Who is this new face in the classroom?

Valentina Perissi
Assistant professor of Biochemistry at Boston University

Perissi Lab website: http://blogs.bu.edu/vperissi/

Questions: vperissi@bu.edu

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: PERISSI, VALENTINA

eRA COMMONS USER NAME (agency login): VPERISSI

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Torino	Laurea (equivalent to BSc/Master)	07/1997	Molecular Biology
University of California San Diego	PHD	06/2004	Molecular Pathology
University of California San Diego	Postdoctoral Fellow	06/2010	Endocrinology and Metabolism

A. PERSONAL STATEMENT

My current research efforts focus on investigating the role of non-proteolytic ubiquitination in the regulation of cell growth, inflammation and metabolism in cellular homeostasis and disease. After postdoctoral training in Endocrinology and Metabolism in Dr. Rosenfeld's laboratory at UCSD, I have established a fully funded and independent research group in the Department of Biochemistry at Boston University. This transition was supported by a K99-R00 career development award from NIH and a Peter Paul Career Development Professorship from Boston University. In 2013, I secured R01 funding from NIDDK to investigate the crosstalk between ubiquitin signaling and inflammation in the adipose tissue and this year I have received a Breast Cancer Breakthrough Award from the Department of Defense to investigate the regulation of breast cancer cells growth and metabolism through non-proteolytic ubiquitination. Since joining Boston University I have mentored three postdoctoral fellows, one PhD, one master and numerous undergraduate students, the last often funded by the Boston University Undergraduate Research program (UROP Awards). Current lab members consist of one research faculty, one postdoctoral fellow, one research technician, two graduate students and two undergraduate students.

B. POSITIONS AND HONORS

<u>Positions and Employment</u>

2005-2006	Fellow in Endocrinology and Metabolism, UCSD, NIH T32 Training Grant
2010-2011	Adjunct Assistant Professor of Medicine, University of California San Diego
2011- present	Assistant Professor of Biochemistry, Boston University
2018- present	Co-Director BNORC Adipocyte Biology Core

C. CONTRIBUTIONS TO SCIENCE

1. My most recent, independent research efforts have focused on investigating the ***role of the nuclear receptor cofactor G Protein Suppressor 2 (GPS2), in regulating non-proteolytic ubiquitin signaling***

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3. My initial work as a graduate student focused on ***understanding the basic molecular mechanisms of nuclear receptors' cofactors actions in modulating chromatin remodeling***. My contribution to the field was particularly significant in dissecting the molecular basis of the interaction between nuclear receptors and corepressors as it led to the identification of a previously unknown signature structural motif that differentiate corepressor from coactivators in their ability to bind to unliganded or liganded nuclear

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4. I became interested in transcription early on during her undergraduate research thesis and along the years I have developed an ***extensive knowledge and vast technical expertise in studying transcriptional regulation in mammalian cells and tissues***. As a result, I significantly contributed to the basic understanding of the molecular mechanisms underlying the regulation of gene expression in a number of cell models, including human breast and prostate cancer, murine retina and macrophage cells, and contributed in a significant manner to the development of Chromatin Immunoprecipitation (ChIP) techniques (first *in vivo* ChIP in mouse early embryonic retina).

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- Who is this new face in the classroom?

- What is she going to teach?
  Module 3 and 4: - transcriptome
  - transcriptional regulation

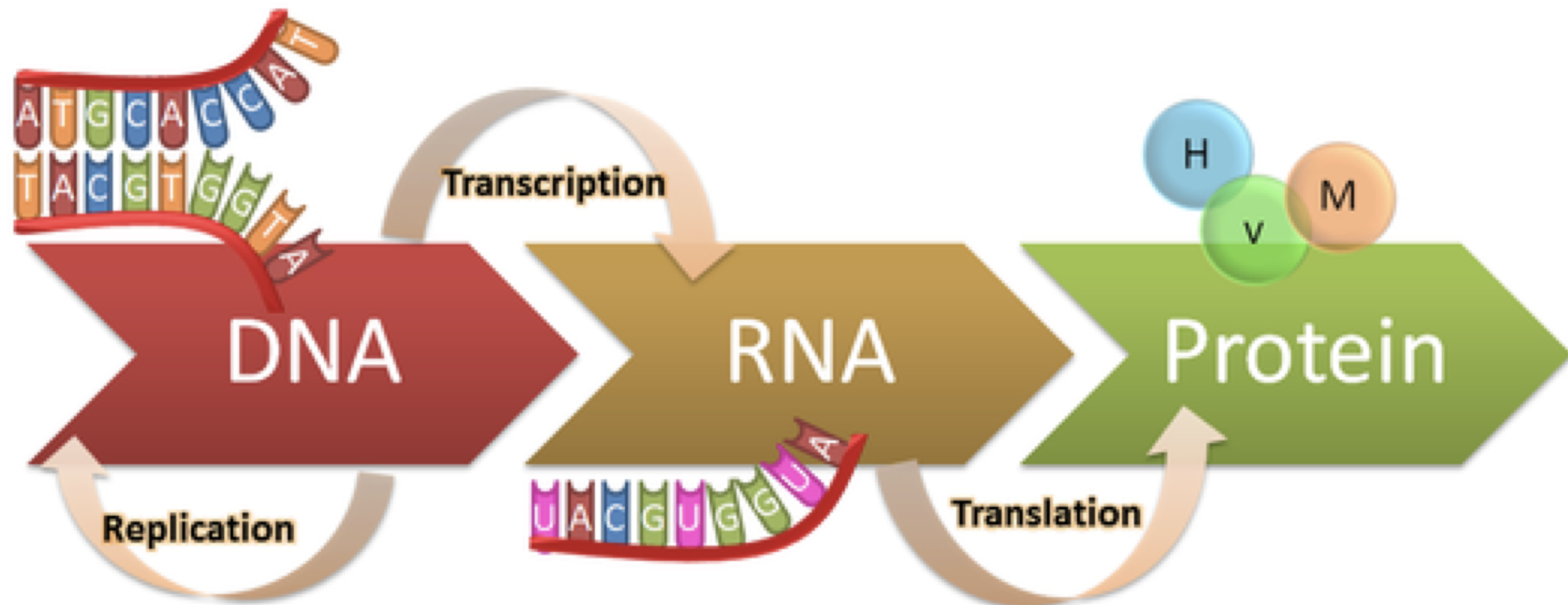
  She needs help – never taught this class before and wasn't here when Michele introduced the previous chapters

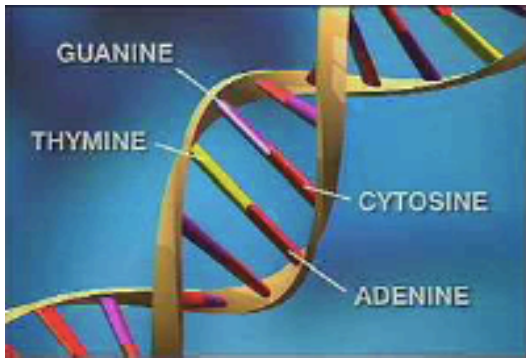
- How to help - Interaction: i) questions
  ii) feedback
  ii) papers discussion

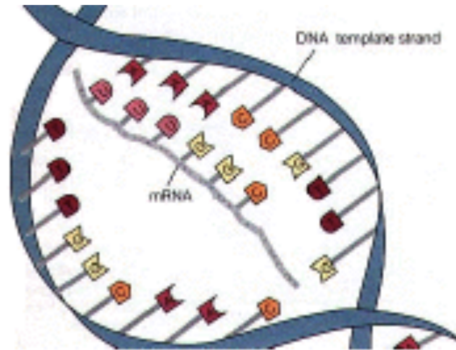
L3.1 – Transcriptomes
(Pre-NGS)

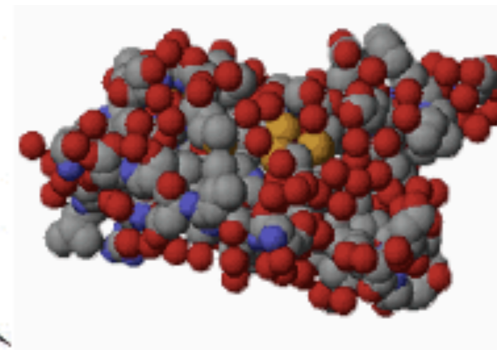
What is the “transcriptome”?

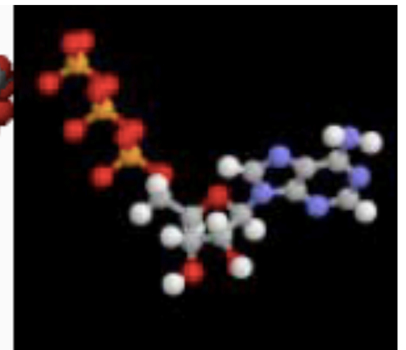
What does “transcriptomics” refer to?


DNA
RNA
Protein
Transcription
Replication
Translation
H
V
M









Epigenetics

DNA → mRNA → Proteins ⇄ Metabolites

Transcription

Differential Splicing
RNA stability etc

Translation

Post Translational
Modification etc

Enzyme
Activity

Genome | Transcriptome | Proteome | Metabolome

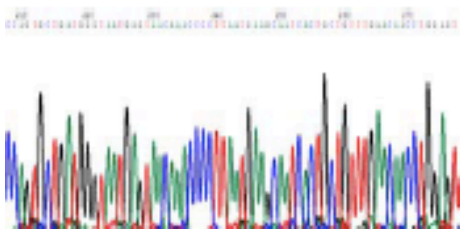
3 billion bases
(*H sapiens*)

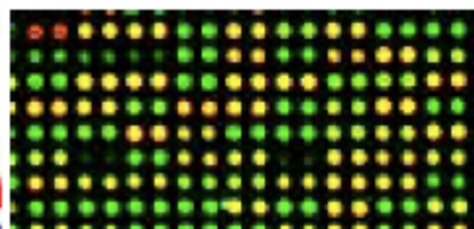
20-30,000
genes

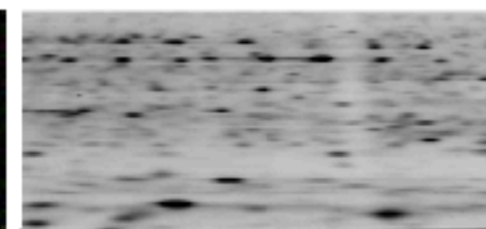
~100,000
proteins

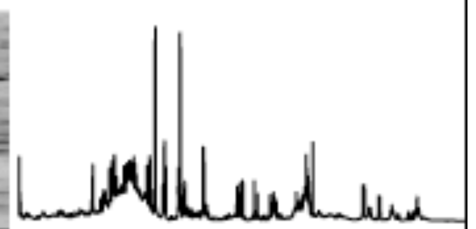
Functional Genomics

Genomics | **Transcriptomics** | **Proteomics** | **Metabolomics**









The key aims of **transcriptomics** are:

1) to catalogue all species of transcript, including mRNAs, non-coding RNAs and small RNAs;
2) to determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other post-transcriptional modifications;
3) to quantify the changing expression levels of each transcript during development and under different experimental or pathological conditions

Two main aspects:

1. Qualitative:
   Assemble a full catalogue of all transcribed sequences
   Describe their function (i.e. are they coding or not)

2. Quantitative:
   Perform absolute or comparative measurement of individual RNA transcripts in different samples

AGENDA

1. Pre-genomic qualitative approaches (Sanger sequencing of cDNA/EST libraries)

2. Pre-genomic quantitative approaches (Northern blotting, RT-PCR..)

3. Microarrays

General

Approaches for accessing to RNA:

1. Hybridization – based methods

2. Sequencing – based methods

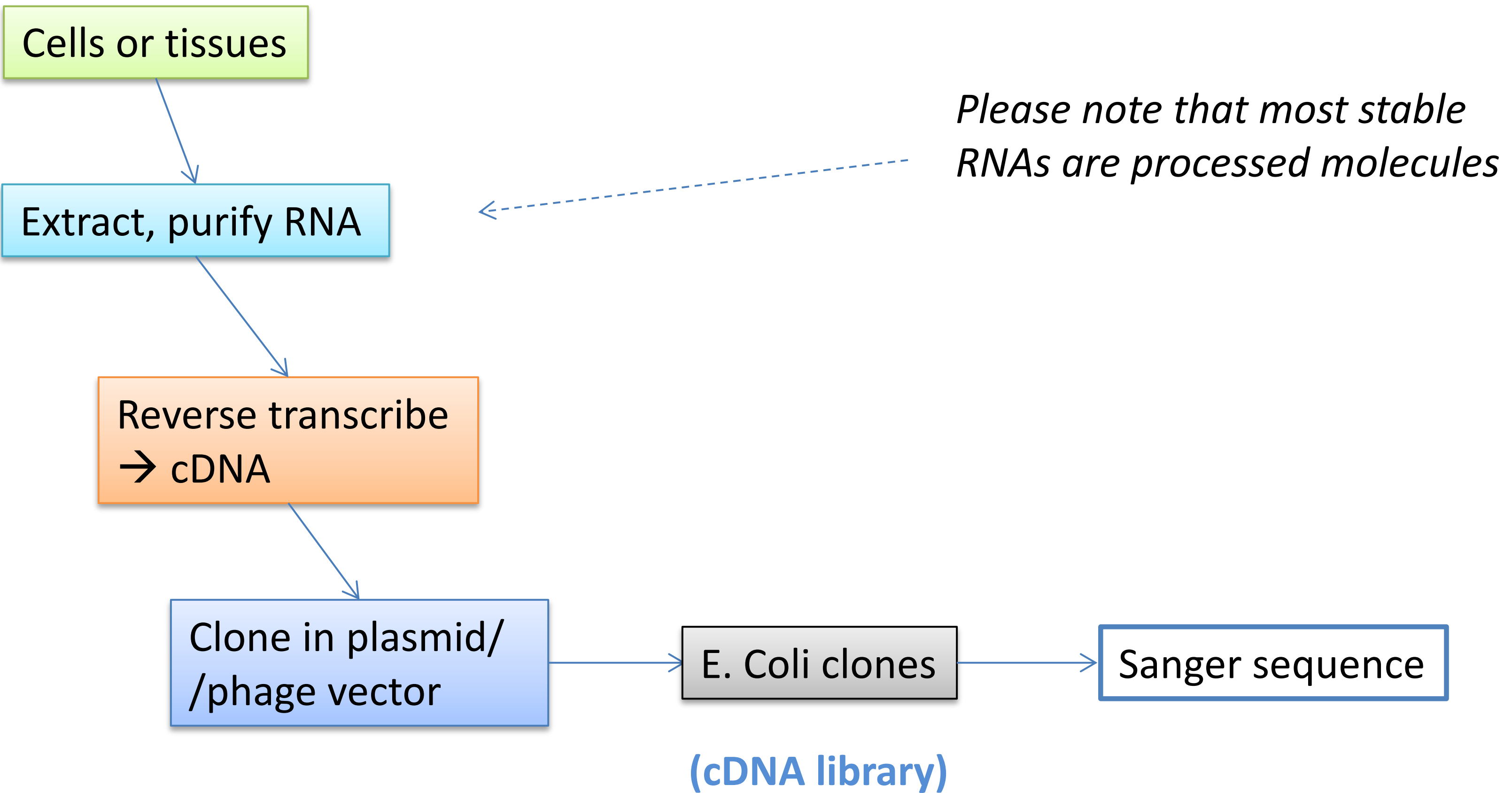
1. The RNA sequence is not observed directly, but it is inferred since it hybridizes with probes or primers.

2. The RNA sequence is converted to DNA (cDNA) and the DNA sequenced (*)

(*) Technologies are being developed to sequence RNA directly without cDNA sysnthesis (Helicos/Nanopore – discussed at the end).

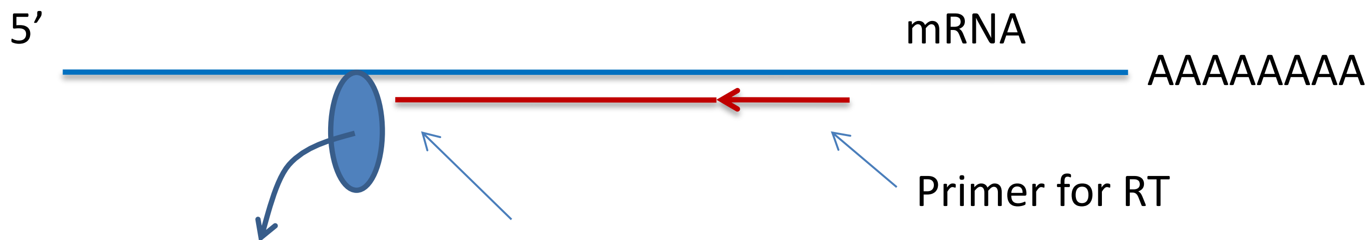
In the **pre-genomic** years, transcriptome was accessed only using single-transcript measurement (or few in parallel)

Individual **clones** of cDNA (complementary DNA) were cloned in plasmid/phage vectors



Limitations:

One common problem is that Reverse Transcriptase is not very “processive” and often terminates before reaching the 5’ end of RNA.
This has led to 3’-end biased RNA databases and difficulty of mapping the true 5’ (and as a consequence, promoters)



Reconstruction of full-lenght cDNA (RNA) required cumbersome analysis.

- RACE
- Primer extension
- Other…

How to complete cDNA structure

RACE or primer extension methods

Pre-genomic

Database is gradually populated with sequences of various kinds:

- full-lenght cDNA (usually recostructed by multiple clones, contain all the RNA sequence, verified)
- Partial cDNA (verified, but not containing all the RNA sequence, due to partial cloning and/or sequencing)
- EST (expressed sequence tags, often without annotation)

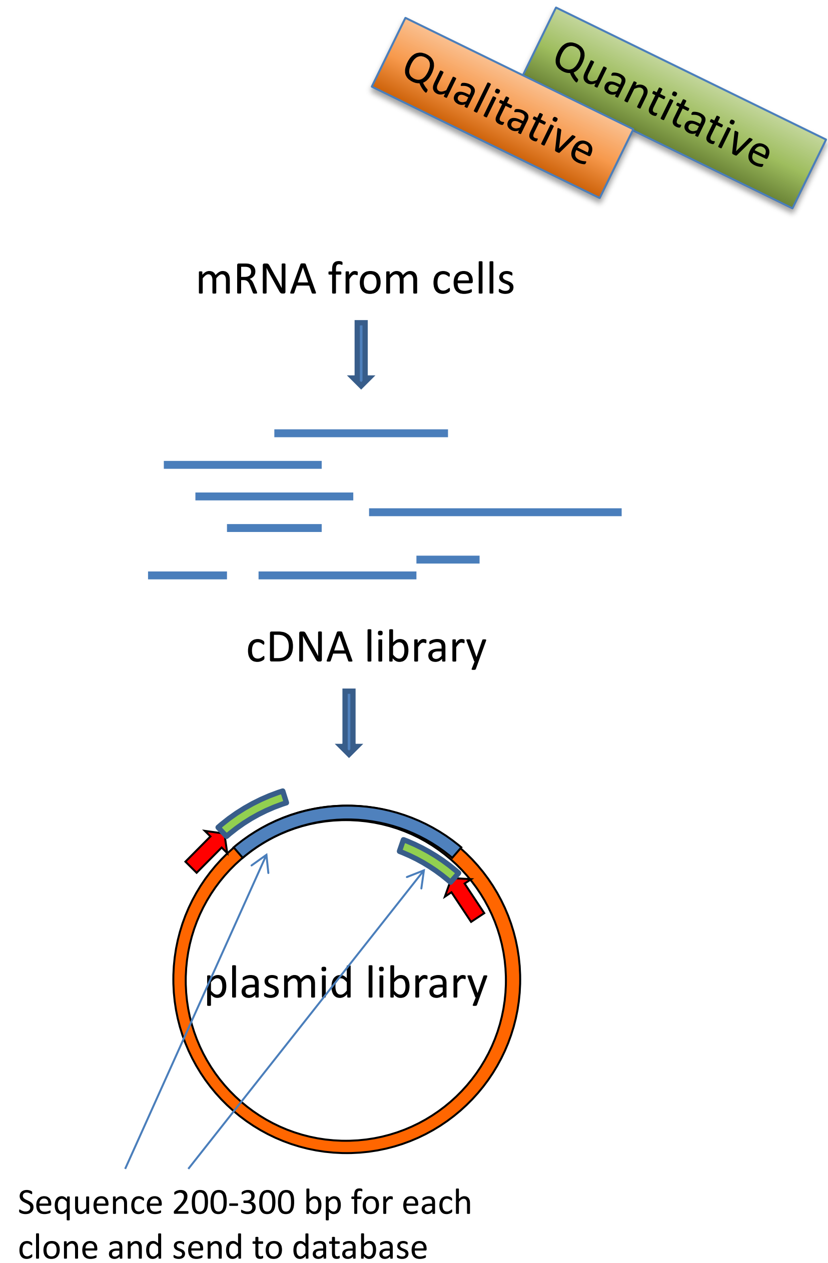
Sequences in database are contributed both by individual scientists and labs, and by Sequencing Centers participating in Genome projects.

Pre-genomic

EST = expressed sequence tags

1. mRNA extraction from cells or tissues
2. cDNA synthesis (oligo-dT or random-primed)
3. cloning into plasmid vectors
4. sequencing from vector primers (200-300nt)
5. Estimate expression from frequency

[Current EST Databases contain millions of EST]



Pre-genomic

Quantitative

Quantitative measurements of single genes:

Northern blotting

RNase Protection Assay (RPA)

RT-PCR

qRT-PCR

Are they sequencing- or hybridization-based methods ?

Gene-by-gene methods to measure gene expression (mRNA)

Post-genomic approaches: goal to simultaneously analyze a large number of transcripts in any particular cell or tissue (Highthroughput)

Making qualitative and quantitative analysis of RNA **highly parallel**

Hybridization methods

DNA microarrays, oligonucleotide microarrays.

Spotted arrays (1996)
In situ synthesized oligo arrays (1999)
Bead-arrays® (2001)

Tiling microarrays (2004)

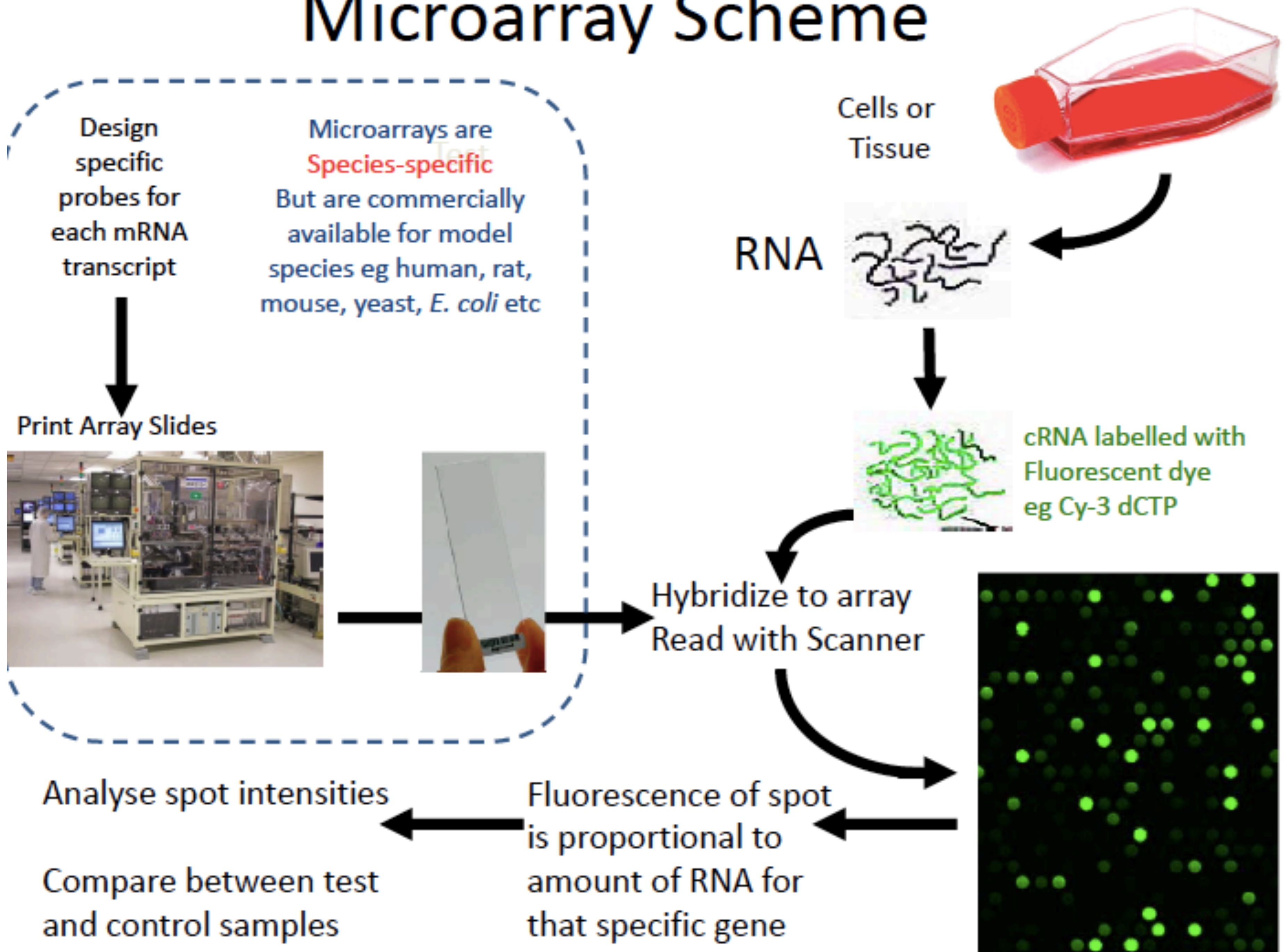
Sequencing methods

EST (1980)

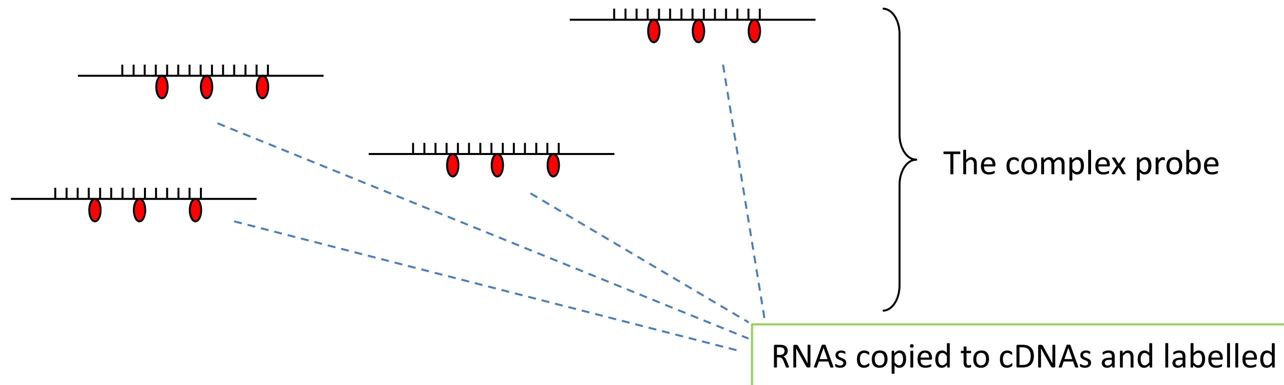
SAGE (1995)(LongSAGE, CAGE)

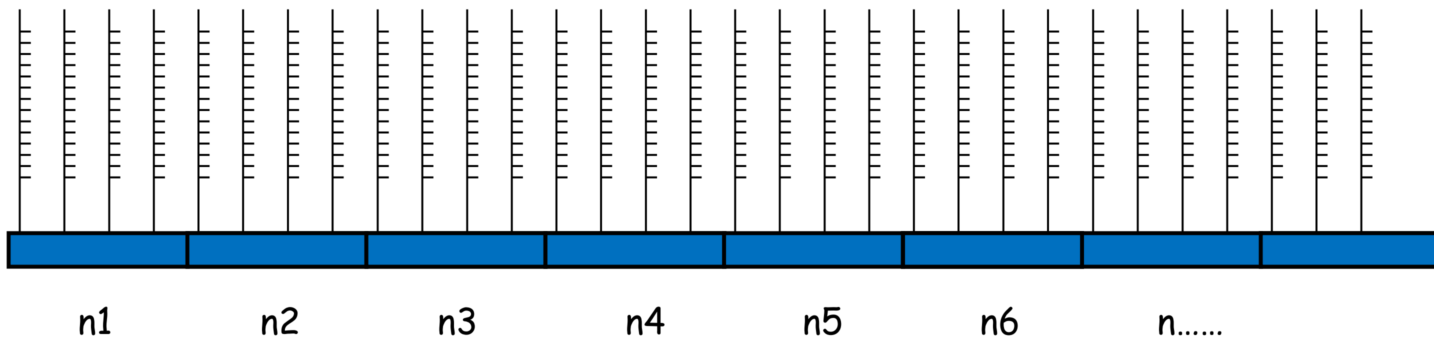
NGS sequencing (2006) (RNA-Seq)

Microarray Scheme



microarrays



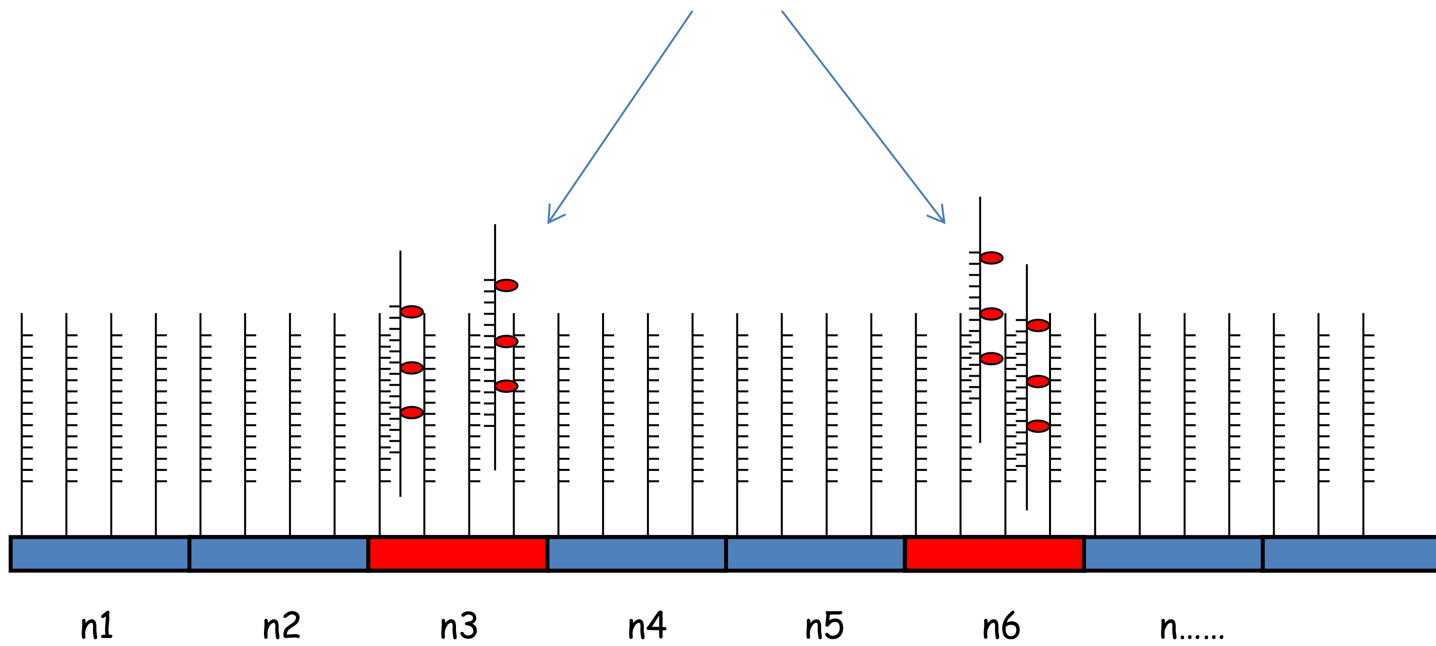


a section of a oligonucleotide microarray at row “n”

microarrays

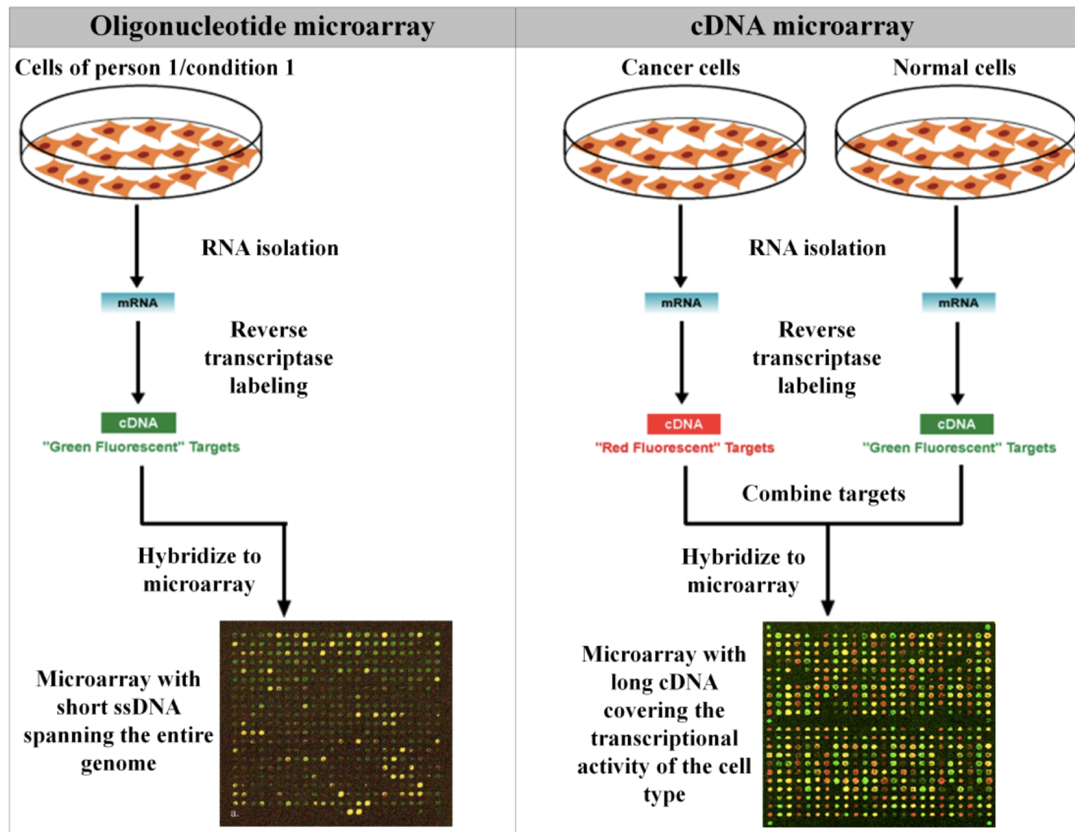
RNAs complementary to
probes n3 and n6 are detected

Two spots fluorescent



a section of a oligonucleotide microarray at row “n”

DNA microarrays



TYPES of DNA MICROARRAYS

1. **Oligo Microarray** (Short oligo probes, multiple probes per gene usually synthesized on chip)
2. **cDNA Microarray** (Longer cDNA probes usually amplified by PCR, spotted or printed on nylon/glass slides)

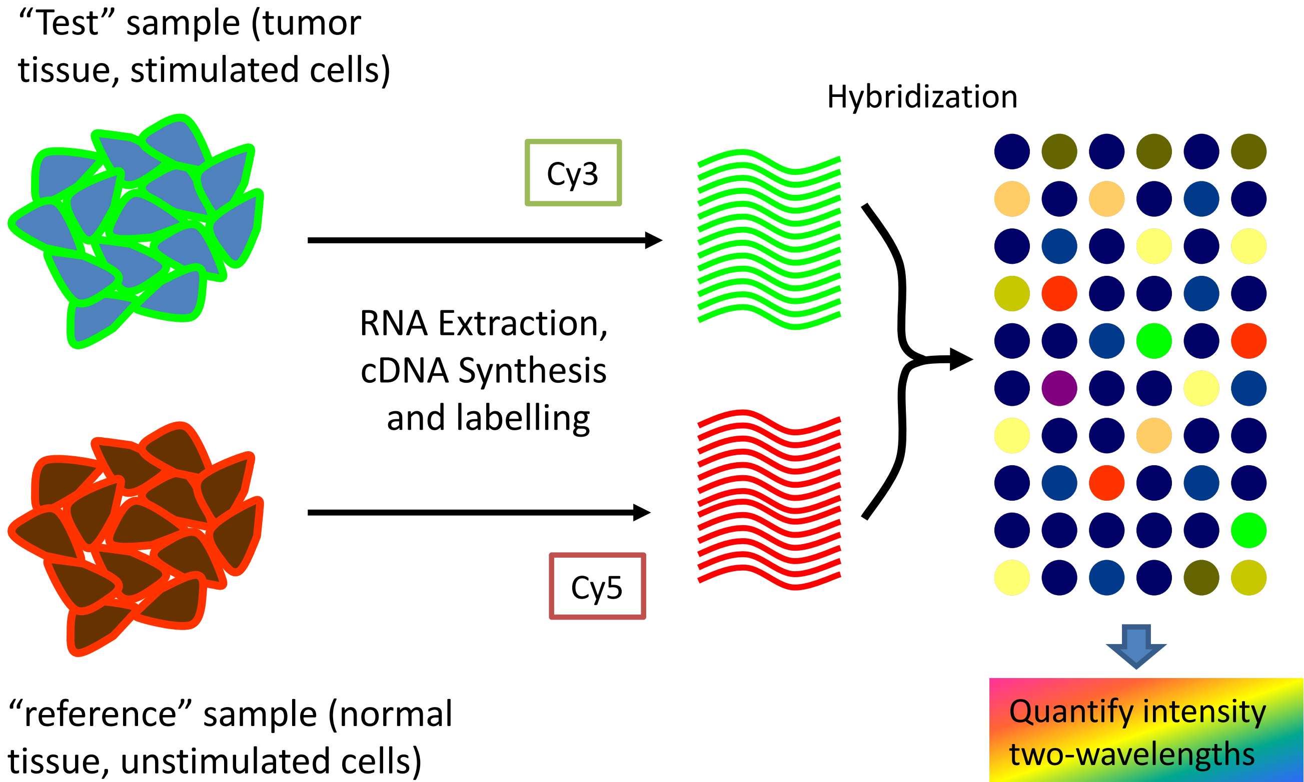
a) **Spotted** (pens, ink-jet, other technologies)

b) **Printed** (ink-jet technology)

c) **In situ**-synthesized

DNA microarrays

Spotted cDNA microarray are best used using double-color, making comparative measures

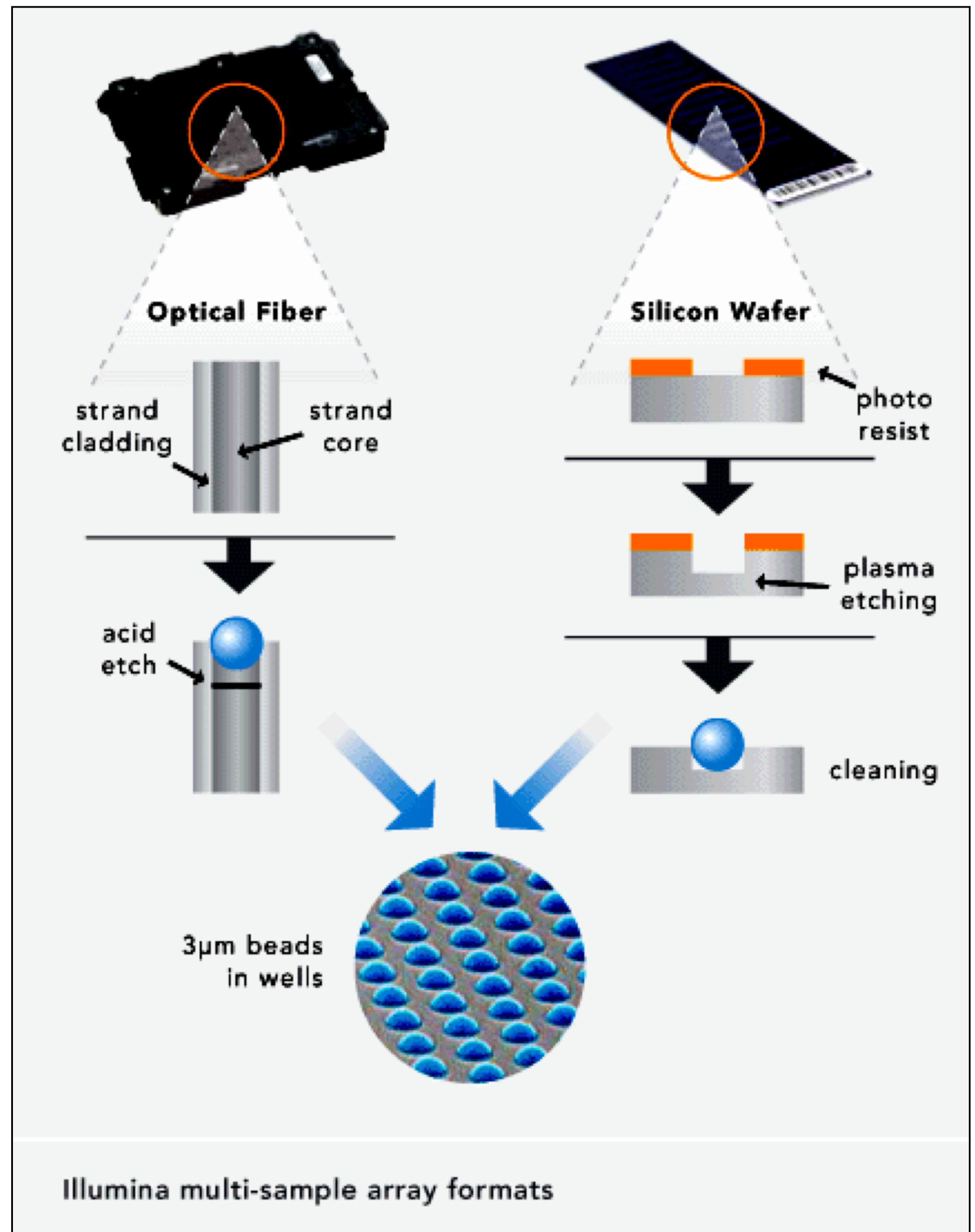


DNA microarrays

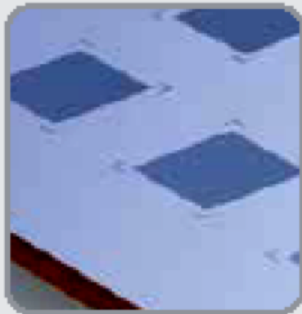
In situ synthesized

Oligonucleotide arrays (Affychip®) Affymetrix	Over 1,000,000 20-25nt long oligonucleotides / cm^2 are sythesized directly on the chip surface, using a photolitographic technique. Each gene is represented by a “probeset” of 12-13 probes.
Bead Arrays® Illumina	Oligonucleotide probes (30-50 nt) are synthesized on beads, with an identification address. Beads are randomly arrayed on surfaces and position of each oligonucleotide determined using addresses.
Agilent sure-print	Probes are synthesize on the microarrays using a proprietary DNA synthesis method based upon inkjet printing technology.

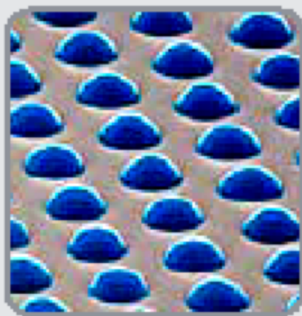
Bead-arrays® are patented by Illumina



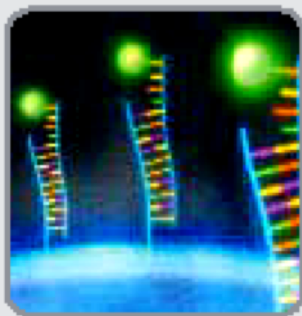
Illumina multi-sample array formats



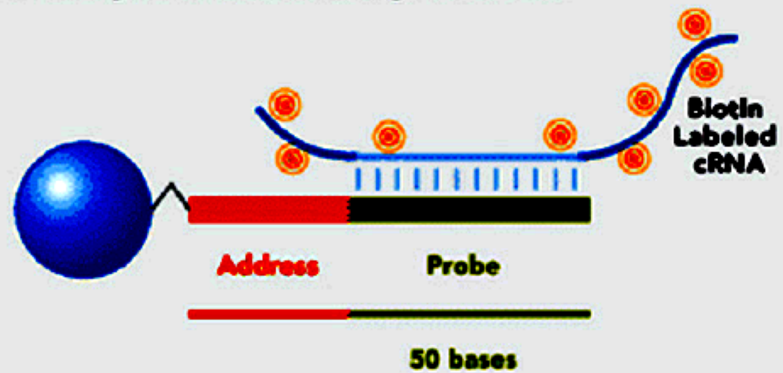
1 Each array cluster contains about 50,000 3-micron beads, or features, assembled in dense geometries.



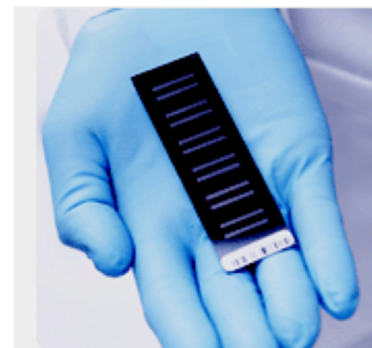
2 Over 1500 probes, or bead types, at >30x average feature redundancy, are represented in each array cluster.



3 Labeled sample targets hybridize to capture probes immobilized on the beads.



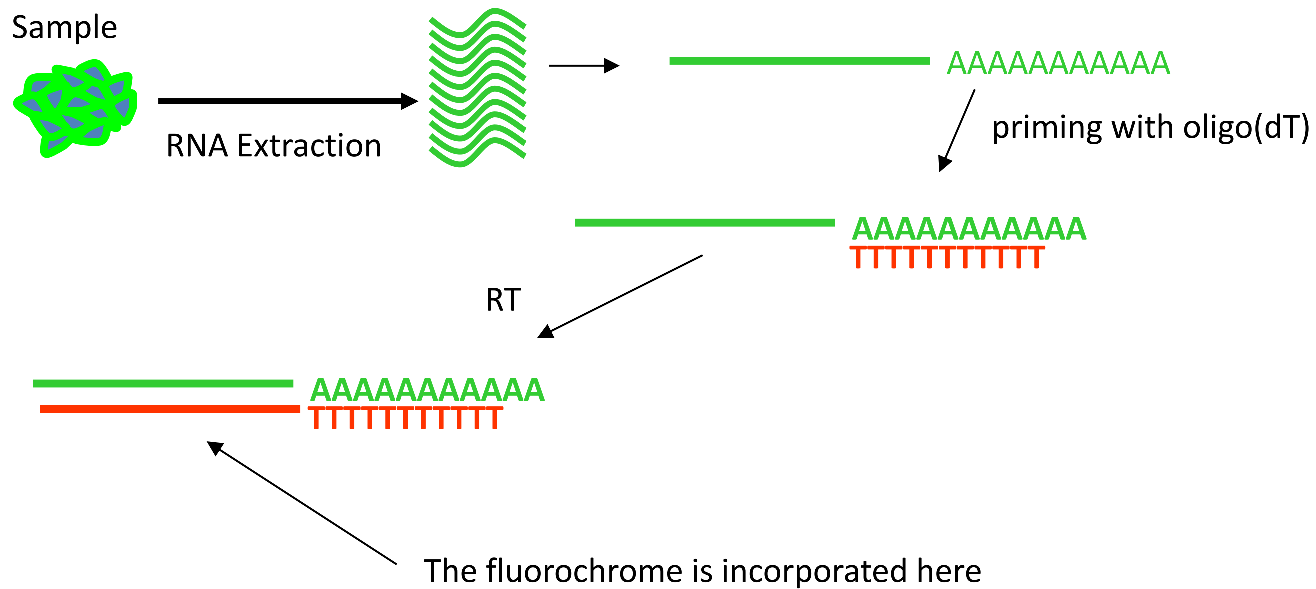
A 50-base gene-specific probe linked to short address. This probe is hybridized to labeled nucleic acid derived from total RNA.



PROBE PREPARATION

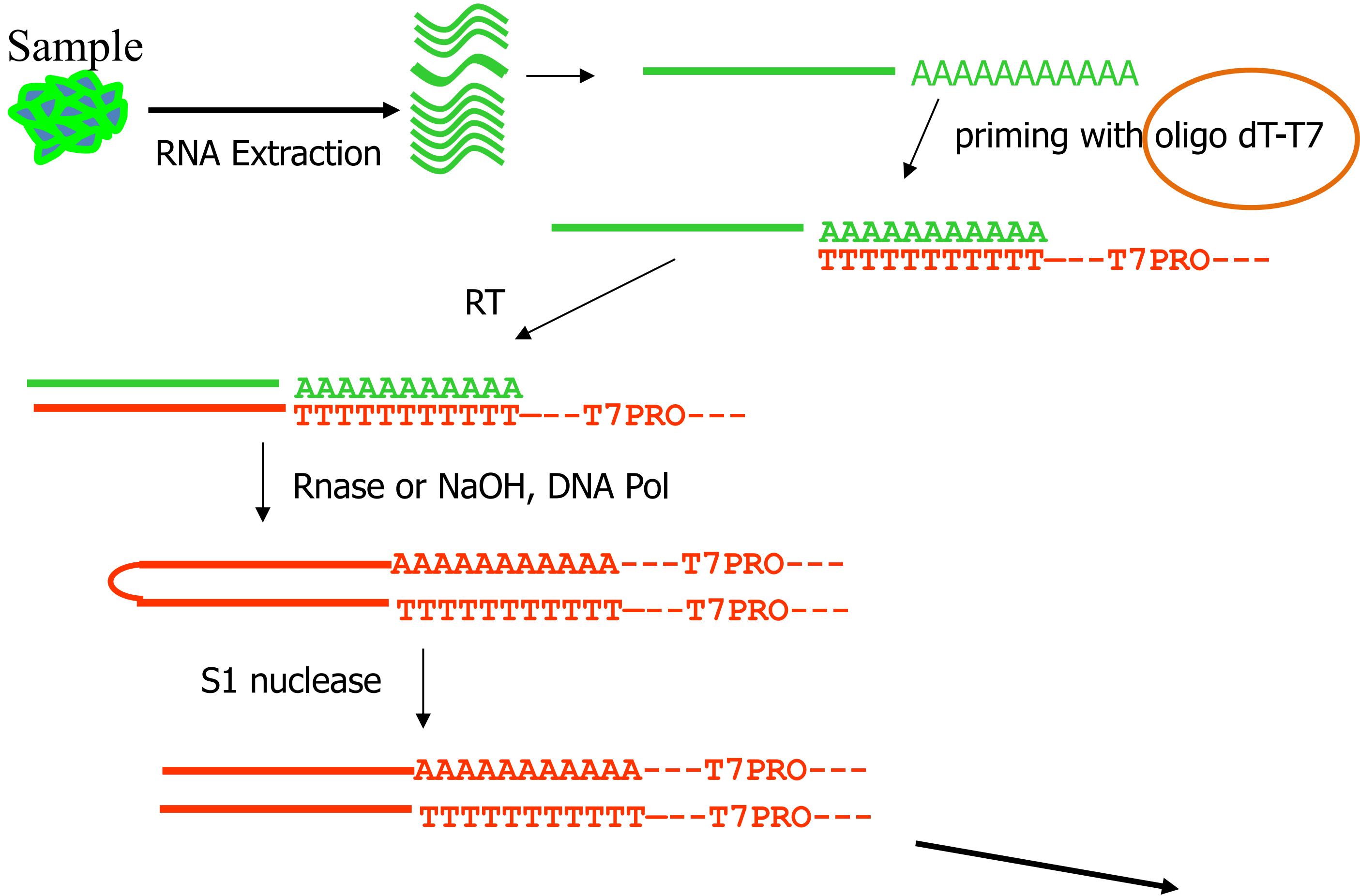
Nonamplified complex probe preparation (very low sensitivity):

RNAs are labelled using fluorochrome-conjugated ribonucleotides (NTP) directly by reverse transcription (RT), priming the synthesis either with oligo-dT or with random primers.



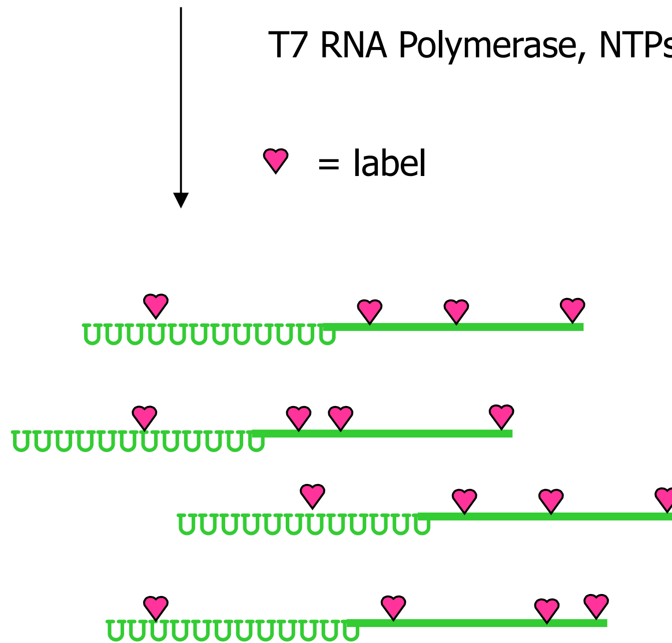
Linearly amplified complex probe preparation using T7 RNA polymerase

Why not using PCR amplification?



---T7PRO---TTTTTTTTTTT

---T7PRO---AAAAAAAAAAA



T7 RNA Polymerase, NTPs, + labelled UTP

Label may be a fluorochrome or a detectable modification, like biotin or digoxigenin, or a chemical group that can be conjugated with fluorochrome after transcription (e.g. allyl-UTP)

TRANSCRIPTION
Linear Amplification of each sequence that was originally present in starting RNA, but **complementary**
= “cRNA”

Quantitative

<u>Read-out</u> <u>of a microarray experiment for analysis of gene expression</u>

Primary read out is a table of **Fluorescence intensities**

Gene name	Probe ID	Fluorescence	Normalized
ABCD	Tor123456	346.78	1
CDH1	Tor123457	111.23	0.324
HRAS	Tor123458	222.46	0.648
TFF1	Tor123459	11.12	0.032

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(continues for N lines, from few hundreds up to 50-60,000 for genome-wide microarrays)

Bioinformatics, Statistics !

Quantitative

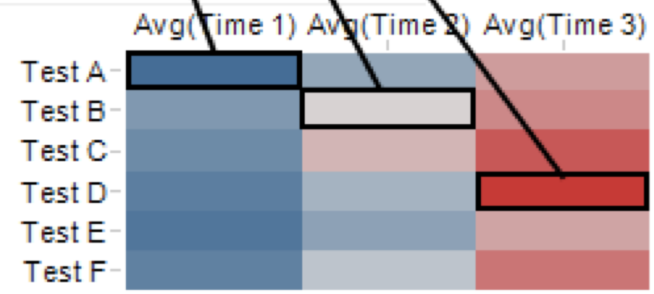
Secondary read-out is a **comparative** expression analysis

In the case of **double-color** assay: For each gene (probe) the ratio between red and green fluorescence is measured.
This is called «ratio» and is usually expressed as a Log in base 2 (Log2ratio)

In the case of **single-color** assay, each gene (probe) is associated to a Normalized value, i.e. the ratio of his normalized fluorescence to a **standard reference** gene(s).

	Nome vetrino	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_1	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_2	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_1	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_2	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4
	Numero vetrino	1	2	3	4	5	6	1	2	3	4	5	6
	Colore	Red	Red	Red	Red	Red	Red	Green	Green	Green	Green	Green	Green
	Stimolo	Tamoxifen	Tamoxifen	Untreated	Untreated	Estrogen	Estrogen	Tamoxifen	Tamoxifen	Untreated	Untreated	Estrogen	Estrogen
	Trattamento	siCtrl	siTab2	siCtrl	siTab2	siCtrl	siTab2	siTab2	siCtrl	siTab2	siCtrl	siTab2	siCtrl
ProbeName	Gene Name	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_1	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_2	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485037705_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_1	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_2	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_3	GEP_TAB2_251485036994_S01_GE2-v5_95_Feb07_2_NO_Spikein_1_4
A_24_P478940	THC2668815	1592.824	997.1755	873.5041	607.8645	6829.532	5040.509	937.3201	1259.797	590.8834	642.9285	4210.666	5307.477
A_32_P38153	AK057709	1014.571	872.695	284.9987	256.1089	2096.175	2135.351	888.3797	1007.321	359.0979	268.0768	2749.321	2277.641
A_32_P49199	PGR	367.4936	431.2933	269.8744	268.6231	1955.63	1716.041	451.8824	339.7921	353.0977	251.629	2050.603	1702.065
A_23_P216225	EGR3	188.7889	117.2561	137.3518	95.94292	849.5961	378.4002	117.6472	166.3025	104.9777	127.9327	439.5834	667.7256
A_23_P73526	CITED1	256.0494	242.8608	100.6554	106.6672	586.6913	574.1368	239.8019	218.5055	122.4987	106.5085	570.5793	502.1365
A_23_P138938	PGR	202.7657	223.1904	166.2498	178.8664	836.921	950.6731	279.9407	197.2315	201.9929	154.4607	911.349	842.6516
A_23_P58407	UGT2B15	290.318	392.5214	159.4434	257.2121	755.6996	1108.057	421.2808	287.7875	276.8222	171.0651	1070.959	898.7676
A_23_P329768	GREB1	387.7765	397.7517	458.2526	438.8445	2063.436	1733.951	792.4646	795.0189	1399.793	1415.548	5009.719	6896.316
A_24_P17691	UGT2B17	225.9956	308.0241	155.5428	244.09	725.6027	934.7345	257.7644	204.1438	185.0093	143.4956	676.1984	573.0021
A_23_P69699	NPY1R	1255.263	1511.697	1073.773	1224.392	4469.823	4513.5	2907.967	2214.892	2590.699	2207.618	10689.16	9776.069
A_23_P202245	RET	1163.705	693.7436	614.1019	302.3615	2485.864	1702.553	696.5159	891.0366	319.0103	493.6255	1659.763	2235.899
A_32_P57877	CD365380	143.9107	149.4294	125.7661	143.0269	922.993	157.3608	157.2323	120.7246	148.6814	135.7534	609.369	151.409
A_23_P353614	C8orf46	117.3481	130.8704	124.6863	124.9572	468.4099	442.0125	123.2606	126.1182	131.7437	110.9967	472.9365	492.9662
A_23_P162579	HSPB8	3419.048	5547.424	1683.745	2566.708	6719.538	8617.95	5334.391	3374.663	2706.644	1632.549	8554.665	6753.329
A_24_P520201	THC2553558	114.9983	107.5519	114.1133	120.1143	796.6127	114.9806	122.9388	114.0368	121.6015	115.0924	508.4736	126.1093
A_23_P43157	MYBL1	1035.348	538.7504	1012.306	501.9906	3764.8							
A_23_P202448	CXCL12	679.2454	719.6995	560.4945	597.2649	2245.09							
A_23_P168351	HEY2	222.4111	362.0376	151.4344	228.0122	550.172							
A_23_P352535	PPP1R16B	257.9374	228.1916	253.6183	312.2321	1657.46							
A_24_P100228	XBP1	13673.57	13781.52	10014.85	10653.04	36387.2							
A_24_P403417	PTGES	128.3569	115.2637	101.5564	91.30175	341.982							
A_24_P511686	CR616845	918.5523	863.9754	521.2184	499.116	1763.46							
A_23_P148609	PLAC1	212.4161	231.6628	172.3428	151.8443	584.15							

Quantitative

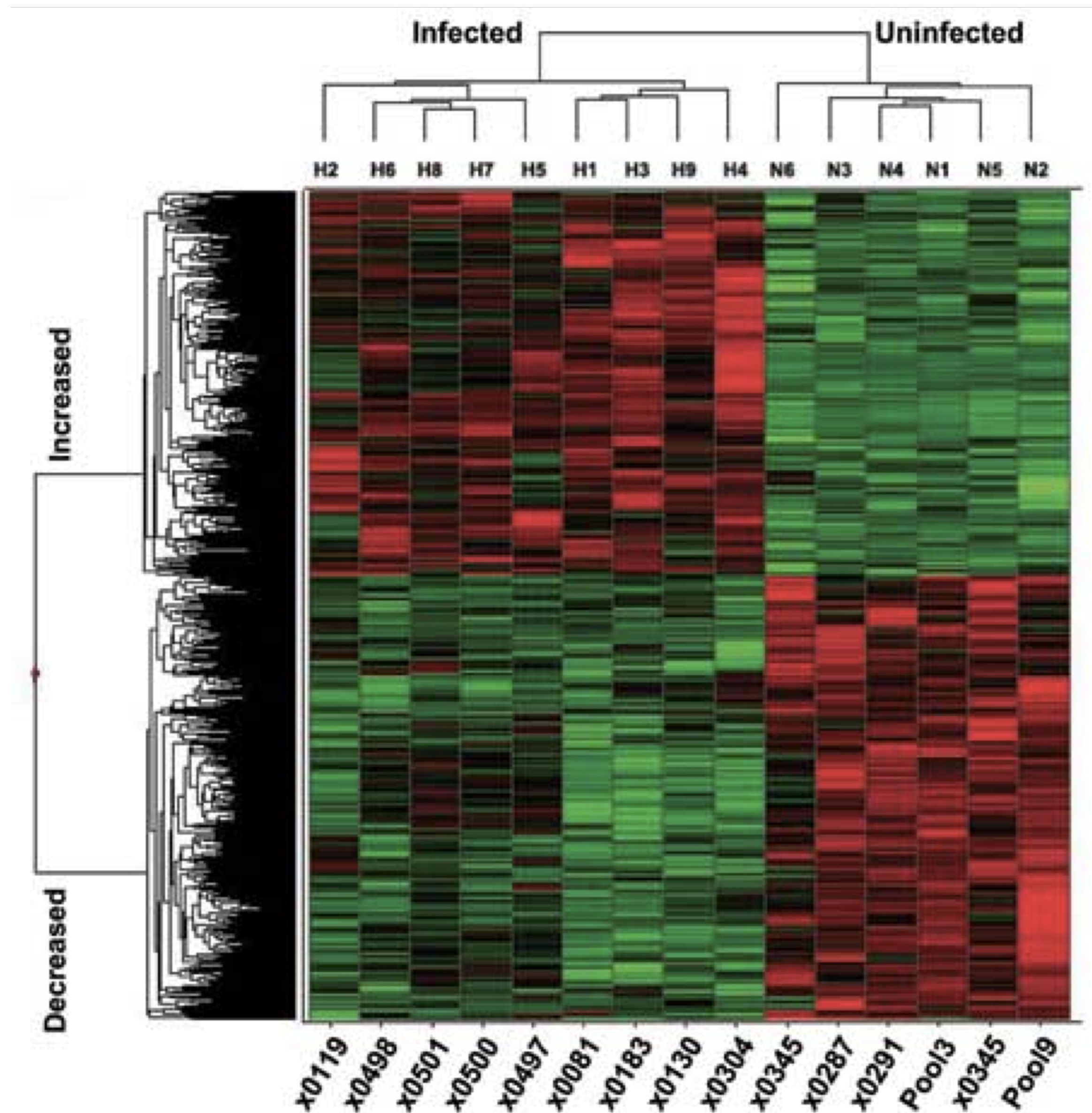


How to best visualize these data?

Gene expression data is often displayed as a heatmap. In heat maps the data is displayed in a grid where each row represents a gene and each column represents a sample.

The heatmap can be combined with clustering methods which group genes and/or samples together based on the similarity of their gene expression pattern (supervised or unsupervised hierarchical clustering).

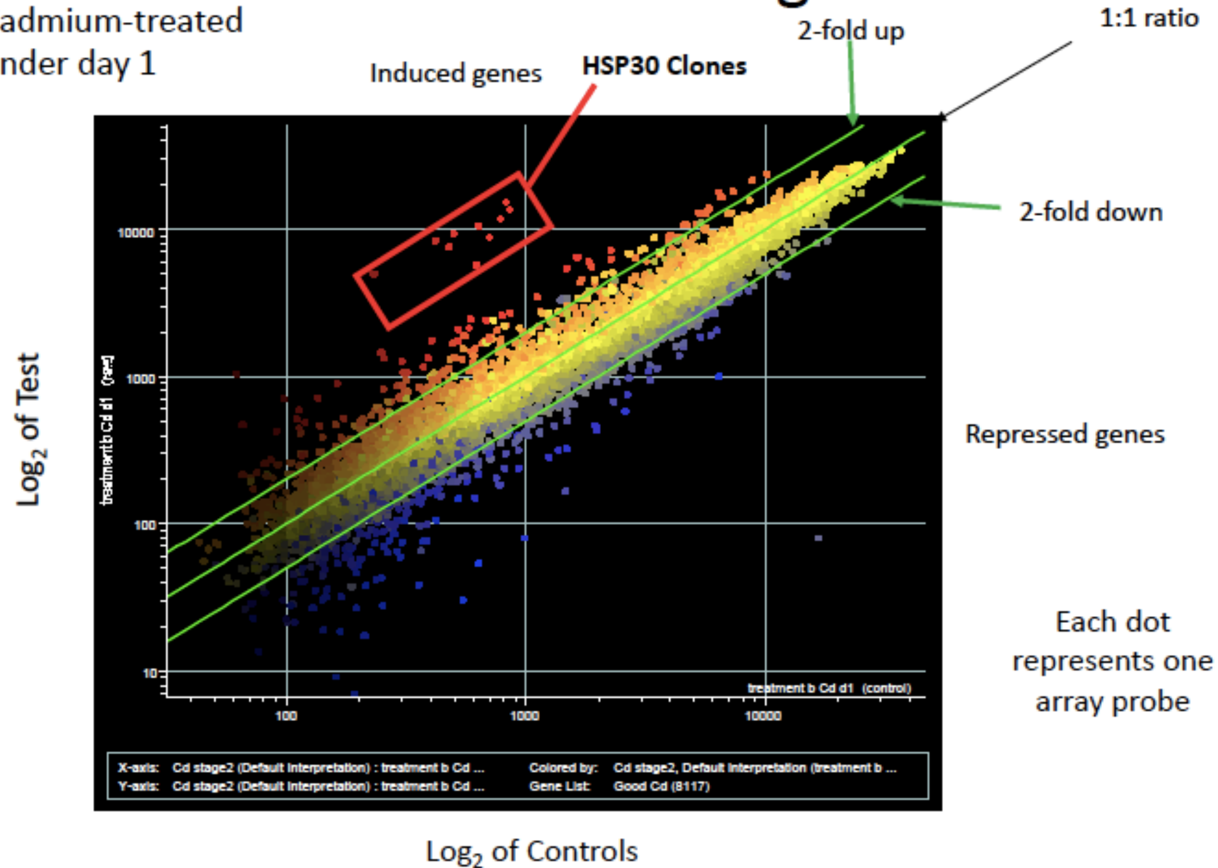
This can be useful for identifying genes that are commonly regulated, or biological signatures associated with a particular condition (GO analysis).



Alternative representation: SCATTER PLOTS

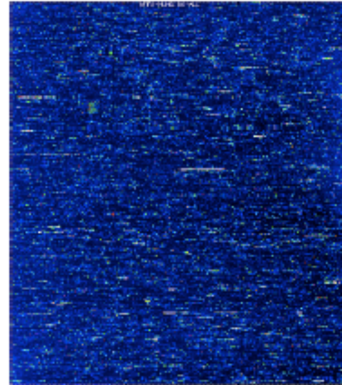
Example scatter plot of Cadmium-treated flounder day 1

Scatter Plot - Logarithmic



An Example Application

- 72 leukemia patients
  - 47 ALL
  - 25 AML
- 1 chip per patient
- 7132 human genes per chip



Golub, et al., Science 286:531-537 (1999).

REPORTS

Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring

T. R. Golub,[1,2*†] D. K. Slonim,[1†] P. Tamayo,[1] C. Huard,[1] M. Gaasenbeek,[1] J. P. Mesirov,[1] H. Coller,[1] M. L. Loh,[2] J. R. Downing,[3] M. A. Caligiuri,[4] C. D. Bloomfield,[4] E. S. Lander[1,5*]

Key Issue:
What's Different?

- What genes are behaving differently between ALL & AML (or other disease/normal states)?
- Potential uses:
  - Diagnosis
  - Prognosis
  - Insight into underlying biology/biologies
  - Treatment

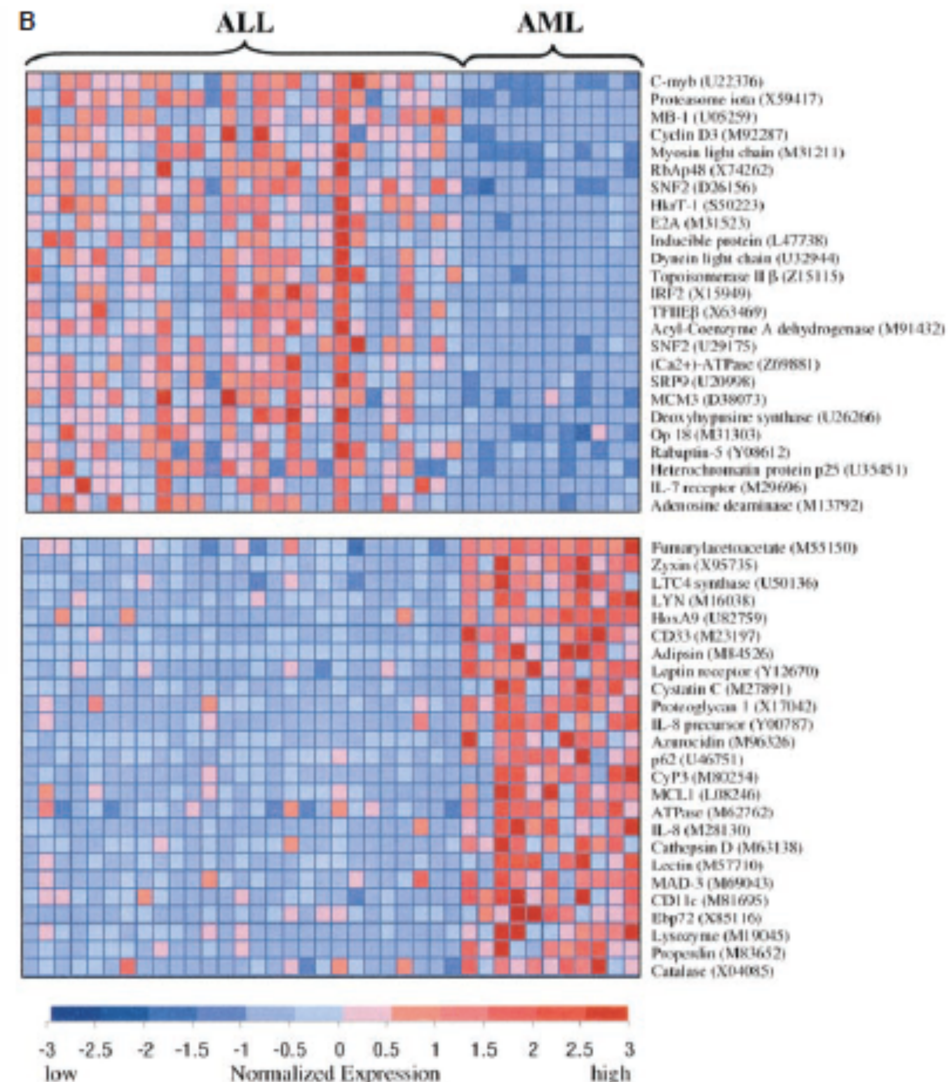


Fig.2: 50 genes with highest correlation with the two diseases

Leukemia (2007) 21, 1984–1991
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www.nature.com/leu

ORIGINAL ARTICLE

Molecular heterogeneity in chronic lymphocytic leukemia is dependent on BCR signaling: clinical correlation

A Rodríguez[1], R Villuendas[1], L Yáñez[2], ME Gómez[3], R Díaz[4], M Pollán[5], N Hernández[3], P de la Cueva[1], MC Marín[1], A Swat[1], E Ruiz[6], MA Cuadrado[2], E Conde[2], L Lombardía[7], F Cifuentes[8], M Gonzalez[9], JA García-Marco[10] and MA Piris[1] for Spanish National Cancer Centre (CNIO)

Chronic lymphocytic leukemia (CLL), the most frequent form of adult leukemia in Western countries, is characterized by a highly variable clinical course. Expression profiling of a series of 160 CLL patients allowed interrogating the genes presumably playing a role in pathogenesis, relating the expression of functionally relevant signatures with the time to treatment.

First, we identified genes relevant to the biology and prognosis of CLL to build a CLL disease-specific oligonucleotide microarray. Second, we hybridized a training series on the CLL-specific chip, generating a biology-based predictive model. Finally, this model was validated in a new CLL series. Clinical

identifies three risk-score groups with treatment-free survival probabilities at 5 years of 83, 50 and 17%. This molecular predictor can be applied to early clinical stages of CLL. This

Genes associated with CLL and progression: gene selection

We used a multistep approach to develop a predictive model for progression in CLL. First, a high-throughout analysis was performed in a previous series of 23 cases of CLL21 using the Agilent Human 1A 22K Oligonucleotide Microarray, identifying 88 genes statistically associated with survival (adjusted P-valueo0.2).

After a comprehensive literature search, we also selected all the genes previously described as being associated with pathogenesis and prognosis of CLL, 409 genes derived from 23 publications. In total, 497 genes (Supplementary Table 5) were obtained from the first step and the literature search and were included in the CLL-specific oligonucleotide microarray.

For control purposes, 58 of these selected genes were printed in duplicate or triplicate on the microarray.

The 526 genes with a low variability of expression in the 23 cases hybridized in the first step were included as normalization genes. Oligonucleotide sequences as internal controls of hybridization (323 sequences) were also incorporated.

Finally, 1900 sequences were printed in known positions on the microarray using an eight-pack microarray format. The array description has been submitted to ArrayExpress (accession number: A-MEXP-328).

Rodriguez et al., Leukemia 2007

We performed genomic-scale gene-expression profiling of a series of 160 untreated CLL patients using the original CLLspecific microarray. This was divided between a training set (98 patients) to create a predictive model and a validation set (62 patients) from a different Institution to corroborate the results.

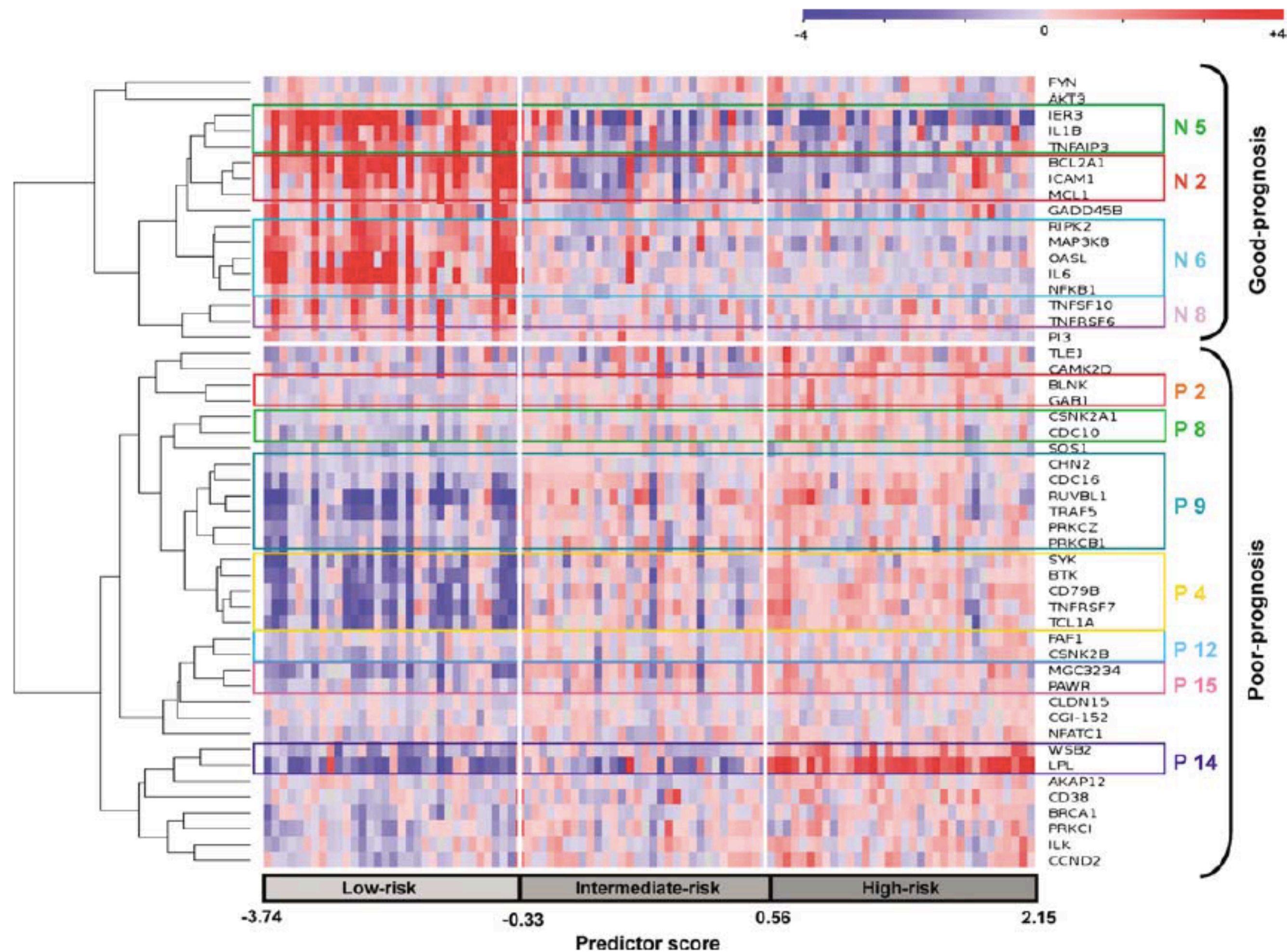


Figure 2 Heat map of prognosis genes. Identification of the signatures associated with variation in time to progression in training series. The series has been divided into terciles. Cases with increased expression of the genes associated with BCR signaling have a shorter time to progression, while cases with greater expression of MAPK/NF-κB genes have a more favorable outcome. Gene expressions are represented as median center values.

Rodriguez et al., Leukemia 2007

Table 3 KEGG pathway annotation for 50 genes associated with TFS

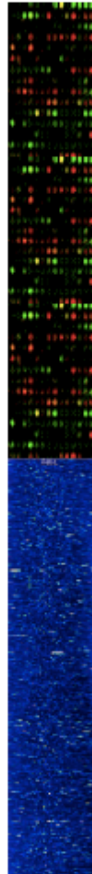
KEGG pathways	*Genes*	*No. of genes*
Genes associated with shorter time to progression		
B-cell receptor signaling pathway	BTK, CD79B, BLNK, NFATC1 and SYK	5
Wnt signaling pathway	CCND2, CSNK2B, RUVBL1, CAMK2D and NFATC1	5
Tight junction	PRKCZ, CSNK2B, PRKCI and CLDN15	4
Focal adhesion	CCND2, SOS1 and ILK	3
Insulin signaling pathway	SOS1, PRKCZ and PRKCI	3
Calcium signaling pathway	CD38, CAMK2D and NFATC1	3
T-cell receptor signaling pathway	SOS1 and NFATC1	2
Jak–STAT signaling pathway	CCND2 and SOS1	2
Genes associated with longer time to progression		
MAPK signaling pathway	GADD45B, MAP3K8, IL-1B, TNFRSF6, NF-κB1 and AKT3	6
Apoptosis	IL-1B, TNFRSF6, TNFSF10, NF-κB1 and AKT3	5
Toll-like receptor signaling pathway	IL-6, IL-1B, NF-κB1 and AKT3	4
Cytokine–cytokine receptor interaction	IL-6, IL-1B, TNFRSF6 and TNFSF10	4
T-cell receptor signaling pathway	FYN, MAP3K8, NF-κB1 and AKT3	4
Focal adhesion	FYN and AKT3	2
Hematopoietic cell lineage	IL-6 and IL-1B	2
Adipocytokine signaling pathway	NF-κB1 and AKT3	2
B-cell receptor signaling pathway	NF-κB1 and AKT3	2
Jak-STAT signaling pathway	IL-6 and AKT3	2

Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; TFS, treatment-free survival.

Tomorrow: How to perform pathway analyses

Rodriguez et al., Leukemia 2007

DNA microarrays have been extensively used in the last decade and are still largely used to gather qualitative and quantitative information on known transcribed sequences.



Practical Applications of Microarrays

Gene Target Discovery
- Diseased vs normal cell comparison suggests sets of genes having key roles.
- Over/underexpressed genes in the diseased cells can suggest drug targets

Pharmacology and Toxicology
- Highly sensitive indicator of a drug's activity (pharmacology) and toxicity (toxicology) in cell culture or test animals.
- Screen or optimize drug candidates prior to costly clinical trials.

Diagnostics
- Potential to diagnose clinical conditions by detecting gene expression patterns associated with disease states in either biopsy samples or peripheral blood cells.

CONCLUSIONS on MICROARRAYS

Microarrays were the first, widely used method to address the transcriptome in many experimental and pathological sistuations

Very important for pathological characterization: guide to prognosis and treatment

Very important experimentally: DE gene have functional significance (G.O.), belong to defined functional pathways and can also be explored to identify regulatory motifs.

<u>Pros:</u>

1. Easy to use and analyze
2. Fast readout
3. Relatively cheap (possibility to limit to specific transcriptomes)

<u>Cons:</u>

1. Probes describe only «part» of the transcript
2. Alternatively spliced exons can be missed
3. Novel transcripts are missed
4. Quantitation is indirect
5. Comparison among different platforms problematic