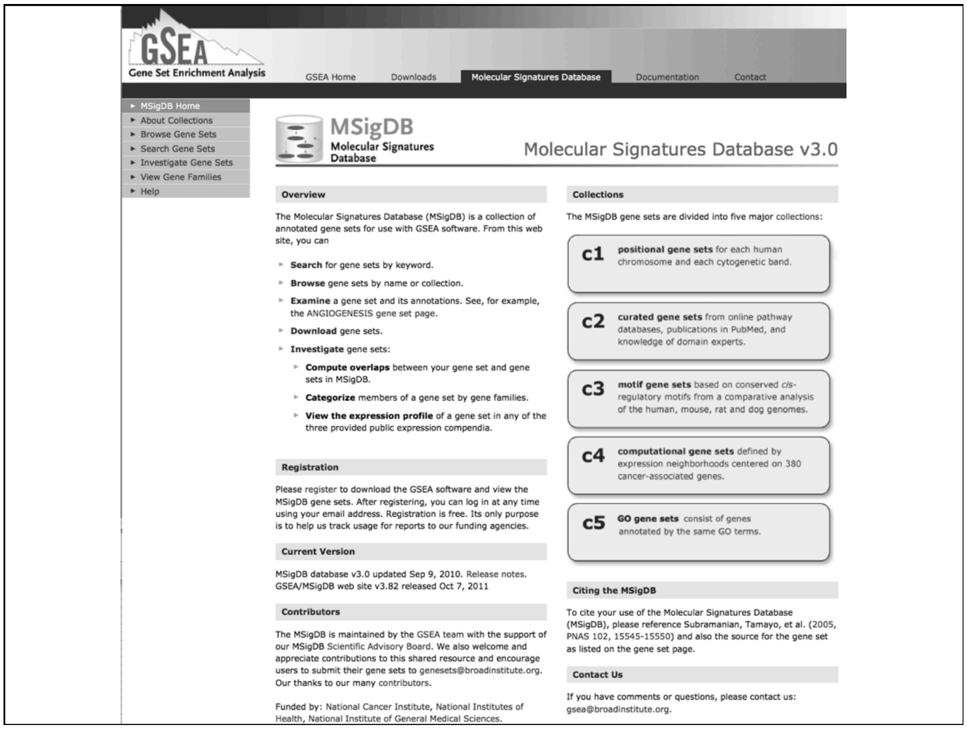
- Often cryptic and hard to interpret.
- Hypothesis generating, but this is often rather subjective.
- Seldom provide strong evidence for a specific mechanism.
- Expression data is intrinsically limited.



GETTING BEYOND GENE LISTS

- Optimal use of gene annotations.
  - Gene Ontology

    (http://david.abcc.ncifcrf.gov/)
- Optimizing use of public data.
  - GEO, ARRAY EXPRESS, ACADEMIC DATA
  - GENE SIGNATURE BASED METHODS (Gene Set Enrichment Analysis).



Molecular Signatures Database v3.0

Overview

The Molecular Signatures Database (MSigDB) is a collection of annotated gene sets for use with GSEA software. From this web site, you can

- **Search** for gene sets by keyword.
- **Browse** gene sets by name or collection.
- **Examine** a gene set and its annotations. See, for example, the ANGIOGENESIS gene set page.
- **Download** gene sets.
- **Investigate** gene sets:
  - **Compute overlaps** between your gene set and gene sets in MSigDB.
  - **Categorize** members of a gene set by gene families.
  - **View the expression profile** of a gene set in any of the three provided public expression compendia.

Registration

Please register to download the GSEA software and view the MSigDB gene sets. After registering, you can log in at any time using your email address. Registration is free. Its only purpose is to help us track usage for reports to our funding agencies.

Current Version

MSigDB database v3.0 updated Sep 9, 2010. Release notes.
GSEA/MSigDB web site v3.82 released Oct 7, 2011

Contributors

The MSigDB is maintained by the GSEA team with the support of our MSigDB Scientific Advisory Board. We also welcome and appreciate contributions to this shared resource and encourage users to submit their gene sets to genesets@broadinstitute.org. Our thanks to our many contributors.

Funded by: National Cancer Institute, National Institutes of Health, National Institute of General Medical Sciences.

Collections

The MSigDB gene sets are divided into five major collections:

c1 **positional gene sets** for each human chromosome and each cytogenetic band.

c2 **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.

c3 **motif gene sets** based on conserved *cis*-regulatory motifs from a comparative analysis of the human, mouse, rat and dog genomes.

c4 **computational gene sets** defined by expression neighborhoods centered on 380 cancer-associated genes.

c5 **GO gene sets** consist of genes annotated by the same GO terms.

Citing the MSigDB

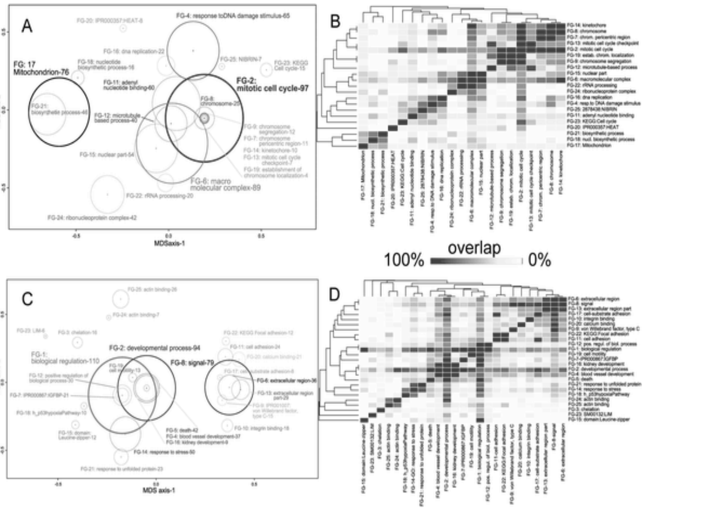
To cite your use of the Molecular Signatures Database (MSigDB), please reference Subramanian, Tamayo, et al. (2005, PNAS 102, 15545-15550) and also the source for the gene set as listed on the gene set page.

Contact Us

If you have comments or questions, please contact us: gsea@broadinstitute.org.

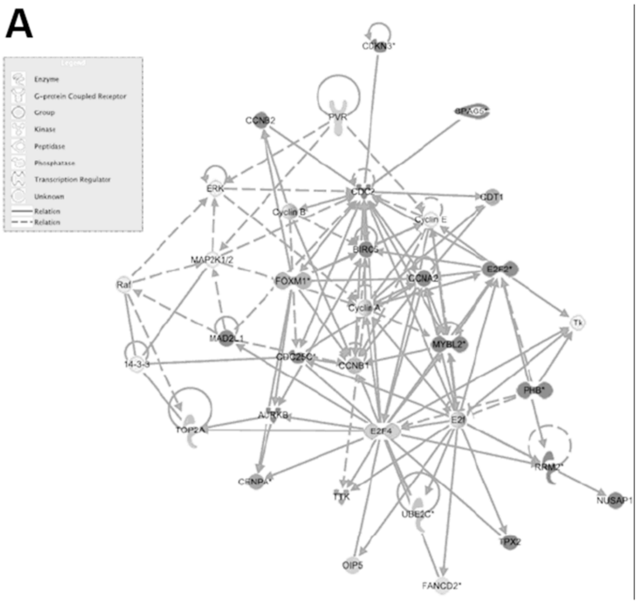
GENE ONTOLOGY AND PROMOTER DATABASES CAN HELP FIND BIOLOGY

GENE ONTOLOGY CATEFORIES AFFECTED BY ONCOGENE KNOCKDOWN IN EWING'S SARCOMA

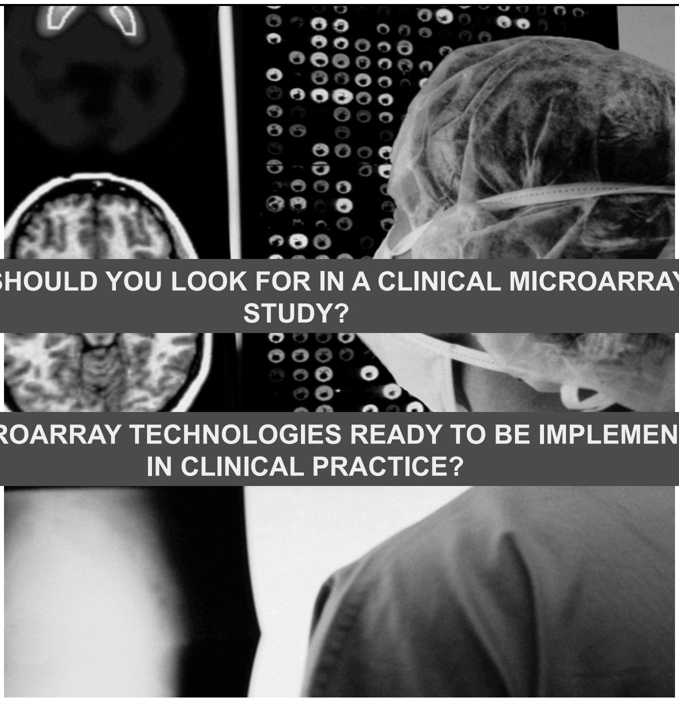


KAUER ET AL. PLOS ONE 4:e5415 2009

Pathway Analysis

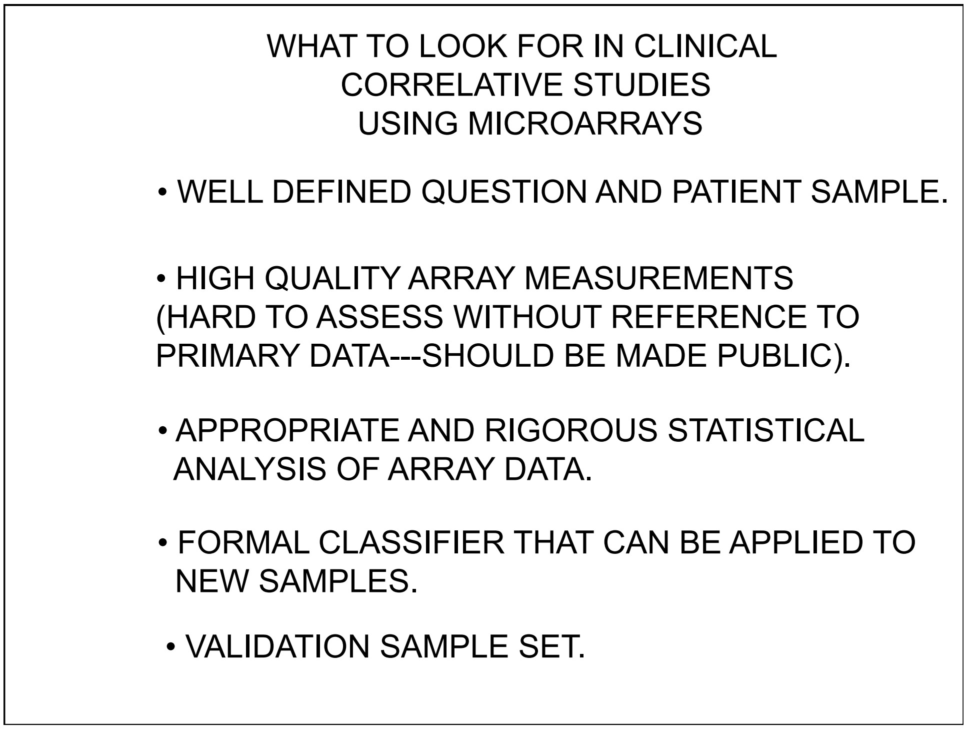


Balleine et al. 14:8244 Clin Can Res 2008



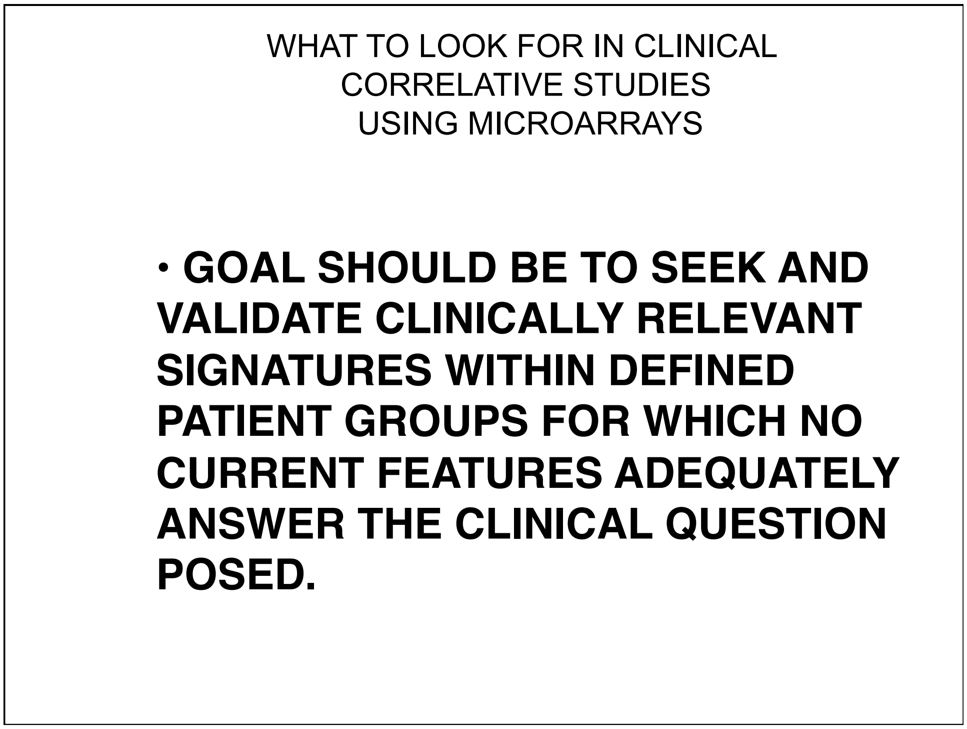
WHAT SHOULD YOU LOOK FOR IN A CLINICAL MICROARRAY STUDY?

ARE MICROARRAY TECHNOLOGIES READY TO BE IMPLEMENTED IN CLINICAL PRACTICE?



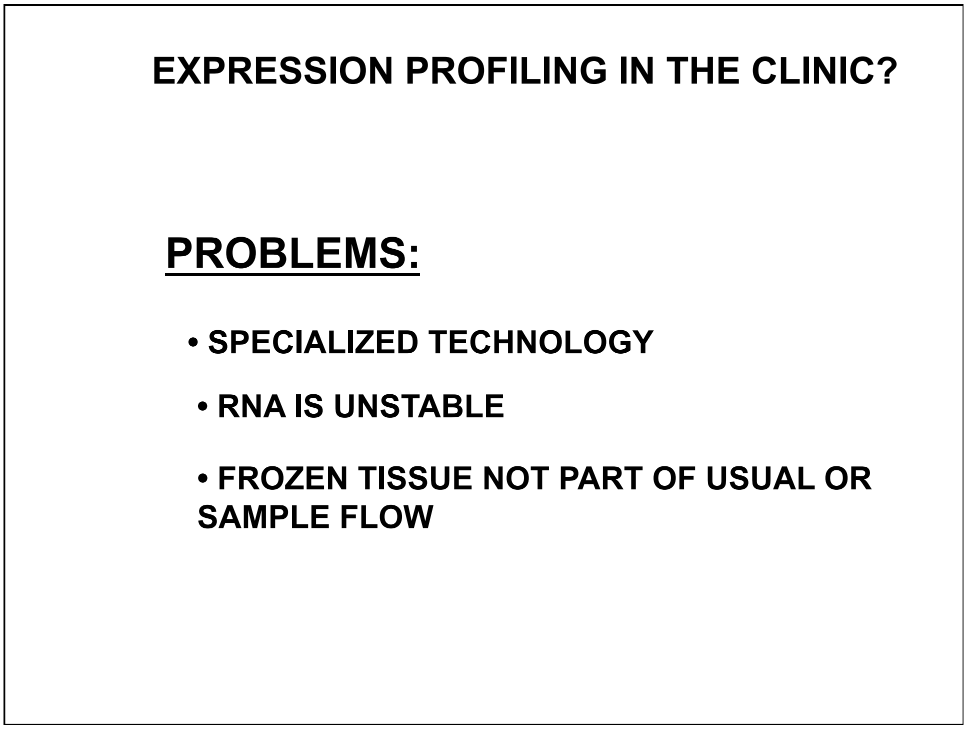
WHAT TO LOOK FOR IN CLINICAL CORRELATIVE STUDIES USING MICROARRAYS

- WELL DEFINED QUESTION AND PATIENT SAMPLE.
- HIGH QUALITY ARRAY MEASUREMENTS (HARD TO ASSESS WITHOUT REFERENCE TO PRIMARY DATA---SHOULD BE MADE PUBLIC).
- APPROPRIATE AND RIGOROUS STATISTICAL ANALYSIS OF ARRAY DATA.
- FORMAL CLASSIFIER THAT CAN BE APPLIED TO NEW SAMPLES.
- VALIDATION SAMPLE SET.



WHAT TO LOOK FOR IN CLINICAL CORRELATIVE STUDIES USING MICROARRAYS

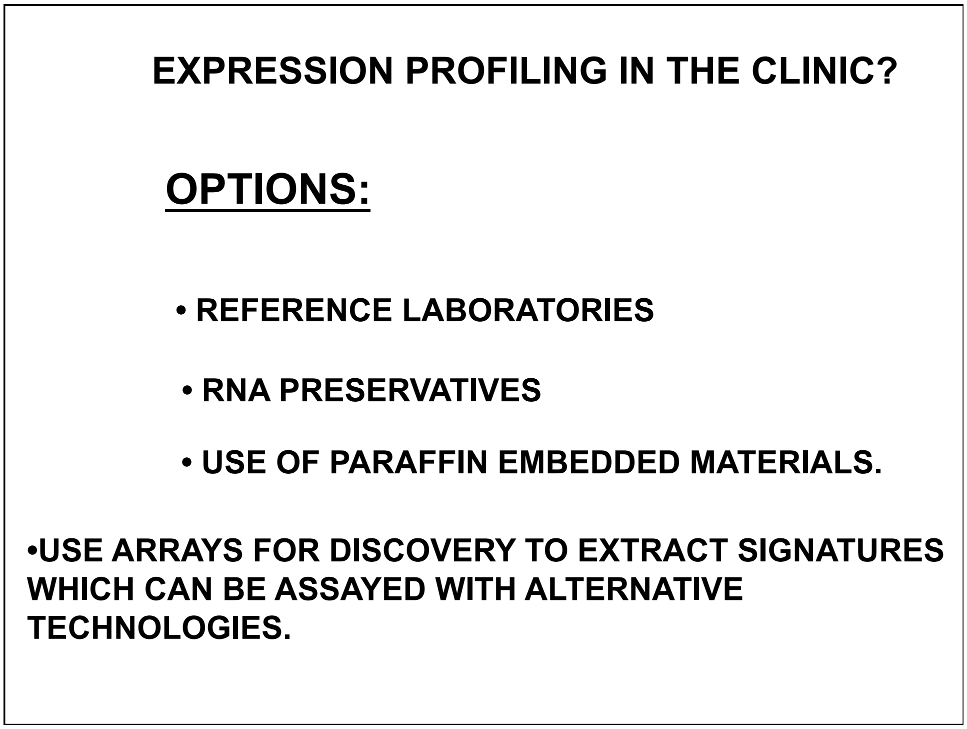
- **GOAL SHOULD BE TO SEEK AND VALIDATE CLINICALLY RELEVANT SIGNATURES WITHIN DEFINED PATIENT GROUPS FOR WHICH NO CURRENT FEATURES ADEQUATELY ANSWER THE CLINICAL QUESTION POSED.**



EXPRESSION PROFILING IN THE CLINIC?

PROBLEMS:

- SPECIALIZED TECHNOLOGY
- RNA IS UNSTABLE
- FROZEN TISSUE NOT PART OF USUAL OR SAMPLE FLOW



EXPRESSION PROFILING IN THE CLINIC?

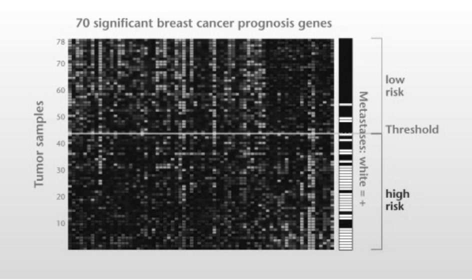
OPTIONS:

- REFERENCE LABORATORIES
- RNA PRESERVATIVES
- USE OF PARAFFIN EMBEDDED MATERIALS.

•USE ARRAYS FOR DISCOVERY TO EXTRACT SIGNATURES WHICH CAN BE ASSAYED WITH ALTERNATIVE TECHNOLOGIES.

FDA APPROVED TESTS FOR BREAST CANCER BASED ON EXPRESSION STUDIES

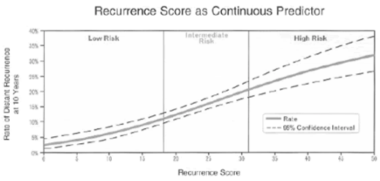
70 GENE MICROARRAY SIGNATURE



Van de Vijver et al

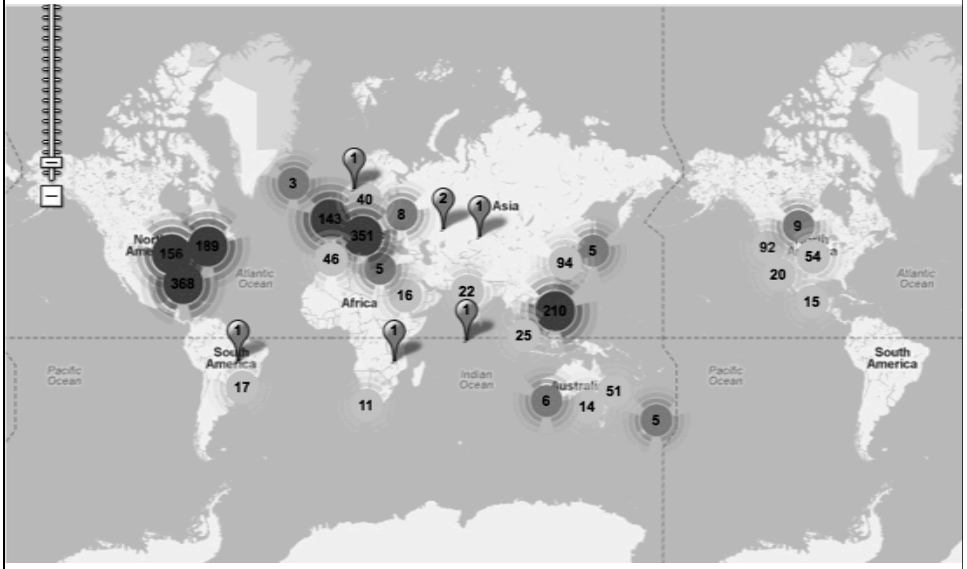
NEJM 347:1999 .

Muiltgene RT-PCR SIgnature

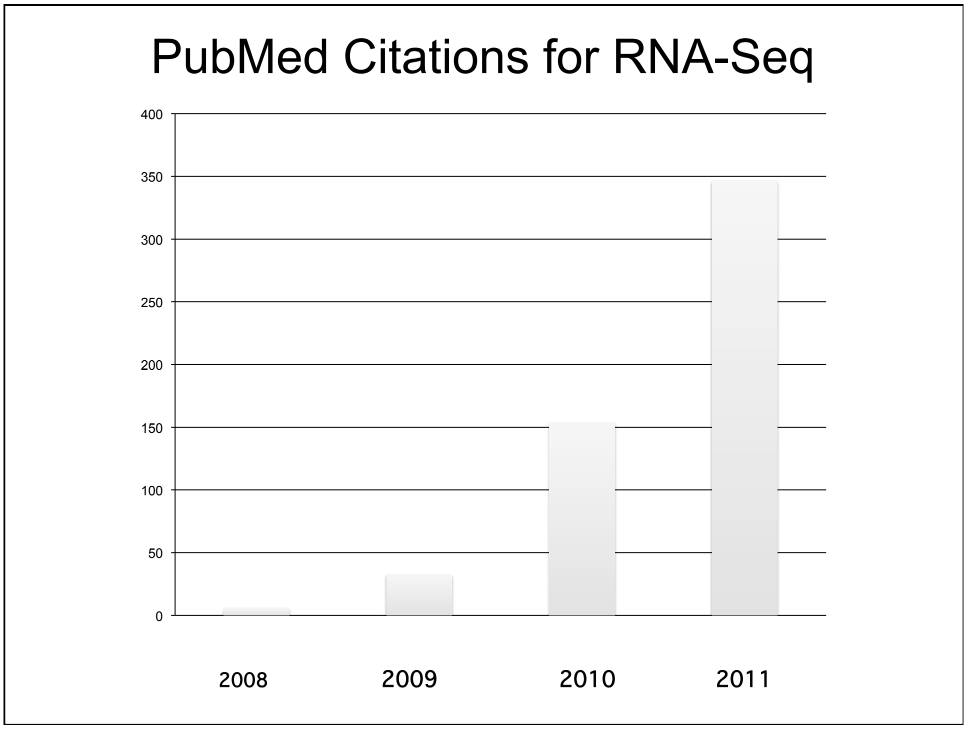


Paik et al NEJM 351:2817

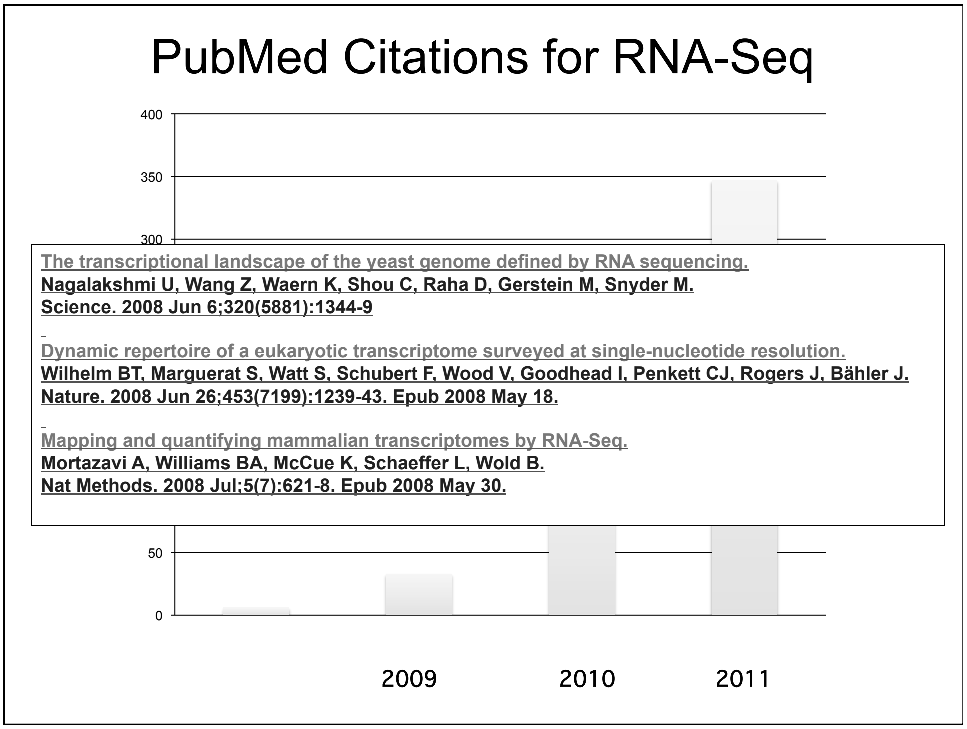
THEY' RE EVERYWHERE!



http://pathogenomics.bham.ac.uk/hts/



PubMed Citations for RNA-Seq

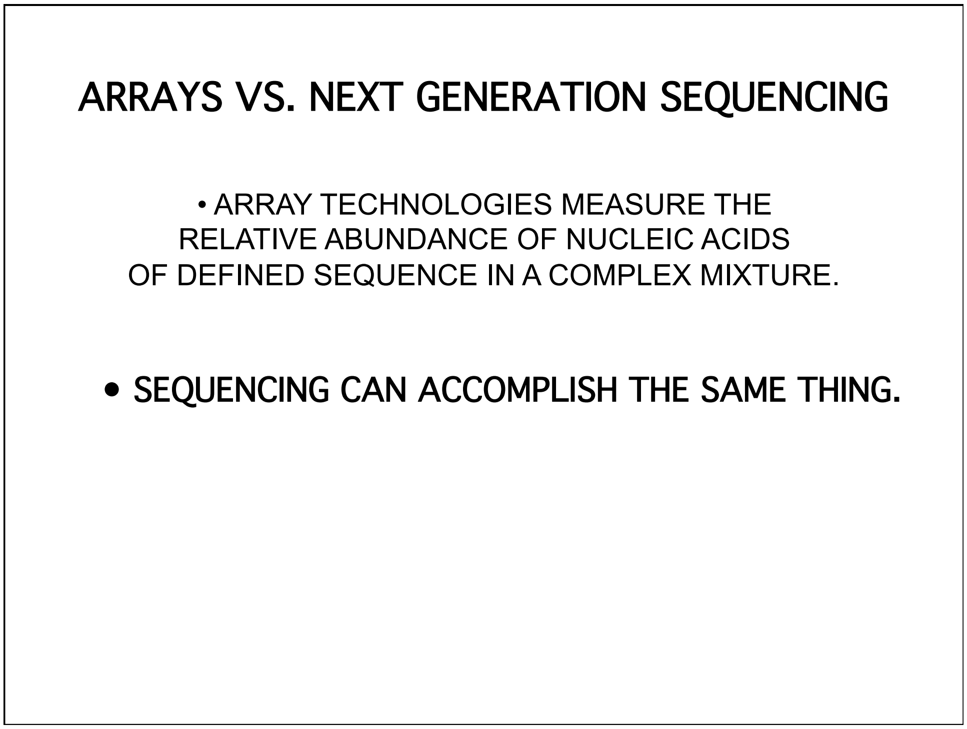


PubMed Citations for RNA-Seq

The transcriptional landscape of the yeast genome defined by RNA sequencing.
Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M.
Science. 2008 Jun 6;320(5881):1344-9

Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution.
Wilhelm BT, Marguerat S, Watt S, Schubert F, Wood V, Goodhead I, Penkett CJ, Rogers J, Bähler J.
Nature. 2008 Jun 26;453(7199):1239-43. Epub 2008 May 18.

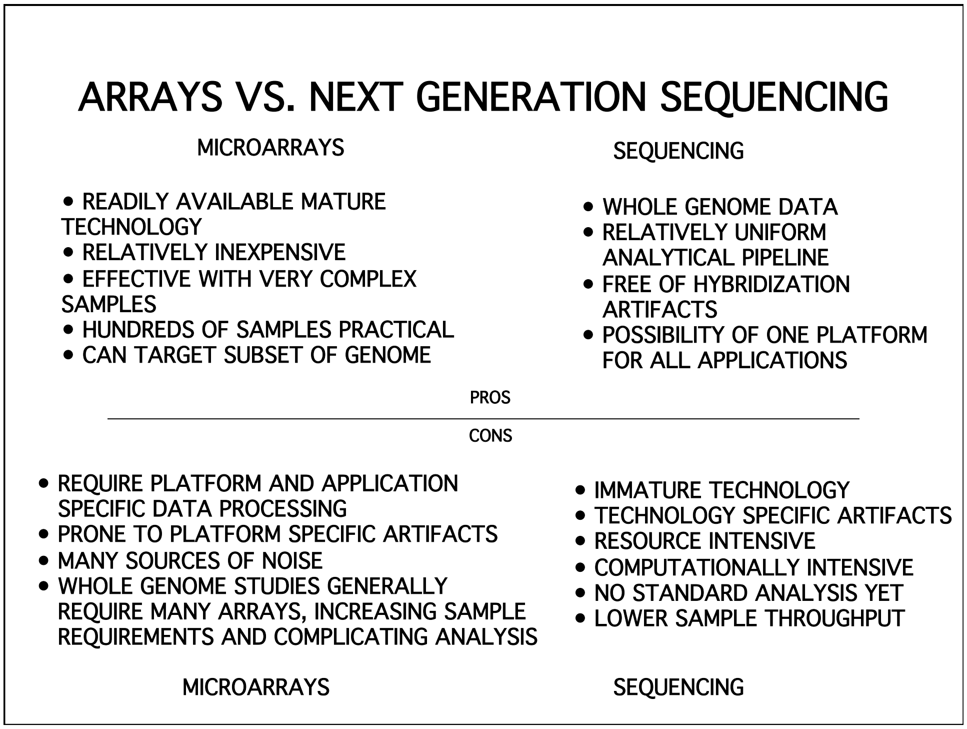
Mapping and quantifying mammalian transcriptomes by RNA-Seq.
Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B.
Nat Methods. 2008 Jul;5(7):621-8. Epub 2008 May 30.



ARRAYS VS. NEXT GENERATION SEQUENCING

- ARRAY TECHNOLOGIES MEASURE THE RELATIVE ABUNDANCE OF NUCLEIC ACIDS OF DEFINED SEQUENCE IN A COMPLEX MIXTURE.

- SEQUENCING CAN ACCOMPLISH THE SAME THING.



ARRAYS VS. NEXT GENERATION SEQUENCING

MICROARRAYS

- READILY AVAILABLE MATURE TECHNOLOGY
- RELATIVELY INEXPENSIVE
- EFFECTIVE WITH VERY COMPLEX SAMPLES
- HUNDREDS OF SAMPLES PRACTICAL
- CAN TARGET SUBSET OF GENOME

SEQUENCING

- WHOLE GENOME DATA
- RELATIVELY UNIFORM ANALYTICAL PIPELINE
- FREE OF HYBRIDIZATION ARTIFACTS
- POSSIBILITY OF ONE PLATFORM FOR ALL APPLICATIONS

PROS

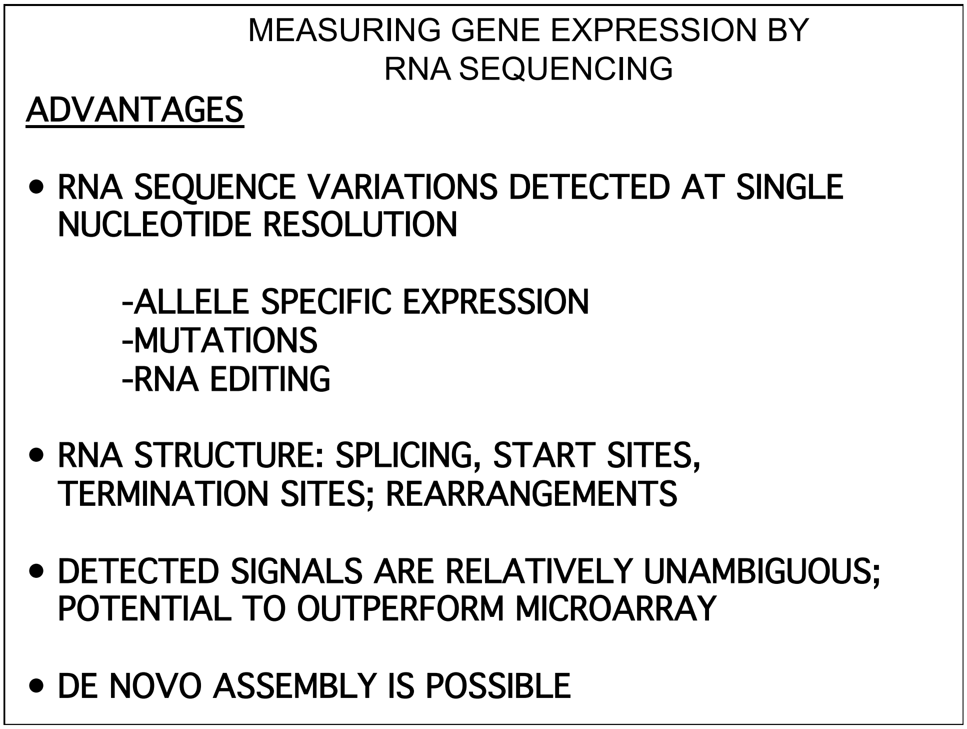
CONS

- REQUIRE PLATFORM AND APPLICATION SPECIFIC DATA PROCESSING
- PRONE TO PLATFORM SPECIFIC ARTIFACTS
- MANY SOURCES OF NOISE
- WHOLE GENOME STUDIES GENERALLY REQUIRE MANY ARRAYS, INCREASING SAMPLE REQUIREMENTS AND COMPLICATING ANALYSIS

- IMMATURE TECHNOLOGY
- TECHNOLOGY SPECIFIC ARTIFACTS
- RESOURCE INTENSIVE
- COMPUTATIONALLY INTENSIVE
- NO STANDARD ANALYSIS YET
- LOWER SAMPLE THROUGHPUT

MICROARRAYS

SEQUENCING



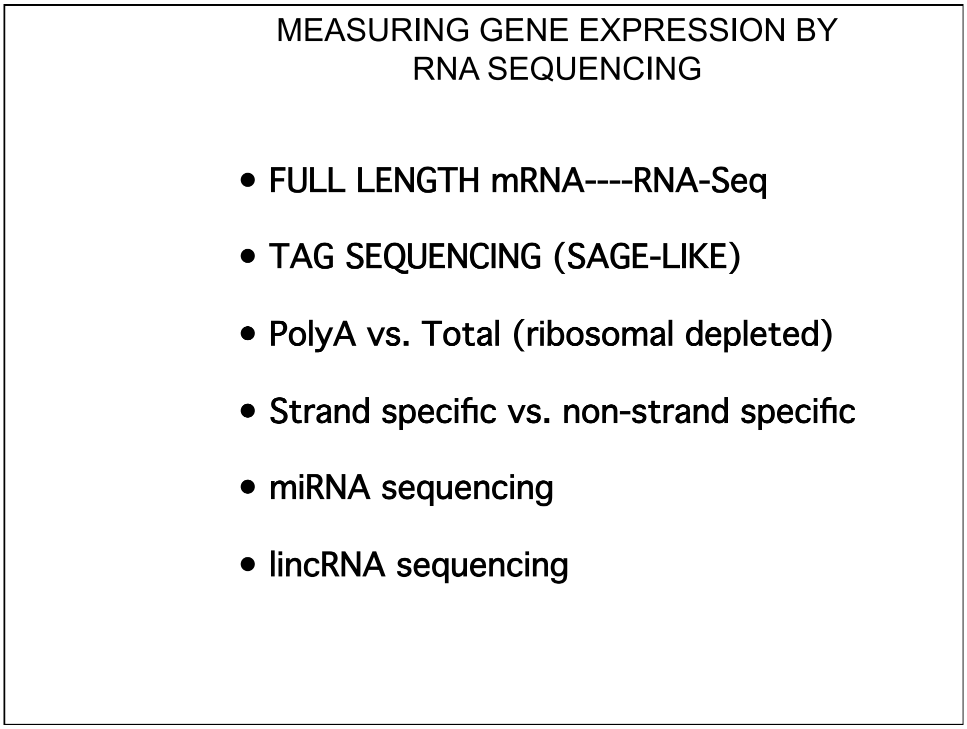
MEASURING GENE EXPRESSION BY RNA SEQUENCING

<u>ADVANTAGES</u>

- RNA SEQUENCE VARIATIONS DETECTED AT SINGLE NUCLEOTIDE RESOLUTION

  -ALLELE SPECIFIC EXPRESSION
  -MUTATIONS
  -RNA EDITING

- RNA STRUCTURE: SPLICING, START SITES, TERMINATION SITES; REARRANGEMENTS
- DETECTED SIGNALS ARE RELATIVELY UNAMBIGUOUS; POTENTIAL TO OUTPERFORM MICROARRAY
- DE NOVO ASSEMBLY IS POSSIBLE



MEASURING GENE EXPRESSION BY RNA SEQUENCING

- FULL LENGTH mRNA----RNA-Seq
- TAG SEQUENCING (SAGE-LIKE)
- PolyA vs. Total (ribosomal depleted)
- Strand specific vs. non-strand specific
- miRNA sequencing
- lincRNA sequencing

MEASURING GENE EXPRESSION BY RNA SEQUENCING: PROS AND CONS

LIMITATIONS

- LOWER LIMIT OF DETECTION IS CONSTRAINED BY THE mRNA ABUNDANCE DISTRIBUTION AND THE NUMBER OF ALIGNED READS PER SAMPLE.

- LARGE SAMPLE NUMBERS DIFFICULT TO ACHIEVE, EXCEPT IN TAG MODE.

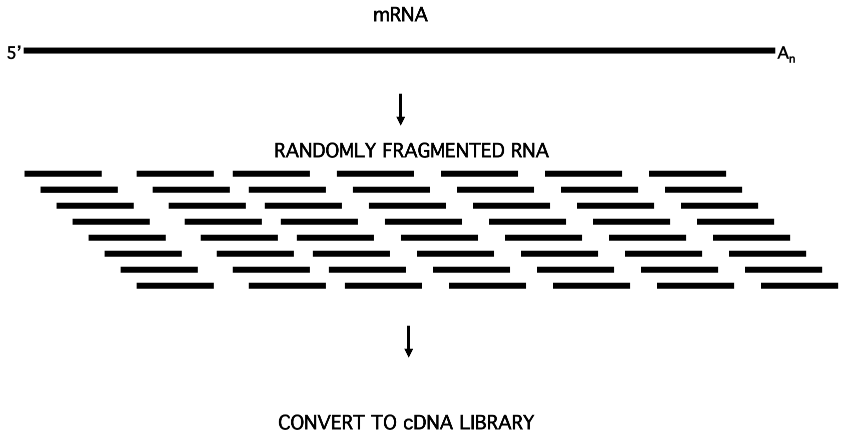
- SOFTWARE IS STILL DEVELOPMENTAL: REQUIRES SOPHISTICATED BIOINFORMATICS COLLABORATION. [For review see Pepke et al. Nat Methods 6:S22 (2009)]

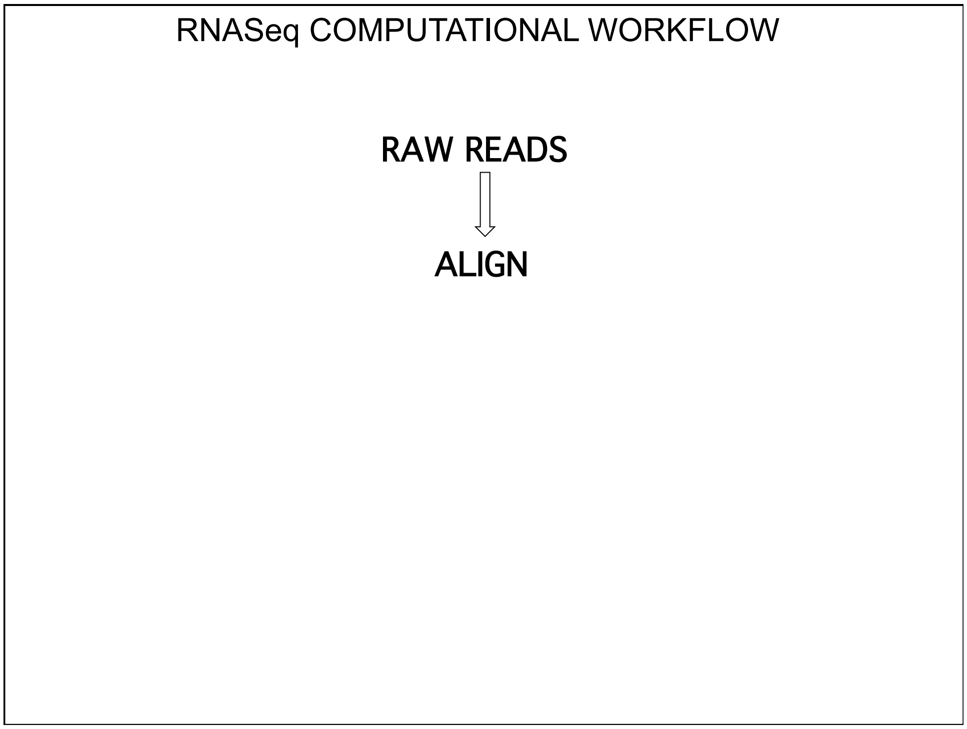
- COMPUTATIONAL HARDWARE REQUIREMENTS ARE SUBSTANTIAL

- LIBRARY PREP METHODS EVOLVING

- DATA MAY NOT MERGE WELL IF NOT GENERATED WITH THE SAME METHOD

MEASURING GENE EXPRESSION BY RNA SEQUENCING



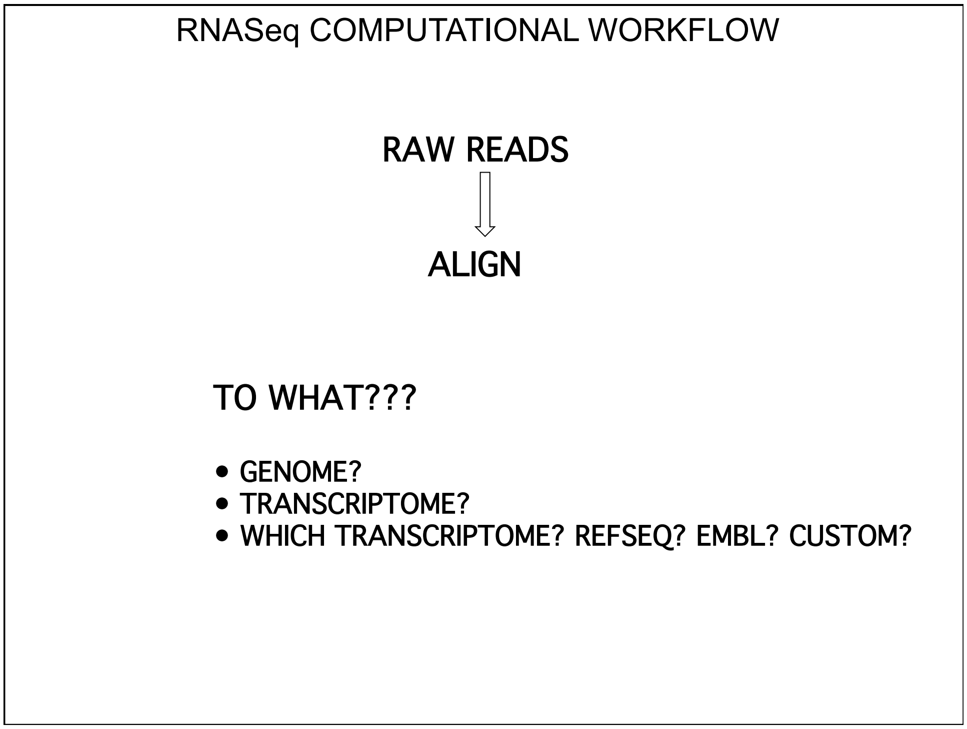


RNASeq COMPUTATIONAL WORKFLOW

RAW READS

⇩

ALIGN



RNASeq COMPUTATIONAL WORKFLOW

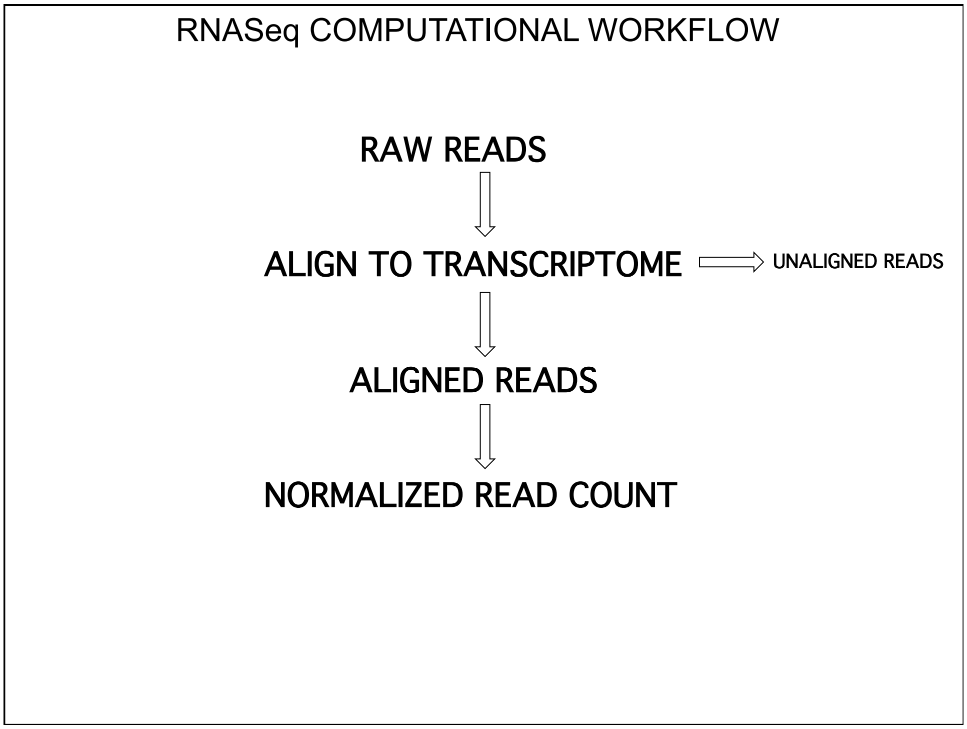
RAW READS

⇩

ALIGN

TO WHAT???

- GENOME?
- TRANSCRIPTOME?
- WHICH TRANSCRIPTOME? REFSEQ? EMBL? CUSTOM?



RNASeq COMPUTATIONAL WORKFLOW

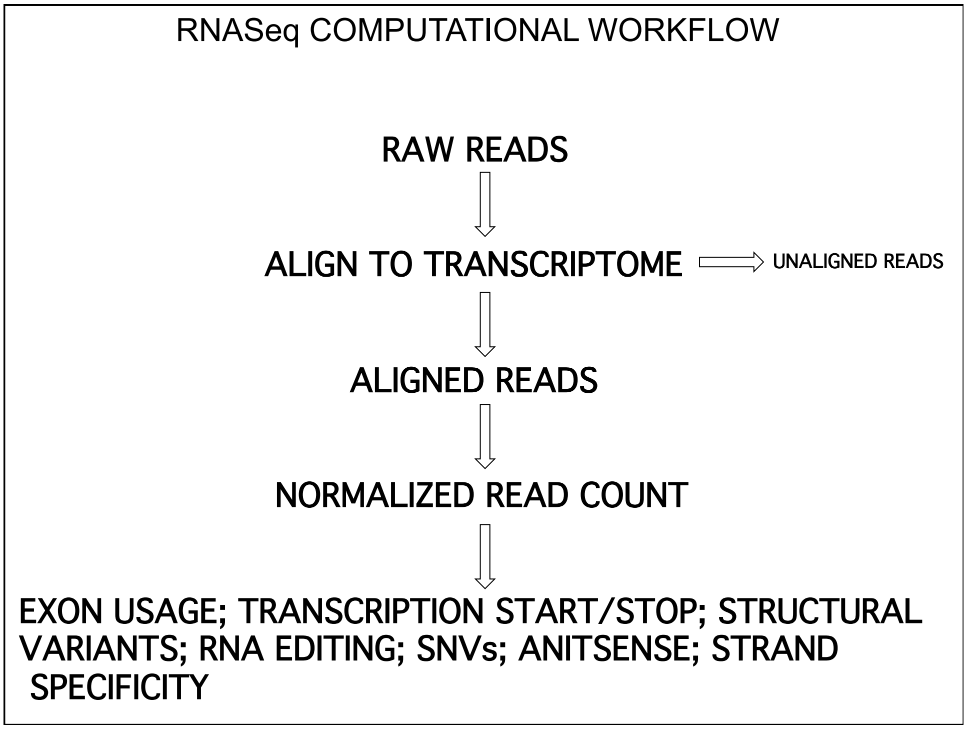
RAW READS

ALIGN TO TRANSCRIPTOME

UNALIGNED READS

ALIGNED READS

NORMALIZED READ COUNT



RNASeq COMPUTATIONAL WORKFLOW

RAW READS

ALIGN TO TRANSCRIPTOME

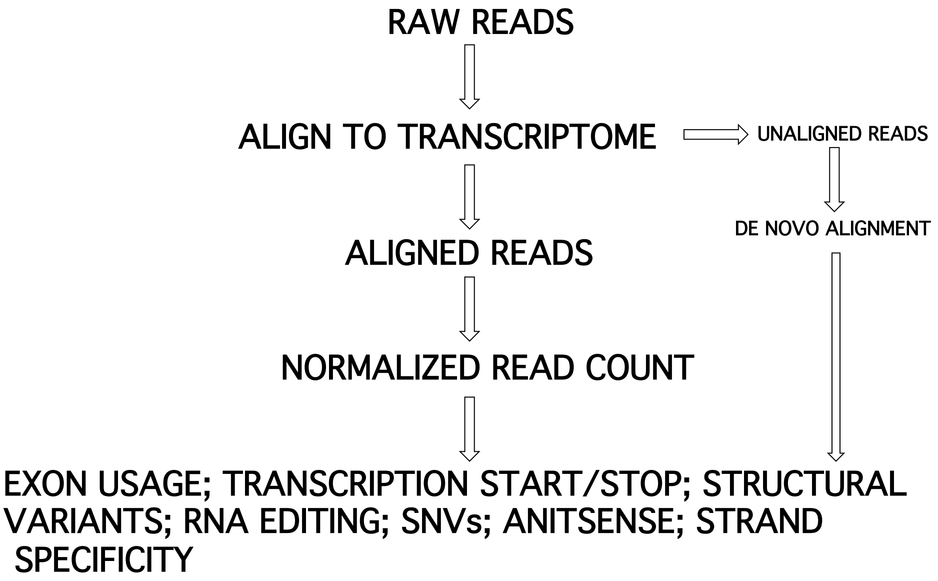
UNALIGNED READS

ALIGNED READS

NORMALIZED READ COUNT

EXON USAGE; TRANSCRIPTION START/STOP; STRUCTURAL VARIANTS; RNA EDITING; SNVs; ANITSENSE; STRAND SPECIFICITY

RNASeq COMPUTATIONAL WORKFLOW



RAW READS

ALIGN TO TRANSCRIPTOME

UNALIGNED READS

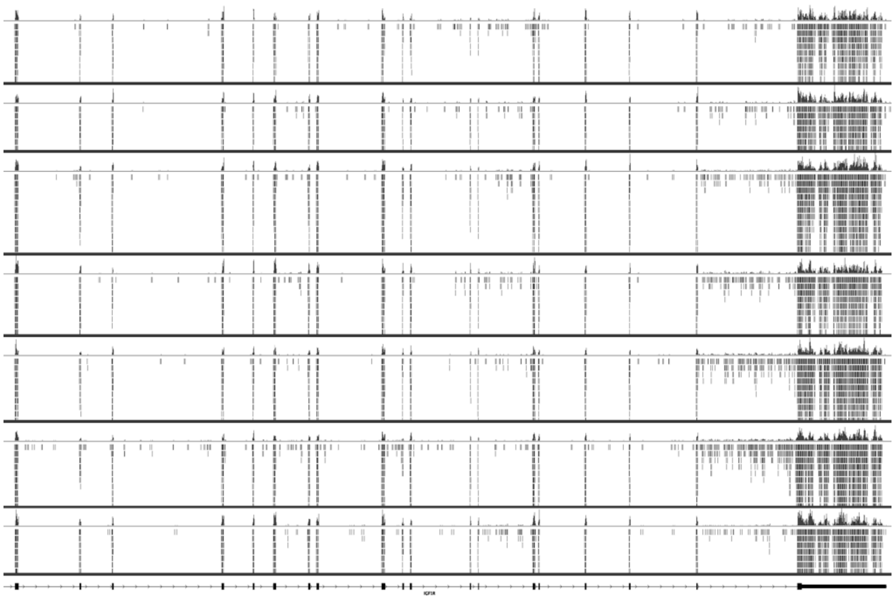
DE NOVO ALIGNMENT

ALIGNED READS

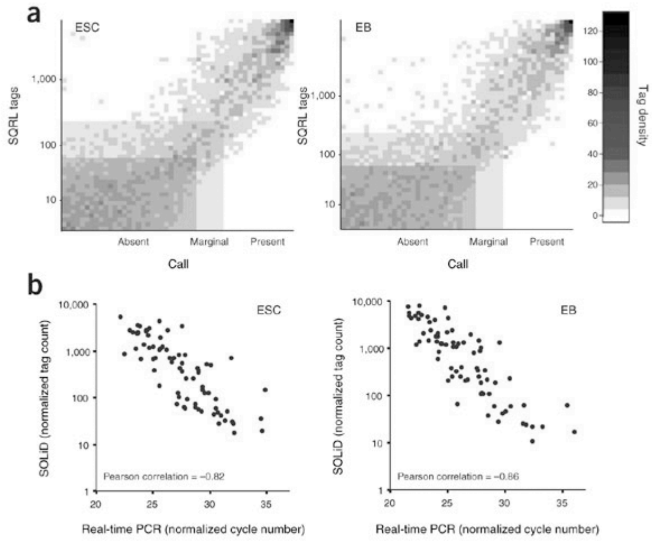
NORMALIZED READ COUNT

EXON USAGE; TRANSCRIPTION START/STOP; STRUCTURAL VARIANTS; RNA EDITING; SNVs; ANITSENSE; STRAND SPECIFICITY

RNA-seq IGF1R



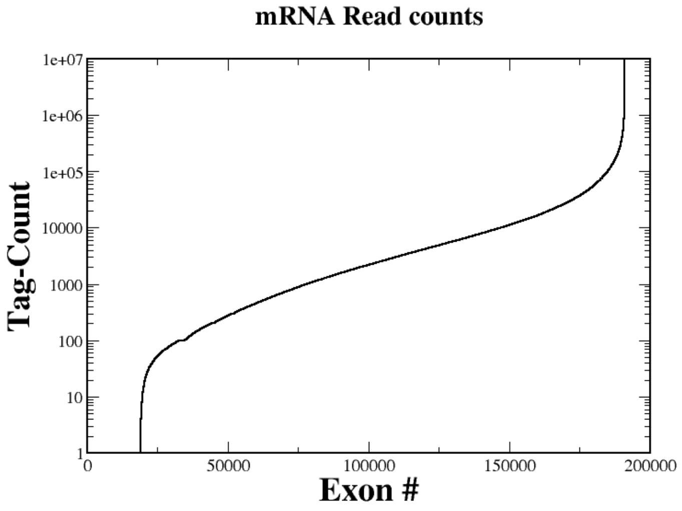
MEASURING GENE EXPRESSION BY RNA SEQUENCING



Cloonan et al.

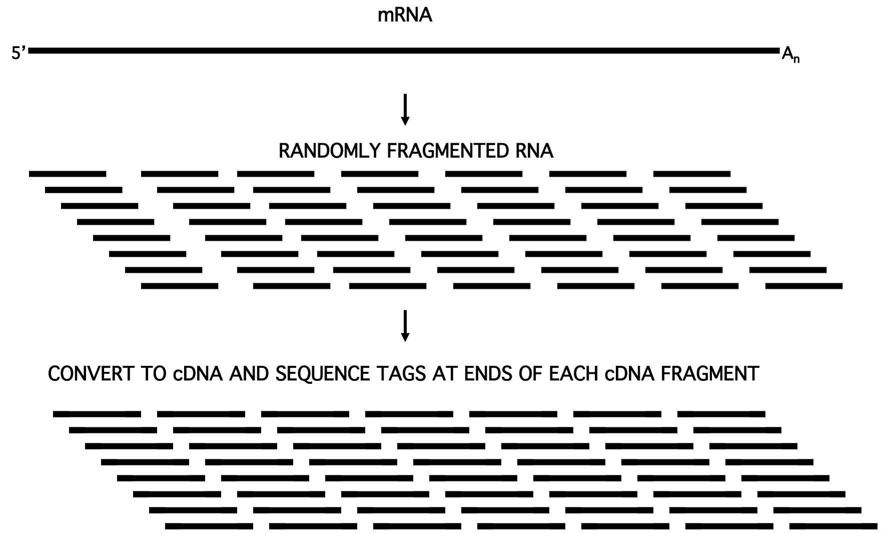
Nature Methods - 5, 613 - 619 (2008)

mRNA ABUNDANCE VARIES OVER A LARGE DYNAMIC RANGE



Sven Bilke

MEASURING GENE EXPRESSION BY RNA SEQUENCING



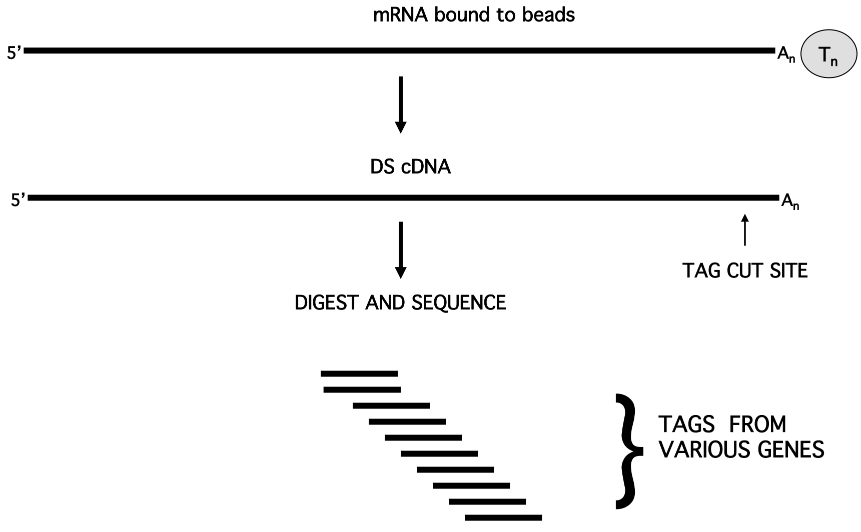
mRNA

5’ A_n

RANDOMLY FRAGMENTED RNA

CONVERT TO cDNA AND SEQUENCE TAGS AT ENDS OF EACH cDNA FRAGMENT

3’ TAG SEQUENCING



mRNA bound to beads

5’ A_n T_n

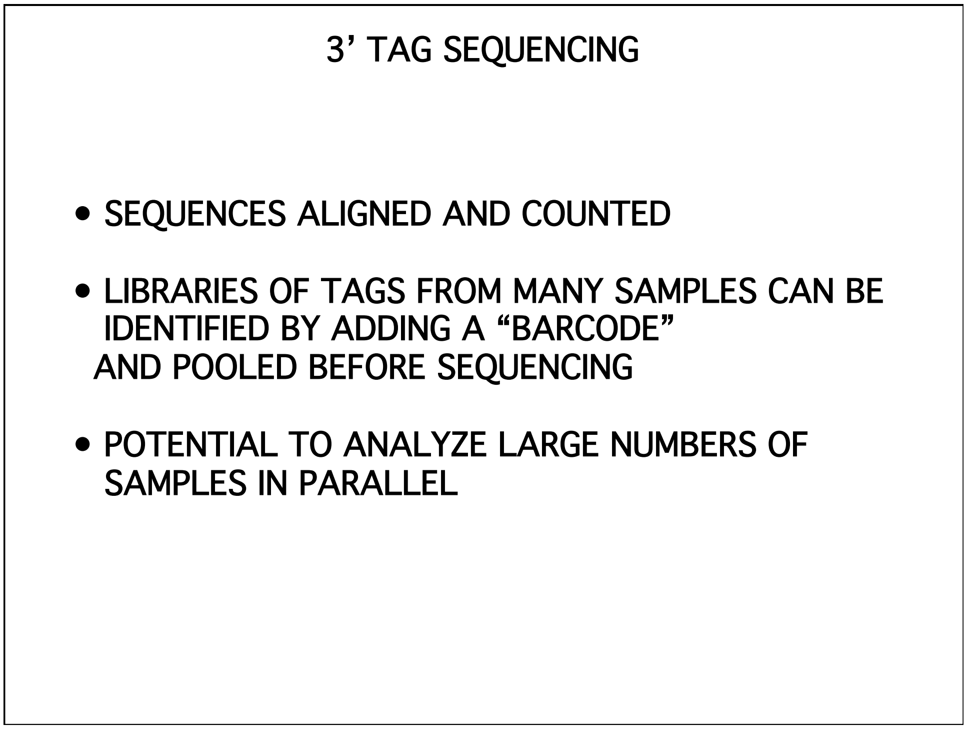
DS cDNA

5’ A_n

TAG CUT SITE

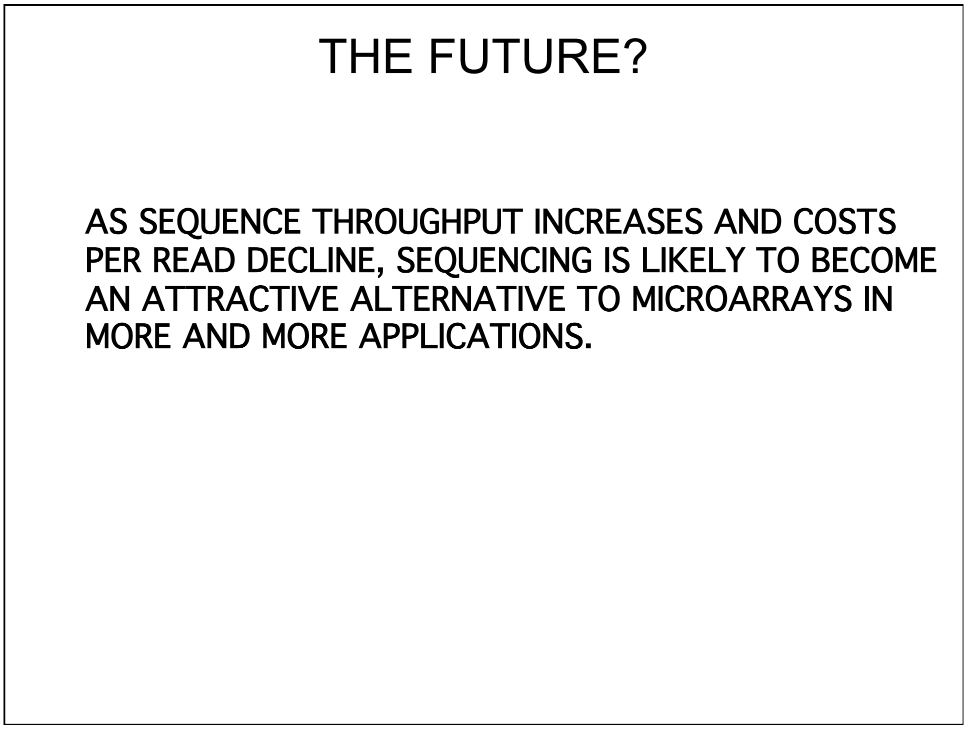
DIGEST AND SEQUENCE

TAGS FROM VARIOUS GENES



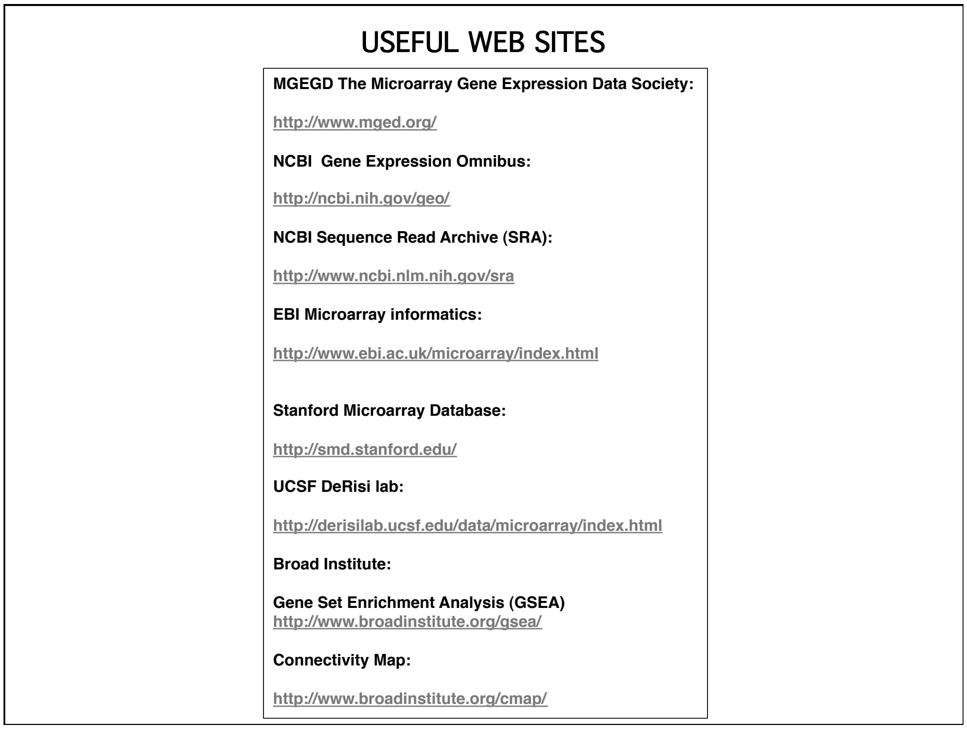
3’ TAG SEQUENCING

- SEQUENCES ALIGNED AND COUNTED
- LIBRARIES OF TAGS FROM MANY SAMPLES CAN BE IDENTIFIED BY ADDING A “BARCODE” AND POOLED BEFORE SEQUENCING
- POTENTIAL TO ANALYZE LARGE NUMBERS OF SAMPLES IN PARALLEL



THE FUTURE?

AS SEQUENCE THROUGHPUT INCREASES AND COSTS PER READ DECLINE, SEQUENCING IS LIKELY TO BECOME AN ATTRACTIVE ALTERNATIVE TO MICROARRAYS IN MORE AND MORE APPLICATIONS.



USEFUL WEB SITES

MGEGD The Microarray Gene Expression Data Society:

http://www.mged.org/

NCBI Gene Expression Omnibus:

http://ncbi.nih.gov/geo/

NCBI Sequence Read Archive (SRA):

http://www.ncbi.nlm.nih.gov/sra

EBI Microarray informatics:

http://www.ebi.ac.uk/microarray/index.html

Stanford Microarray Database:

http://smd.stanford.edu/

UCSF DeRisi lab:

http://derisilab.ucsf.edu/data/microarray/index.html

Broad Institute:

Gene Set Enrichment Analysis (GSEA)
http://www.broadinstitute.org/gsea/

Connectivity Map:

http://www.broadinstitute.org/cmap/