Introduction to microarrays (1)

Basic concepts

Software

Annotations

Databases

Presentation

Content

- Introduction
- Production and use of microarrays
- From Images to expression matrices
- Microarray bioinformatics
    - Software for the analysis of microarray data
    - Annotations and annotations databases
    - More microarrays databases.

Introduction

Some history

- Molecular biology has many techniques to measure RNA, DNA, proteins or metabolites.
  - Northern blot, differential display, SAGE
  - Southern blot: [similar to microarray]
- What characterizes the post genomic era is not what can be measured but the number of simultaneous measurements that can be performed.

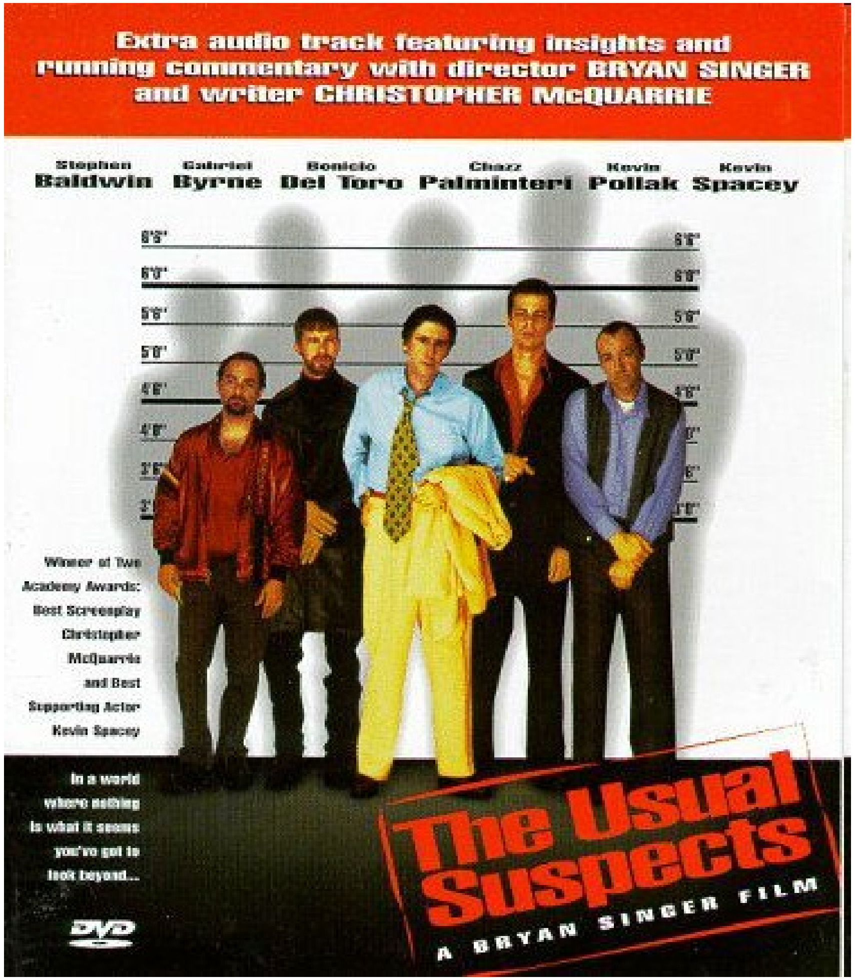
A CSI approach to gene selection

- A crime has been commited (tumour)
- You’re CSI –Horatio Fisher- and want to find who’s responsible for this.
- Let’s see how you would act …
  - In the old times,
  - In the microarray age,
  - In the next generation age.

In the old times …

- You would chase the “Usual Suspects” and make an in deep interrogation.
  - If guilty you might make them talk,
  - But if not you might miss the bad guy.

- *That is looking at specific genes may yield great or awful results.*


Extra audio track featuring insights and running commentary with director BRYAN SINGER and writer CHRISTOPHER McQUARRIE
Stephen Baldwin
Gabriel Byrne
Benicio Del Toro
Chazz Palminteri
Kevin Pollak
Kevin Spacey
Winner of Two Academy Awards: Best Screenplay Christopher McQuarrie and Best Supporting Actor Kevin Spacey
In a world where nothing is what it seems you've got to look beyond...
DVD
The Usual Suspects
A BRYAN SINGER FILM

In the microarray age…

- You have the census of most people and their fingerprints.
  - If you find a fingerprint in your database that is clean enough you may find the bad guy.
    - What about bad prints?
    - What about those who are not censed.
    - And those no-fingerprints?

- *That is you may look at all known genes but you*
  - *do it Indirectly and noisly*
  - *miss genes/forms that are uncensed.*

In the NGS epoch (now)

- If the crime scene had had cameras you would have directly known who the criminal was.

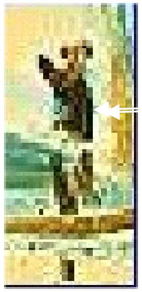
- *Sequencing allows you to access* ***everything***
  - *Known and unknown forms are sequenced.*
  - *The technique is less noisy and the resolution higher.*

Microrrays represent a *paradigm shift*

With the same resources

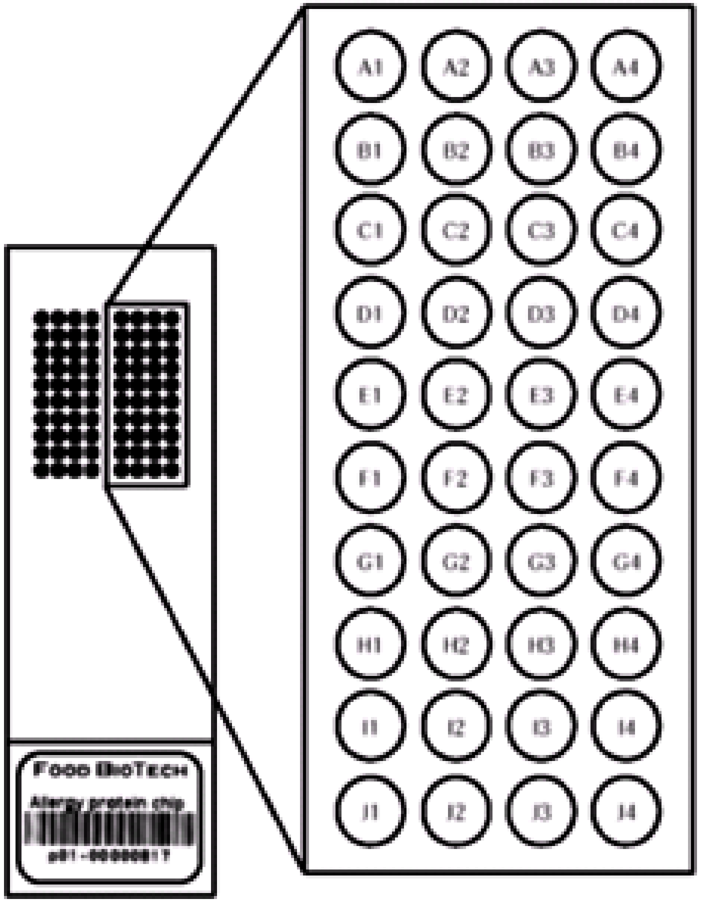
we obtain an image of lower

resolution but wider scope



So, What is a microarray?

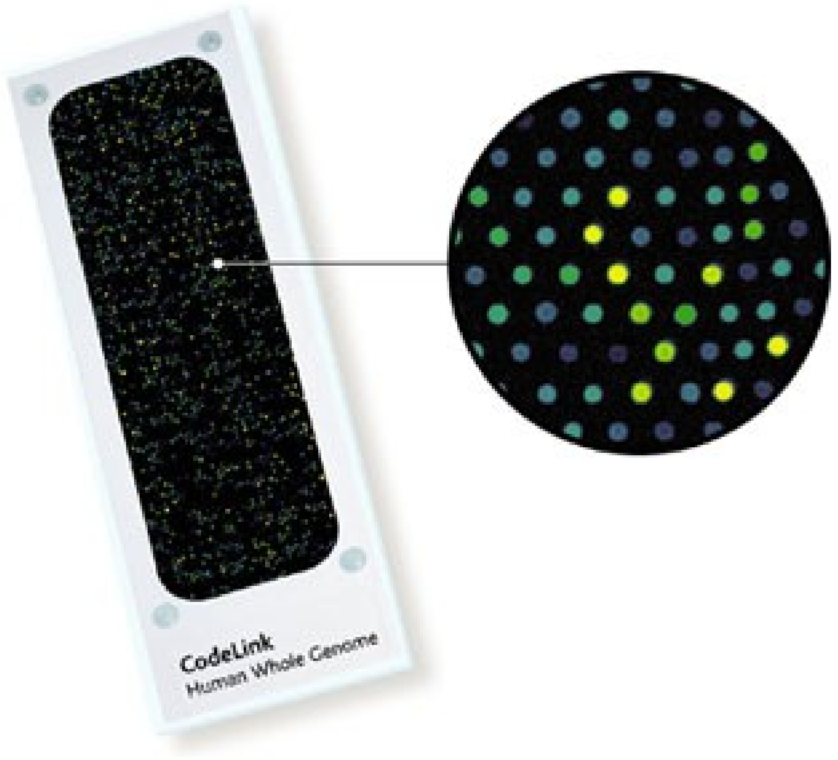
- An experimental format,
- based on the synthesis or attachment of probes, which represent genes (or proteins, or metabolites ...),
- on a solid substrate (glass, plastic, silica ...),
- Intended to be exposed to the target molecules (the sample).



How does it work?

- The level of hybridization between
  - □ specific probes and
  - □ target molecules
- is generally indicated by means of fluorescence and
- is measured by image analysis.

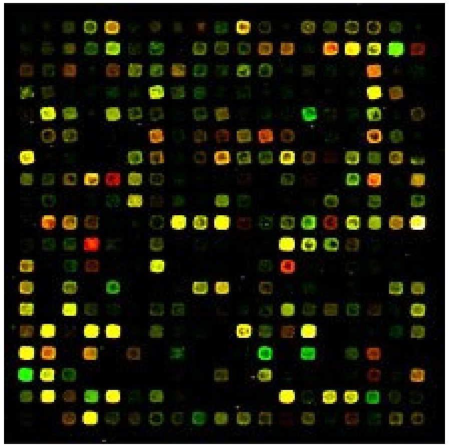
- The measure obytained indicates
  - □ the level of expression of the gene corresponding to the probe
  - □ in the test sample

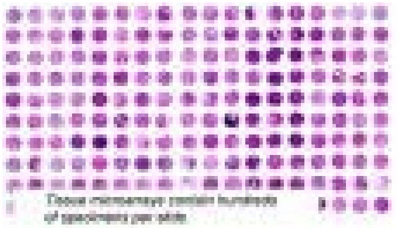


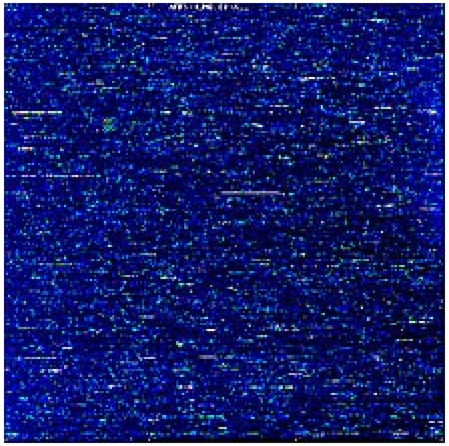
Types of microarrays

Protens

- Tissues
- DNA
  - □ CGH arrays
  - □ SNP arrays
- RNA (expression)
  - □ Two color (or cDNA)
    - e.g. Agilent
  - □ One color (or Affymetrix or oligonucleótidos)
    - GeneChip® Affymetrix
    - Illumina bead arrays







Microarray applications

- Study of genes that are differentially expressed between various conditions (Healthy / sick, mutant / wild treated / untreated)
- Molecular classification of complex diseases
- Identify sets of genes characterizing a disease (signature or "signature")
- Predicting the response to treatment
- Detection of mutations and single polymorphisms (SNP)

But also

- Circadian clock analysis,
- Plant defence mechanisms,
- Environmental stress responses,
- Fruit ripening,

Production and use of microarrays

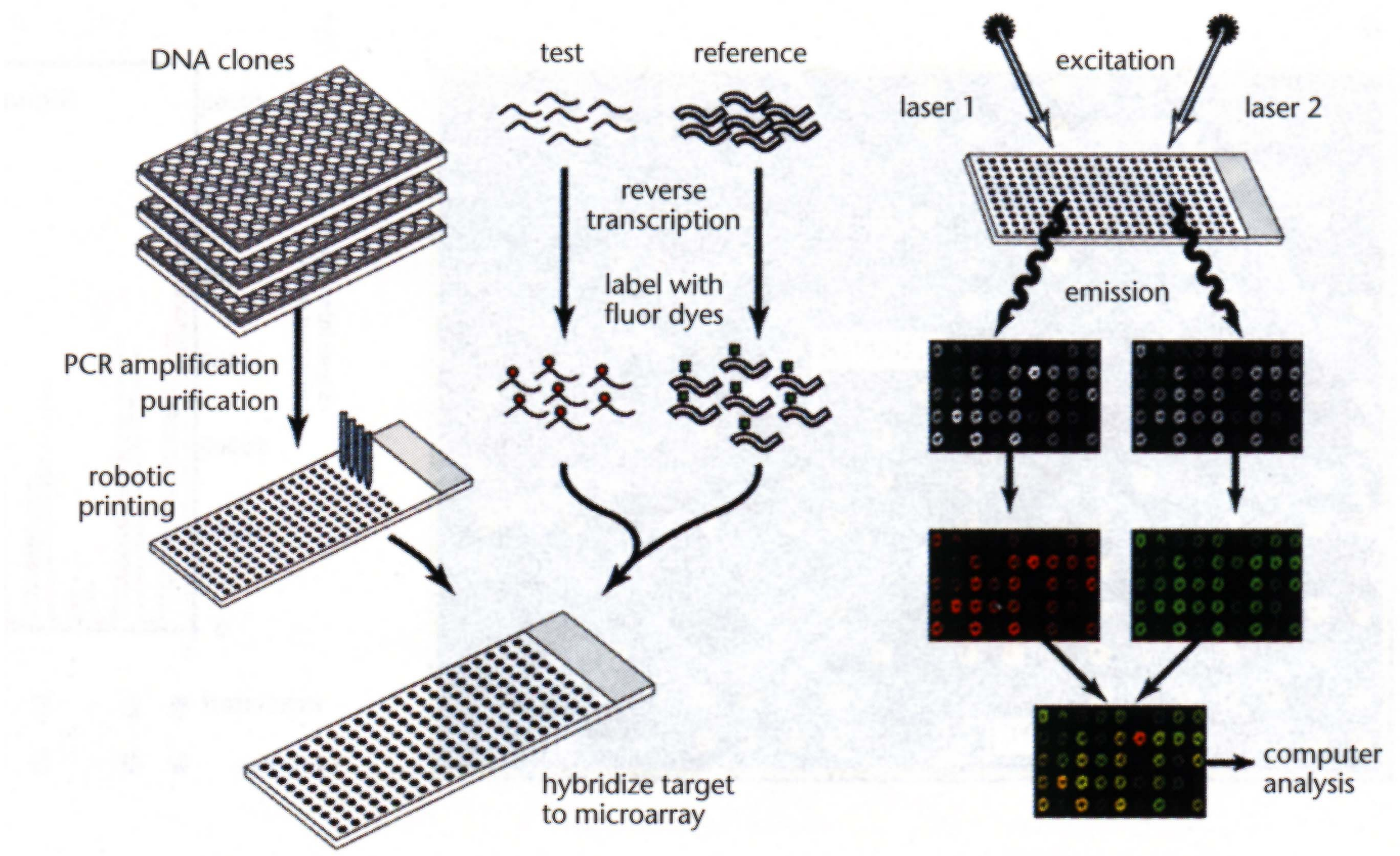
Expression microarrays

- There are many types of microarrays
- They rely on similar principles but
- the details of its operation change from one to another case
- Here we focus on expression arrays
  - 2-color arrays (spotted)
  - Oligonucleotide arrays (in situ synthesized).

Two colour microarrays (spotted)

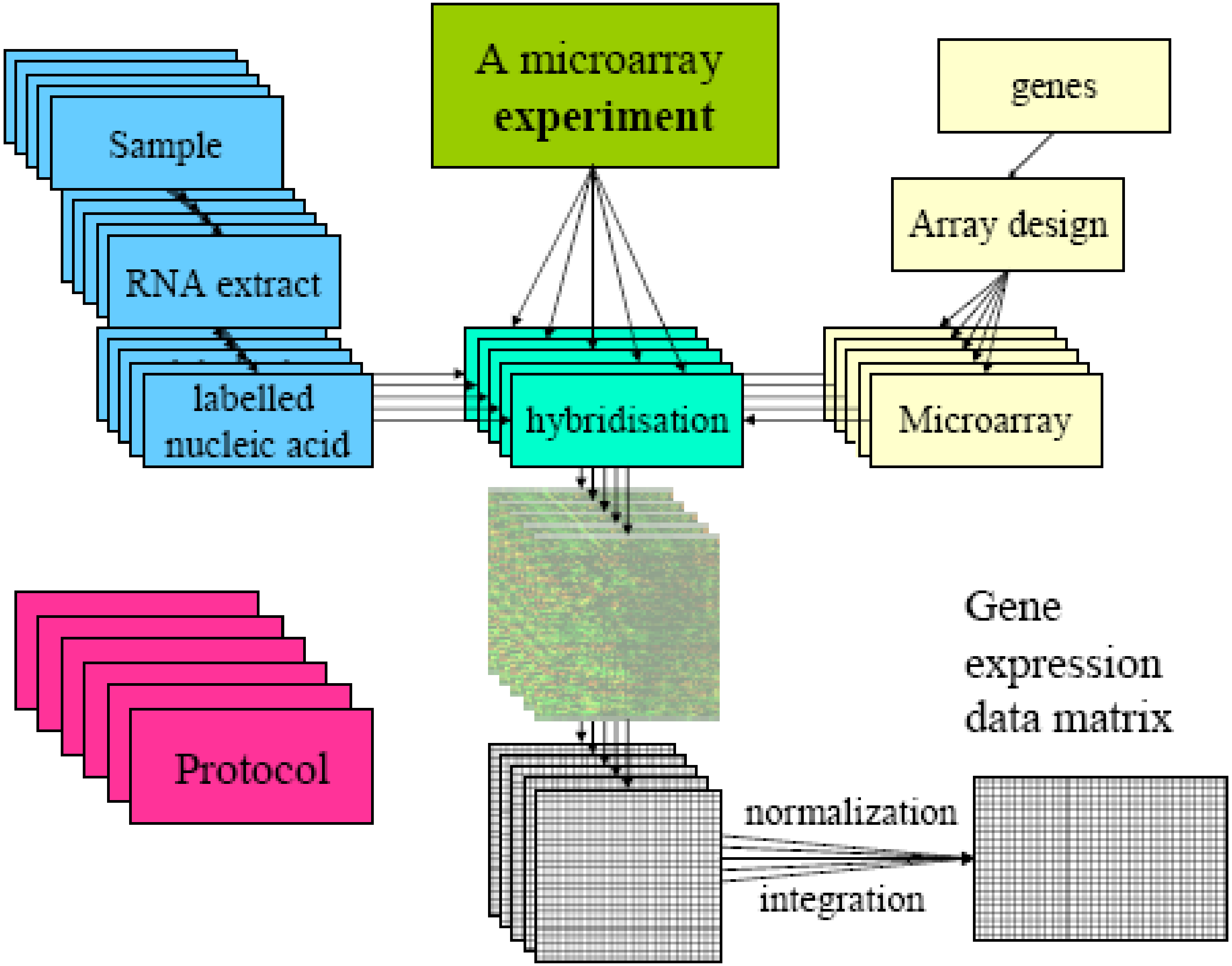
- Chip design and production
- Sample Preparation
- Hybridization
- Scanning the chip
- Image analysis

General overview of the process



To visualize an animation go to:

http://www.bio.davidson.edu/courses/genomics/chip/chip.html



A microarray experiment

Sample

RNA extract

labelled nucleic acid

hybridisation

genes

Array design

Microarray

Protocol

Gene expression data matrix

normalization

integration

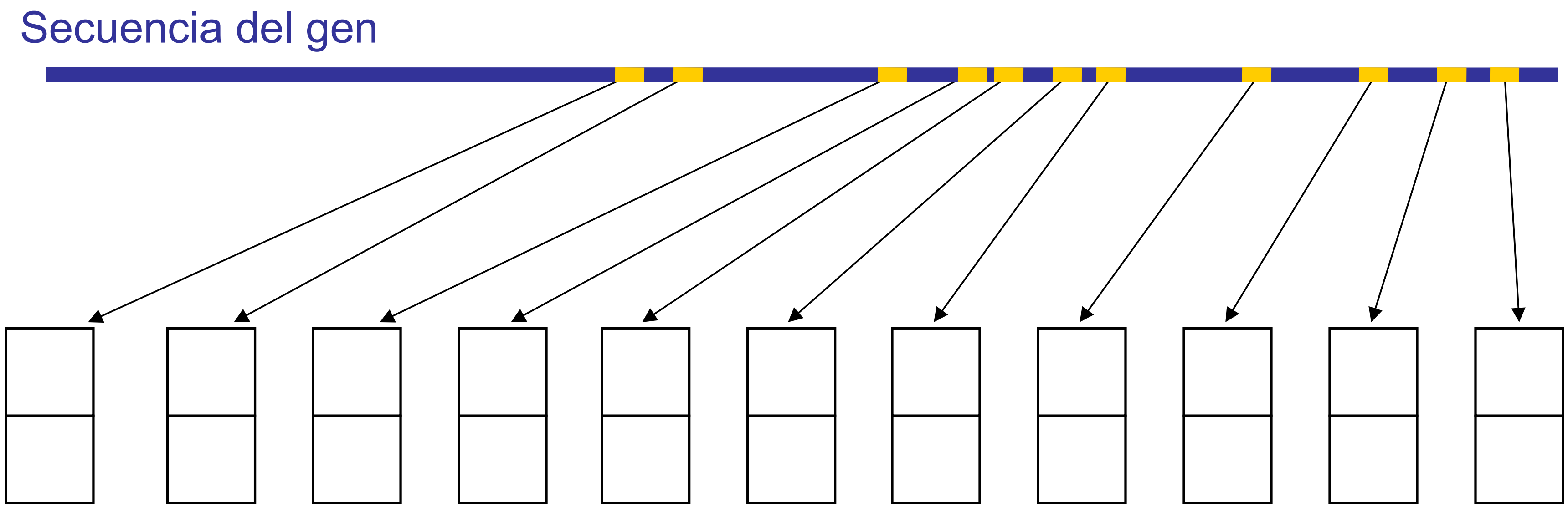
Oligo microarrays sinthesized *in situ*

- More advanced design than 2 colors
- Rely on technologies developed for microelectronics
- Some distinctive features
  - Not based on competitive hybridization: each chip containing samples from a single type (aka "1 color")
  - Probes are synthesized directly on the chip instead of in vitro synthesized and then attached to slides
  - Each gene is represented by a group of short probes rather than a single long probe

Probesets, probes, PM & MM

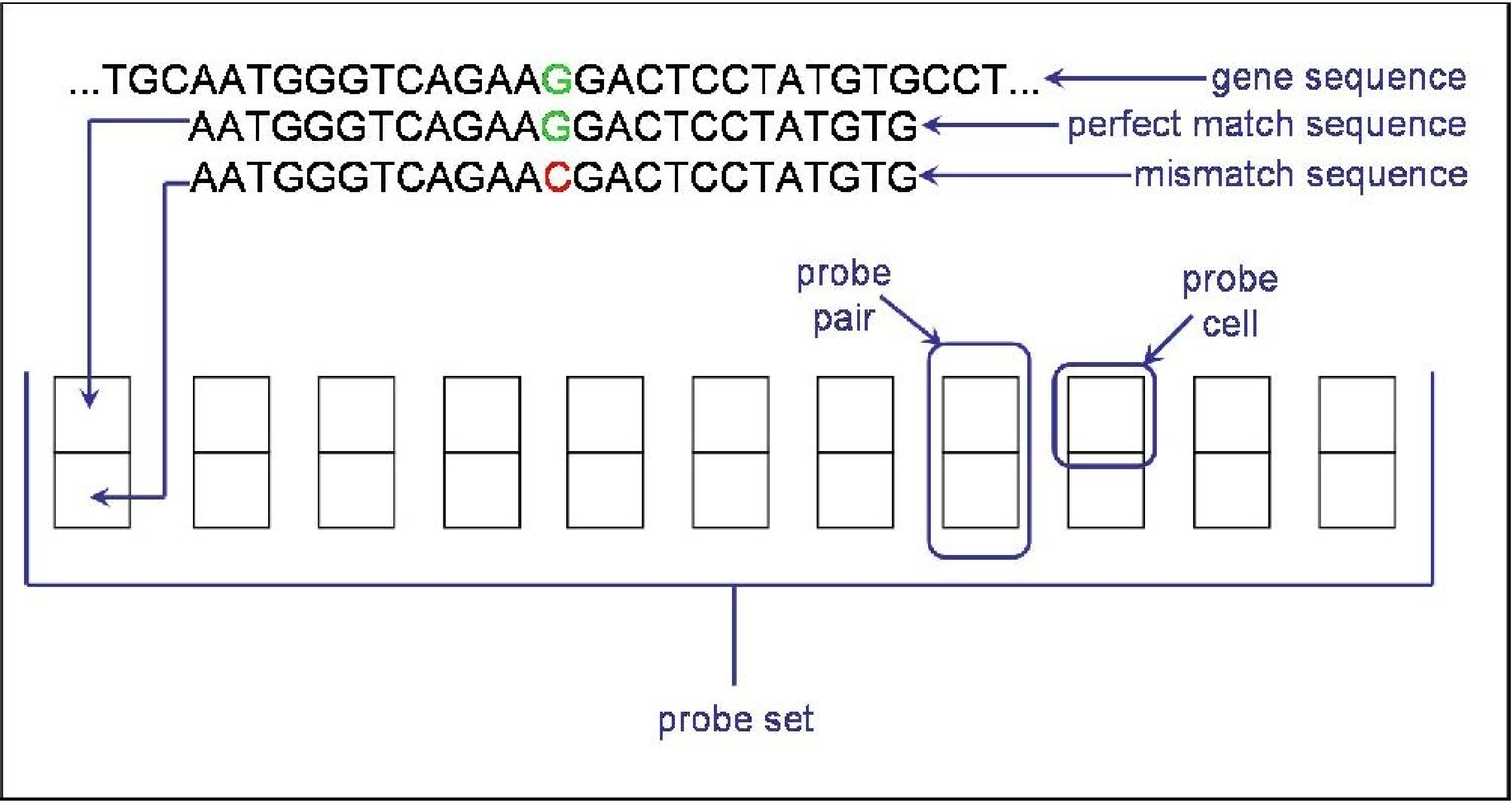
- A set of probes is used to measure mRNA level of a single gene.
- Each group (probeset) consists of multiple pairs of cells (probe cells)
  - with millions of copies of a 25bp oligo.
- Pairs consist of
  - a Perfect Match (PM) which coincides exactly with a portion of the gene
  - A Mismatch (MM) identical to PM except in the central nucleotide replaced with its complementary

Probesets are made of "Probe pairs" which represent different parts of same gene(1 gene =1 probeset)

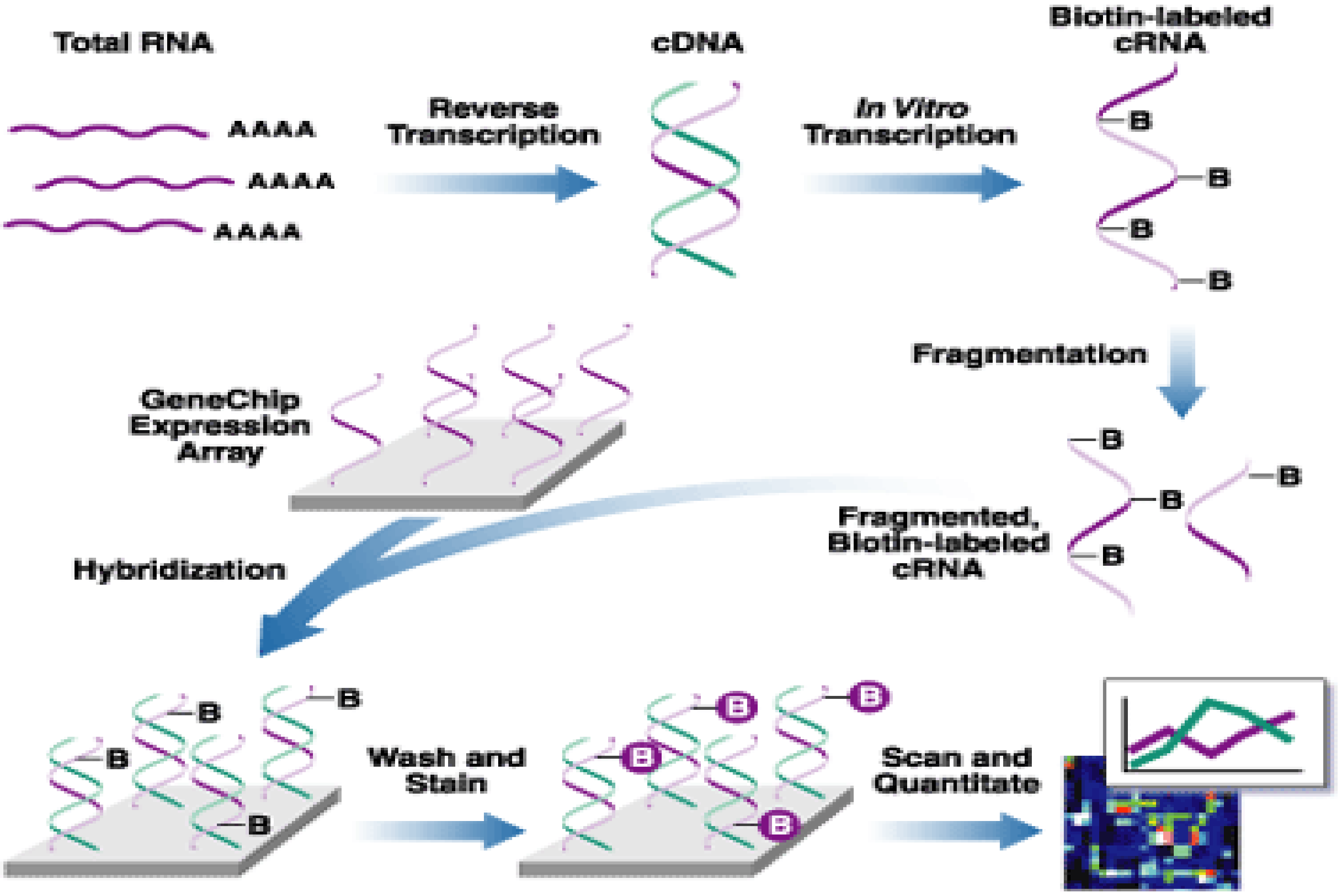


Probes are selected to be specific to the gene they represent and to have good hybridization properties

Probe pairs = PM and MM
An attempt to detect noise

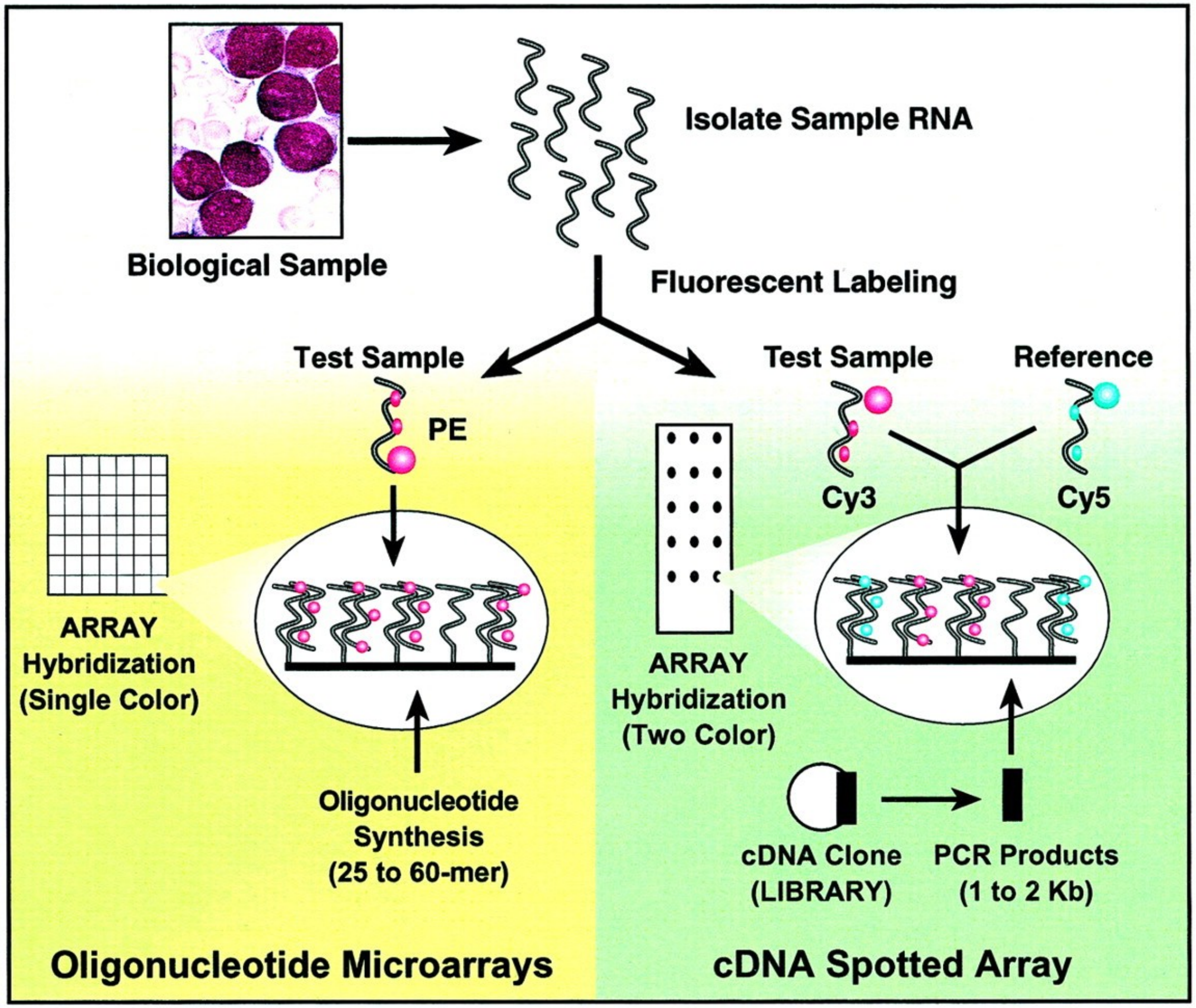

...TGCAATGGGTCAGAAGGACTCCTATGTGCCT...
gene sequence
AATGGGTCAGAAGGACTCCTATGTG
perfect match sequence
AATGGGTCAGAACGACTCCTATGTG
mismatch sequence
probe pair
probe cell
probe set

Proces overview (Affy)



@Affymetrix

Comparison between two types



Ramaswamy S , Golub T R JCO 2002;20:1932-1941

JOURNAL OF CLINICAL ONCOLOGY

Comparison between two types

cDNA Microarrays

ADVANTAGES

Cheaper (not anymore)

Flexibility in experimental design

High signal intensity (long secs)

DISADVANTAGES

Low reproducibility

Cross-hybridization (low specificity)

Need more manual handling (possibility of contamination)

Oligonucleotide Microarrays

ADVANTAGES

Quick and robotic manufacturing

High Reproducibility

High specificity (short sequences)

Use many probes / gene

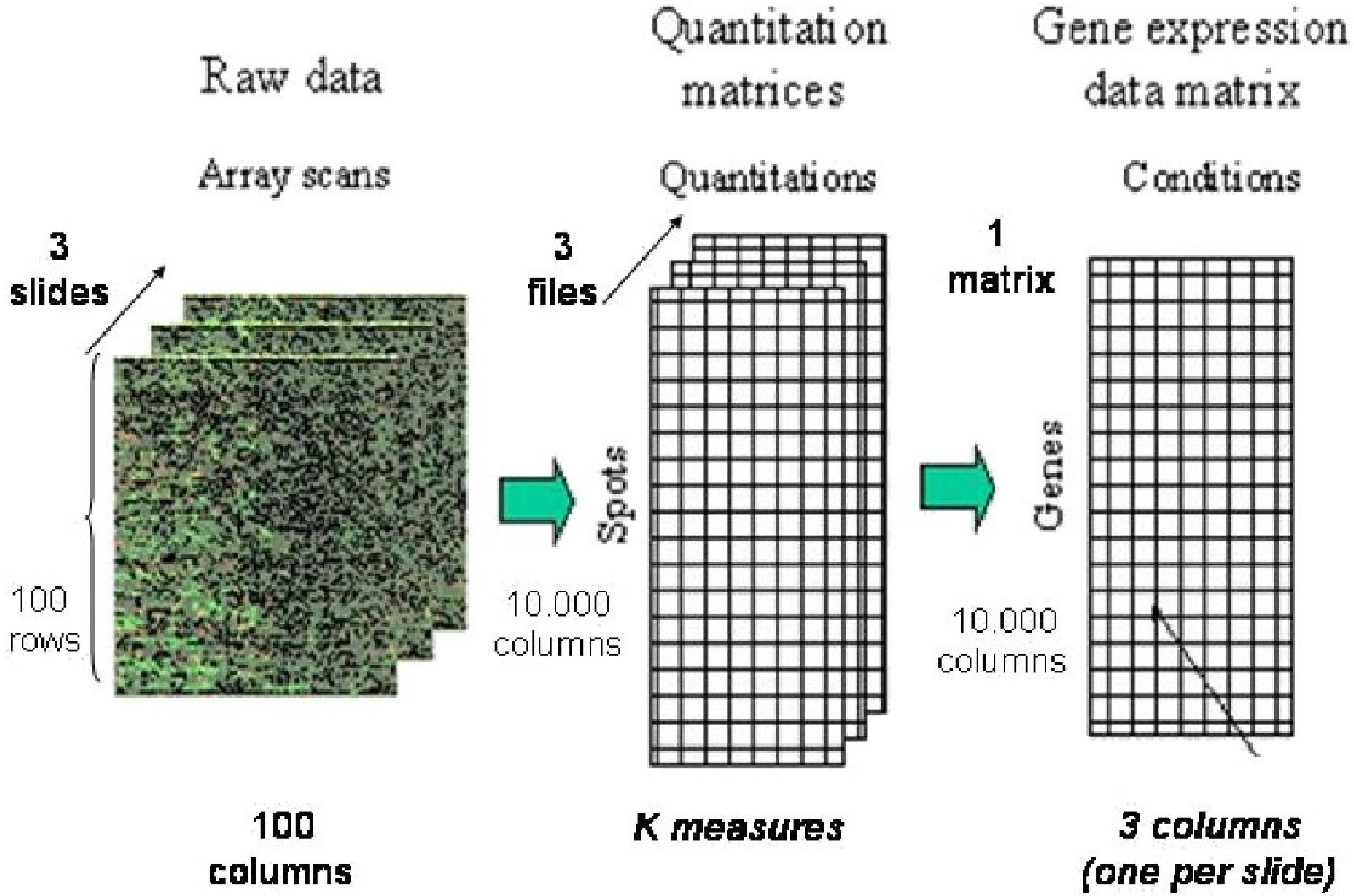
DISADVANTAGES

Requires more specialized equipment

Expensive

Less flexible (genes on the chip cannot be selected)

From image to expression matrix



Expression measures (cDNA)

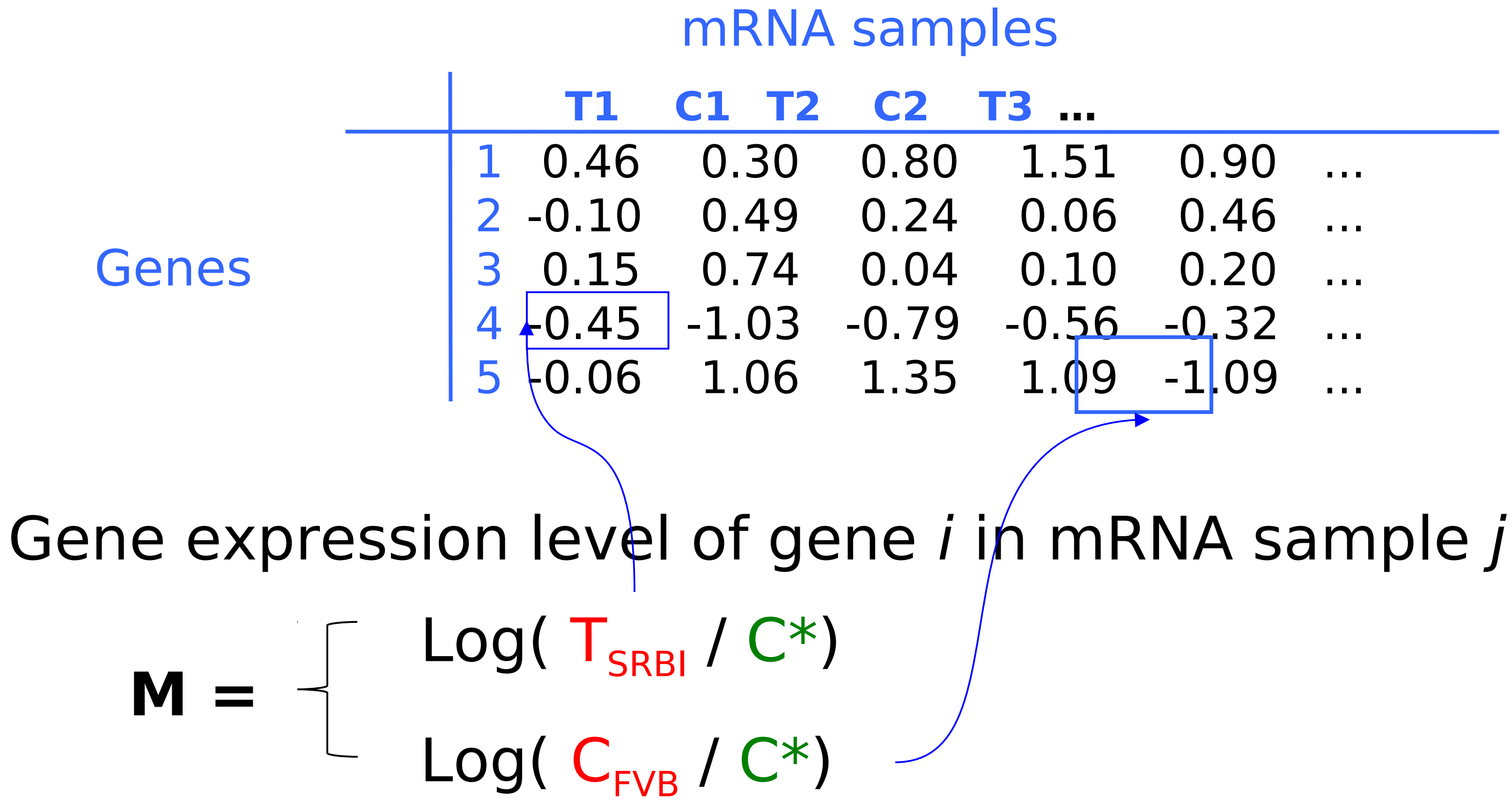
Gene expression is measured from intensity measures as the (corrected) relative intensity of one dye vs. the (corrected) relative intensity of the other.

$$M = \frac{R_g}{G_g}, \text{ or } M_{CORR} = \frac{R_g - bgR_g}{G_g - bgG_g}$$

Background correction may be needed, or not, according to the array quality.

Example: gene expression data

Gene expression data on *6348* genes for *16* samples.



mRNA samples

Genes

	T1	C1	T2	C2	T3	...
1	0.46	0.30	0.80	1.51	0.90	...
2	-0.10	0.49	0.24	0.06	0.46	...
3	0.15	0.74	0.04	0.10	0.20	...
4	-0.45	-1.03	-0.79	-0.56	-0.32	...
5	-0.06	1.06	1.35	1.09	-1.09	...

Gene expression level of gene *i* in mRNA sample *j*

$$\mathbf{M} = \begin{cases} \text{Log}(\, T_{\text{SRBI}} \,/\, C^{*}) \\ \text{Log}(\, C_{\text{FVB}} \,/\, C^{*}) \end{cases}$$

Expression measures (affy)

- Obtaining expression measures for affymetrix arrays is less straightforward.
  - Background correction and normalization is required.
  - There are multiple *PM* and *MM* values which must be integrated into one single expression value.
- The resulting summarized values are absolute expression measures which are more difficult to interpret than relative expression values.

$$Avg.Diff = \frac{1}{|A|} \sum_{j \in A} \left(PM_j - MM_j \right)$$

Example: absolute expression values

```
             C01-001.CEL C02-001.CEL C03-001.CEL
1415670_at      8.954387    9.088924    8.833863
1415671_at     10.700876   10.639307   10.610953
1415672_at     10.377266   10.510106   10.461701
1415673_at      7.320335    7.252635    7.112313
1415674_a_at    8.381129    8.332256    8.393718
1415675_at      8.120937    8.082713    8.051514
1415676_a_at   10.322229   10.287371   10.282812
1415677_at      9.038344    8.979641    8.905711
```

Partek®
turning data into discovery®

GeneSpring®

Software for microarray data analysis

TM4
MICROARRAY SOFTWARE SUITE

MeV
MultiExperiment
Viewer

BRB-ArrayTools

Which software for the analysis?

- Microarray experiments generate huge quantities of data which have to be.
  - Stored, managed, visualized, processed …
- Many options available. However…
- No tool satisfies all user’s needs.
- Trade-off. A tool must be.
  - Powerful but user friendly.
  - Complete but without too many options,
  - Flexible but easy to start with and go further.
  - Available, to date, well documented but affordable.

We picked up some options…

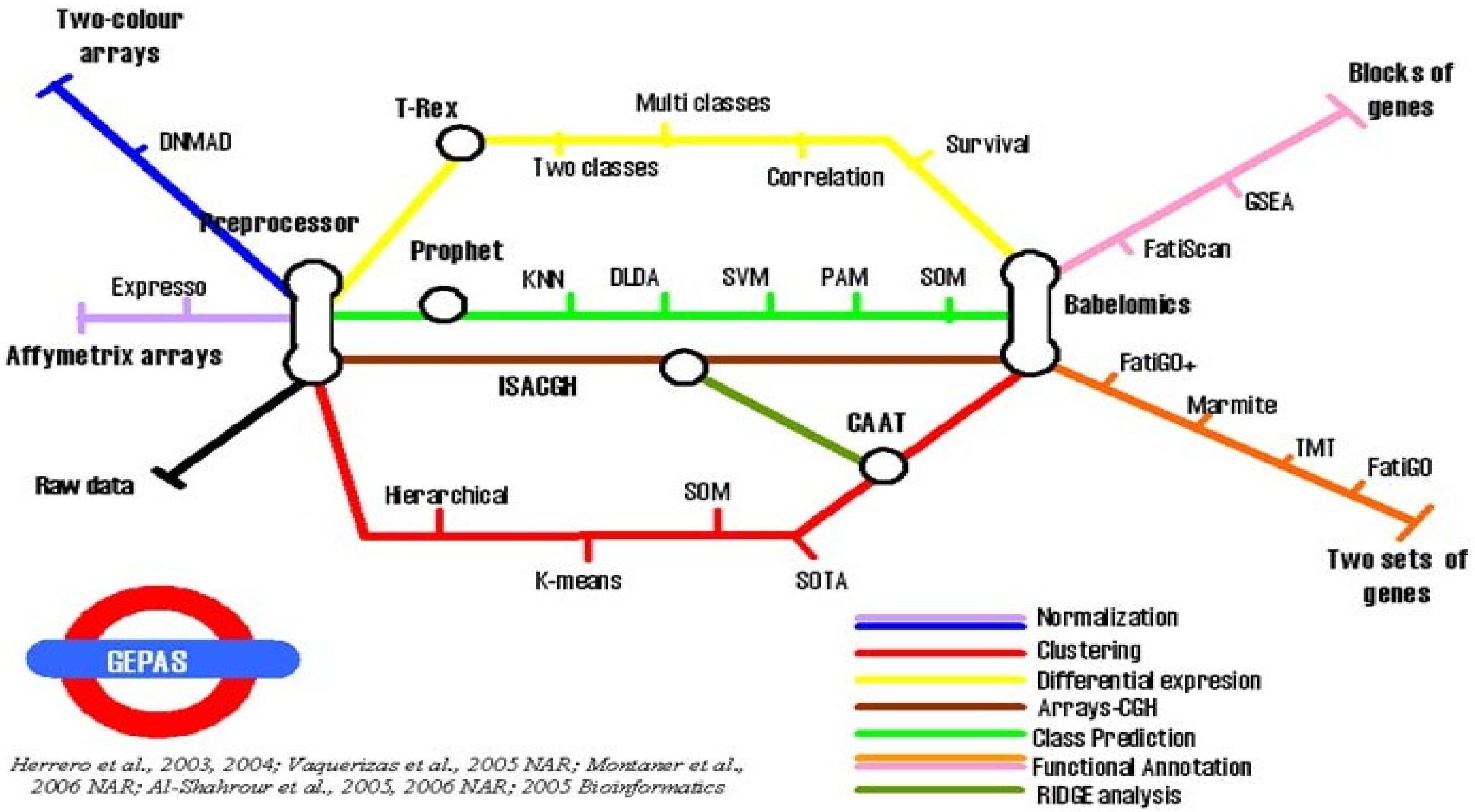
- Many tools
  - Free / Commercial
    - [R, BRB, MeV, dChip…] / [Partek, GeneSpring, Ingenuity]
  - Downloadable / On-line
    - [R, BRB, MeV…] / [Gepas,…]
  - Standalone / As part of suites
    - [BRB, dChip] / [MeV (TM4), OntoTools]
- A survey of free microarray data analysis tools:
  - http://chagall.med.cornell.edu/I2MT/MA-tools.pdf

Open source analysis tools

Programa	☺	☹
R/Bioconductor	Powerful, flexible, updated Unix/Windows/Mac	Console-based, hard to use
BRB tools	Excel based User-friendly	Hard to recover from error Hard to extend
dChip	Expresión & SNP’s User-friendly	Windows Les options
GEPAS	Web-based, Many options Good tutorials	Web-based A little rigid

…

GEPAS (currently Babelomics)



Herrero et al., 2003, 2004; Vaquerizas et al., 2005 NAR; Montaner et al., 2006 NAR; Al-Shahrour et al., 2005, 2006 NAR; 2005 Bioinformatics

So, what you need is “R”?

R is an open-source system for statistical computation and graphics. It consists of.

A language.

A run-time environment with.

Graphics, a debugger, and.

Access to certain system functions,

It can be used.

Interactively, through a command language.

Or running programs stored in script files.

R and Microarrays

- R is a popular tool between statisticians.
- Once they started to work with microarrays they continued using it.
  - To perform the analysis.
  - To implement new tools.
- This gave rise very fast to lots of free R-based software to analyze microarrays.
- The Bioconductor project groups many of these (but not all) developments.

The Bioconductor project

http://www.bioconductor.org

Open source and open development software project for the analysis and comprehension of genomic data.

Most early developments as R packages.

Extensive documentation and training material from short courses.

Has reached some stability but still evolving !!!
→ *what is now a standard may not be so in a future.*

Some pro's & con's

- Powerful,
- Used by statisticians
- Easy to extend
  - Creating add-on **packages**
  - Many already available
- Freely available
- Unix, windows & Mac
- Lot of documentation

- Not very easy to learn
- Command-based
- Documentation sometimes cryptic
- Memory intensive
  - Worst in windows
- Slow at times

We believe the effort is worth the pity!!!

- *If you "just want to do statistical analysis"*
  → Easy to find alternatives
- *If you intend to do microarray data analysis*
  → Probably one of best options

BRB-ArrayTools

Integrated software package using Excel-based user interface but state-of-the art analysis methods programmed in R, Java & Fortran.

Publicly available for non-commercial use.

http://linus.nci.nih.gov/BRB-ArrayTools.html

Selected Features of BRB-ArrayTools

- Multivariate permutation tests for class comparison to control false discovery proportion with any specified confidence level
- SAM
- Find Gene Ontology groups and signaling pathways that are differentially expressed
- Survival analysis
- Analysis of variance
- Class prediction models (7) with prediction error estimated by LOOCV, k-fold CV or .632 bootstrap, and permutation analysis of cross-validated error rate
  - DLDA, SVM, CCP, Nearest Neighbor, Nearest Centroid, Shrunken Centroids, Random Forests
- Clustering tools for class discovery with reproducibility statistics on clusters
  - Built in access to Eisen's Cluster and Treeview
- Visualization tools including rotating 3D principal components plot exportable to Powerpoint with rotation controls
- Import of Affy CEL files and apply RMA probe processing and quantile normalization
- Extensible via R plug-in feature
- Links genes to annotations in genomic databases
- Tutorials and datasets

Some pro’s & con’s

- Much easier to learn
- User friendly (Excel interface)
- Freely available
- Good, uniform, documentation

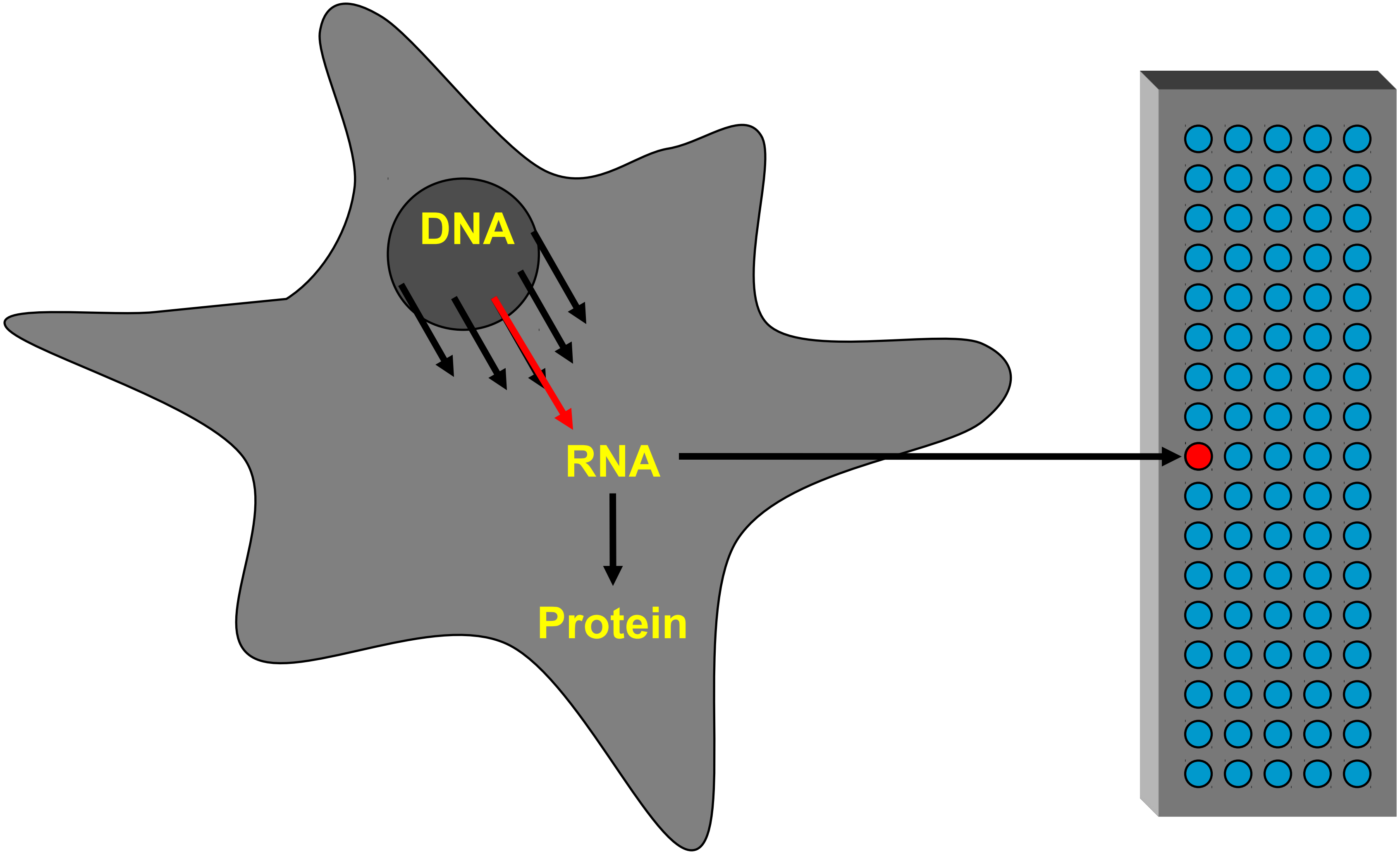
- Less powerful than "raw" R and Bioconductor,
- Difficult to extend
  - If you miss something it's hard to get it
- Only available for Windows

Gene Annotations in Genomics Experiments

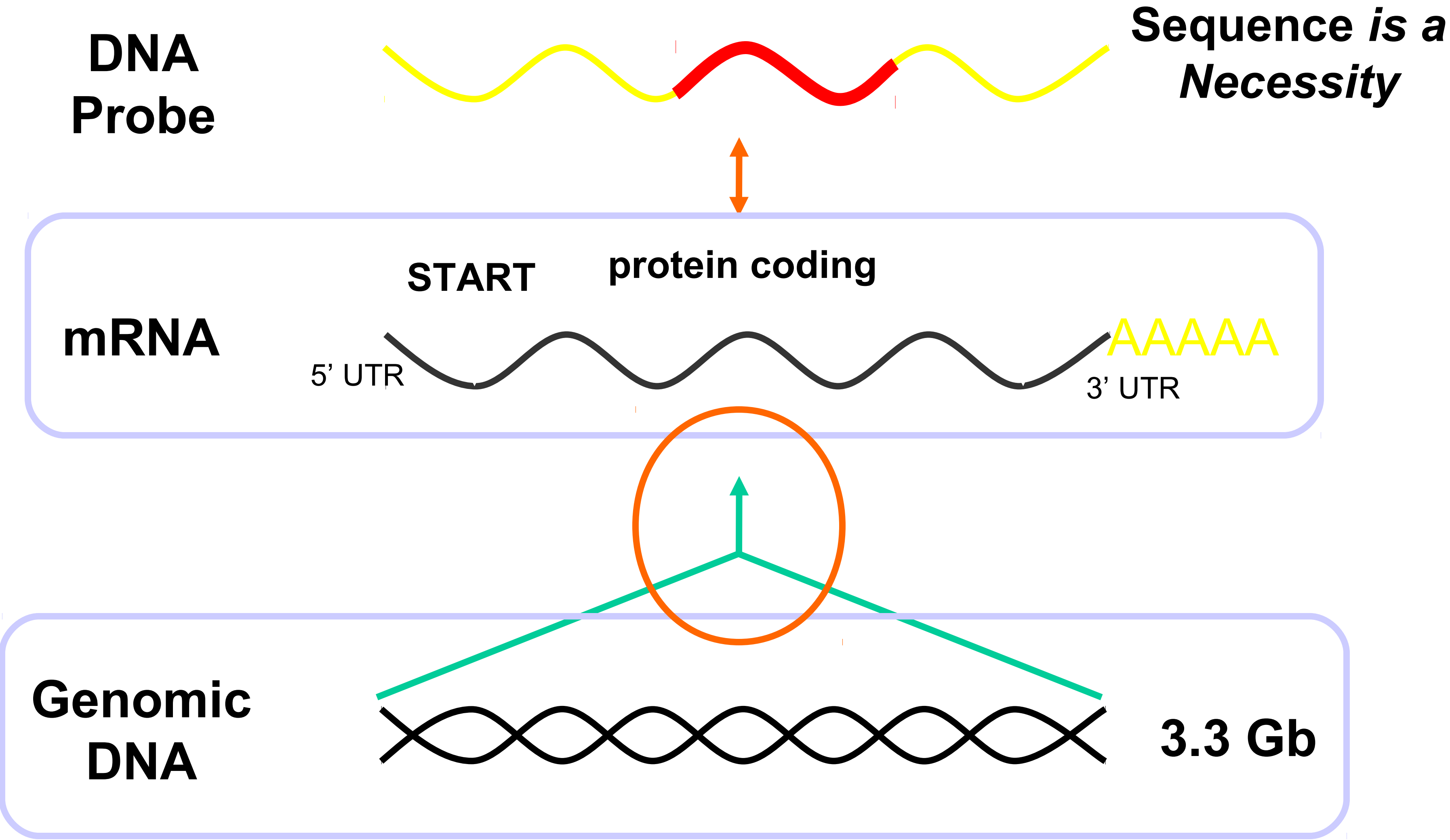
Biological preliminaries

- Every cell in the human body contains the entire human genome: 3.3 Gb or ~30K genes.
- The investigation of gene expression is meaningful because different cells, in different environments, doing different jobs express different genes.
- To-do list to *create plattforms* for gene expression analysis:
  - Define what a gene is.
  - Identify genes in a sea of genomic DNA where <3% of DNA is contained in genes.
  - Design and implement probes that will effectively assay expression of ALL (most? many?) genes simultaneously.
  - Cross-reference these probes.

Cell Biology, Gene Expression and Microarray analysis



Gene: *Protein coding unit of genomic DNA with an mRNA intermediate.*



DNA Probe

Sequence *is a Necessity*

mRNA

START

protein coding

5’ UTR

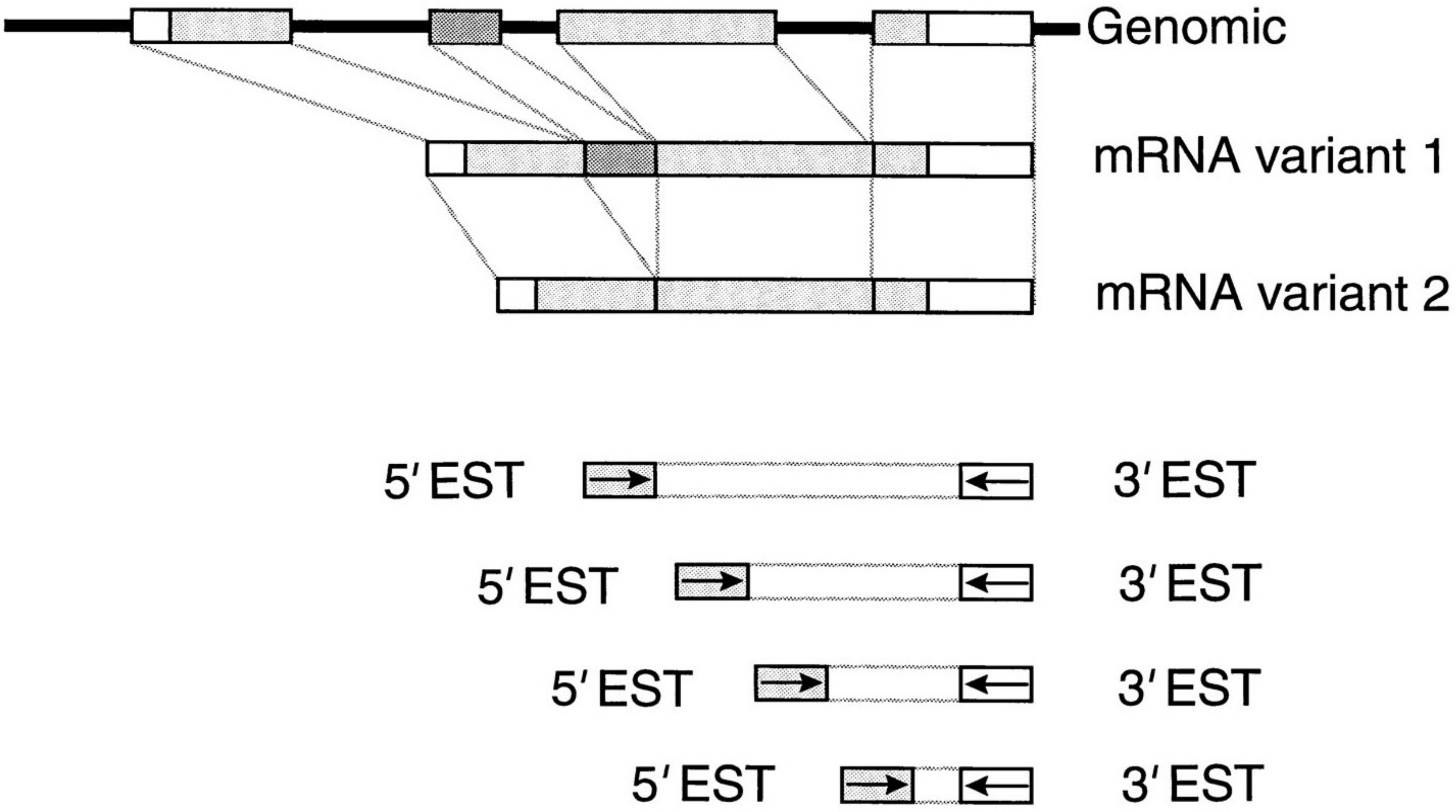
AAAAA

3’ UTR

Genomic DNA

3.3 Gb

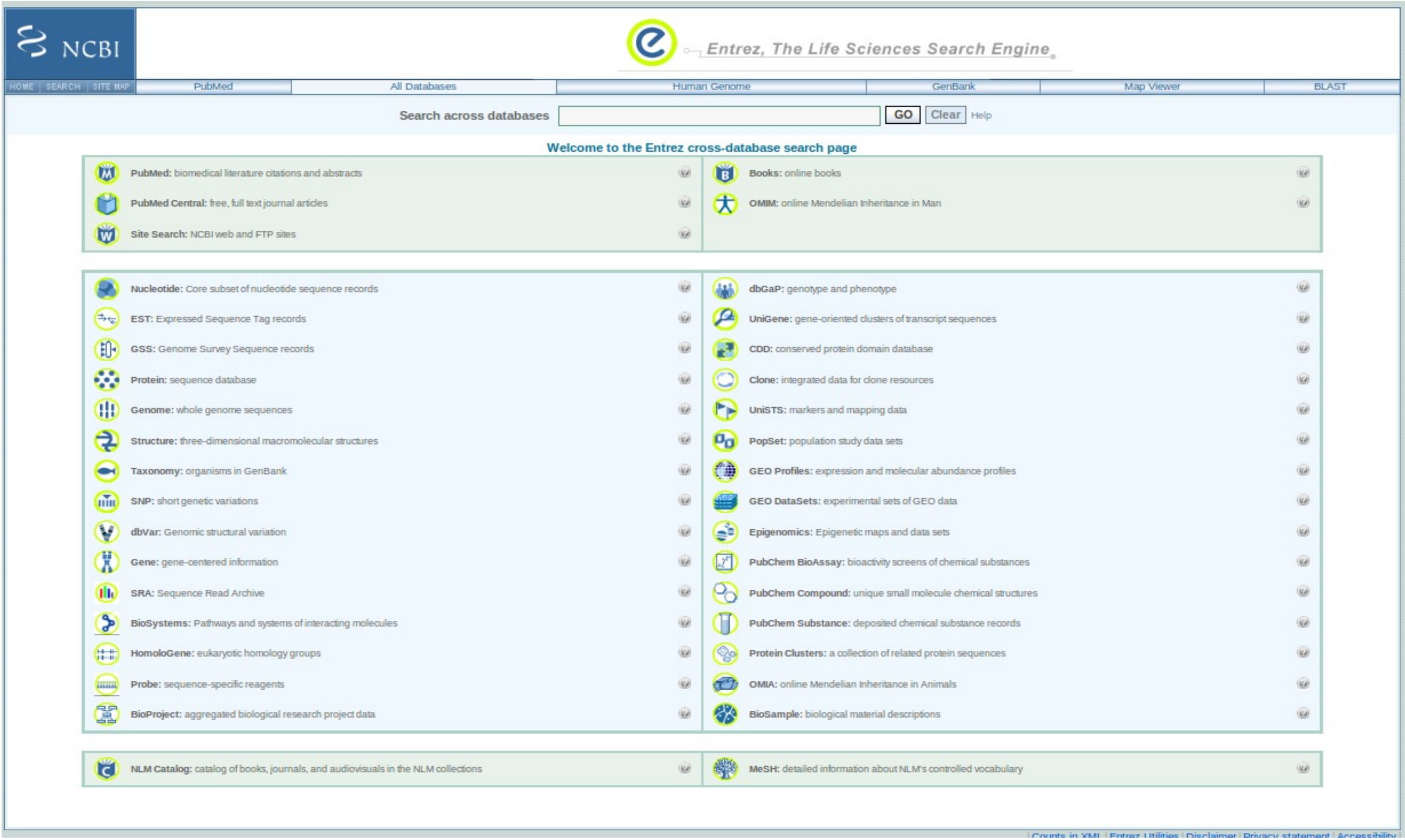
From Genomic DNA to mRNA Transcripts



Sequence and Gene databases

- Probes have to be mapped to databases.
- These may be either
    - gene or sequence databases
- Sequence databases:
    - From which sequence has the probe been synthesized?
- Gene databases
    - Which gene is the probe intended to interrogate

NCBI-Entrez



Welcome to the Entrez cross-database search page

- **PubMed:** biomedical literature citations and abstracts
- **PubMed Central:** free, full text journal articles
- **Site Search:** NCBI web and FTP sites
- **Books:** online books
- **OMIM:** online Mendelian Inheritance in Man

- **Nucleotide:** Core subset of nucleotide sequence records
- **EST:** Expressed Sequence Tag records
- **GSS:** Genome Survey Sequence records
- **Protein:** sequence database
- **Genome:** whole genome sequences
- **Structure:** three-dimensional macromolecular structures
- **Taxonomy:** organisms in GenBank
- **SNP:** short genetic variations
- **dbVar:** Genomic structural variation
- **Gene:** gene-centered information
- **SRA:** Sequence Read Archive
- **BioSystems:** Pathways and systems of interacting molecules
- **HomoloGene:** eukaryotic homology groups
- **Probe:** sequence-specific reagents
- **BioProject:** aggregated biological research project data
- **dbGaP:** genotype and phenotype
- **UniGene:** gene-oriented clusters of transcript sequences
- **CDD:** conserved protein domain database
- **Clone:** integrated data for clone resources
- **UniSTS:** markers and mapping data
- **PopSet:** population study data sets
- **GEO Profiles:** expression and molecular abundance profiles
- **GEO DataSets:** experimental sets of GEO data
- **Epigenomics:** Epigenetic maps and data sets
- **PubChem BioAssay:** bioactivity screens of chemical substances
- **PubChem Compound:** unique small molecule chemical structures
- **PubChem Substance:** deposited chemical substance records
- **Protein Clusters:** a collection of related protein sequences
- **OMIA:** online Mendelian Inheritance in Animals
- **BioSample:** biological material descriptions

- **NLM Catalog:** catalog of books, journals, and audiovisuals in the NLM collections
- **MeSH:** detailed information about NLM's controlled vocabulary

Counts in XML | Entrez Utilities | Disclaimer | Privacy statement | Accessibility

Entrez Gene

- Entrez database was constructed to replace the widely known and used LocusLink database in the year 2004.
- Entrez Gene integrates information from LocusLink and from genes annotated on Reference Sequences from completely sequenced genomes.

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

RefSeq

- The Reference Sequence (RefSeq) collection aims to provide a comprehensive, integrated, non-redundant, well-annotated set of sequences, including genomic DNA, transcripts, and proteins.
- Similar to a review article, a RefSeq is a synthesis of information integrated across multiple sources at a given time. RefSeqs provide a foundation for uniting sequence data with genetic and functional information.
- They are generated to provide reference standards for multiple purposes ranging from genome annotation to reporting locations of sequence variation in medical records.

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

Unigene

- UniGene is an experimental system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters.
- Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location.
- In addition to sequences of well-characterized genes, hundreds of thousands novel expressed sequence tag (EST) sequences have been included. Consequently, the collection may be of use to the community as a resource for gene discovery.

http://www.bioinfo.org.cn/relative/NCBI-UniGene.htm

Databases for microarrays

Microarray Data in a Nutshell

- Lots of data to be managed before and after the experiment.
- Data to be stored before the experiment .
  - Description of the *array* and the *sample*.
  - Direct access to all the cDNA and gene sequences, annotations, and physical DNA resources.
- Data to be stored after the experiment
  - Raw Data - scanned images.
  - Gene Expression Matrix - Relative expression levels observed on various sites on the array.
- Hence we can see that ***database software capable of dealing with larger volumes of numeric and image data is required.***

Why Databases?

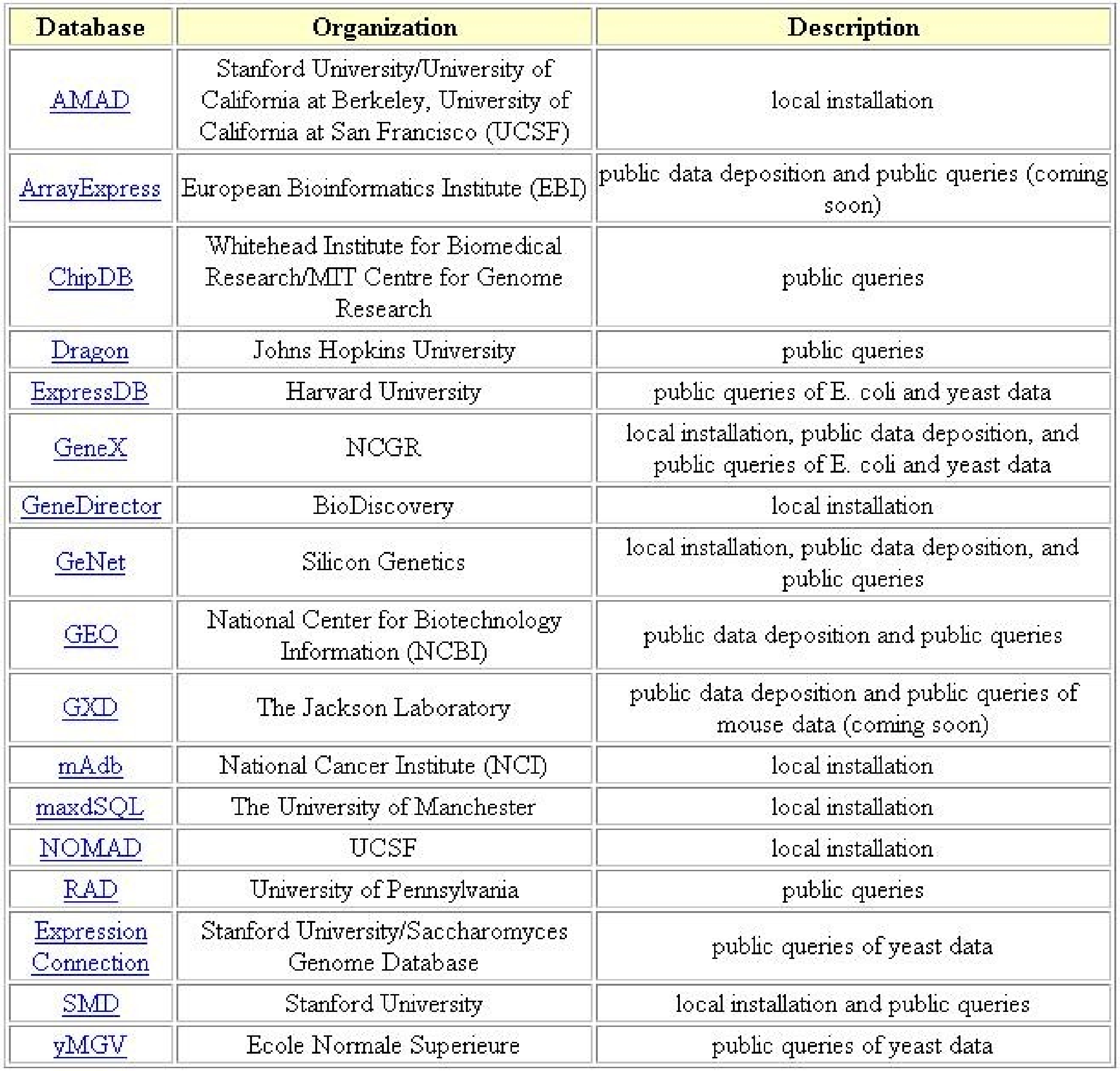
- Tailored to datatype
- Tailored to the Scientists
- Intuitive ways to query the data
  - Diagrams, forms, point and click, text etc.
- Support for efficient answering of queries.
  - Query optimisation, indexes, compact physical storage.

Gene Expression Databases Require Integration

- There are many different types of data presenting numerous relationships.
- There are a number of Databases with lots of information.
- Experiments need to be compared because the experiments are very difficult to perform and very expensive.
- Solution: Make all the databases talk the same language.
- XML was the choice of data interchange format.

Existing Microarray Databases

- Several gene expression databases exist:Both commercial and non-commercial.
- Most focus on either a particular technolgy or a particular organism or both.
- Commercial databases:
  - *Rosetta Inpharmatics* and *Genelogic*, the specifics of their internal structure is not available for internal scrutiny due to their proprietary nature.
- Some non-commercial efforts to design more general databases merit particular mention.
- We will discuss few of the most promising ones
  - ArrayExpress - EBI
  - The Gene expression Omnibus (GEO) - NLM
  - The Standford microarray Database
  - ExpressDB - Harvard
  - Genex - NCGR



Database	Organization	Description
AMAD	Stanford University/University of California at Berkeley, University of California at San Francisco (UCSF)	local installation
ArrayExpress	European Bioinformatics Institute (EBI)	public data deposition and public queries (coming soon)
ChipDB	Whitehead Institute for Biomedical Research/MIT Centre for Genome Research	public queries
Dragon	Johns Hopkins University	public queries
ExpressDB	Harvard University	public queries of E. coli and yeast data
GeneX	NCGR	local installation, public data deposition, and public queries of E. coli and yeast data
GeneDirector	BioDiscovery	local installation
GeNet	Silicon Genetics	local installation, public data deposition, and public queries
GEO	National Center for Biotechnology Information (NCBI)	public data deposition and public queries
GXD	The Jackson Laboratory	public data deposition and public queries of mouse data (coming soon)
mAdb	National Cancer Institute (NCI)	local installation
maxdSQL	The University of Manchester	local installation
NOMAD	UCSF	local installation
RAD	University of Pennsylvania	public queries
Expression Connection	Stanford University/Saccharomyces Genome Database	public queries of yeast data
SMD	Stanford University	local installation and public queries
yMGV	Ecole Normale Superieure	public queries of yeast data

ArrayExpress

- Public repository of microarray based gene expression data.
- Implemented in Oracle at EBI.
- Contains:
  - several curated gene expression datasets
  - possible introduction of an image server to archive raw image data associated with the experiments.
- Accepts submissions in MAGE-ML format via a web-based data annotation/submission tool called MIAMExpress.
  - A demo version of MIAMExpress is available at: http://industry.ebi.ac.uk/~parkinso/subtool/subtype.html
- Provides a simple web-based query interface and is directly linked to the Expression Profiler data analysis tool which allows expression data *clustering* and other types of data exploration directly through the web.

Gene Express Omnibus

- The Gene Expression Omnibus ia a gene expression database hosted at the National library of Medicine
- It supports four basic data elements
  - Platform (the physical reagents used to generate the data)
  - Sample (information about the mRNA being used)
  - Submitter (the person and organisation submitting the data)
  - Series (the relationship among the samples).
- It allows download of entire datasets, it has not ability to query the relationships
- Data are entered as tab delimited ASCII records,with a number of columns that depend on the kind of array selected.
- Supports Serial Analysis of Gene Expression (SAGE) data.

ExpressDB

ExpressDB is a relational database containing yeast and E.coli RNA expression data.

It has been conceived as an example on how to manage that kind of data.

It allows web-querying or SQL-querying.

It is linked to an integrated database for functional genomics called Biomolecule Interaction Growth and Expression Database (BIGED).

BIGED is intended to support and integrate RNA expression data with other kinds of functional genomics data

Survey of existing microarray systems

- A comparison of microarray databases

BRIEFINGS IN BIOINFORMATICS, Vol 2, No 2, pp 143-158, May 2001.

- http://mybio.wikia.com/wiki/Microarray_databases

The Microarray Gene Expression Database Group (MGED)

History and Future:

Founded at a meeting in November, 1999 in Cambridge, UK.

In May 2000 and March 2001: development of recommendations for microarray data annotations (MAIME, MAML).

MGED 2nd meeting:

- establishment of a steering committee consisting of representatives of many of the worlds leading microarray laboratories and companies

MGED 4th meeting in 2002:

- MAIME 1.0 will be published
- MAML/GEML and object models will be accepted by the OMG
- concrete ontology and data normalization recommendations will be published.

information can be obtained from http://www.mged.org

The Microarray Gene Expression Database Group (MGED)

Goals:

- Facilitate the adoption of standards for DNA-array experiment annotation and data representation.
- Introduce standard experimental controls and data normalization methods.
- Establish gene expression data repositories.
- Allow comparision of gene expression data from different sources.

MGED Working Groups

Goals:

- MIAME: Experiment description and data representation standards - Alvis Brazma

- MAGE: Introduce standard experimental controls and data normalization methods - Paul Spellman. This group includes the MAGE-OM and MAGE-ML development.

- OWG: Microarray data standards, annotations, ontologies and databases - Chris Stoeckert

- NWG: Standards for normalization of microarray data and cross-platform comparison - Gavin Sherlock

References

URL:

- Tutorial on Information Management for Genome Level Bioinformatics, Paton and Goble, at VLDB 2001: http://www.dia.uniroma3.it/~vldbproc/#tutEuropea
- European Molecular Biology Network http://www.embnet.org/
- Univ. Manchester site (with relational version of Microarray data representation, and links to other sites)

  http://www.bioinf.man.ac.uk

Database textbook with absolutely no bioinformatics coverage

For Microarray Data

- http://linkage.rockefeller.edu/wli/microarray/