CIBERER NGS course: from reads to candidate genes

Introduction

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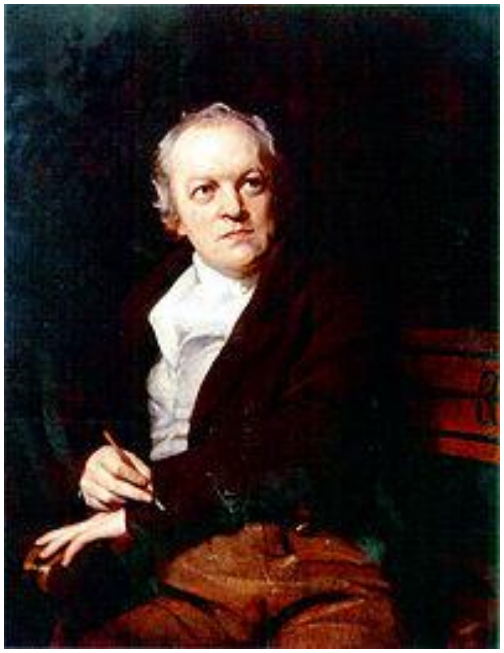
http://bioinfo.cipf.es
http://www.babelomics.org

@xdopazo

CIPF, 28-30 September2015

Background



The road of excess leads to the palace of wisdom

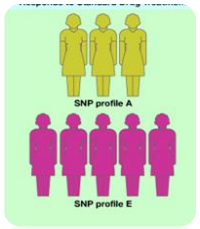
(*William Blake, 28 November 1757 – 12 August 1827, poet, painter, and printmaker*)

The introduction and popularisation of high-throughput techniques has drastically changed the way in which biological problems **can** be addressed and hypotheses **can** be tested.

But not necessarily the way in which we really address or test them…

Where do we come from?
The pre-genomics paradigm

Genes in the DNA...



...produces the final phenotype

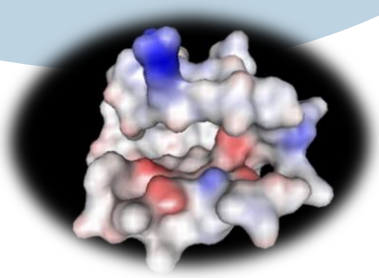
...code for proteins...

```
>protein kunase
acctgttgatggcgacagggactgtatgctg
atctatgctgatgcatgcatgctgactactga
tgtgggggctattgacttgatgtctatc....
```

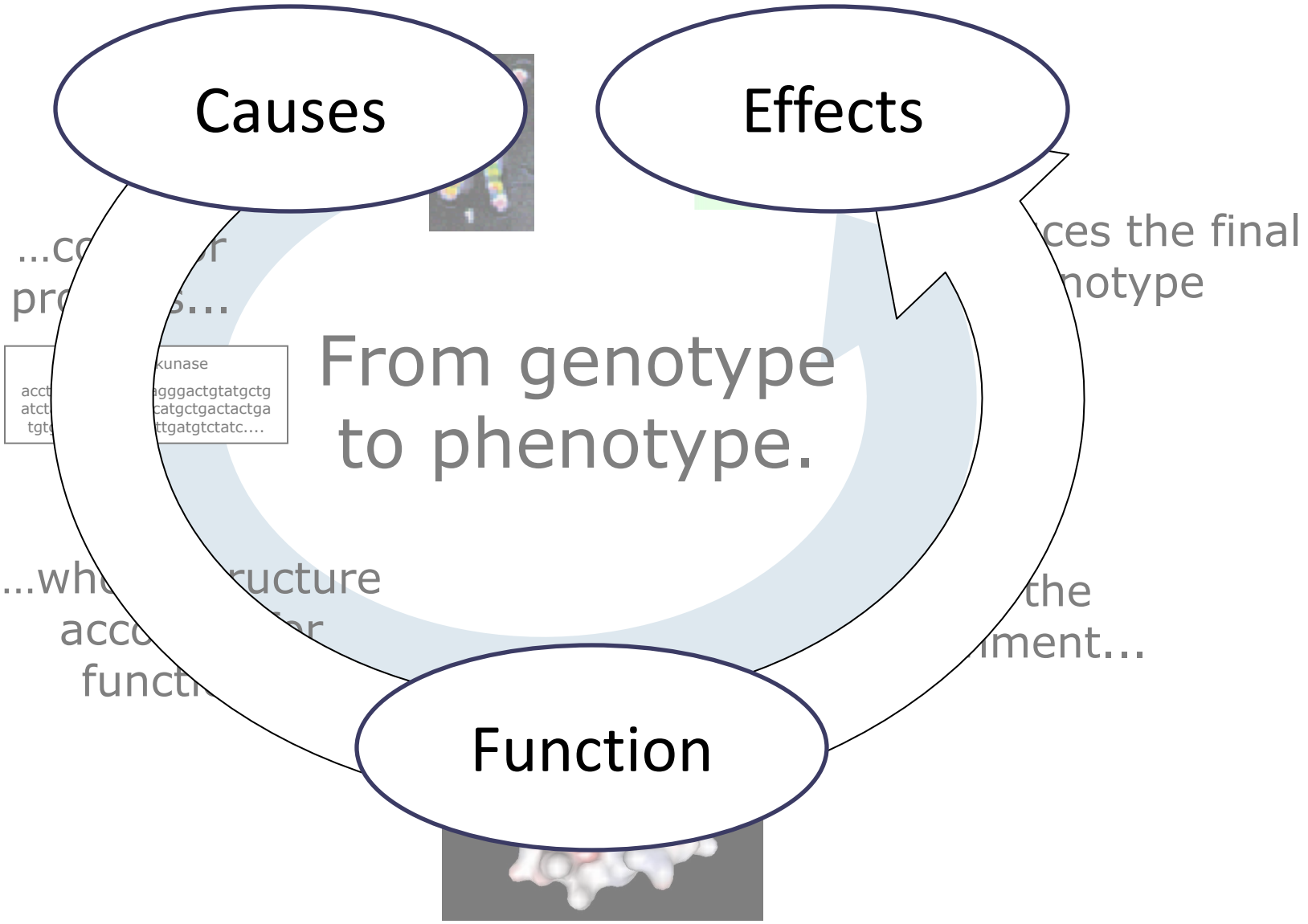
From genotype to phenotype.

...whose structure accounts for function...

...plus the environment...

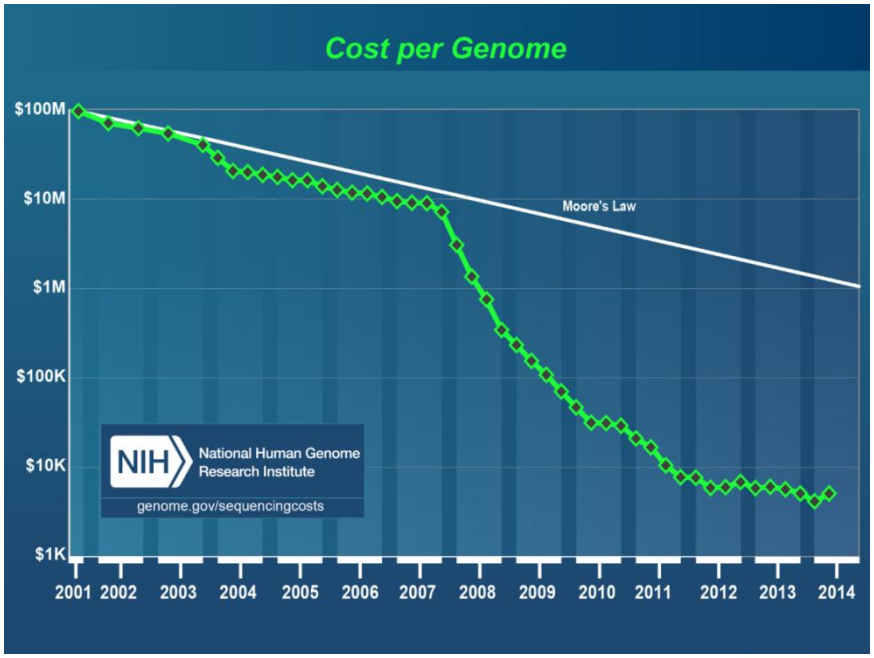


Reduccionistic approach to link causes (genome) to effects (phenotype) through actions (function)

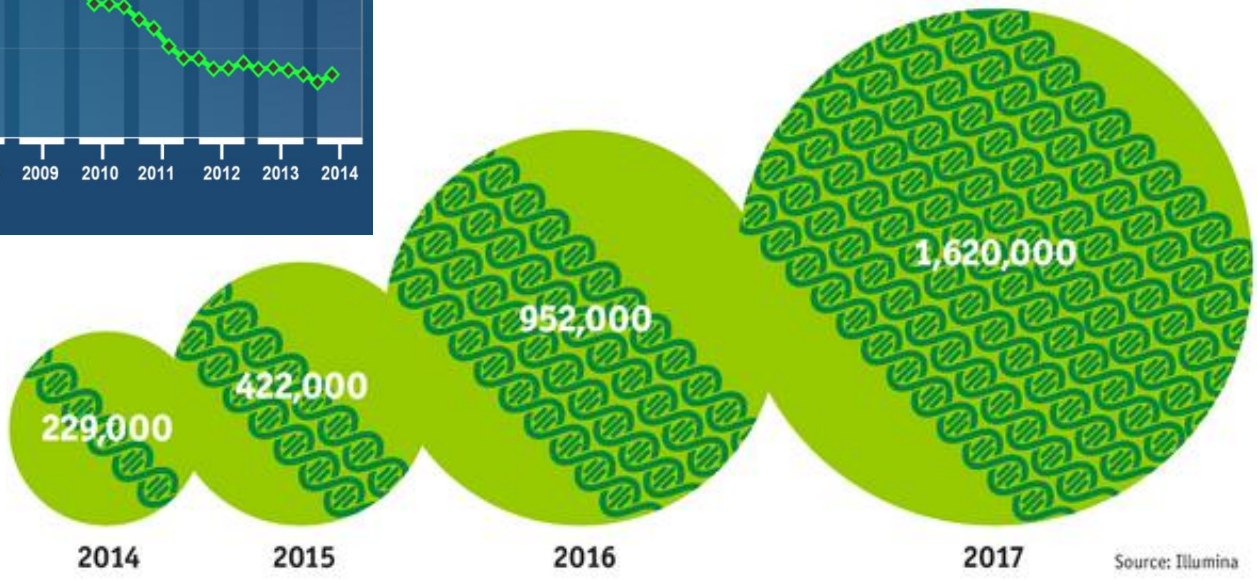


The genome sequencing pace

http://www.genome.gov/sequencingcosts/



NGS is matching the cost of many conventional clinical tests



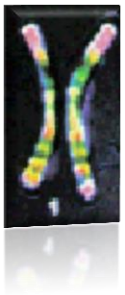
http://www.economist.com/news/21631808-so-much-genetic-data-so-many-uses-genes-unzipped

Next Generation Sequencing
10^9bp per round

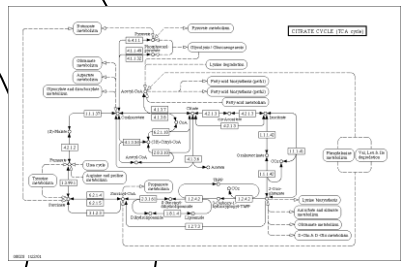
Genes in the DNA...

12 million SNPs in exonic regions

...with its complex variability...



...whose final effect configures the phenotype...

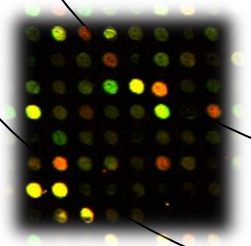


From genotype to phenotype.

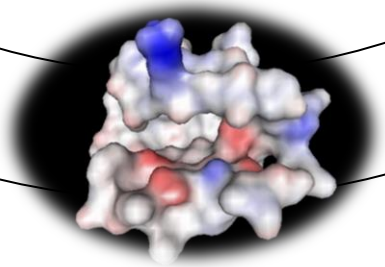
(in the post-genomics scenario)

...conforming complex interaction networks...

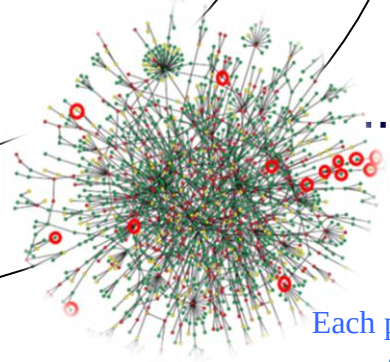
...when they are expressed in the proper moment and place...



...code for proteins...



That undergo post-translational modifications, somatic recombination...
100K-500K proteins

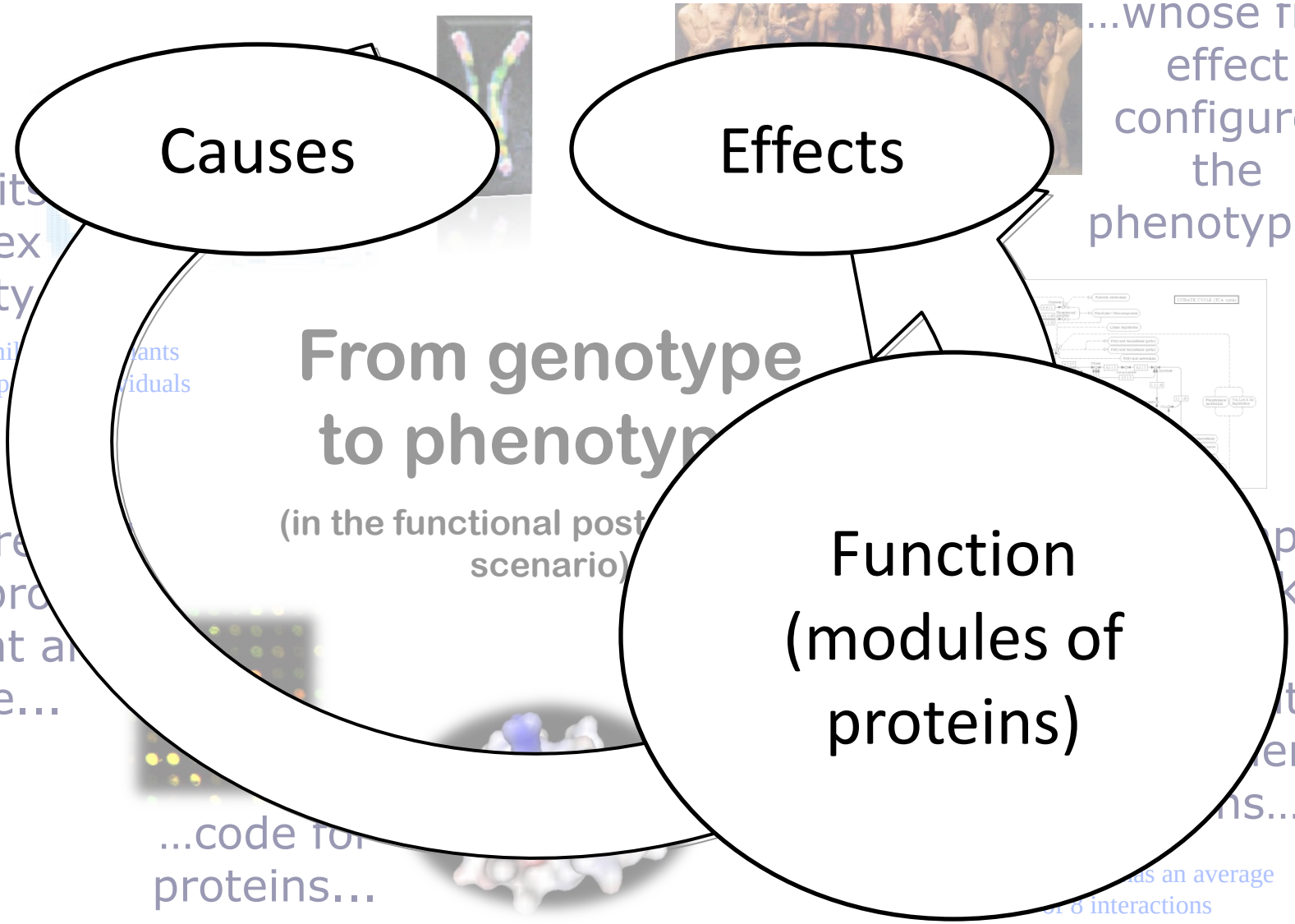


...in cooperation with other proteins...

Each protein has an average of 8 interactions

...that account for function if...

Holistic approach. Causes and effects remain essentially the same. The concept of function has changed



High-throughput data for functional genomics

Next Generation Sequencing
10^9bp per round

Genes in the DNA...

...whose final effect configures the phenotype...

Genome wide

Genotyping

...with its complete variability...

Half a million of variants between pairs of individuals

From genotype to phenotype.

(in the functional post-genomics scenario)

Metabolomics

...conforming complex interaction networks...

...when they are expressed in the proper moment and place...

Transcriptomics

...code for proteins...

Proteomics

...in cooperation with other proteins...

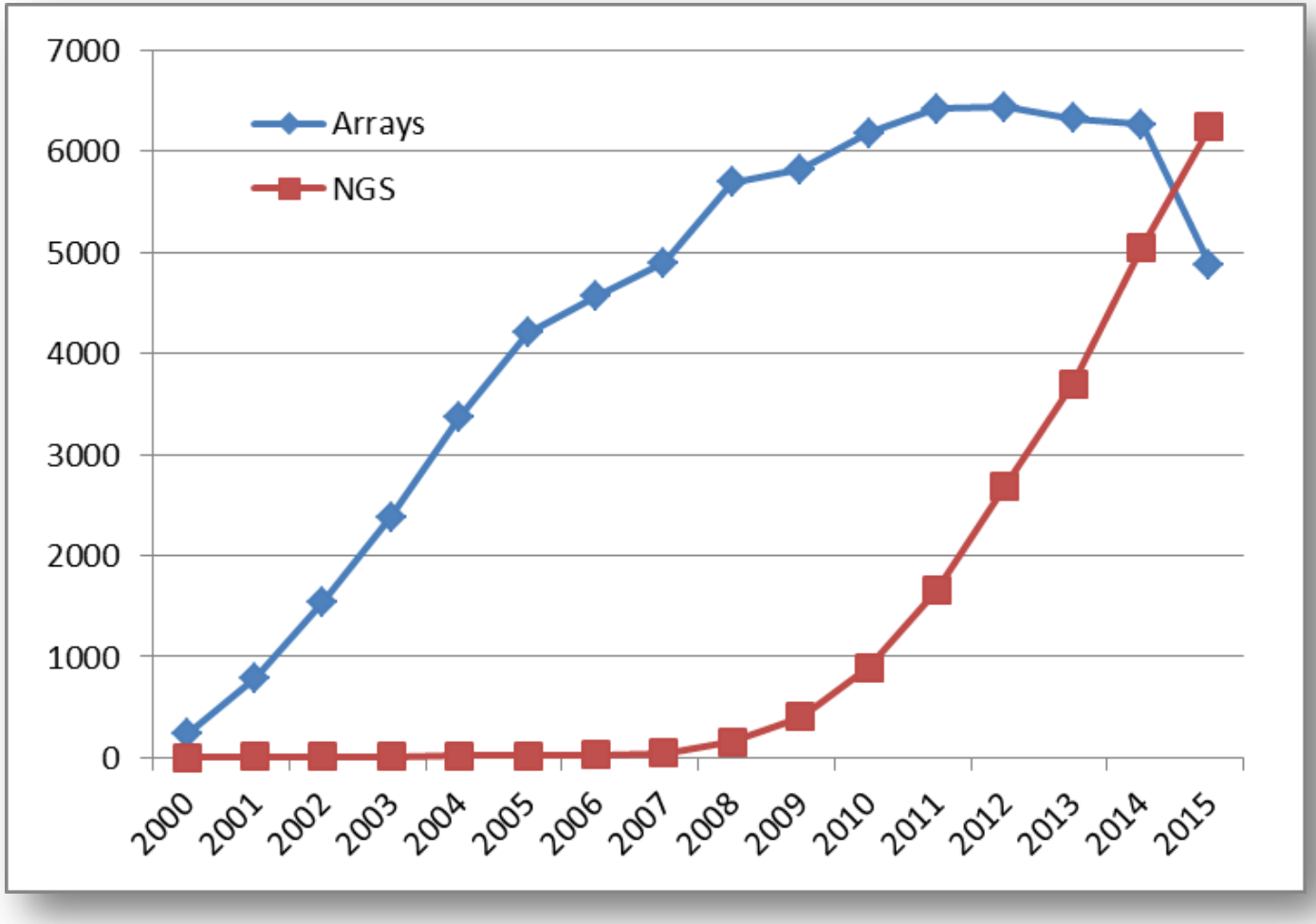
Each protein has an average of 6 interactions

That undergo post-translational modifications, somatic recombination...
100K-500K proteins

...that account for function if...

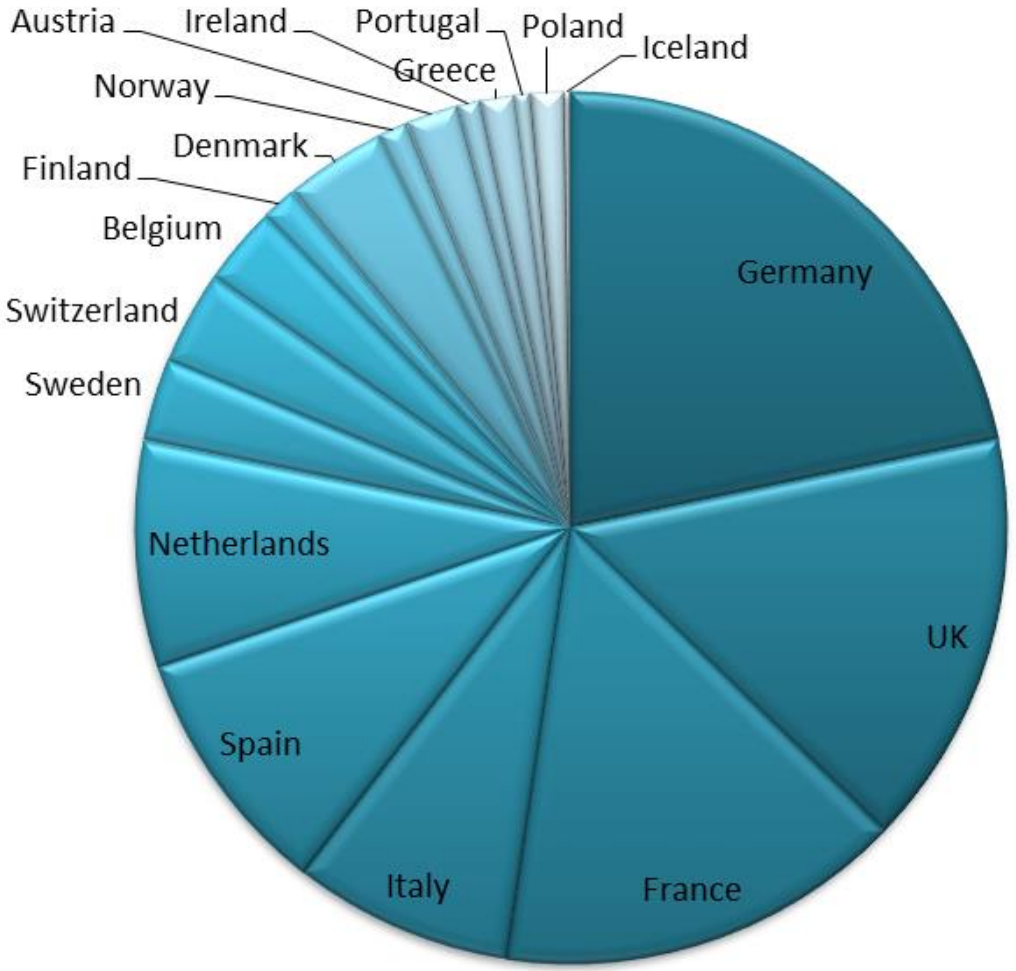
Almost-omics

Evolution of the papers published in microarray and NGS technologies



Source Pubmed. Query: "high-throughput sequencing"[Title/Abstract] OR "next generation sequencing"[Title/Abstract] OR "rna seq"[Title/Abstract]) AND year[Publication Date]

Some bibliographic data: NGS publications



2015 Europe

Source Pubmed. Query:
("high-throughput sequencing"[Title/Abstract] OR "next generation sequencing"[Title/Abstract] OR "rna seq"[Title/Abstract]) AND "2015"[Publication Date] AND country[Affiliation]

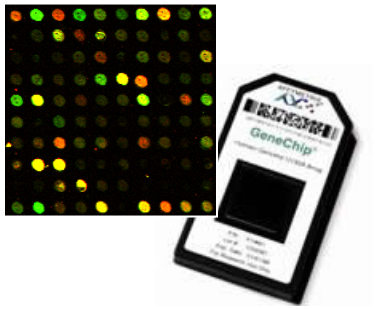
Transcriptomics

The double challenge:
Data processing and interpretation

acggcgt
aggtcat
tgcattca
tactatca
tcacagg
cgggagt
tctatcag
tcgtatct
gatctata
aaat
aaat
aaat

FastQ files

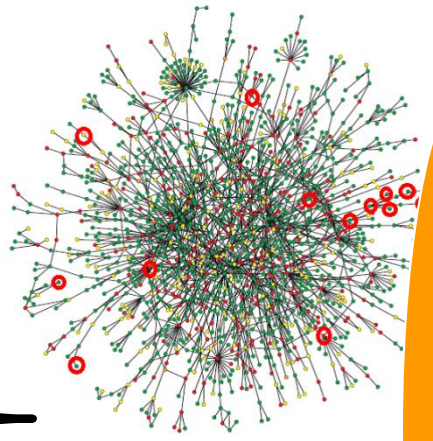
Read mapping and quantification

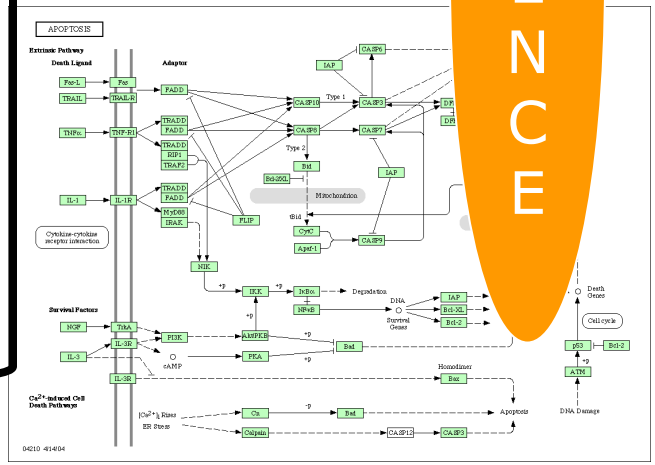


Raw image generation

Intensity summary

Transcriptomics





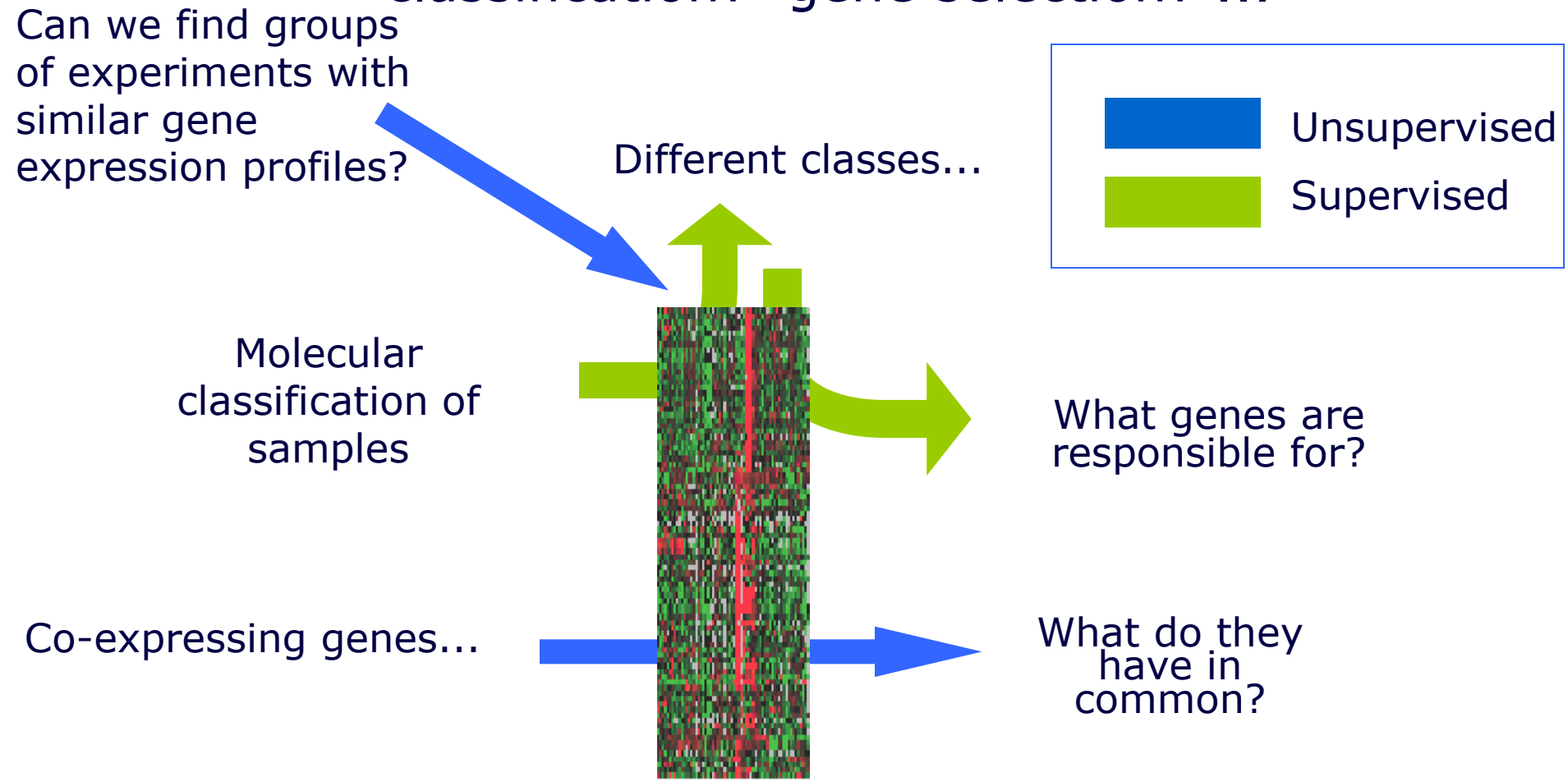
SCIENCE

Technology driven

Hypothesis driven

Before analysing your data you must know what is your question.

What is the aim? Class discovery? sample classification? gene selection? ...

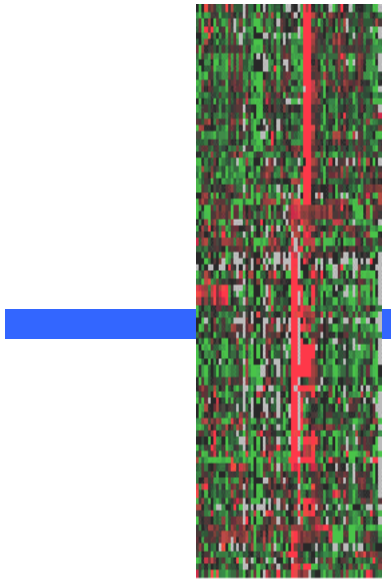


Unsupervised problem: class discovery

Our interest is in discovering clusters of items (genes or experiments) which we do not know beforehand

Can we find groups of experiments with similar gene expression profiles?

Co-expressing genes...



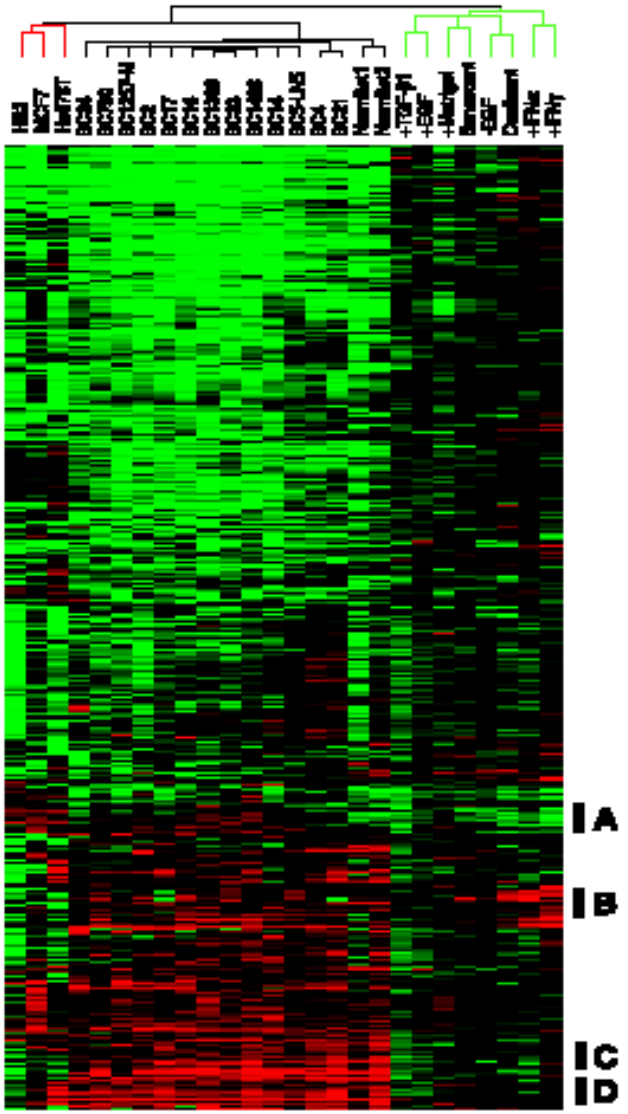
- What genes co-express?
- How many different expression patterns do we have?
- What do they have in common?
- Etc.

Clustering of experiments: The rationale

If enough genes have their expression levels altered in the different experiments, we might be able of finding these classes by comparing gene expression profiles.

Distinctive gene expression patterns in human mammary epithelial cells and breast cancers

Overview of the combined *in vitro* and breast tissue specimen cluster diagram. A scaled-down representation of the 1,247-gene cluster diagram The black bars show the positions of the clusters discussed in the text: (*A*) proliferation-associated, (*B*) IFNregulated, (*C*) B lymphocytes, and (*D*) stromal cells.



Perou et al., PNAS (1999)

Supervised problems.

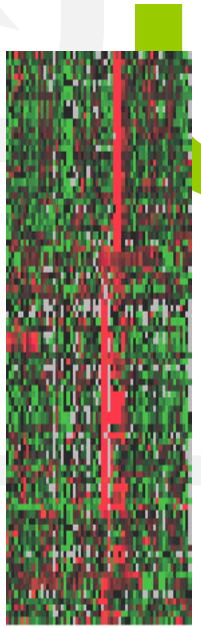
Differential gene expression

Can we find groups of experiments with similar gene expression profiles?

Molecular classification of samples

Co-expressing genes...

Different classes...



What genes are responsible for?

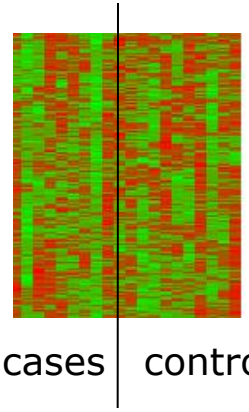
What do they have in common?

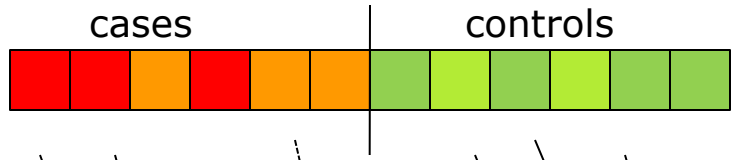
Differential gene expression

The simplest way: univariant gene-by-gene.
Other multivariant approaches can be used

- **Two classes**
  - T-test
  - Limma
  - Fold-change
- **Multiclass**
  - Anova
  - Limma
- **Continuous variable (e.g. level of a metabolite)**
  - Pearson
  - Spearmam
  - Regression
- **Survival**
  - Cox model
- **Time Course**

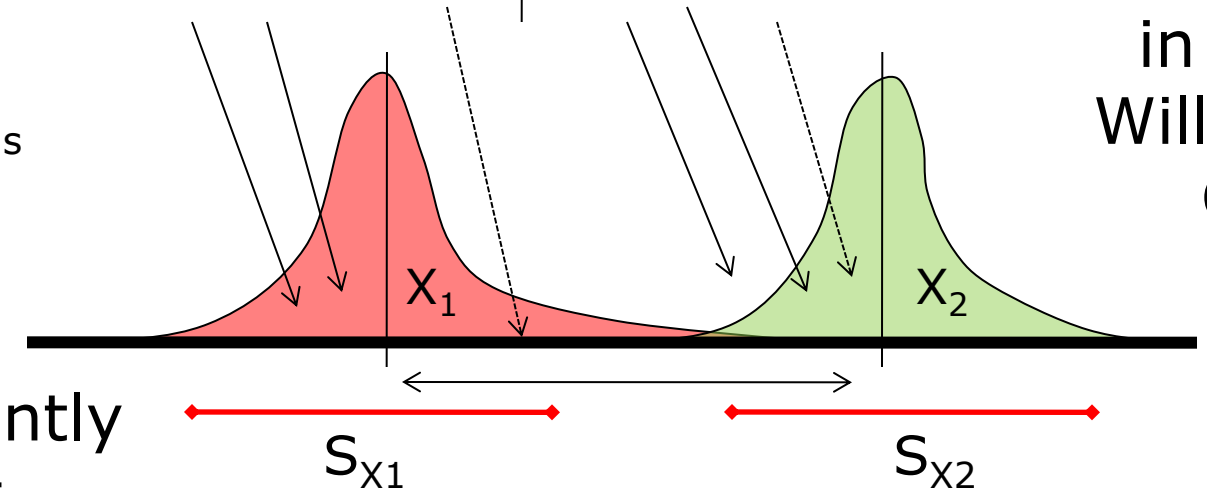
Differential gene expression



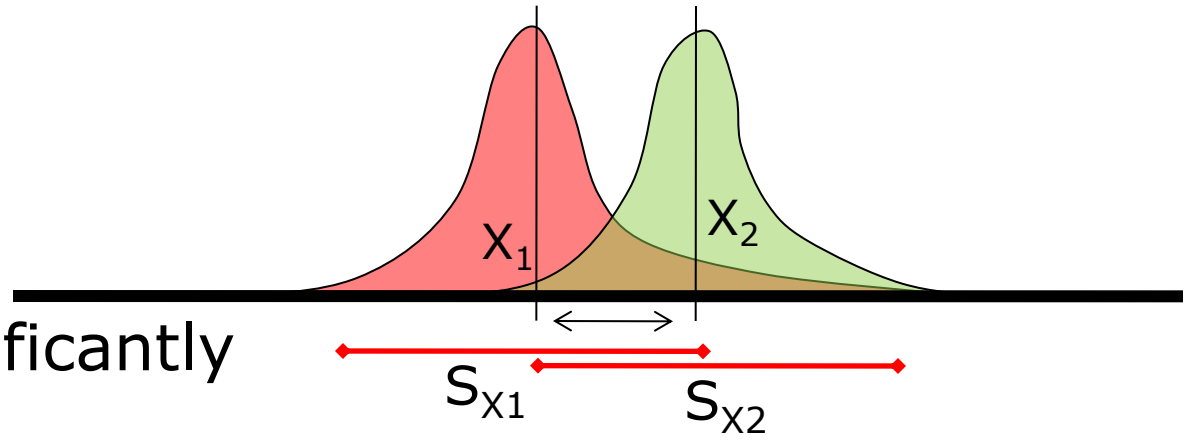


The t-statistic was introduced in 1908 by William Sealy Gosset

Significantly different



Non significantly different



$$t = \frac{\bar{X}_1 - \bar{X}_2}{S_{X_1X_2} \cdot \sqrt{\frac{2}{n}}} \quad \text{being} \quad S_{X_1X_2} = \sqrt{\frac{S^2_{X_1} + S^2_{X_2}}{2}}.$$

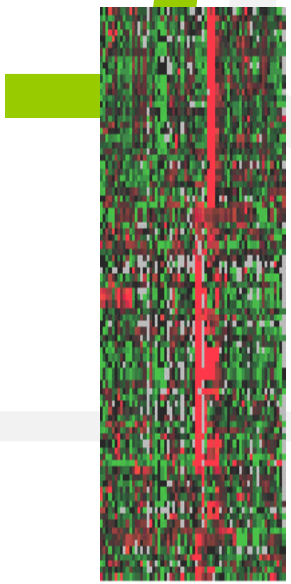
Supervised problems.

sample classification

Can we find groups of experiments with similar gene expression profiles?

Different classes...

Molecular classification of samples



What genes are responsible for?

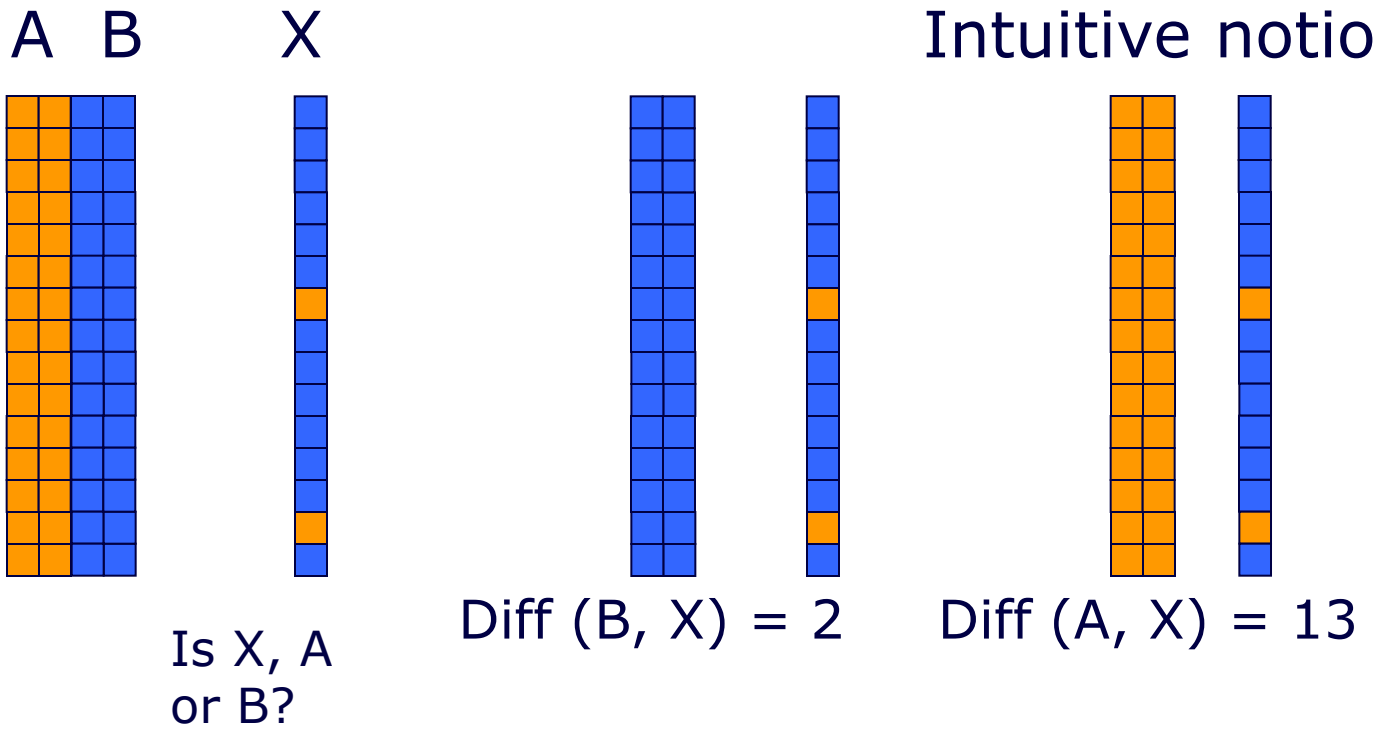
Co-expressing genes...

What do they have in common?

Predictors and molecular signatures

What is a predictor?

Intuitive notion:



Most probably X belongs to class B

Algorithms: DLDA, KNN, SVM, random forests, PAM, etc.

Predictor of clinical outcome in breast cancer

Genes are arranged to their correlation eith the pronostic groups

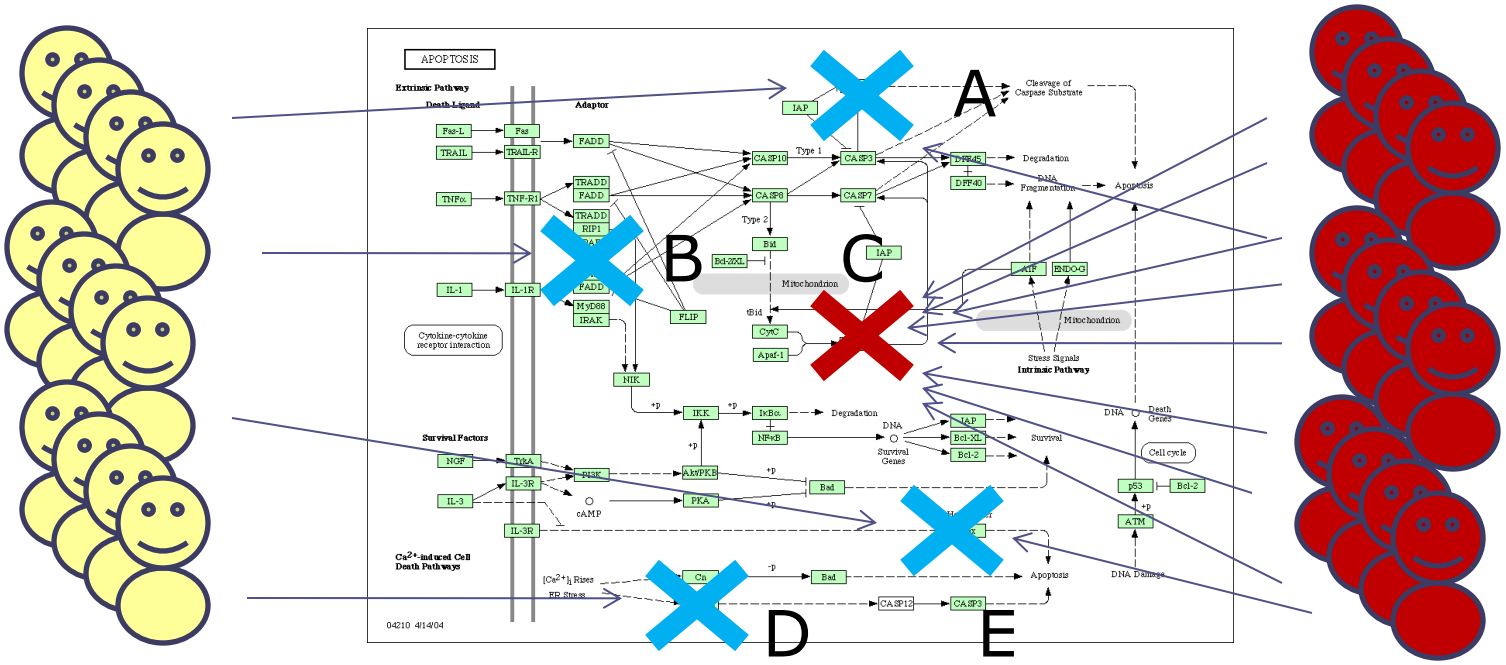
Pronostic classifier with optimal accuracy

van't Veer et al., Nature, 2002

Genotyping/Resequencing:

Finding mutations associated to diseases

The simplest case: monogenic disease



Controls

Cases

Gene A	1 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 1 0 0 0 0 0 0 0
Gene B	0 0 0 1 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0
Gene C	0 0 0 0 0 0 0 0 0 0 0 0	1 1 1 1 1 1 1 1 1 1 1 1
Gene D	0 0 0 0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 0 0 0 0 0
Gene E	0 0 0 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 0 1 0 0 0 0

Primary data analysis tools

Fastq file, up to hundreds of GB per run

QC and preprocessing

QC stats, filtering and preprocessing options

HPG Aligner, short read aligner

Double mapping strategy:
Burrows-Wheeler Transform (*GPU Nvidia CUDA*) + Smith-Waterman (*CPU OpenMP+SSE/AVX*)

SAM/BAM file

QC and preprocessing

QC stats, filtering and preprocessing options

Variant calling analysis

GATK and SAM mPileup HPC Implementation.
Statistics genomic tests

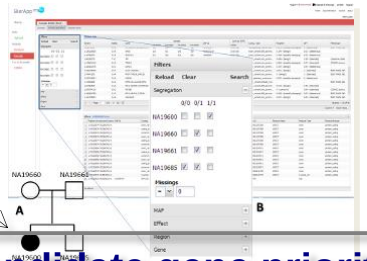
VCF file

QC and preprocessing

QC stats, filtering and preprocessing options

Genome Maps. Variant VCF viewer

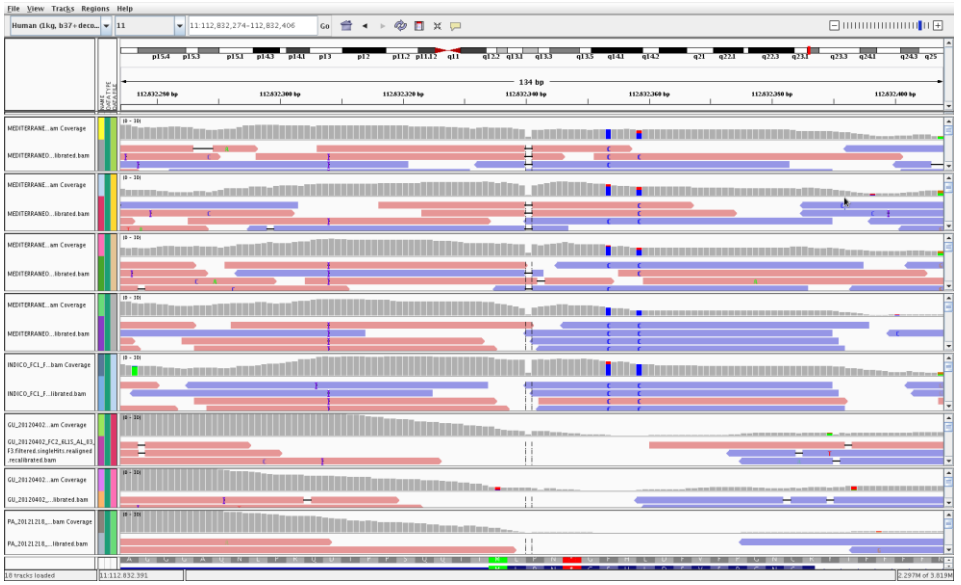
HTML5+SVG Web based viewer



BiERApp candidate gene prioritization

Consequence type, pathogenicity, Population frequencies, etc

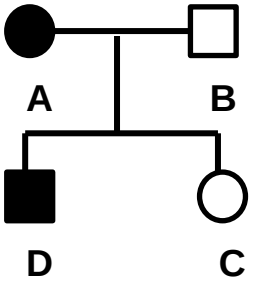
The principle: comparison of patients to reference controls or segregation within families

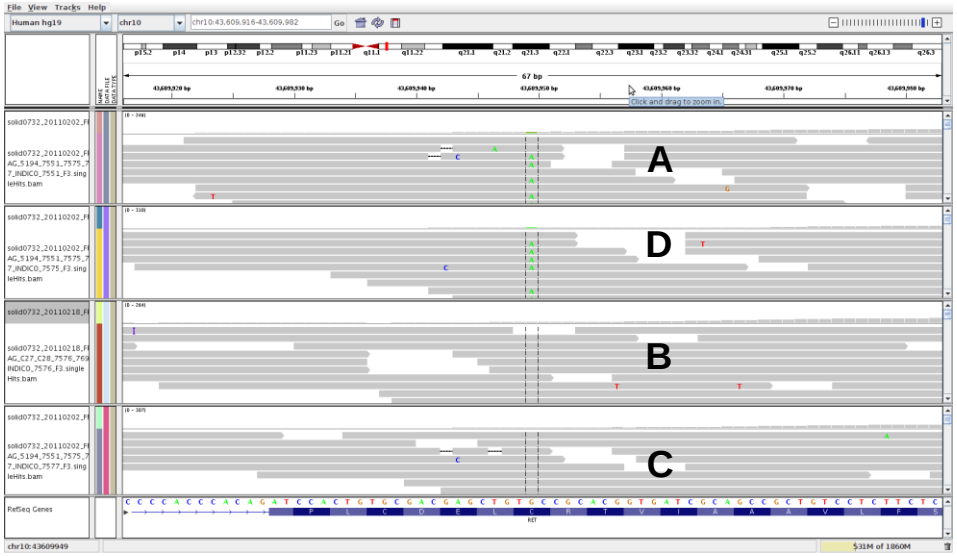


Cases

Controls

Segregation within a pedigree





Variant/gene prioritization by successive filtering

Variant level

Potential impact of the variant

Population frequencies

Experimental design level

Family(es)
Trios
Case / control

Functional (system) level

Gene set
Network analysis
Pathway analysis

Control of sequencing errors (missing values)

Testing strategies

Pipeline of data analysis

Initial QC

Sequence cleansing

Base quality

Remove adapters

Remove duplicates

FASTQ file

Mapping + QC

Mapping (HPG)

Remove multiple mapping reads

Remove low quality mapping reads

Realigning

Base quality recalibrating

BAM file

Variant calling + QC

Calling and labeling of missing values

Calling SNVs and indels (GATK) using 6 statistics based on QC, strand bias, consistence (poor QC callings are converted to missing values as well)

Create multiple VCF with missing, SNVs and indels

VCF file

Variant and gene prioritization + QC

Counts of sites with variants

Variant annotation (function, putative effect, conservation, etc.)

Inheritance analysis (including compound heterozygotes in recessive inheritance)

Filtering by frequency with external controls (**Spanish controls**, dbSNP, 1000g, 5500g) and annotation

Multi-family intersection of genes and variants

Network-based prioritization

Report

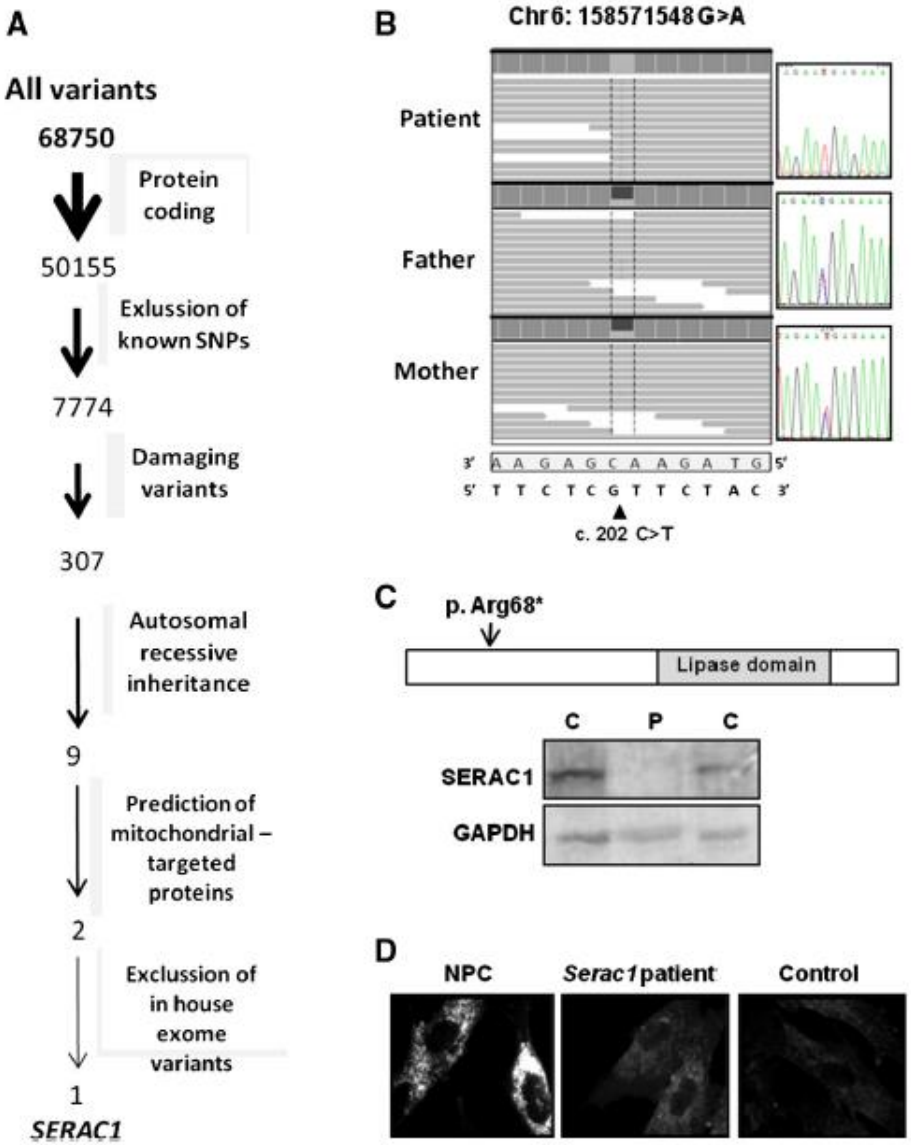
Primary analysis

Gene prioritization

Successive Filtering approach

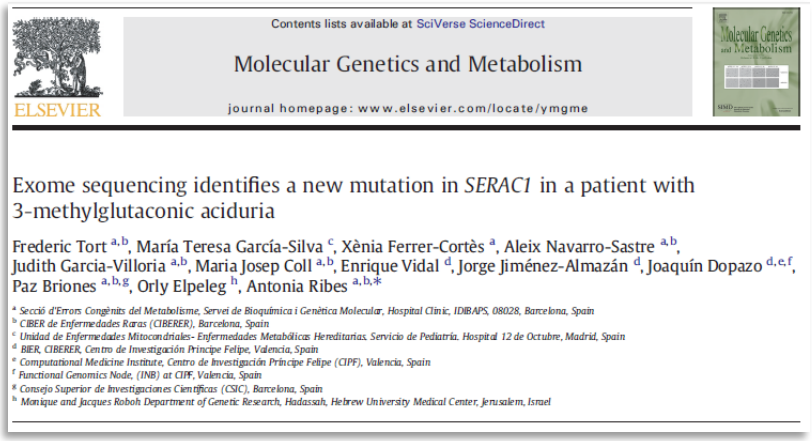
An example with 3-Methylglutaconic aciduria syndrome

F. Tort et al. / Molecular Genetics and Metabolism xxx (2013) xxx–xxx



3-Methylglutaconic aciduria (3-MGA-uria) is a heterogeneous group of syndromes characterized by an increased excretion of 3-methylglutaconic and 3-methylglutaric acids.

WES with a consecutive filter approach is enough to detect the new mutation in this case.



Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

ELSEVIER

Exome sequencing identifies a new mutation in *SERAC1* in a patient with 3-methylglutaconic aciduria

Frederic Tort [a,b], María Teresa García-Silva [c], Xènia Ferrer-Cortès [a], Aleix Navarro-Sastre [a,b], Judith Garcia-Villoria [a,b], Maria Josep Coll [a,b], Enrique Vidal [d], Jorge Jiménez-Almazán [d], Joaquín Dopazo [d,e,f], Paz Briones [a,b,g], Orly Elpeleg [h], Antonia Ribes [a,b,*]

[a] Secció d'Errors Congènits del Metabolisme, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, IDIBAPS, 08028, Barcelona, Spain
[b] CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain
[c] Unidad de Enfermedades Mitocondriales- Enfermedades Metabólicas Hereditarias. Servicio de Pediatría, Hospital 12 de Octubre, Madrid, Spain
[d] BIER, CIBERER, Centro de Investigación Príncipe Felipe, Valencia, Spain
[e] Computational Medicine Institute, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain
[f] Functional Genomics Node, (INB) at CIPF, Valencia, Spain
[g] Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
[h] Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Center, Jerusalem, Israel

Exome sequencing has been systematically used to identify Mendelian disease genes

ARTICLES

nature genetics

Exome sequencing identifies the cause of a mendelian disorder

Sarah B Ng[1,10], Kati J Buckingham[2,10], Choli Lee[1], Abigail W Bigham[2], Holly K Tabor[2,3], Karin M Dent[4], Chad D Huff[5], Paul T Shannon[6], Ethylin Wang Jabs[7,8], Deborah A Nickerson[1], Jay Shendure[1] & Michael J Bamshad[1,2,9]

We demonstrate the first successful application of exome sequencing to discover the gene for a rare mendelian disorder of unknown cause, Miller syndrome (MIM%263750). For four affected individuals in three independent kindreds, we captured and sequenced coding regions to a mean coverage of 40× and sufficient depth to call variants at ~97% of each targeted exome. Filtering against public SNP databases and eight HapMap exomes for genes with two previously unknown variants in each of the four individuals identified a single candidate gene, *DHODH*, which encodes a key enzyme in the pyrimidine *de novo* biosynthesis pathway. Sanger sequencing confirmed the presence of *DHODH* mutations in three additional families with Miller syndrome. Exome sequencing of a small number of unrelated affected individuals is a powerful, efficient strategy for identifying the genes

REVIEWS

TRANSLATIONAL GENETICS

Exome sequencing as a tool for Mendelian disease gene discovery

Michael J. Bamshad[*‡], *Sarah B. Ng*[‡], *Abigail W. Bigham*[*§], *Holly K. Tabor*[*||], *Mary J. Emond*[¶], *Deborah A. Nickerson*[‡] *and Jay Shendure*[‡]

Abstract | Exome sequencing — the targeted sequencing of the subset of the human genome that is protein coding — is a powerful and cost-effective new tool for dissecting the genetic basis of diseases and traits that have proved to be intractable to conventional gene-discovery strategies. Over the past 2 years, experimental and analytical approaches relating to exome sequencing have established a rich framework for discovering the genes underlying unsolved Mendelian disorders. Additionally, exome sequencing is being adapted to explore the extent to which rare alleles explain the heritability of complex diseases and health-related traits. These advances also set the stage for applying exome and whole-genome sequencing to facilitate clinical diagnosis and personalized disease-risk profiling.

PLoS GENETICS

Whole-Exome Re-Sequencing in a Family Quartet Identifies *POP1* Mutations As the Cause of a Novel Skeletal Dysplasia

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Abstract

Recent advances in DNA sequencing have enabled mapping of genes for monogenic traits in families with small pedigrees and even in unrelated cases. We report the identification of disease-causing mutations in a rare, severe, skeletal dysplasia,

European Journal of Human Genetics (2011) 19, 115–117

www.nature.com/ejhg

SHORT REPORT

Next generation sequencing in a family with autosomal recessive Kahrizi syndrome (OMIM 612713) reveals a homozygous frameshift mutation in *SRD5A3*

Kimia Kahrizi[1], Cougar Hao Hu[2], Masoud Garshasbi[2], Seyedeh Sedigheh Abedini[1], Shirin Ghadami[1], Roxana Kariminejad[1], Reinhard Ullmann[2], Wei Chen[2], H-Hilger Ropers[2], Andreas W Kuss[2], Hossein Najmabadi[1] and Andreas Tzschach[*,2]

As part of a large-scale, systematic effort to unravel the molecular causes of autosomal recessive mental retardation, we have previously described a novel syndrome consisting of mental retardation, coloboma, cataract and kyphosis (Kahrizi syndrome

Molecular Vision 2013; 19:2187-2195 <http://www.molvis.org/molvis/v19/2187>
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Whole-exome sequencing identifies novel compound heterozygous mutations in *USH2A* in Spanish patients with autosomal recessive retinitis pigmentosa

Cristina Méndez-Vidal,[1,2] María González-del Pozo,[1,2] Alicia Vela-Boza,[3] Javier Santoyo-López,[3] Francisco J. López-Domingo,[3] Carmen Vázquez-Marouschek,[4] Joaquin Dopazo,[3,5,6] Salud Borrego,[1,2] Guillermo Antiñolo[1,2,3]

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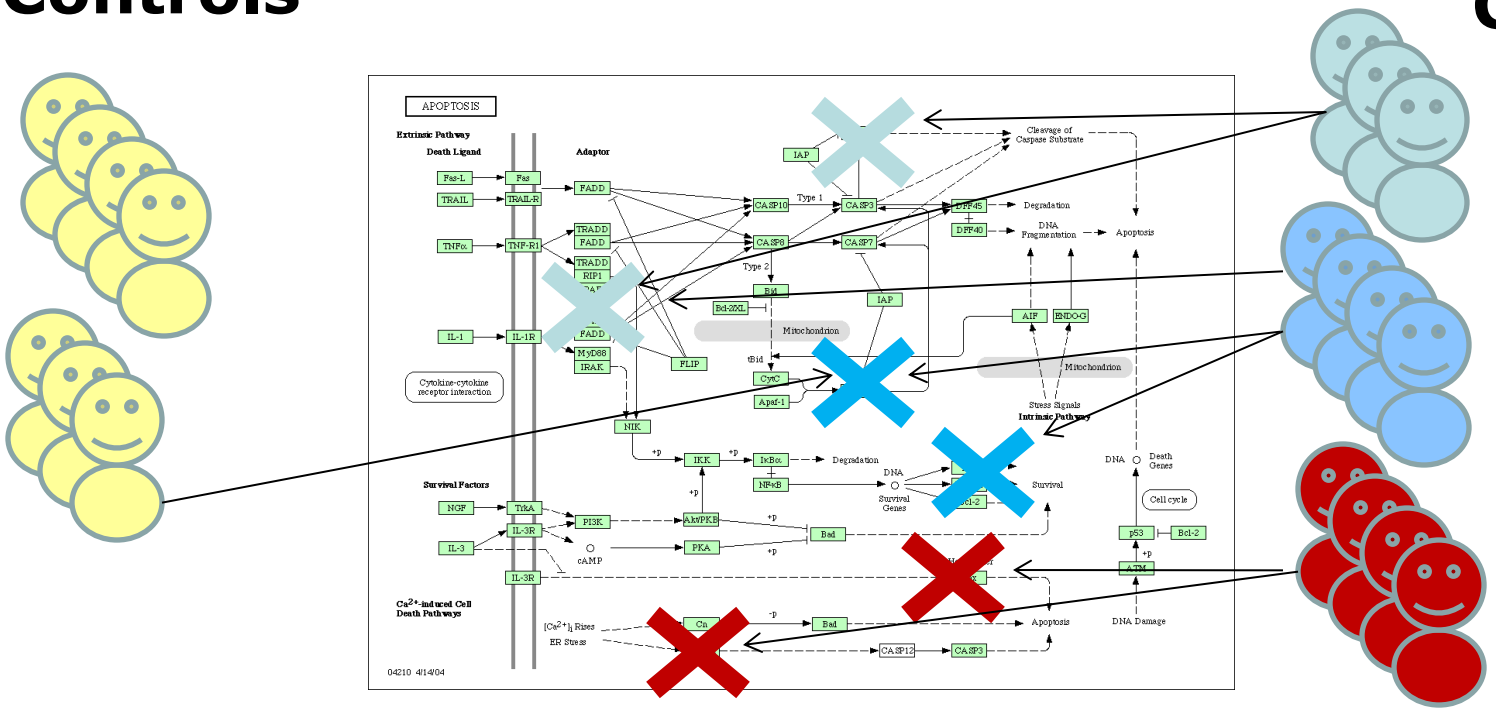
An approach inspired on systems biology can help in detecting causal genes

Controls

Cases



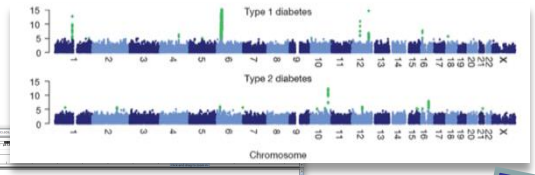
Affected **cases** in complex diseases will be a **heterogeneous** population with different mutations (or combinations).

Many cases and controls are needed to obtain significant associations.

The only **common element** is the (know or unknown) **pathway affected**.

Disease understood as the failure of a functional module

From gene-based to function-based perspective



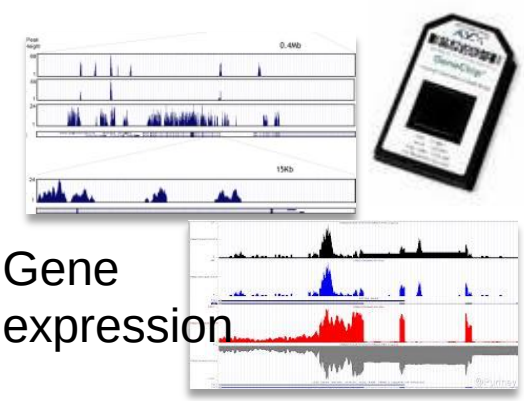
SNPs

WES/WGS

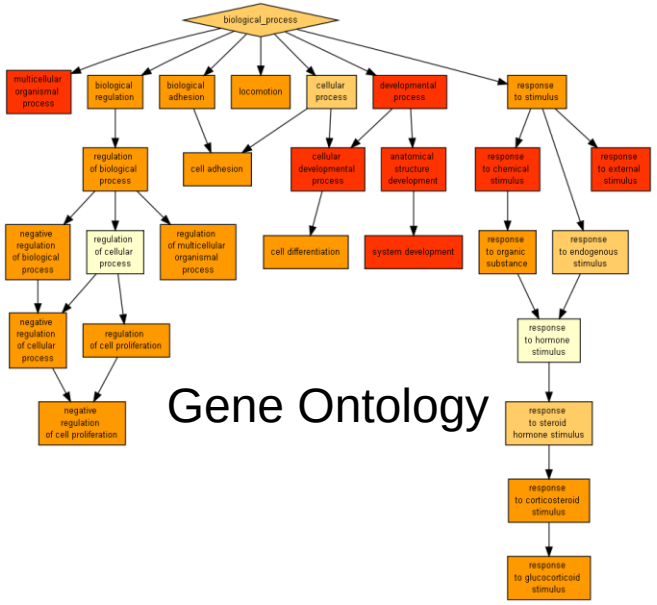
Gene integrity

AND/OR

Gene activity



Gene expression



Gene Ontology

Gene Ontology are **labels** to genes that describe, by means of a controlled vocabulary (ontology), the **functional role(s)** played by the genes in the cell.
A set of genes **sharing** a **GO** annotation can be considered a **functional module**.

An example of GWAS

GWAS in Breast Cancer.

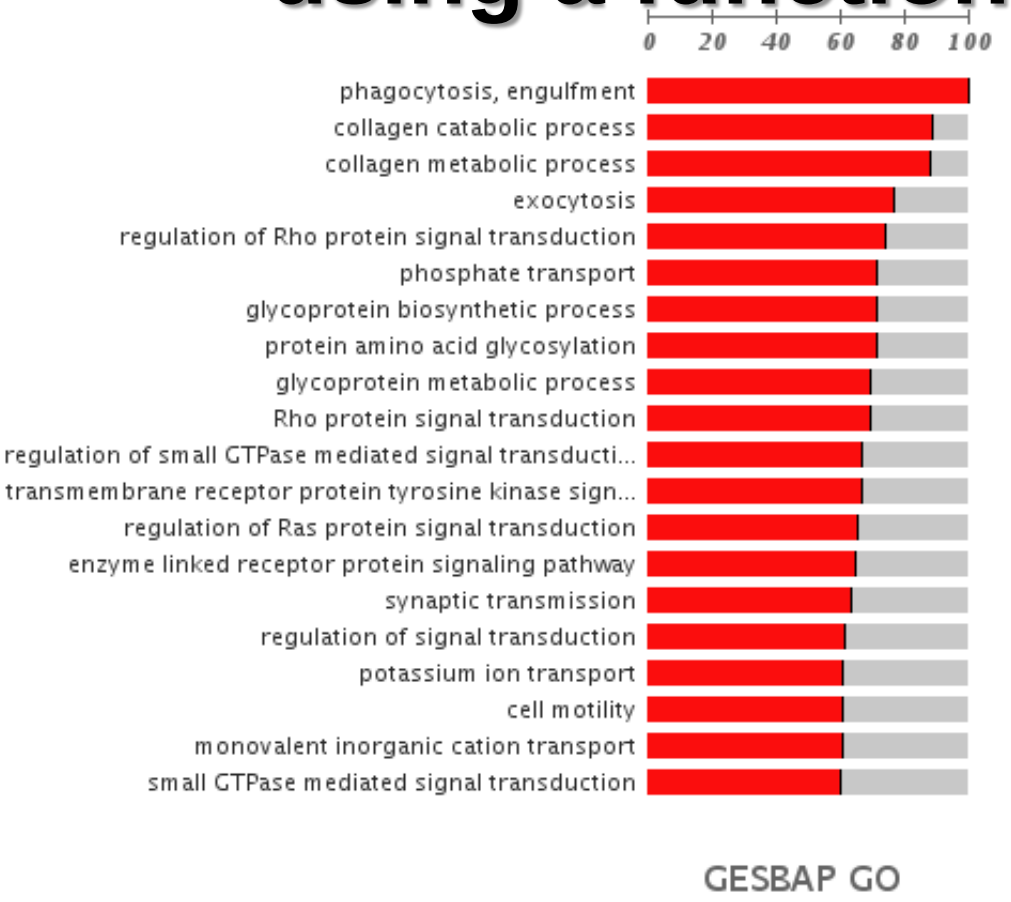
The CGEMS initiative. (Hunter et al. Nat Genet 2007)

1145 cases 1142 controls. Affy 500K

Conventional association test reports only 4 SNPs significantly mapping only on one gene: FGFR2

Conclusions: **conventional SNP-based** or **gene-based tests** are not providing much resolution.

The same GWAS data re-analyzed using a function-based test



Breast Cancer

CGEMS initiative.
(Hunter et al. Nat Genet 2007)

1145 cases 1142 controls. Affy 500K

Only 4 SNPs were significantly associated, mapping only in one gene: FGFR2

PBA reveals 19 GO categories including *regulation of signal transduction* (FDR-adjusted p-value=4.45×10^{-03}) in which FGFR2 is included.

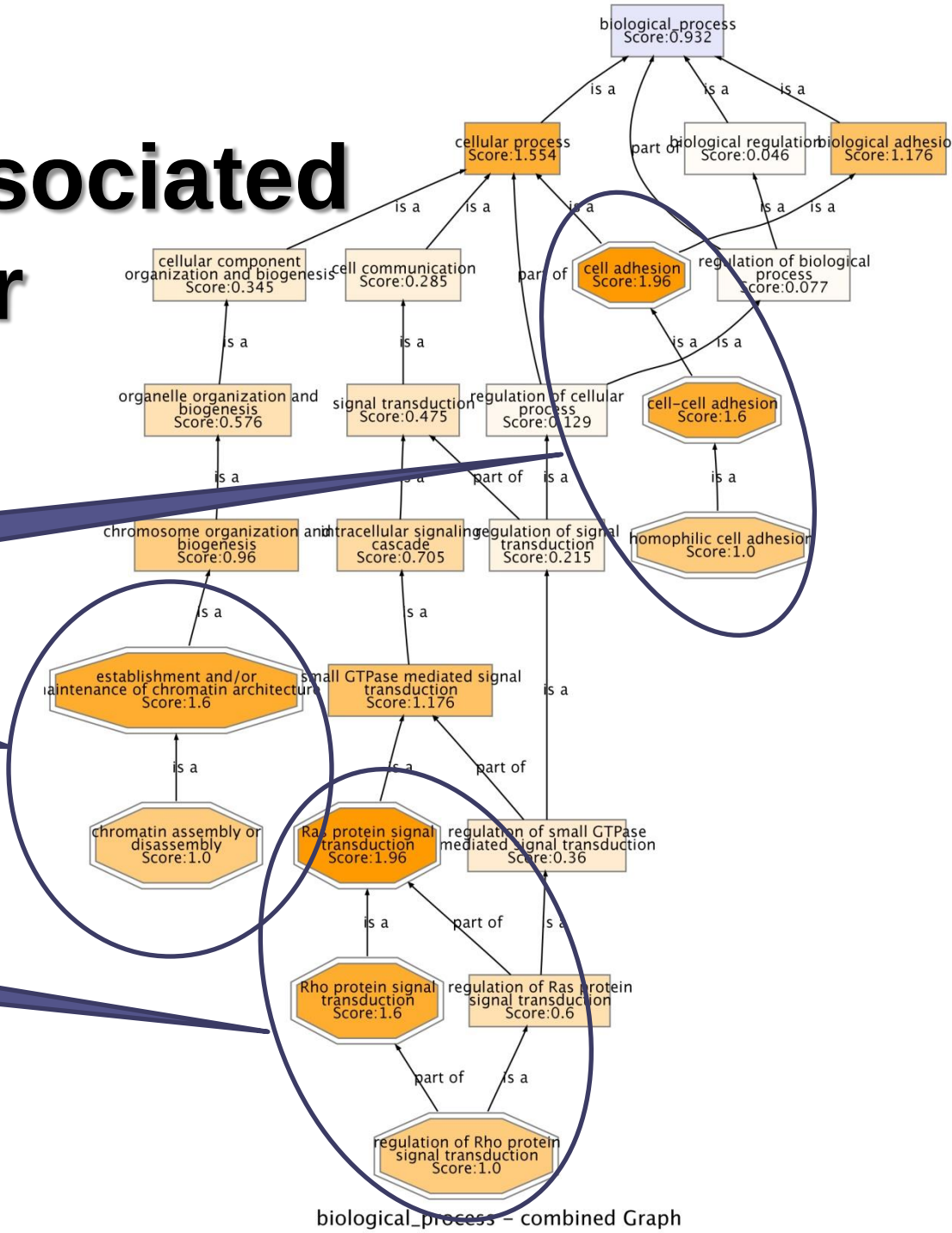
Bonifaci et al., BMC Medical Genomics 2008; Medina et al., 2009 NAR

GO processes significantly associated to breast cancer

Metastasis

Chromosomal instability

Rho pathway

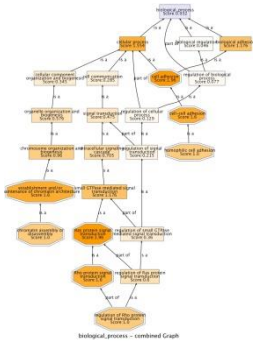


From gene-based to function-based perspective

SNPs,
Gene expression

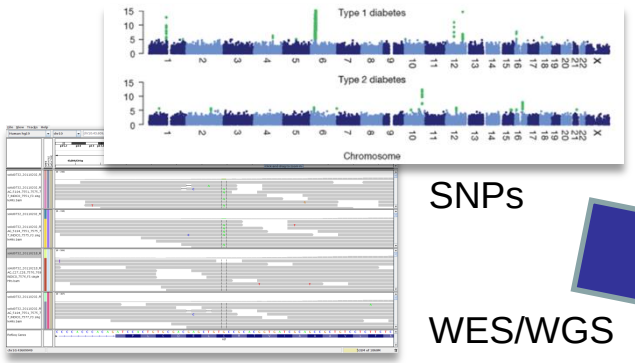
$Gene_1$
$Gene_2$
$Gene_3$
$Gene_4$
:
:
:
$Gene_{22000}$

Gene Ontology



	SNPs, gene exp.	GO
Detection power	Low (only very prevalent genes)	high
Annotations available	many	many
Use	Biomarker	Illustrative, give hints

From gene-based to function-based perspective

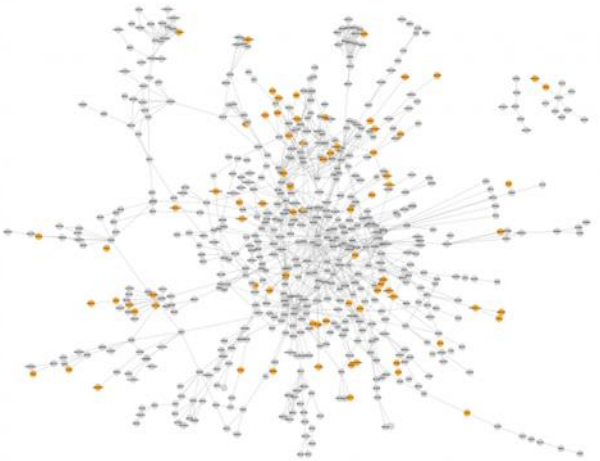


SNPs

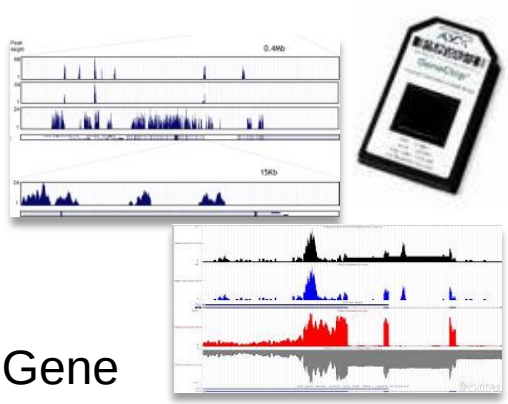
WES/WGS

Gene integrity

Using protein interaction networks as an scaffold to interpret the genomic data in a functionally-derived context



AND/OR



Gene expression

Gene activity

What part of the interactome is active and/or is damaged

Network analysis helps to find disease genes in complex diseases

Research

Open Access

Four new loci associations discovered by pathway-based and network analyses of the genome-wide variability profile of Hirschsprung's disease

Raquel Ma Fernández[1,2], Marta Bleda[2,3], Rocío Núñez-Torres[1,2], Ignacio Medina[3,4], Berta Luzón-Toro[1,2], Luz García-Alonso[3], Ana Torroglosa[1,2], Martina Marbà[3,4], Ma Valle Enguix-Riego[1,2], David Montaner[3], Guillermo Antiñolo[1,2], Joaquín Dopazo[2,3,4]* and Salud Borrego[1,2]*

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Nucleic Acids Research, 2009, 1–6
doi:10.1093/nar/gkp402

SNOW, a web-based tool for the statistical analysis of protein–protein interaction networks

Pablo Minguez[1], Stefan Götz[1,2], David Montaner[1], Fatima Al-Shahrour[1] and Joaquin Dopazo[1,2,3,*]

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Published online 27 July 2012 *Nucleic Acids Research, 2012, Vol. 40, No. 20 e158*
doi:10.1093/nar/gks699

Discovering the hidden sub-network component in a ranked list of genes or proteins derived from genomic experiments

Luz García-Alonso[1], Roberto Alonso[1], Enrique Vidal[1], Alicia Amadoz[1], Alejandro de María[1], Pablo Minguez[2], Ignacio Medina[1,3] and Joaquín Dopazo[1,3,4,*]

[1]Department of Bioinformatics, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain, [2]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany, [3]Functional Genomics Node (INB) at CIPF, Valencia and [4]CIBER de Enfermedades Raras (CIBERER), Valencia, Spain

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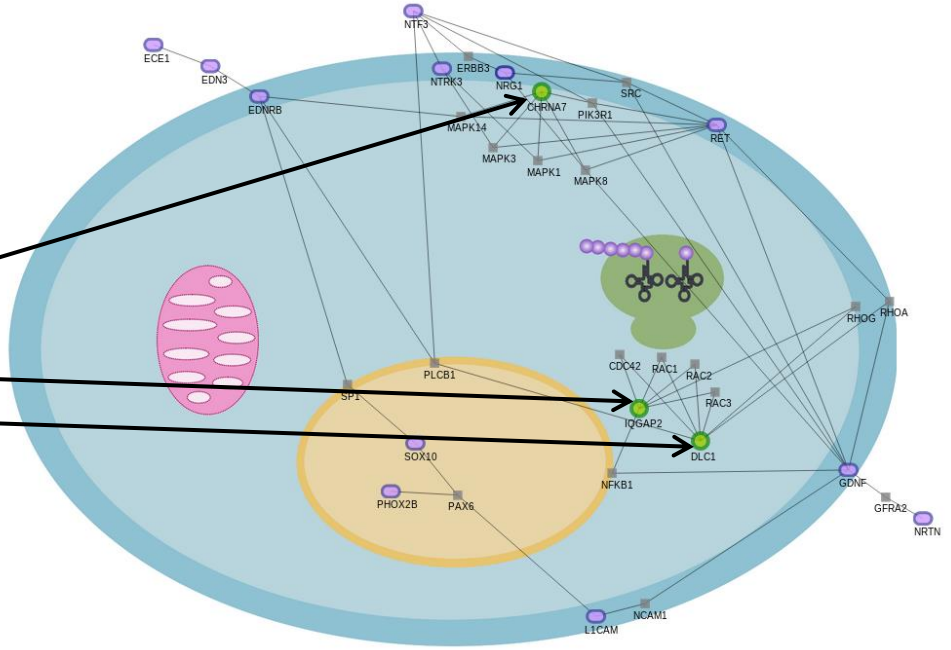


CHRNA7 (rs2175886 p = 0.000607)
IQGAP2 (rs950643 p = 0.0003585)
DLC1 (rs1454947 p = 0.007526)
*RASGEF1A** (rs1254964 p = 3.856×10^{-05})
**no interactions known (yet)*

SNPs validated in independent cohorts

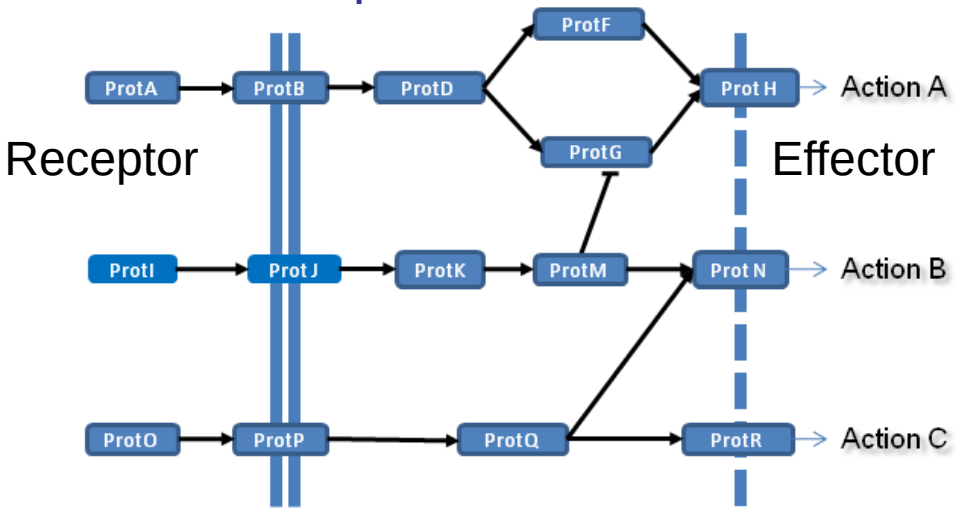
From gene-based to function-based perspective

	SNPs, gene expression, etc.	GO	Protein interaction networks
Detection power	Low (only very prevalent genes)	High	High
Information coverage	Almost all	Almost all	Less (~9000 genes in human)
Use	Biomarker	Illustrative, give hints	Biomarker*

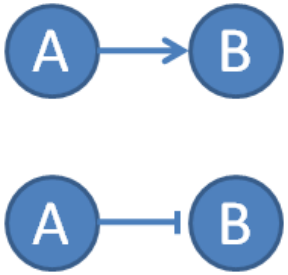
**Need of extra information (e.g. GO) to provide functional insights in the findings*

From gene-based to mechanism-based perspective

Transforming gene expression values into another value that **accounts for a function**. Easiest example of modeling function: **signaling pathways**. Function: transmission of a signal from a receptor to an effector



	ProtH	ProtN	ProtR
ProtA	1	0	0
ProtI	1	1	0
ProtQ	0	1	1
function	Action A	Action B	Action C



Activations and repressions occur

Modeling pathways

Sebastian-Leon *et al. BMC Systems Biology* 2014, **8**:121
http://www.biomedcentral.com/1752-0509/8/121

BMC Systems Biology

METHODOLOGY ARTICLE Open Access

Understanding disease mechanisms with models of signaling pathway activities

Patricia Sebastian-Leon[1], Enrique Vidal[1,2,3], Pablo Minguez[1,4], Ana Conesa[1], Sonia Tarazona[1], Alicia Amadoz[1], Carmen Armero[5], Francisco Salavert[1,2], Antonio Vidal-Puig[6], David Montaner[1] and Joaquín Dopazo[1,2,7*]

Published online 8 June 2013 *Nucleic Acids Research, 2013, Vol. 41, Web Server issue* **W213–W217**
doi:10.1093/nar/gkt451

Inferring the functional effect of gene expression changes in signaling pathways

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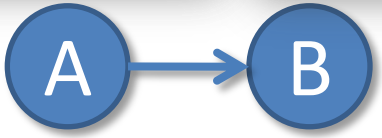
[1]Department of Computational Genomics, Centro de Investigación Príncipe Felipe (CIPF), Valencia 46012, Spain, [2]CIBER de Enfermedades Raras (CIBERER), Valencia 46012, Spain, [3]Genometra S.L., Valencia, Spain and [4]Functional Genomics Node (INB) at CIPF, Valencia 46012, Spain

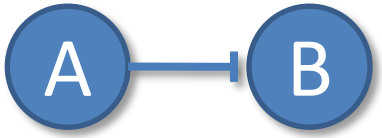
Received March 3, 2013; Revised April 18, 2013; Accepted May 2, 2013

Activation



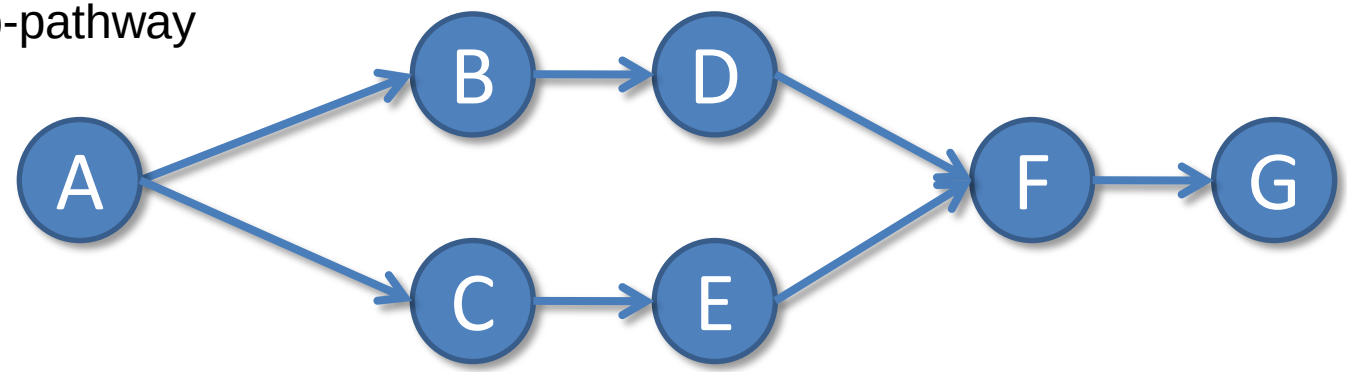
Prob. = P(A activated)P(B activated)

Inhibition



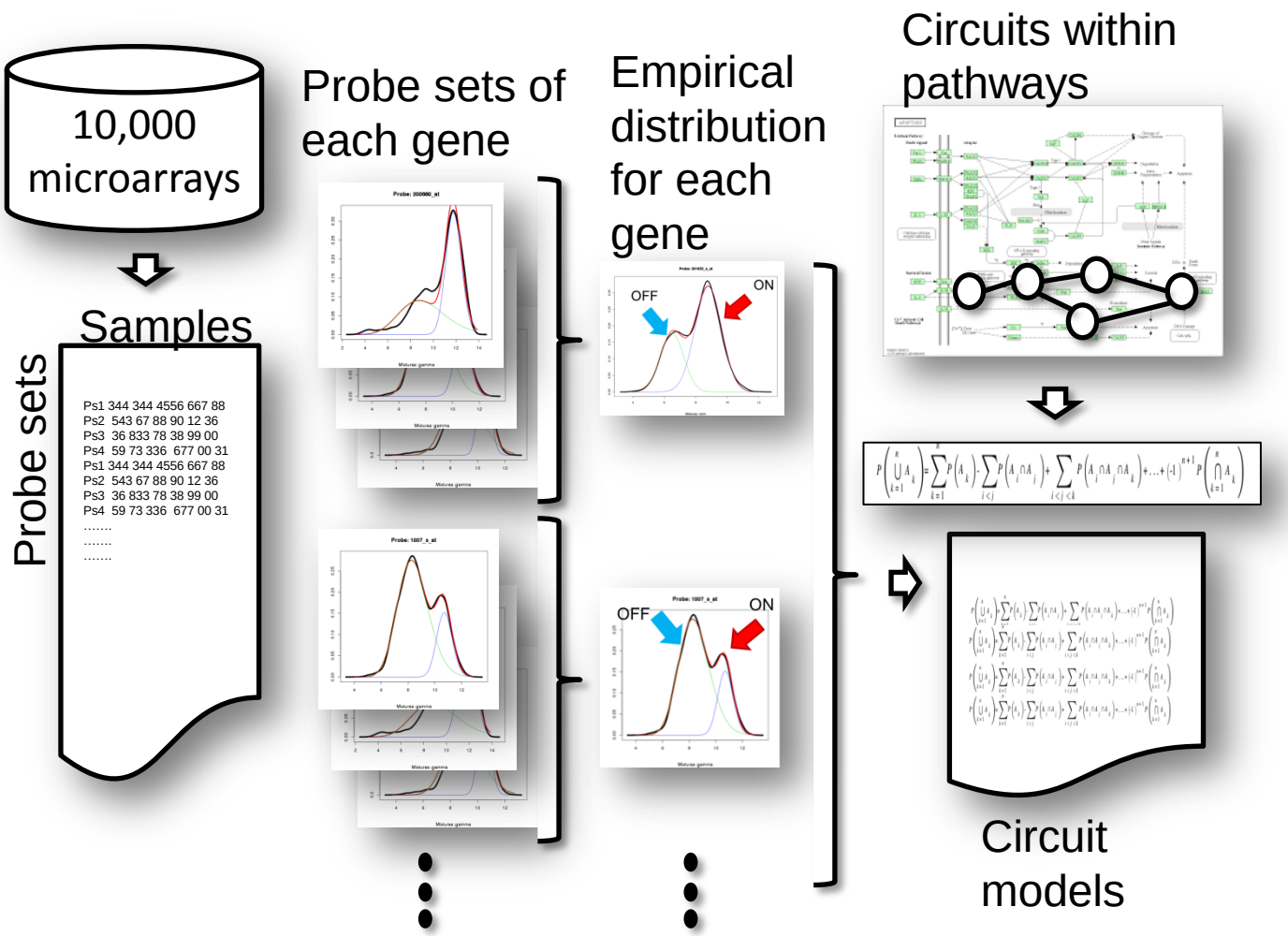
Prob. = [1-P(A activated)]P(B activated)

Sub-pathway



P(A→G activated) = P(A)P(B)P(D)P(F)P(G) + P(A)P(C)P(E)P(F)P(G) - P(A)P(F)P(G)P(B)P(C)P(D)P(E)

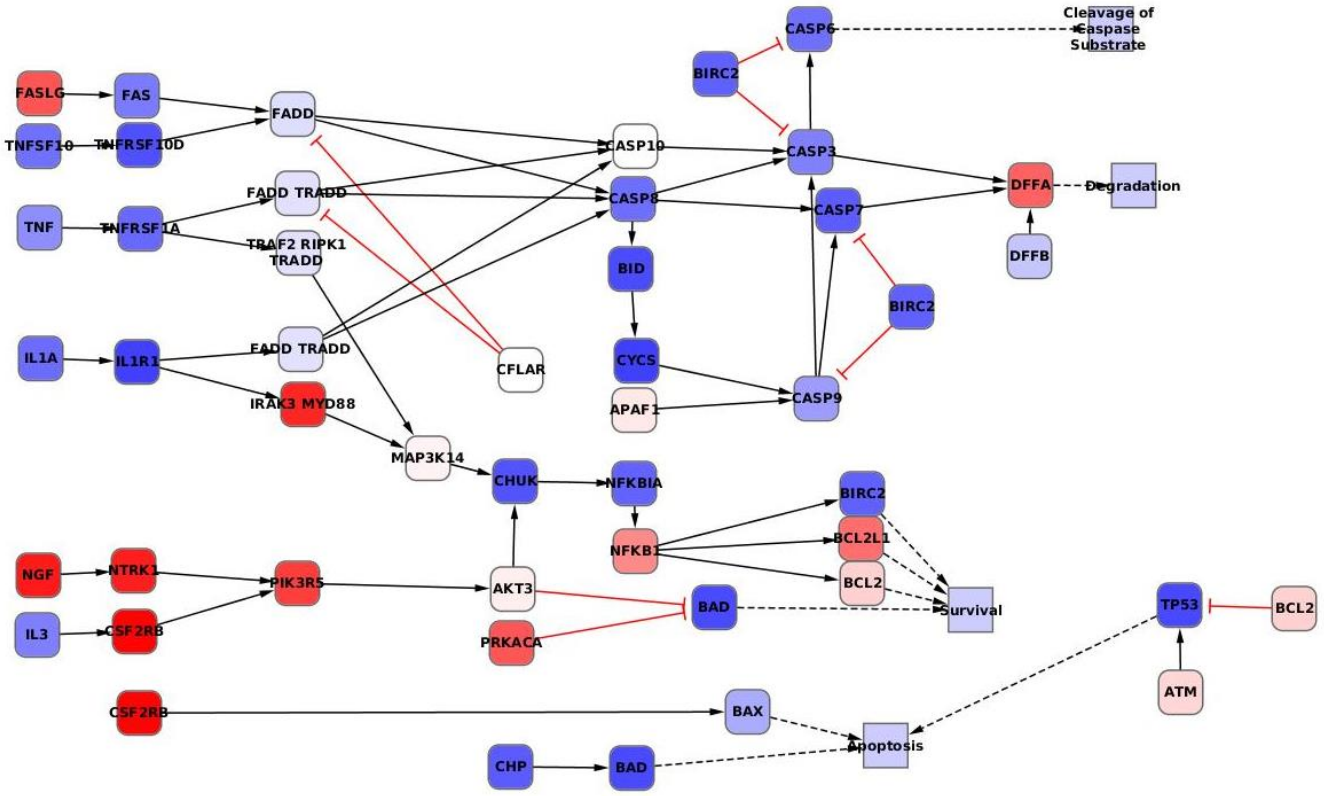
Modeling activation probabilities of circuits



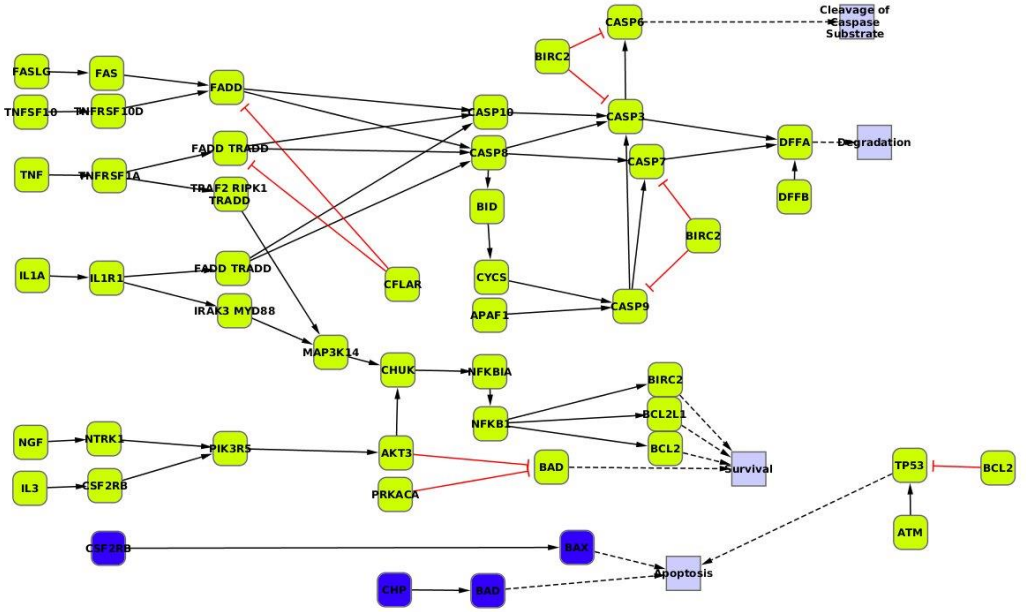
The effects of changes in gene activity are not obvious

What would you predict about the consequences of gene activity changes in the apoptosis pathway in a case control experiment of colorectal cancer?

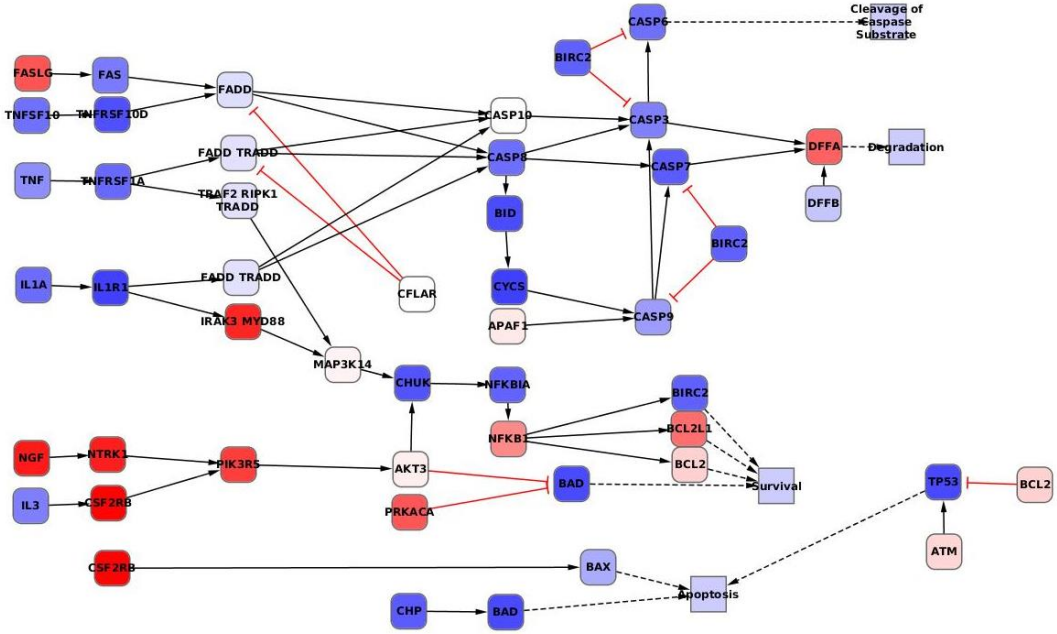
The figure shows the gene up-regulations (red) and down-regulations (blue)



Apoptosis inhibition is not obvious from gene expression



Two of the three possible sub-pathways leading to apoptosis are inhibited in colorectal cancer. Upper panel shows the inhibited sub-pathways in blue. Lower panel shows the actual gene up-regulations (red) and down-regulations (blue) that justify this change in the activity of the sub-pathways



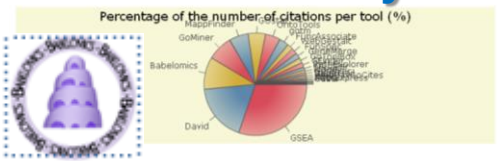
From gene-based to function-based perspective

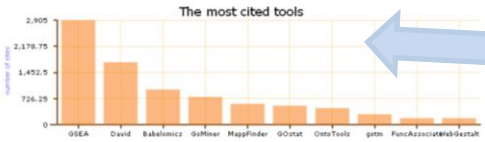
	SNPs, gene expression, etc.	GO	Protein interaction networks	Models of cellular functions
Detection power	Low (only very prevalent genes)	High	High	Very high
Information coverage	Almost all	Almost all	Low (~9000 genes in human)	Low (~6700 genes in human)*
Use	Biomarker	Illustrative, give hints	Biomarker	Biomarker that explain disease mechanism

*Only ~800 genes in human signaling pathways

Software development

Functional analysis

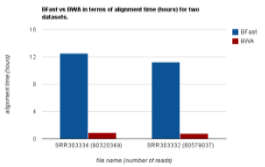




Babelomics is the third most cited tool for functional analysis. Includes more than 30 tools for advanced, systems-biology based data analysis

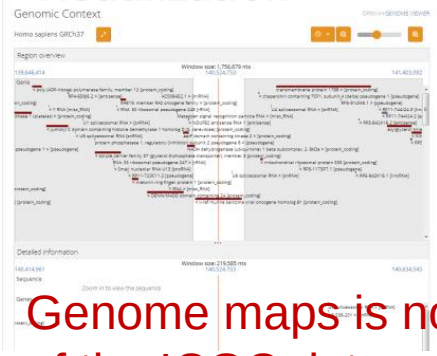
See interactive map of for the last 24h use http://bioinfo.cipf.es/toolsusage

Mapping



HPC on CPU, SSE4, GPUs on NGS data processing
Speedups up to 40X

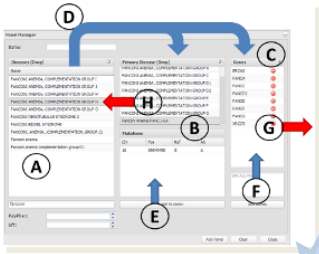
Visualization



Ultrafast genome viewer with google technology

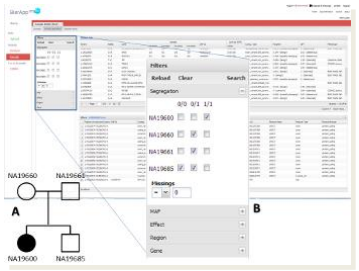
Genome maps is now part of the ICGC data portal

Diagnostic

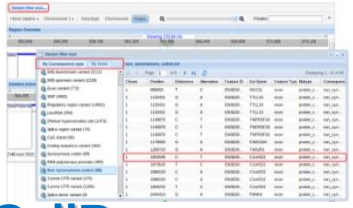


NGS panels

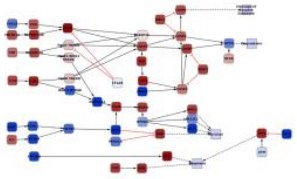
Variant prioritization



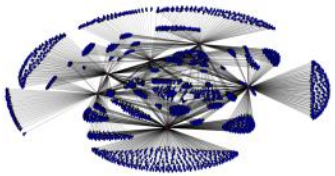
Variant annotation



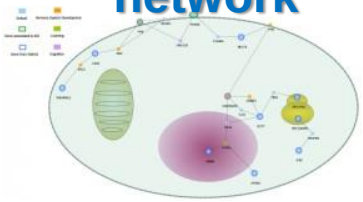
Signaling network



Regulatory network



Interaction network



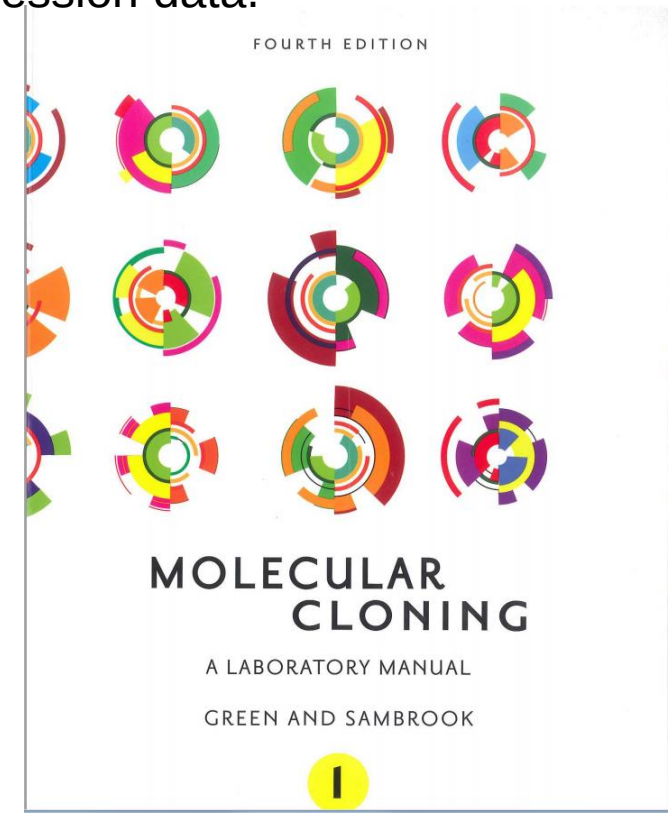
CellBase

Knowledge database

More than 150.000 experiments were analyzed in our tools during the last year

Babelomics in the Maniatis

The Babelomics suite of programs becomes a classic. Now is cited as a method in the last edition of **Molecular Cloning**, the popular **Maniatis**. The protocol 4 of chapter 8, Expression Profiling by Microarray and RNA-seq, contains a description on how to use Babelomics to analyze expression data.



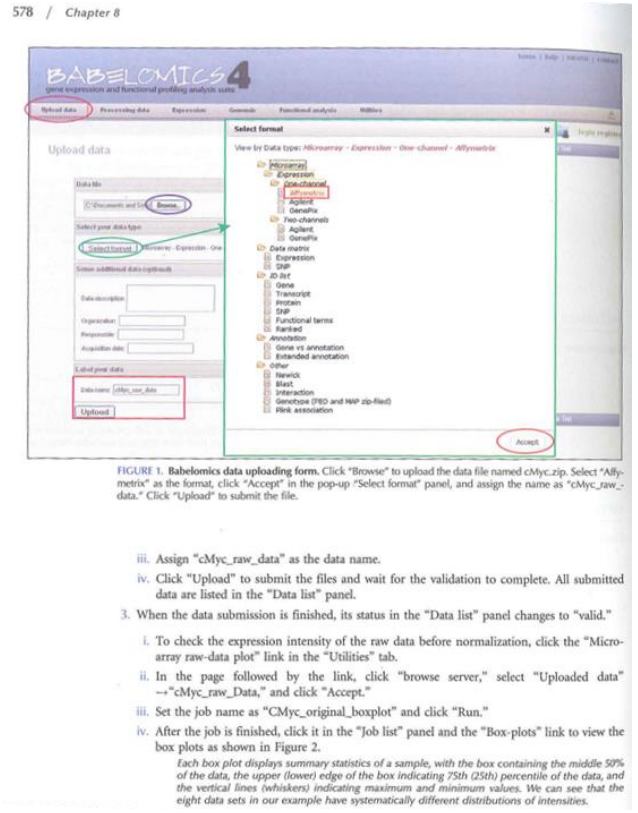


FIGURE 1. **Babelomics data uploading form.** Click "Browse" to upload the data file named cMyc.zip. Select "Affymetrix" as the format, click "Accept" in the pop-up "Select format" panel, and assign the name as "cMyc_raw_data." Click "Upload" to submit the file.

iii. Assign "cMyc_raw_data" as the data name.
iv. Click "Upload" to submit the files and wait for the validation to complete. All submitted data are listed in the "Data list" panel.

3. When the data submission is finished, its status in the "Data list" panel changes to "valid."
   i. To check the expression intensity of the raw data before normalization, click the "Microarray raw-data plot" link in the "Utilities" tab.
   ii. In the page followed by the link, click "browse server," select "Uploaded data" → "cMyc_raw_Data," and click "Accept."
   iii. Set the job name as "CMyc_original_boxplot" and click "Run."
   iv. After the job is finished, click it in the "Job list" panel and the "Box-plots" link to view the box plots as shown in Figure 2.

Each box plot displays summary statistics of a sample, with the box containing the middle 50% of the data, the upper (lower) edge of the box indicating 75th (25th) percentile of the data, and the vertical lines (whiskers) indicating maximum and minimum values. We can see that the eight data sets in our example have systematically different distributions of intensities.

High impact developments

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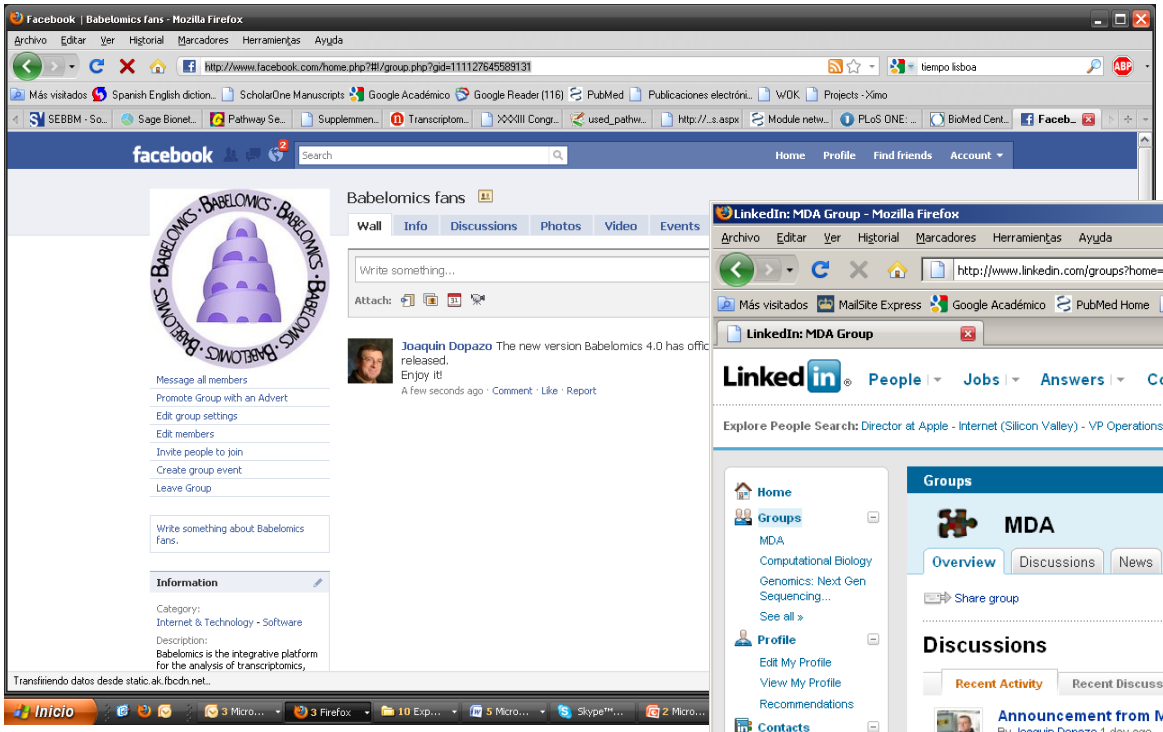

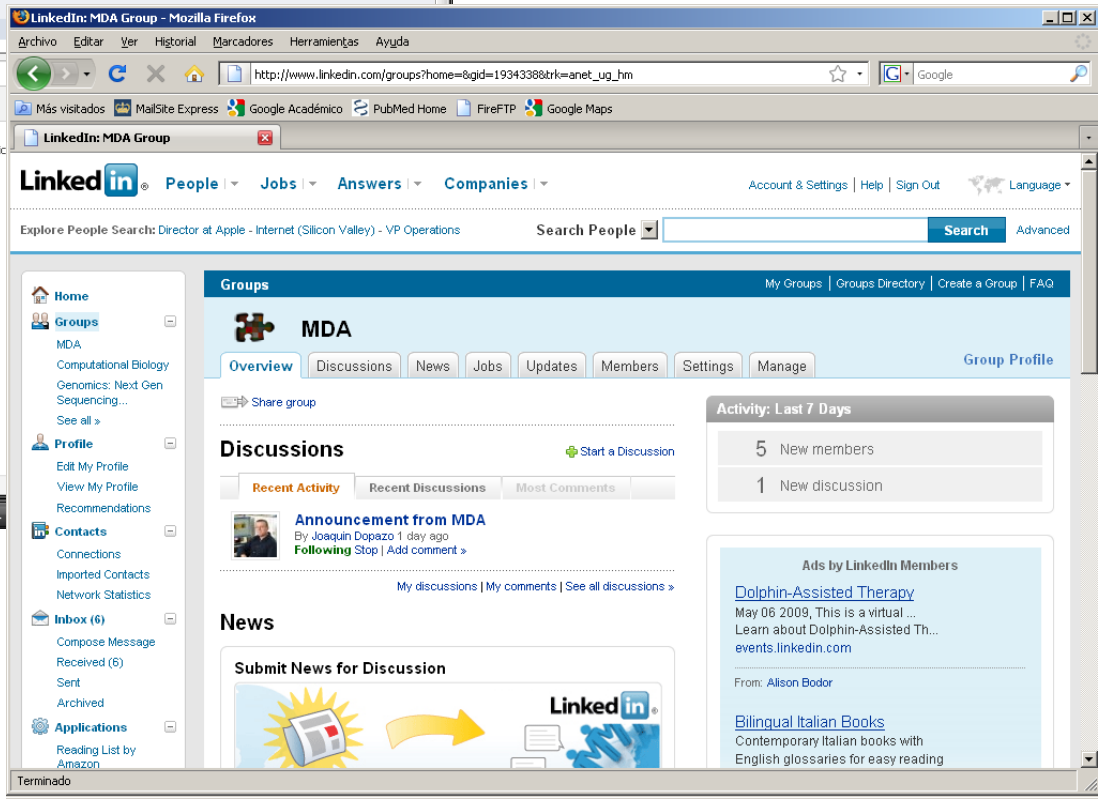

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…the INB, National Institute of Bioinformatics (Functional Genomics Node) and the BiER (CIBERER Network of Centers for Rare Diseases)

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