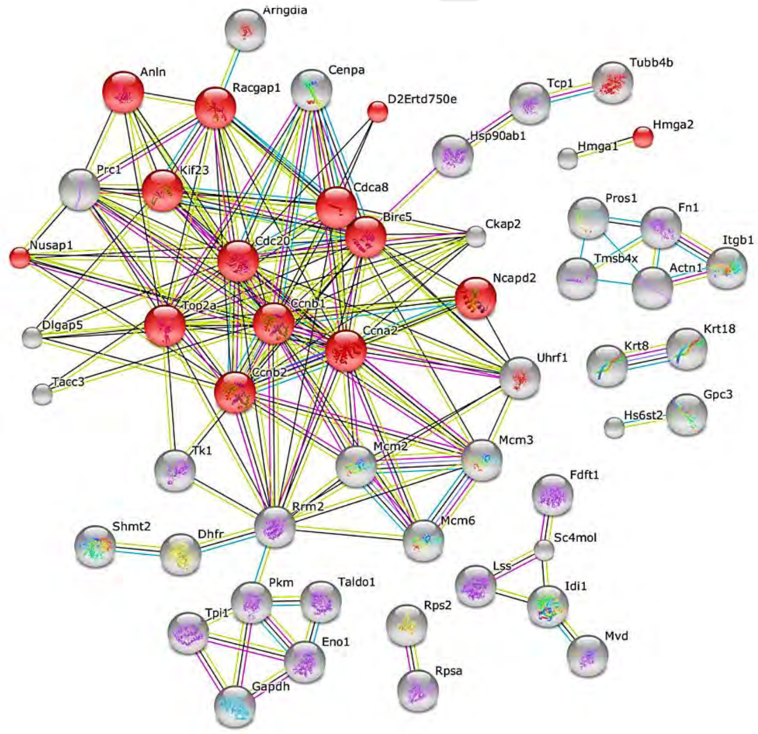
Transcriptómica y coexpresión génica



Prof. Ulises Urzúa
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Universidad de Chile

uurzua@med.uchile.cl

Temario

PARTE 1

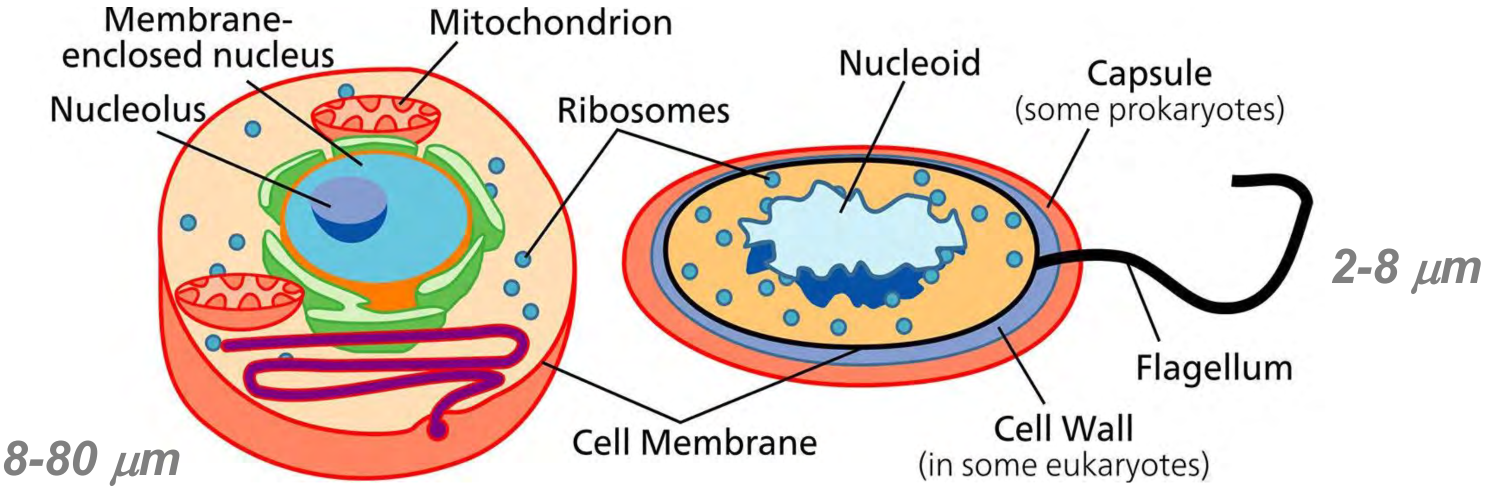
- Introducción. Genómica
- Microarreglos de DNA. Fundamentos
- Transcriptomica
- Co-expresion de genes. Casos

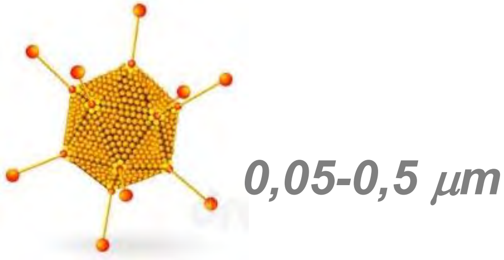
PARTE 2

- Aplicaciones en cancer (4)
- Microarray-CGH
- Cancer ovarico y envejecimiento

Genómica - material genético

- *Genoma*: secuencia completa del material genético de un organismo.



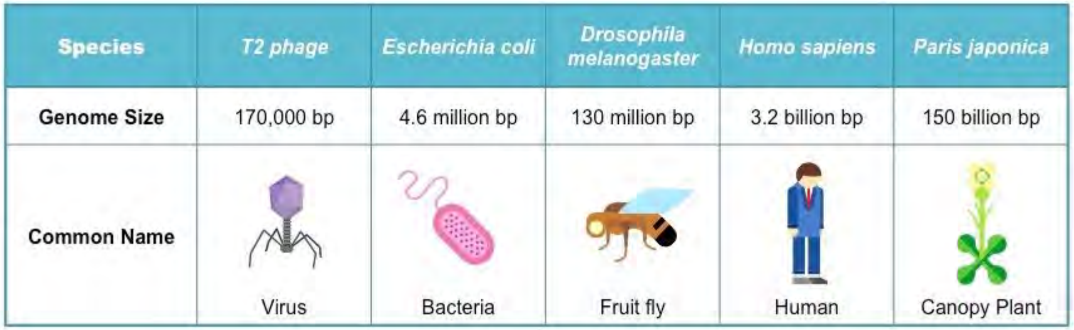


https://en.wikipedia.org/wiki/Prokaryote#Relationship_to_eukaryotes

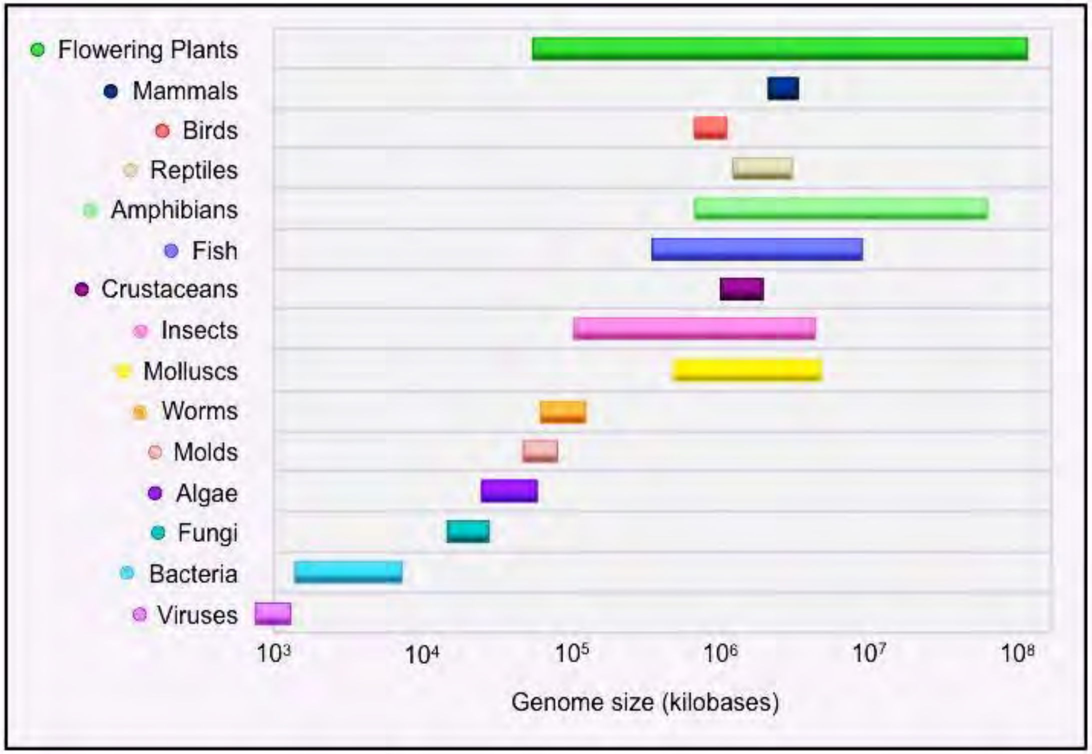
Genómica - tamaño genómico

1 bp = un par de bases.
(A-T, C-G)

1 Kb = 1000 bp
1 Mb = 1.000.000 pb

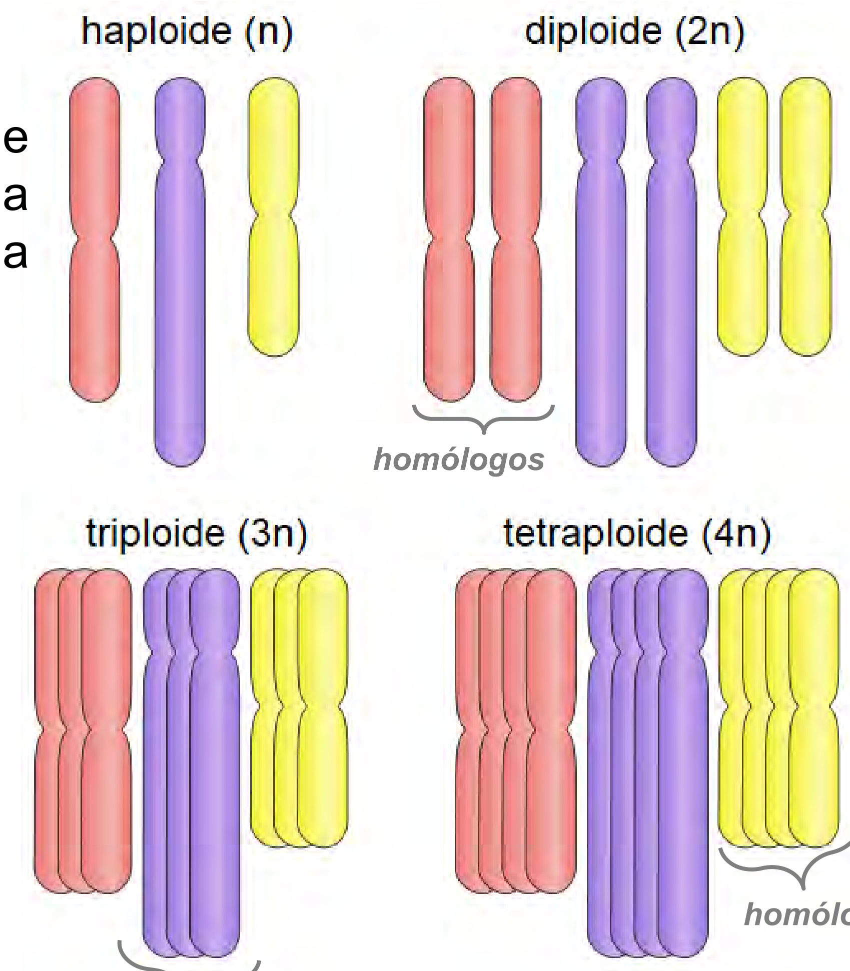


Species	*T2 phage*	*Escherichia coli*	*Drosophila melanogaster*	*Homo sapiens*	*Paris japonica*
Genome Size	170,000 bp	4.6 million bp	130 million bp	3.2 billion bp	150 billion bp
Common Name	Virus	Bacteria	Fruit fly	Human	Canopy Plant



http://ib.bioninja.com.au/

Genómica - cromosomas



1 copia de cada cromosoma

2 copias

homólogos

3 copias

4 copias

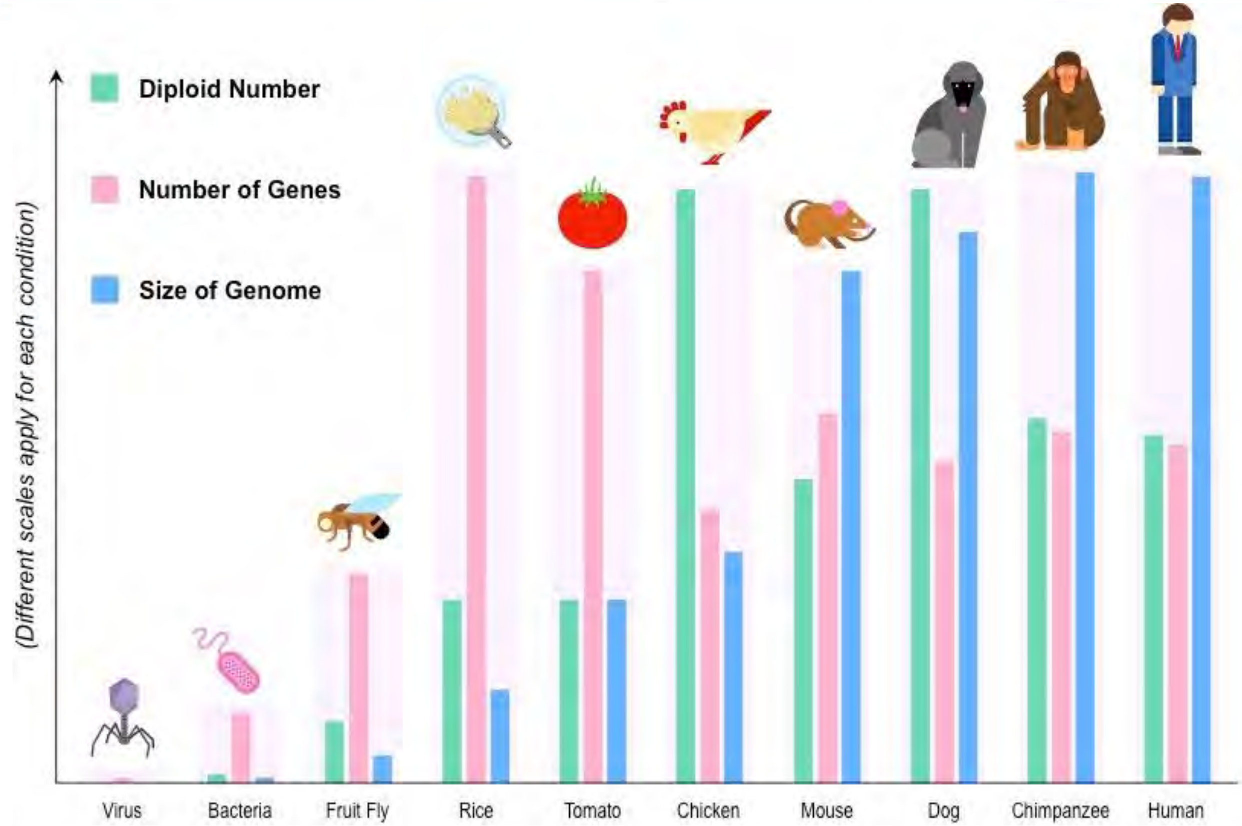
homólogos

homólogos

https://commons.wikimedia.org/

Genómica - cromosomas

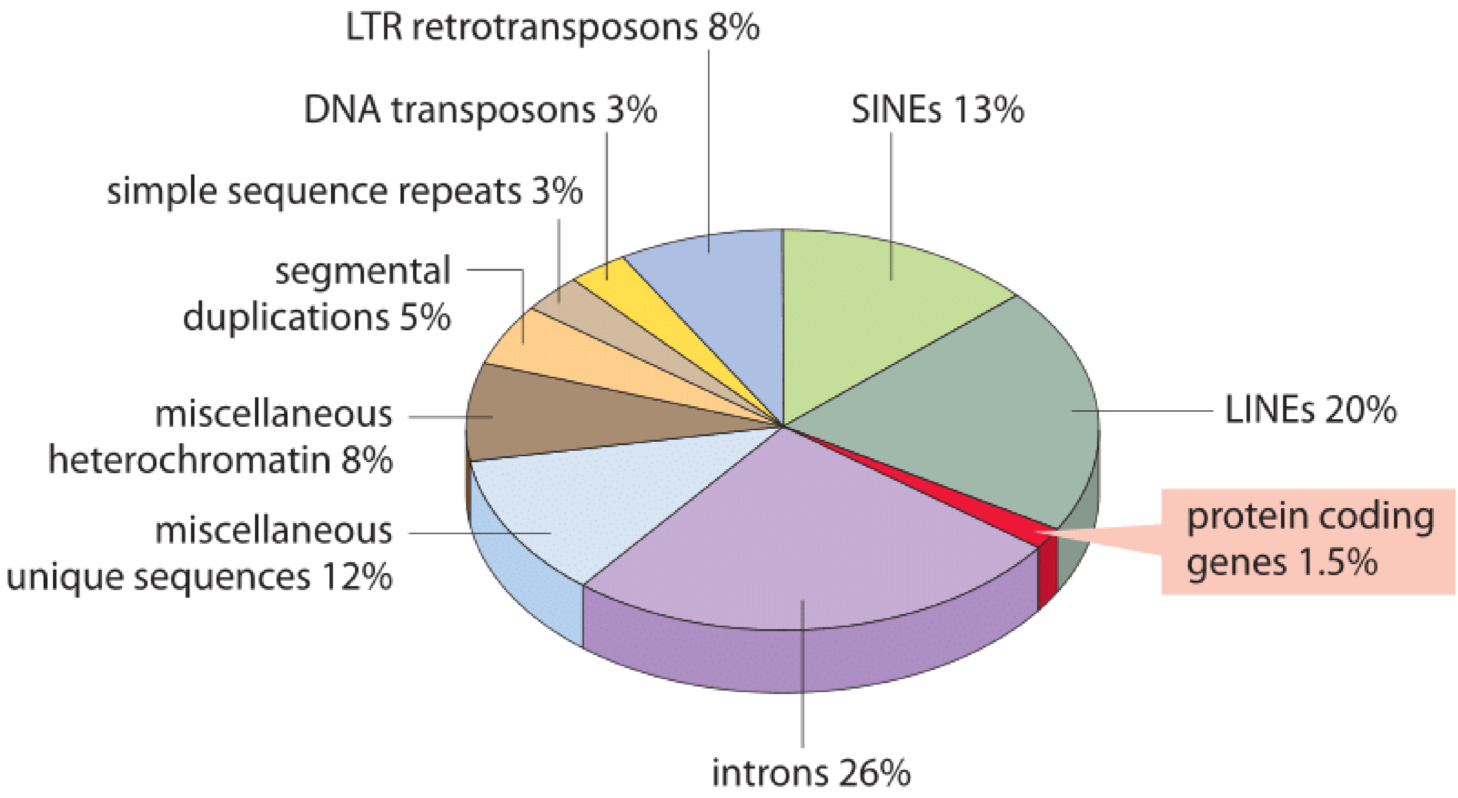
Species	***Parascaris equorum***	***Oryza sativa***	***Homo sapiens***	***Pan troglodytes***	***Canis familiaris***
Chromosome #	4	24	46	48	78
Common Name	 Roundworm	 Rice	 Human	 Chimpanzee	 Dog



http://ib.bioninja.com.au/

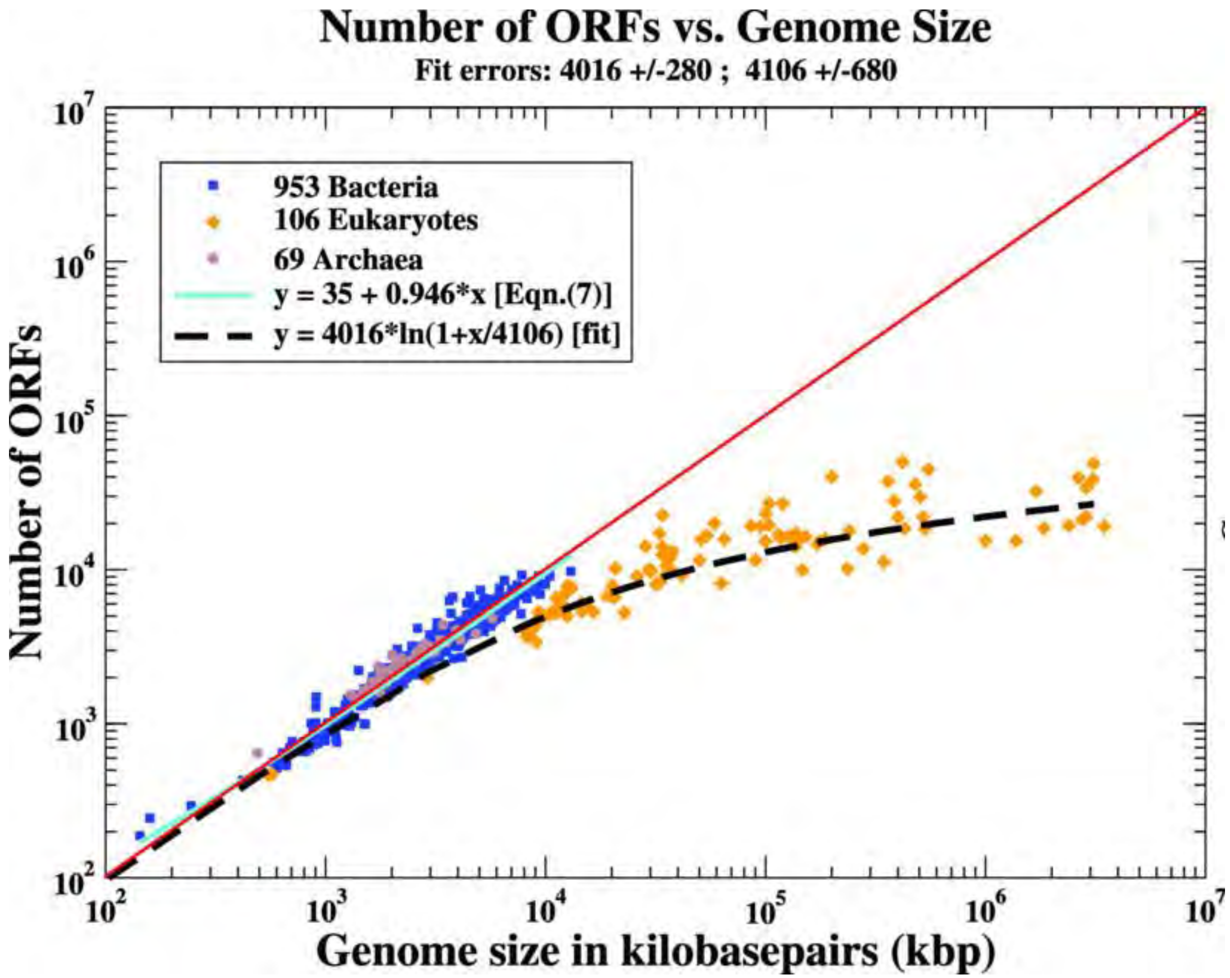
Genómica - composición del genoma

- Tipos de secuencia del genoma humano.



http://book.bionumbers.org/

Genómica - tamaño genómico vs # genes



Friar et al PLoS ONE: 2012, 7(5);e36624

Genómica - gen

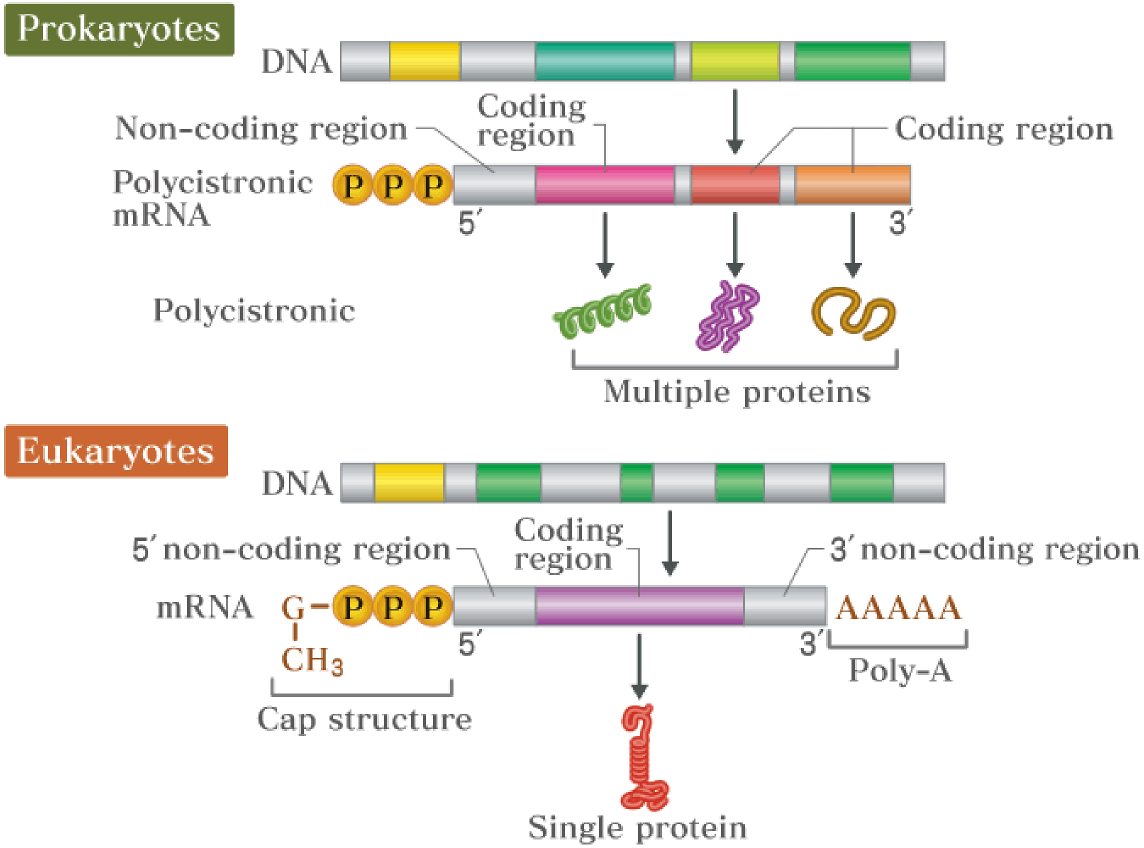
Gen: i) secuencia parcial del genoma que *codifica* para un producto (transcrito, proteína);
ii) unidad hereditaria física y funcional que es transferida desde una célula parental a una célula hija.

Genes H sapiens	***Número***
Codificantes	20,376
No-codificantes	22,305
- Pequeños	5,363
- Largos	14,720
- Misceláneos	2,222
Pseudogenes	14,692
Transcritos	203,903

http://www.ensembl.org/Homo_sapiens/Location/Genome

Genómica - gen

Gen: i) secuencia parcial del genoma que *codifica* para un producto (transcrito, proteína);
ii) unidad hereditaria física y funcional que es transferida desde una célula parental a una célula hija.

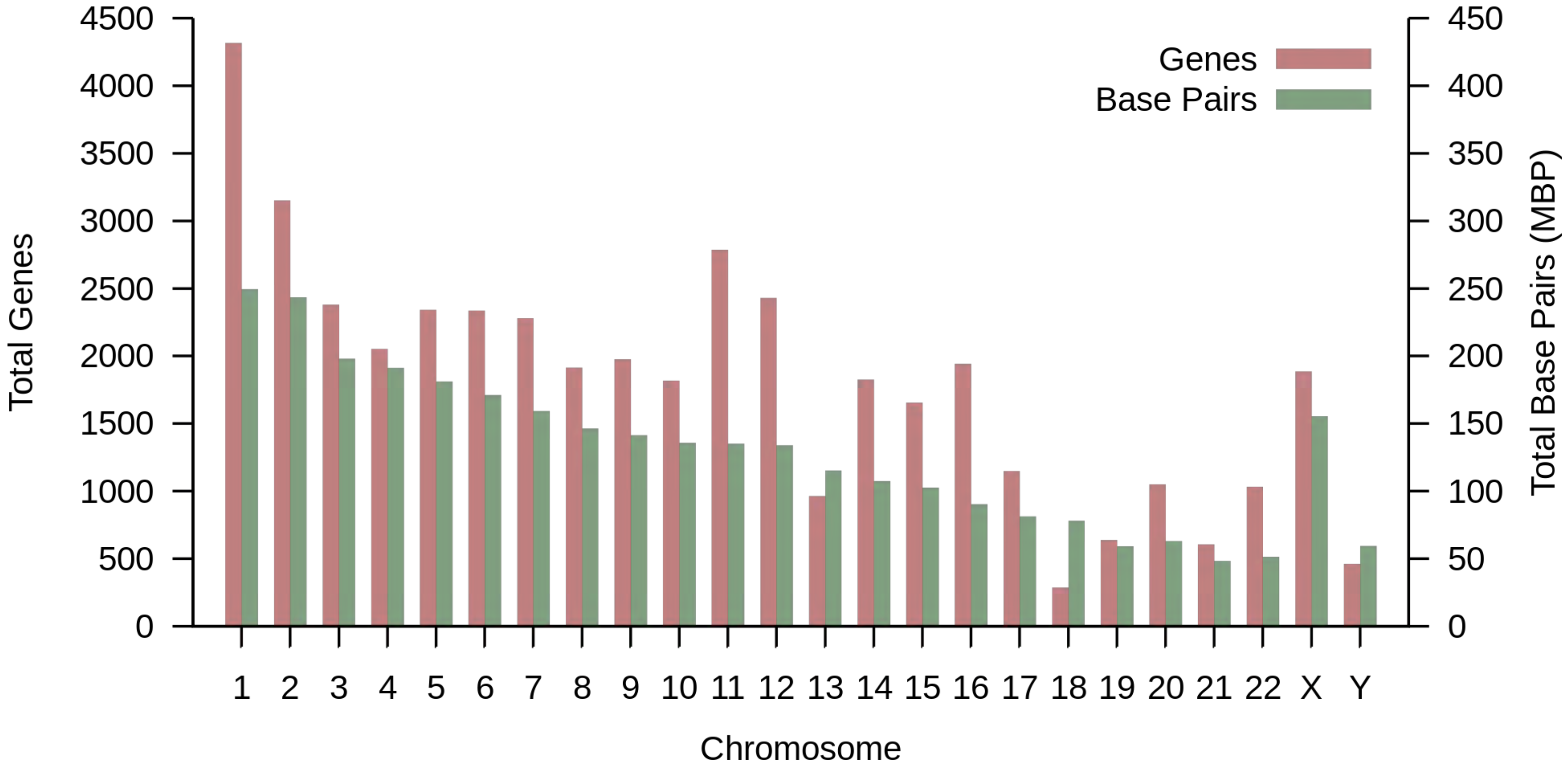


©CSLS / The University of Tokyo

Genómica - tamaño genes H sapiens

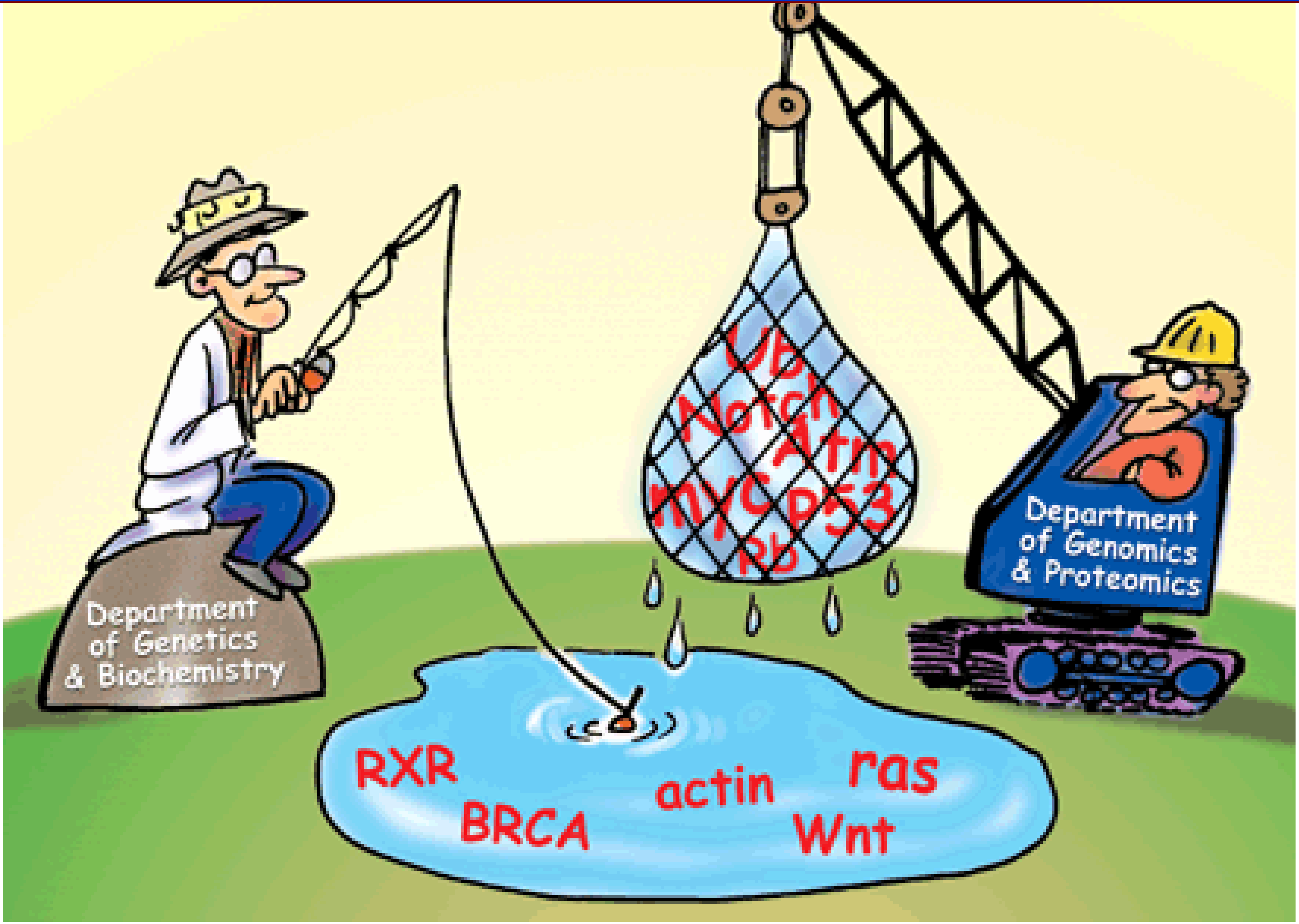
Protein	Chrom	Gene	Length	Exons	Exon length	Intron length	Alt splicing
Histone H1A	6	HIST1H1A	781	1	781	0	no
Cytochrome b	MT	MTCYB	1,140	1	1,140	0	no
Hemoglobin beta subunit	11	HBB	1,605	3	626	979	no
Glyceraldehyde-3-phosphate dehydrogenase	12	GAPDH	4,444	9	1,425	3,019	yes
Breast cancer type 2 susceptibility protein	13	BRCA2	83,736	27	11,386	72,350	yes
Cystic fibrosis transmembrane conductance regulator	7	CFTR	202,881	27	4,440	198,441	yes
Titin	2	TTN	281,434	364	104,301	177,133	yes
Dystrophin	X	DMD	2,220,381	79	10,500	2,209,881	yes
Promedio			***26,288***	***11***	***309***		

Genómica - gen/cromosoma

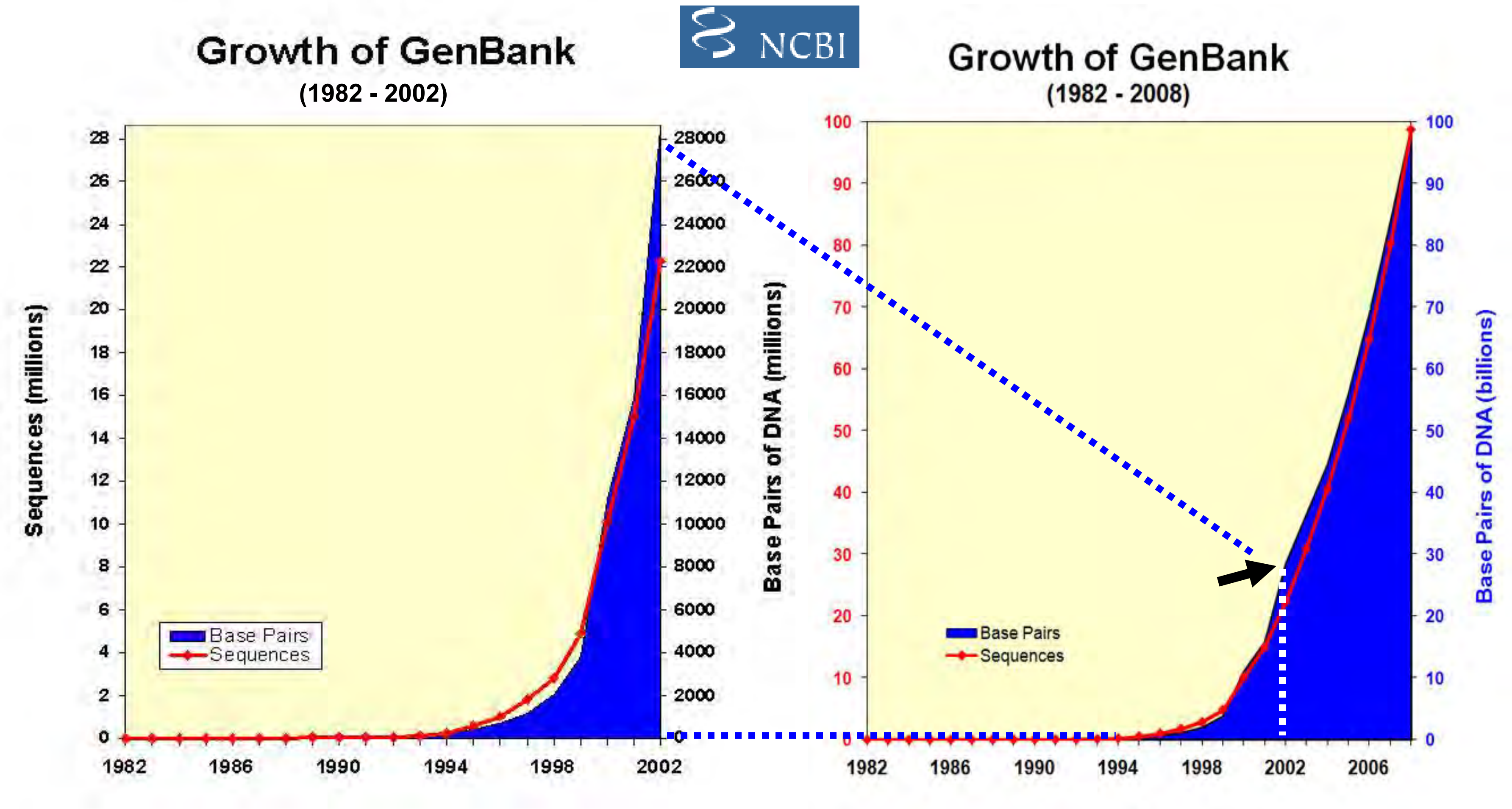


http://vega.sanger.ac.uk/Homo_sapiens/index.html

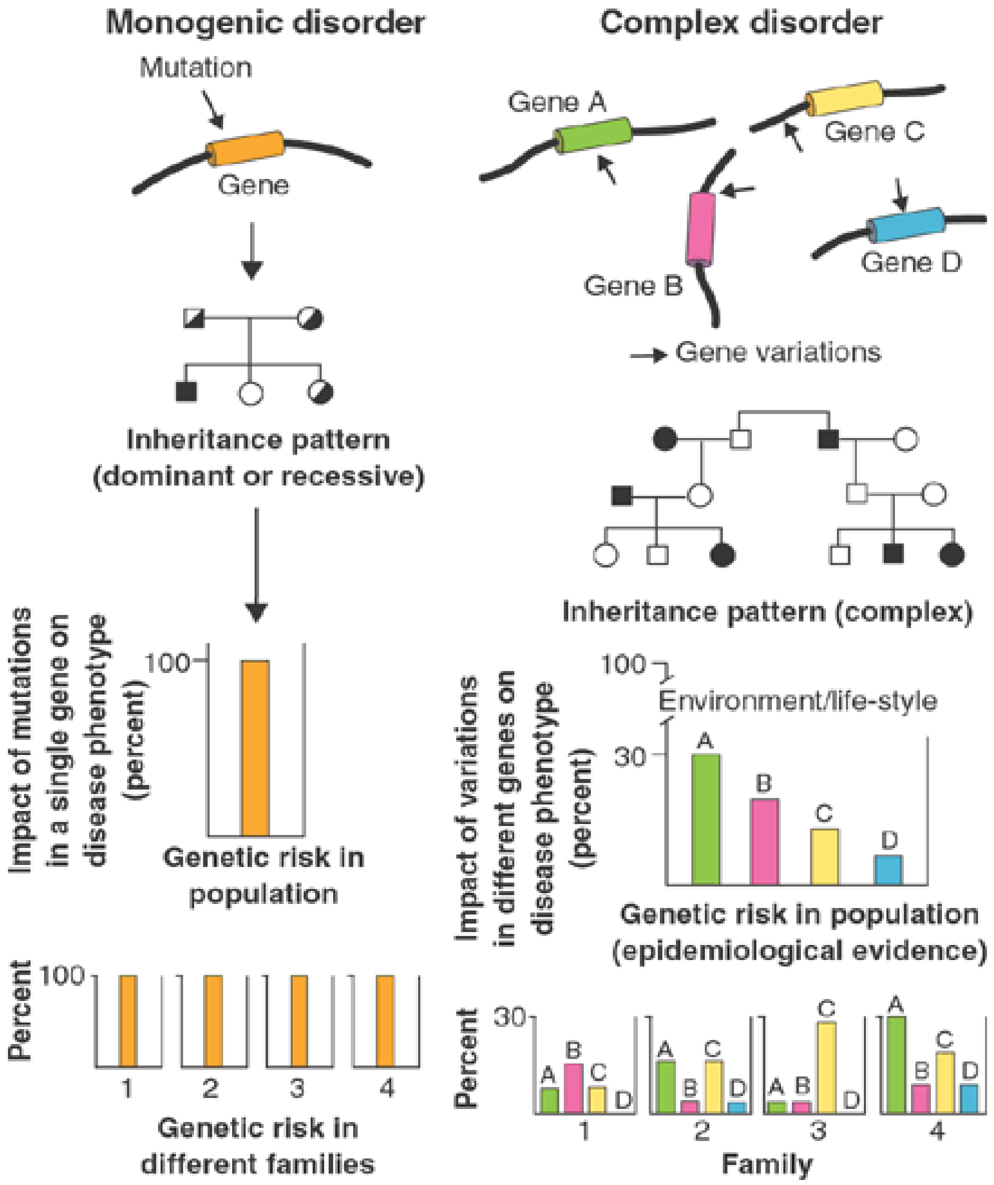
Genes, genomas y genómica



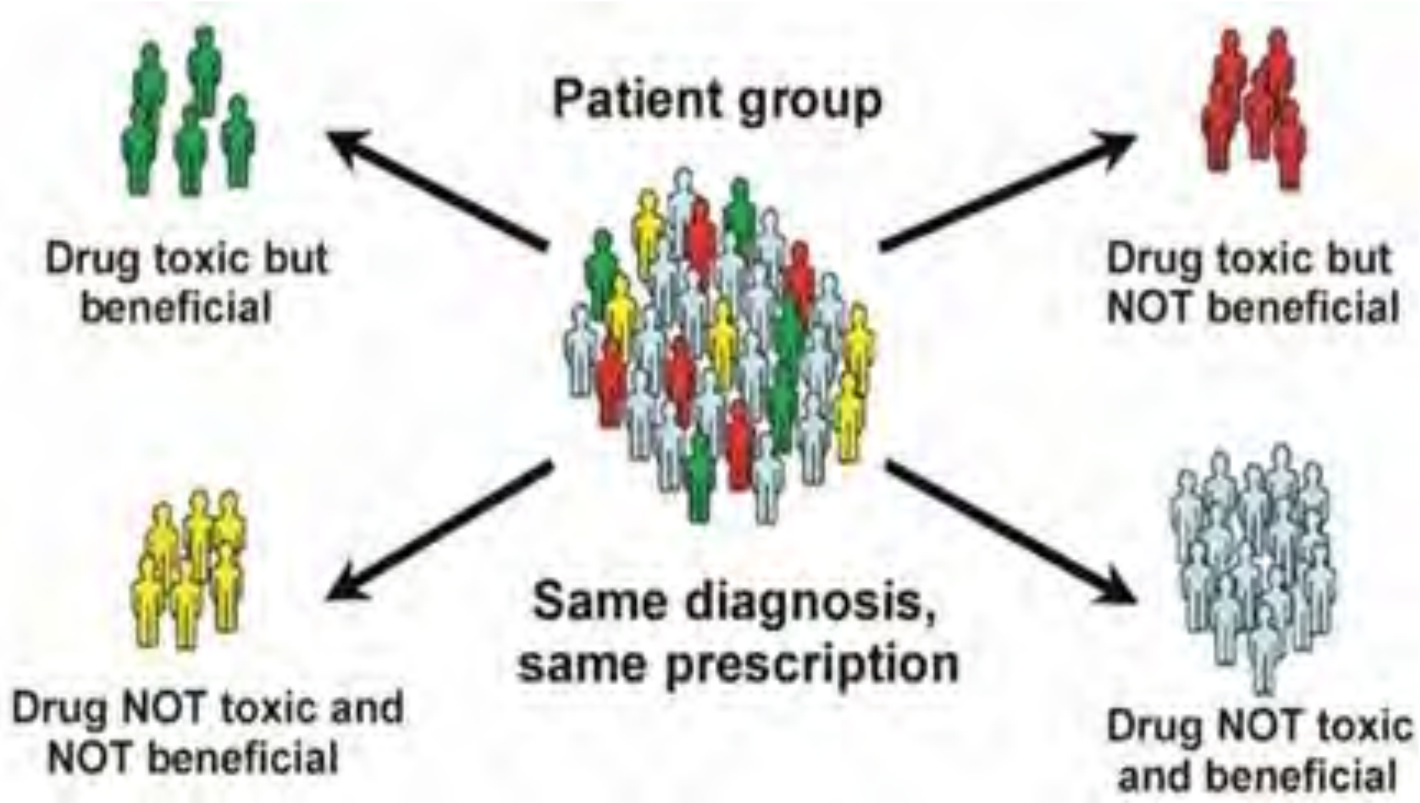
Genes, genomas y genómica



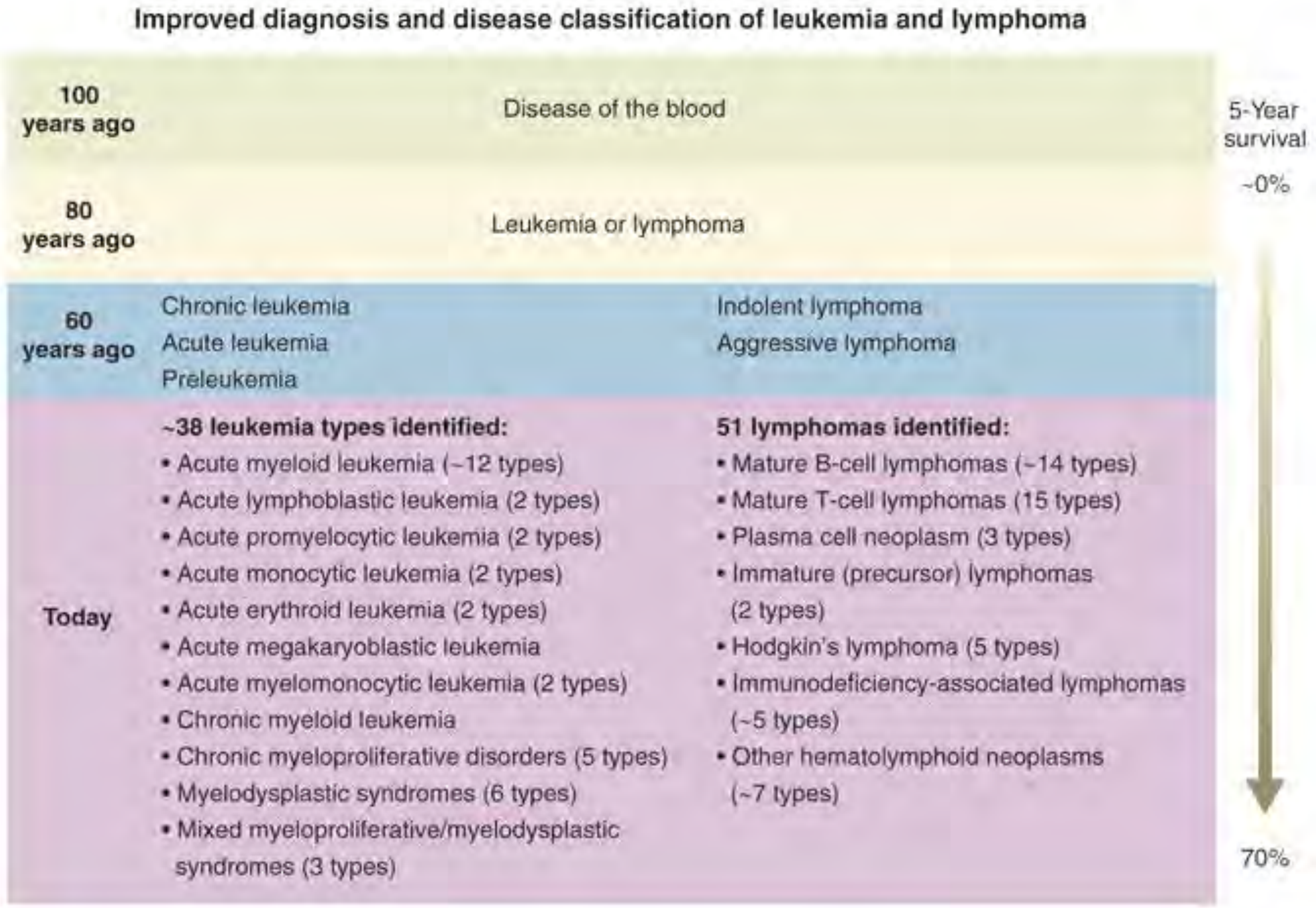
Genes, genomas y genómica



Medicina personalizada



Genes, genomas y genómica

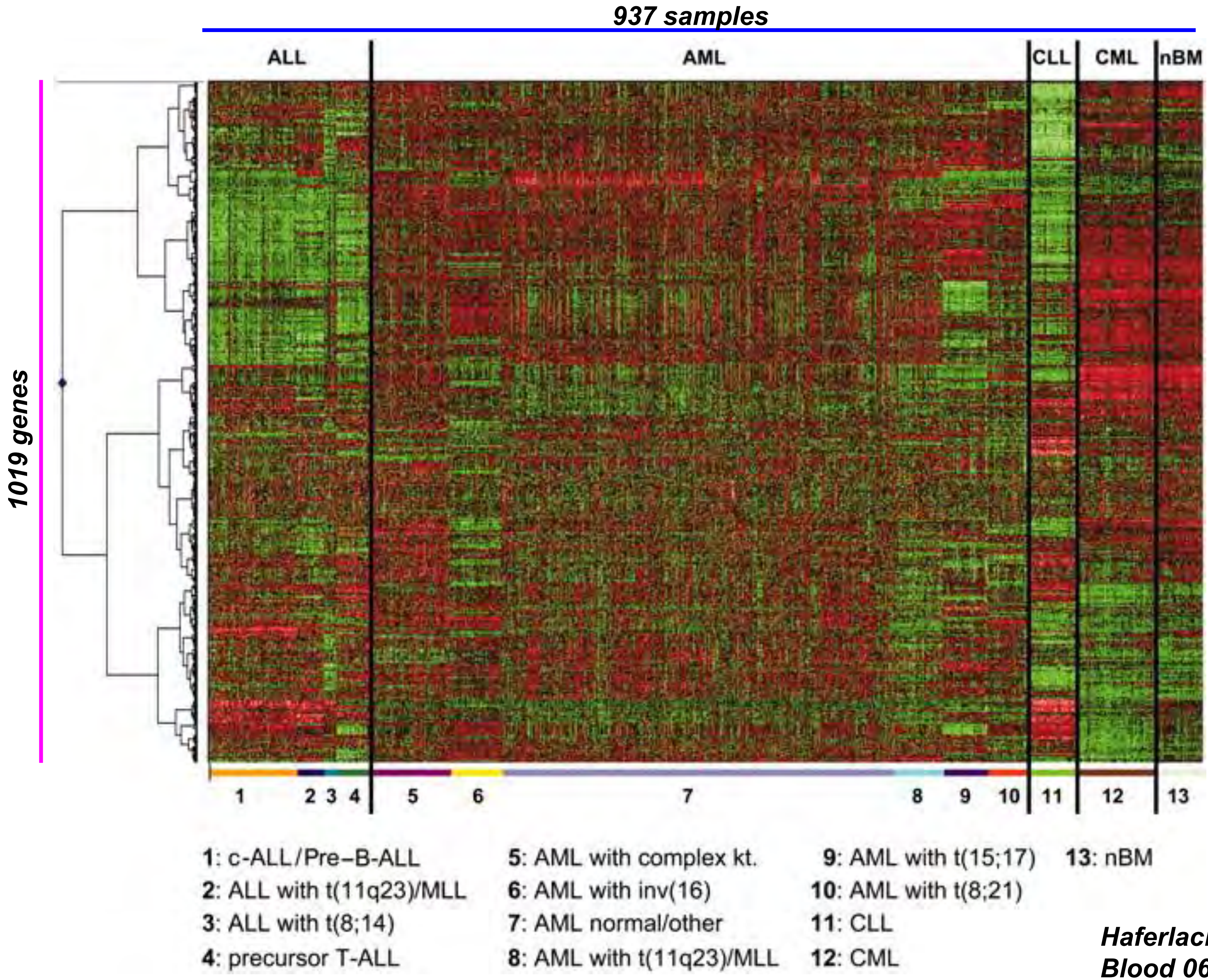


Improved diagnosis and disease classification of leukemia and lymphoma

100 years ago	Disease of the blood		5-Year survival ~0%
80 years ago	Leukemia or lymphoma		
60 years ago	Chronic leukemia Acute leukemia Preleukemia	Indolent lymphoma Aggressive lymphoma	
Today	**~38 leukemia types identified:** • Acute myeloid leukemia (~12 types) • Acute lymphoblastic leukemia (2 types) • Acute promyelocytic leukemia (2 types) • Acute monocytic leukemia (2 types) • Acute erythroid leukemia (2 types) • Acute megakaryoblastic leukemia • Acute myelomonocytic leukemia (2 types) • Chronic myeloid leukemia • Chronic myeloproliferative disorders (5 types) • Myelodysplastic syndromes (6 types) • Mixed myeloproliferative/myelodysplastic syndromes (3 types)	**51 lymphomas identified:** • Mature B-cell lymphomas (~14 types) • Mature T-cell lymphomas (15 types) • Plasma cell neoplasm (3 types) • Immature (precursor) lymphomas (2 types) • Hodgkin's lymphoma (5 types) • Immunodeficiency-associated lymphomas (~5 types) • Other hematolymphoid neoplasms (~7 types)	70%

Allison M (2008) Nat Biotechnol;26(5):509-17

PEG en leucemias

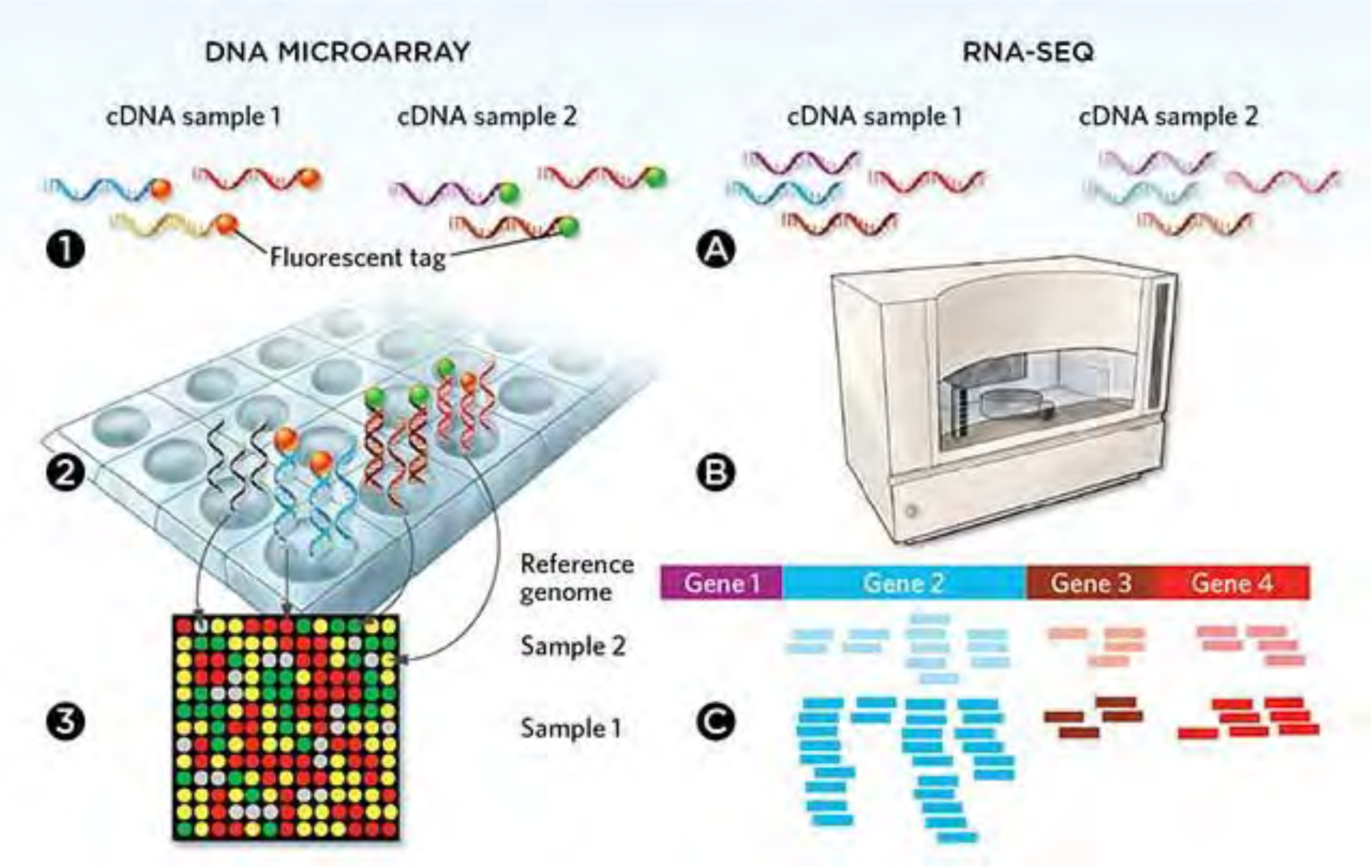


Haferlach et al (2005)
Blood 06(4):1189-98 .

Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

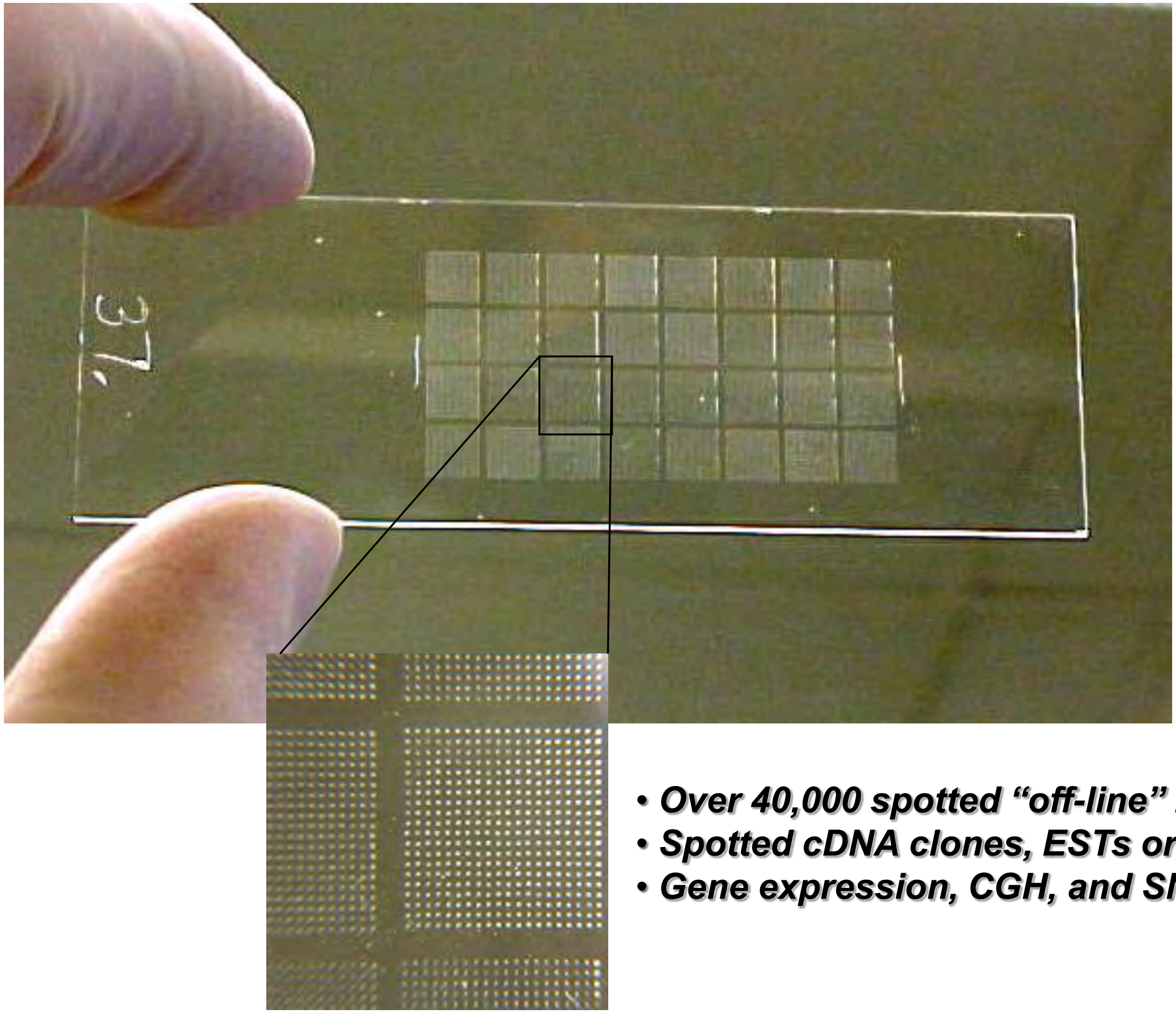
Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

Microarreglos vs RNAseq



https://www.the-scientist.com

Glass slide microarrays



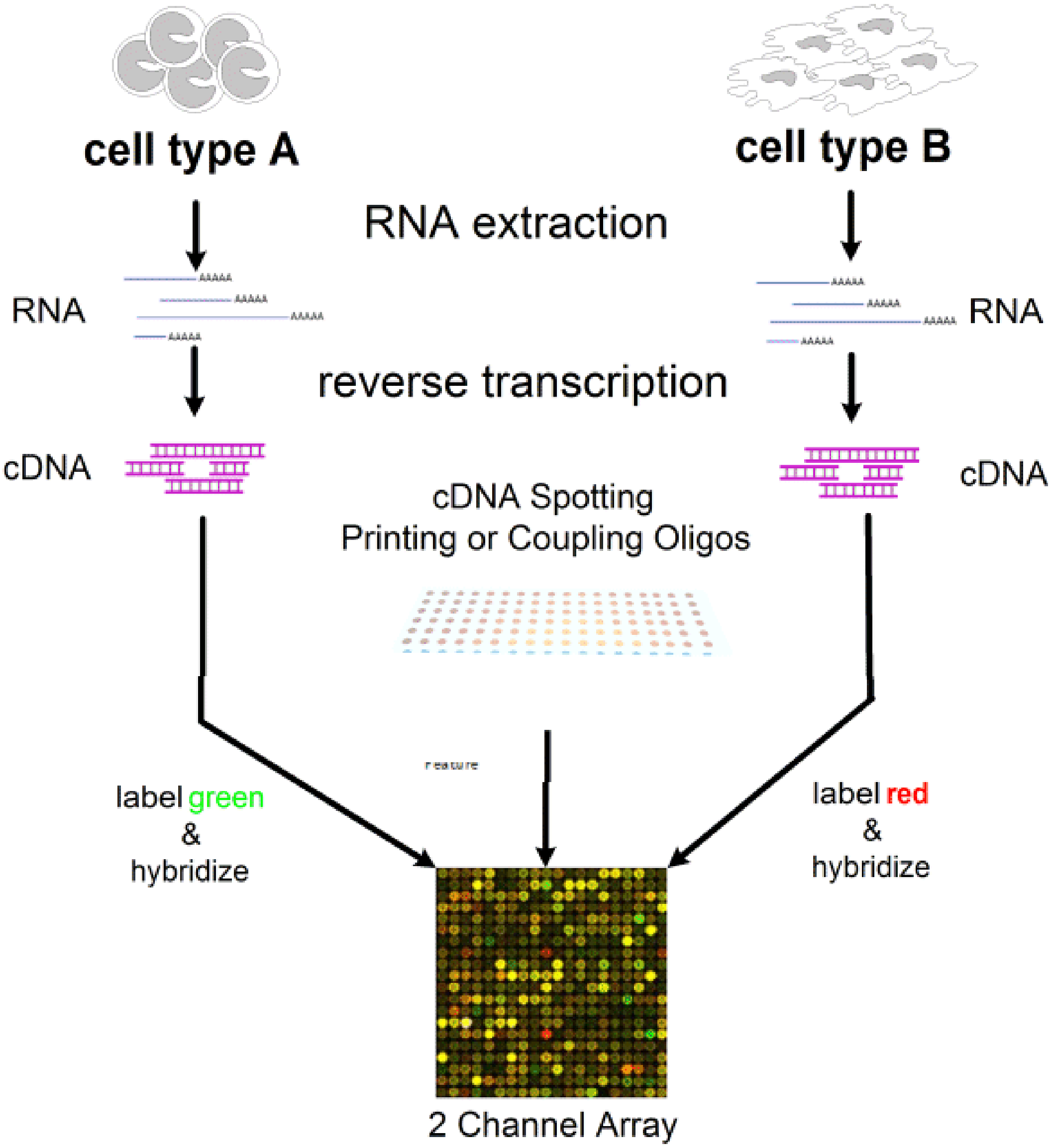
- ***Over 40,000 spotted "off-line" DNA probes***
- ***Spotted cDNA clones, ESTs or oligos***
- ***Gene expression, CGH, and SNPs***

Microarreglos de DNA

- ***Microarray**: ordered arrangement of known DNA sequences on a solid-planar substrate which allows the hybridization binding of labeled sample RNAs or DNAs.*
- A single microarray contains from few hundreds to 400.000 microscopic elements of uniform size and spacing.
- Immobilized DNAs are ***oligonucleotides*** (20-80 mer), ***cDNAs*** (0.5-5 Kb) or ***BAC*** clones (10-50 Kb). Substrates are rigid, thermostable, optically flat surfaces like nylon, glass or silica.
- DNAs are spotted onto chemically modified substrates and then immobilized using UV. Oligonucleotides can be either spotted or synthesized *in situ*.

Microarreglos de DNA

A comparative hybridization experiment



cell type A

cell type B

RNA extraction

RNA

RNA

reverse transcription

cDNA

cDNA

cDNA Spotting
Printing or Coupling Oligos

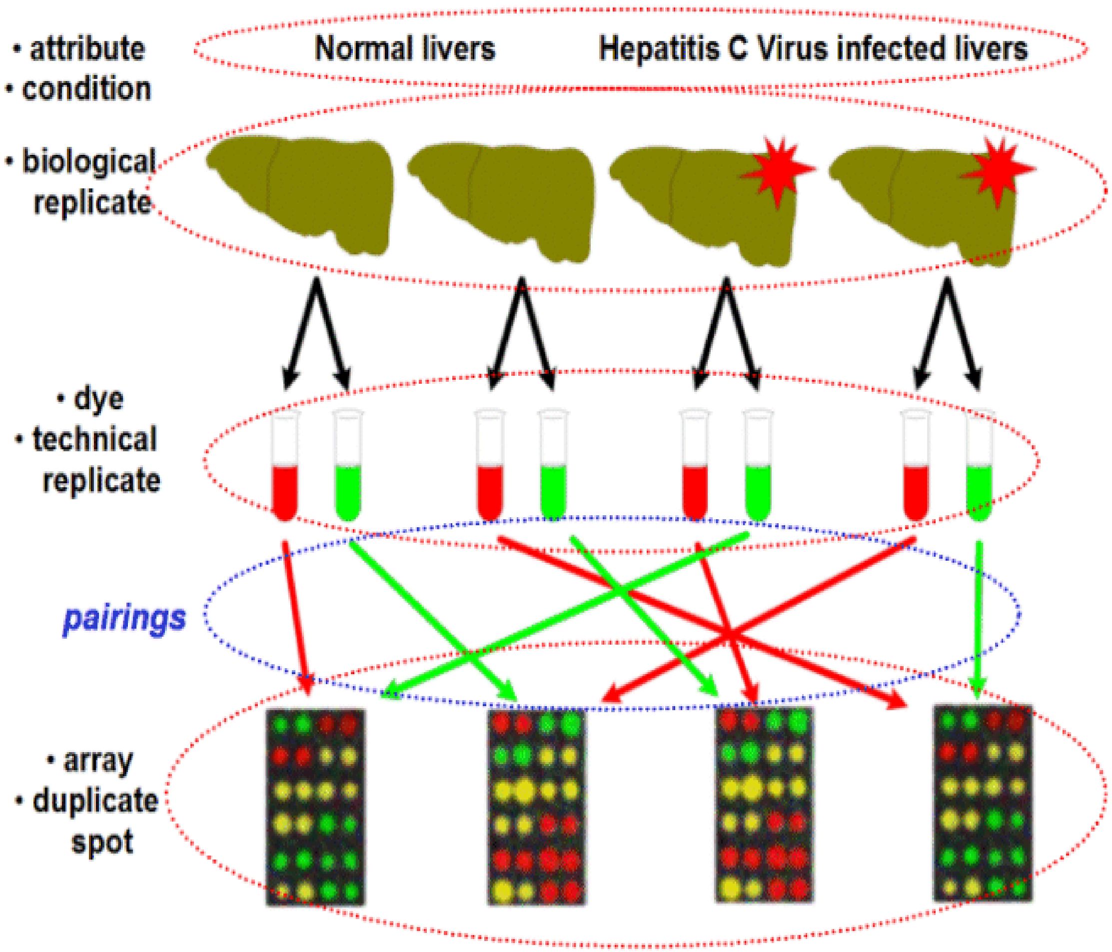
reature

label green
&
hybridize

label red
&
hybridize

2 Channel Array

Experimental design and variability



Sources of variability:

- Due to ***attributes*** or ***conditions***

- ***Biological variation*** is intrinsic; influenced by genetic & environmental factors, as well as whether samples are from populations or individuals

- Due to ***technical issues,*** results during sample extraction (***quality***), labeling and hybridization

- Due to ***fluorophore stability*** during laser scanning and fluorescence detection

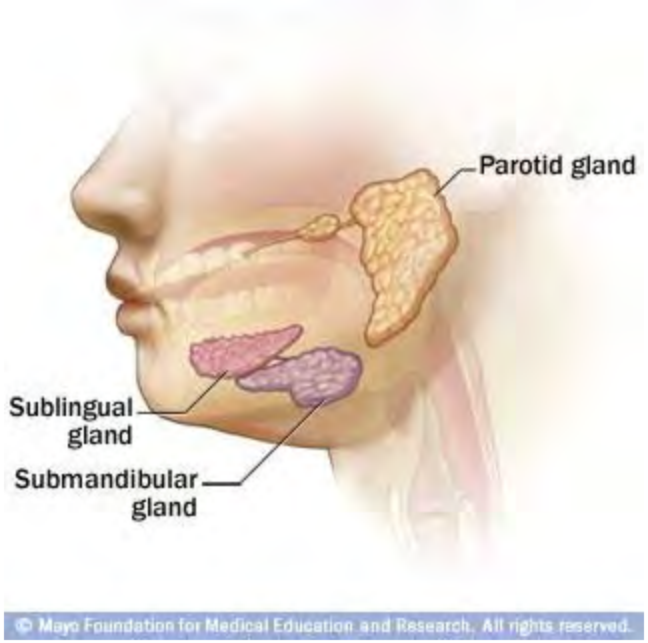
Ejemplo 1

Transcriptome of Sjögren syndrome

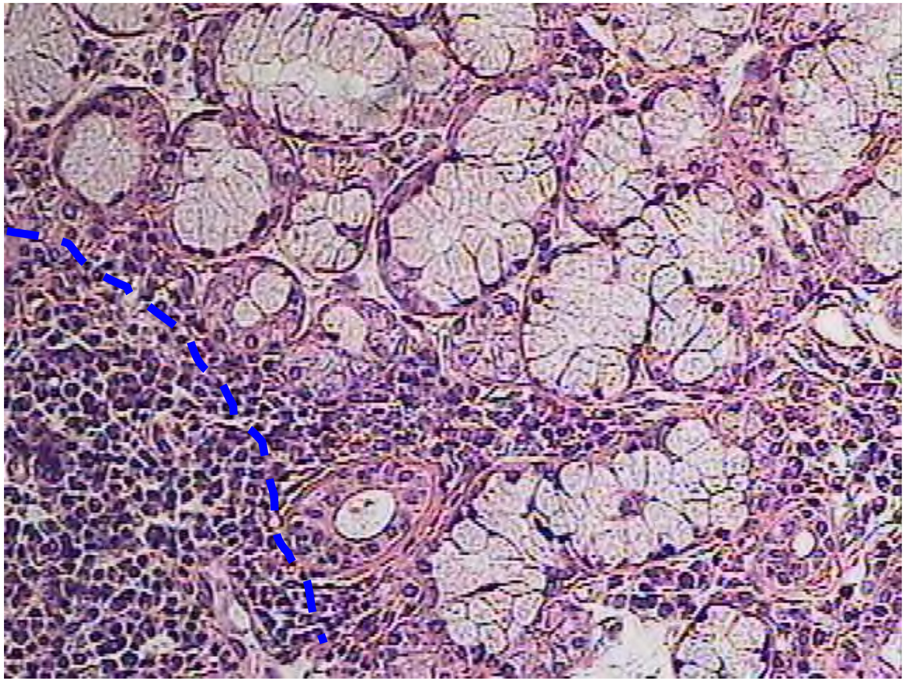
Julieta González - ICBM

Paola Pérez – NIDCR/ NIH

Focal lip sialadenitis in Sjogren`s syndrome

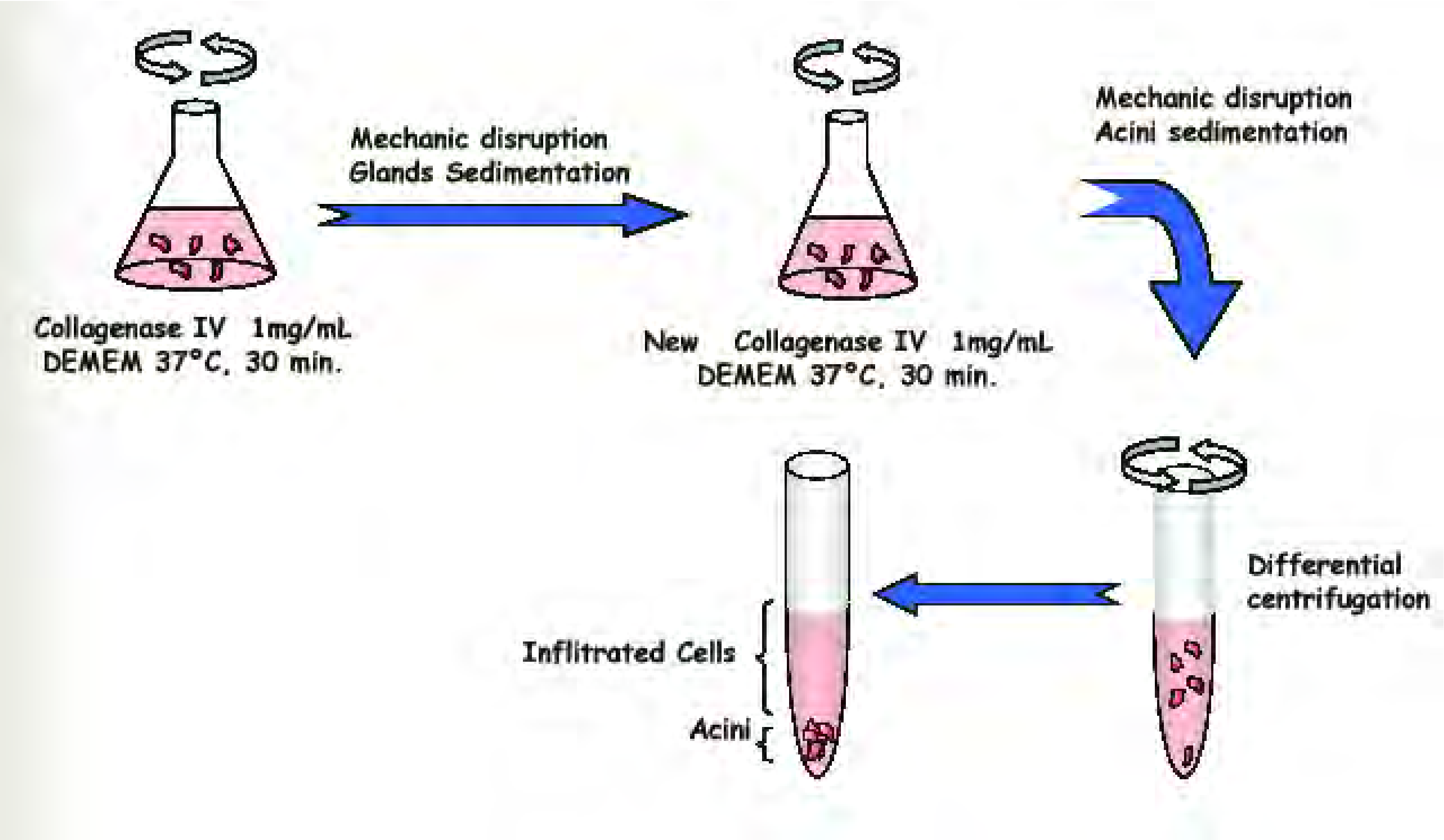


© Mayo Foundation for Medical Education and Research. All rights reserved.



Focus: clusters of > 50 lymphocytes
Focus score: number of focus/40mm^2 glandular tissue (0 → 4)

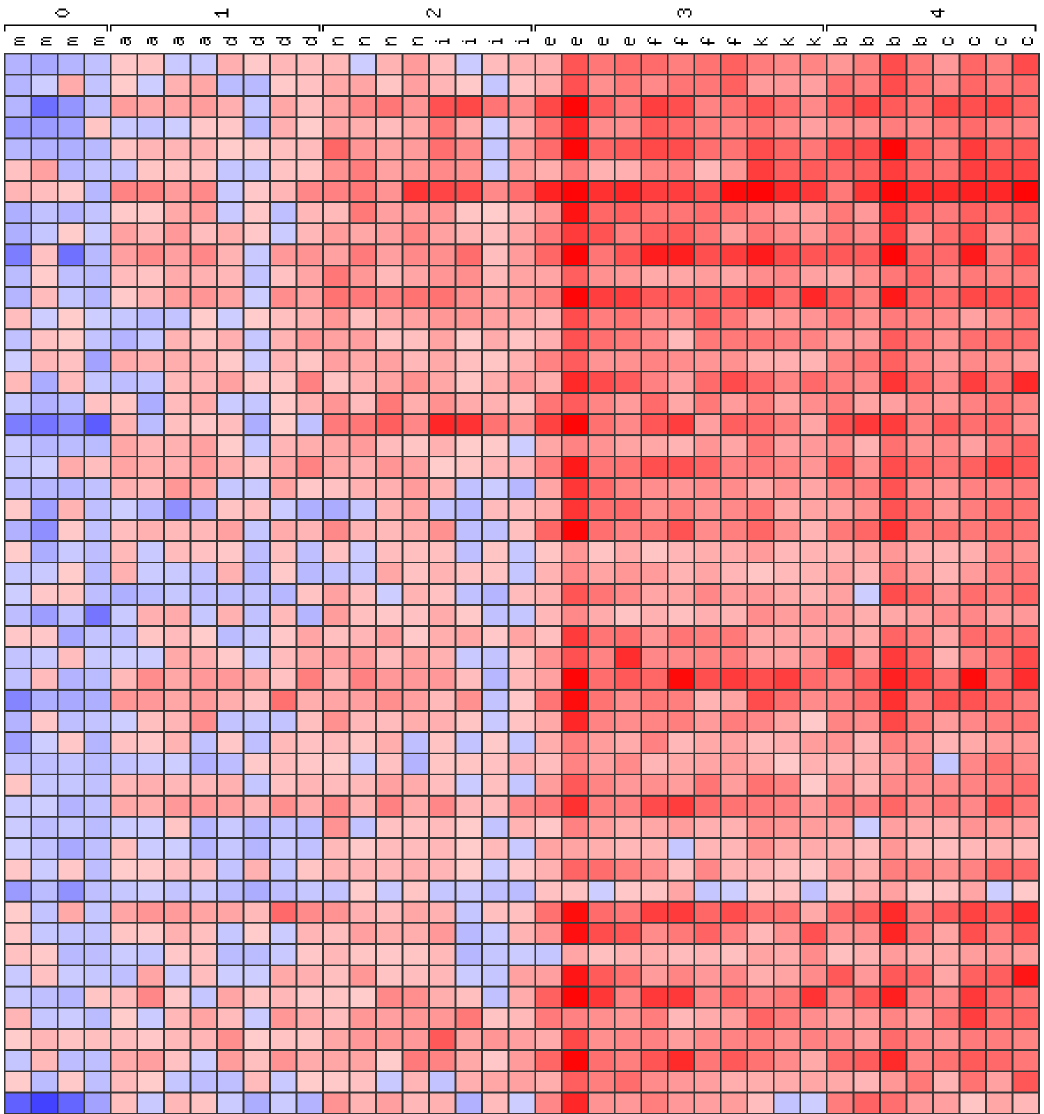
Isolation of epithelial cells



POMELO II
(THE RETURN OF THE POMELO)

http://pomelo2.iib.uam.es/

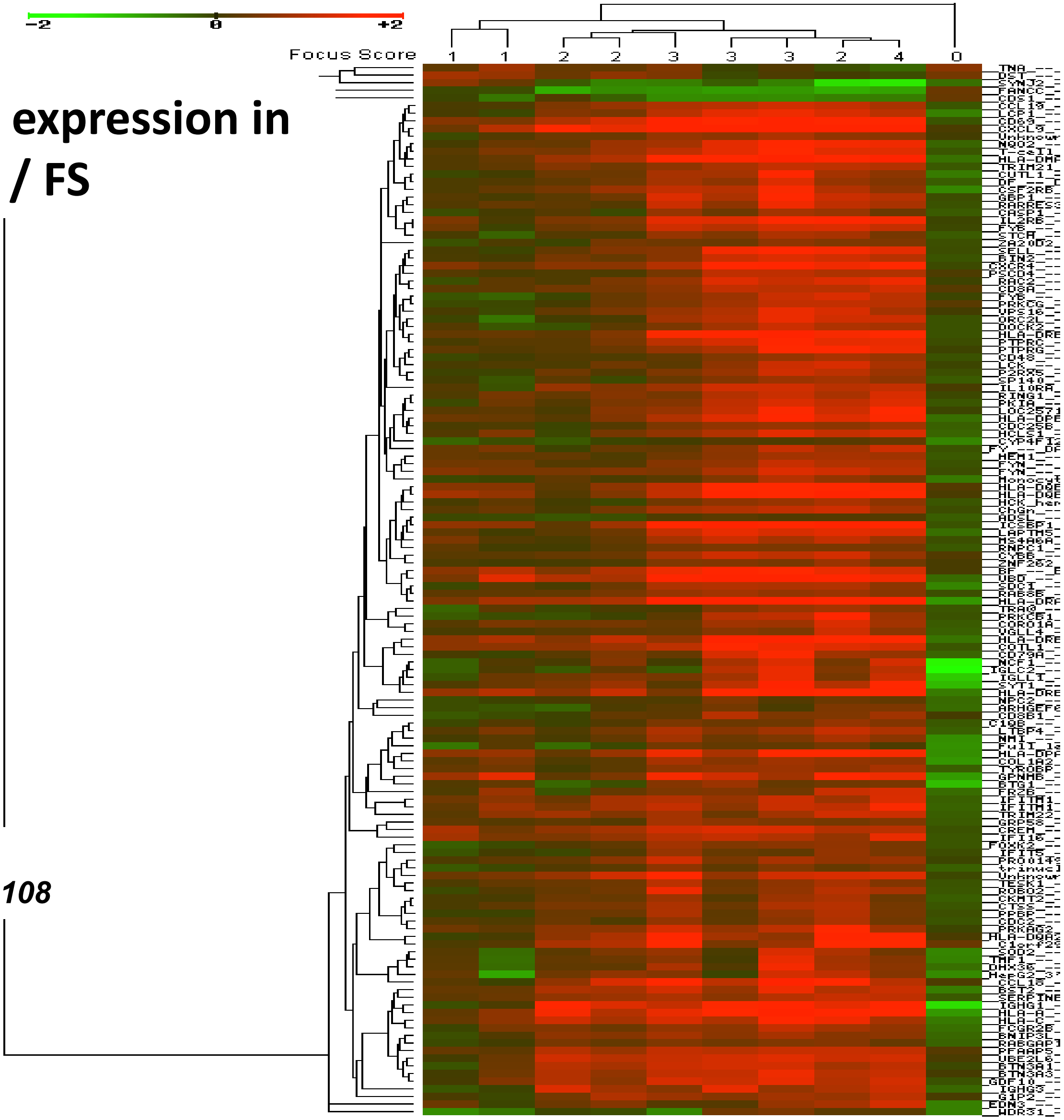
10K cDNA microarray 2-channels



Adj. p-value	FDR indep.	
0	0.000245706	IMAGE_753313_
0	0.000245706	IMAGE_827132_
0	0.000245706	IMAGE_853906_
0	0.000245706	IMAGE_344589_
0	0.000245706	IMAGE_183337_
0	0.000245706	IMAGE_80109__
0	0.000245706	IMAGE_503617_
0	0.000245706	IMAGE_824024_
5e-06	0.000245706	IMAGE_306841_
5e-06	0.000245706	IMAGE_153411_
5e-06	0.000245706	IMAGE_154482_
5e-06	0.000245706	IMAGE_704459_
5e-06	0.000245706	IMAGE_757440_
5e-06	0.000245706	IMAGE_194965_
5e-06	0.000245706	IMAGE_773246_
1e-05	0.000245706	IMAGE_489079_
1e-05	0.000245706	IMAGE_430465_
1e-05	0.000245706	IMAGE_855745_
1e-05	0.000245706	IMAGE_491460_
3e-05	0.000245706	IMAGE_854444_
3.5e-05	0.000245706	IMAGE_767183_
6e-05	0.000245706	IMAGE_380263_
6e-05	0.000245706	IMAGE_840942_
7.5e-05	0.000245706	IMAGE_85128__
7.5e-05	0.000245706	IMAGE_125134_
7.5e-05	0.000245706	IMAGE_293325_
7.5e-05	0.000245706	IMAGE_704532_
7.5e-05	0.000245706	IMAGE_128150_
0.00011	0.000245706	IMAGE_206544_
0.00011	0.000245706	IMAGE_290230_
0.00011	0.000245706	IMAGE_868332_
0.00011	0.000245706	IMAGE_712378_
0.00013	0.000245706	IMAGE_786067_
0.00013	0.000245706	IMAGE_229723_
0.000135	0.000245706	IMAGE_487151_
0.000135	0.000245706	IMAGE_144849_
0.000155	0.000245706	IMAGE_40781__
0.000175	0.000245706	IMAGE_246722_
0.000215	0.000245706	IMAGE_730410_
0.000225	0.000245706	IMAGE_129446_
0.00023	0.000245706	IMAGE_809598_
0.000285	0.000245706	IMAGE_229365_
0.00029	0.000245706	IMAGE_310105_
0.00029	0.000245706	IMAGE_137531_
0.000305	0.000245706	IMAGE_417711_
0.00032	0.000245706	IMAGE_210646_
0.0004	0.000245706	IMAGE_415529_
0.000405	0.000245706	IMAGE_79629__
0.00041	0.000245706	IMAGE_486678_
0.000415	0.000245706	IMAGE_824340_

Perfiles de expresión génica

Epithelial gene expression in salivary glands / FS



Pérez et al, 2009 –
J Autoimmun 33(2):99-108

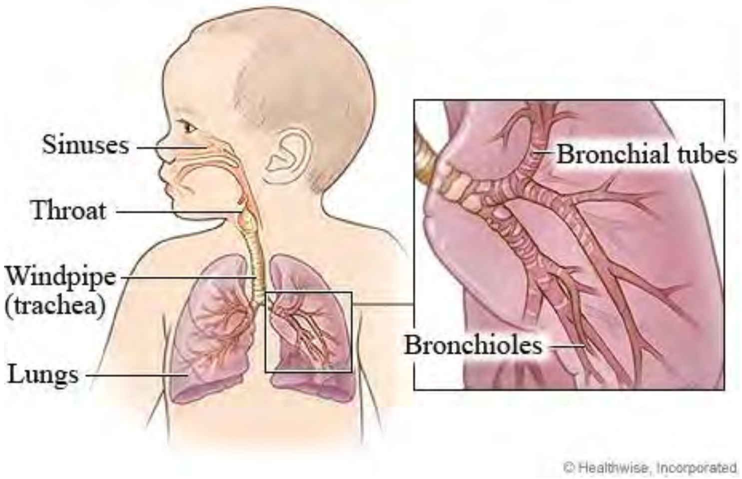
Ejemplo 2

Genomic response to RSV infection

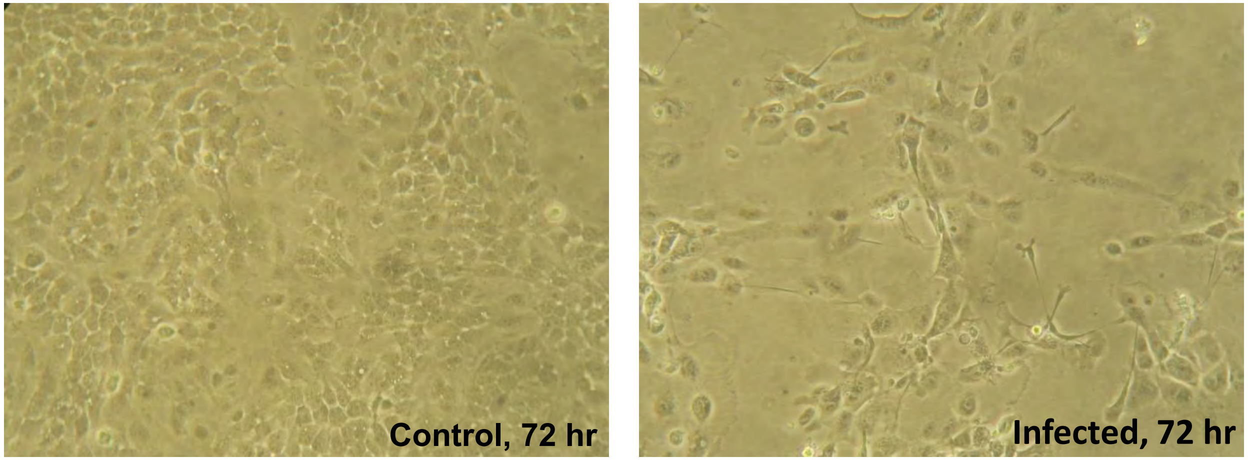
Sandra Ampuero – ICBM

Carmen Larrañaga – ICBM

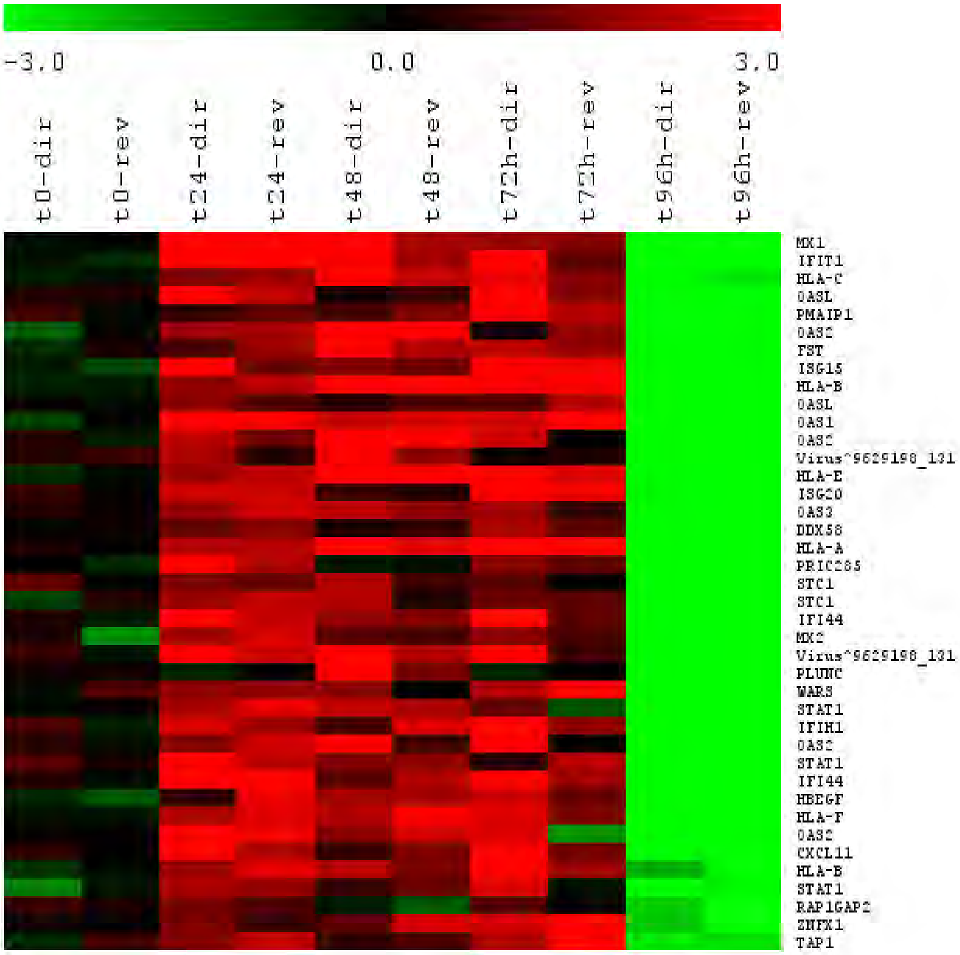
Respiratory syncytial virus (RSV)



NCI-H292 cells



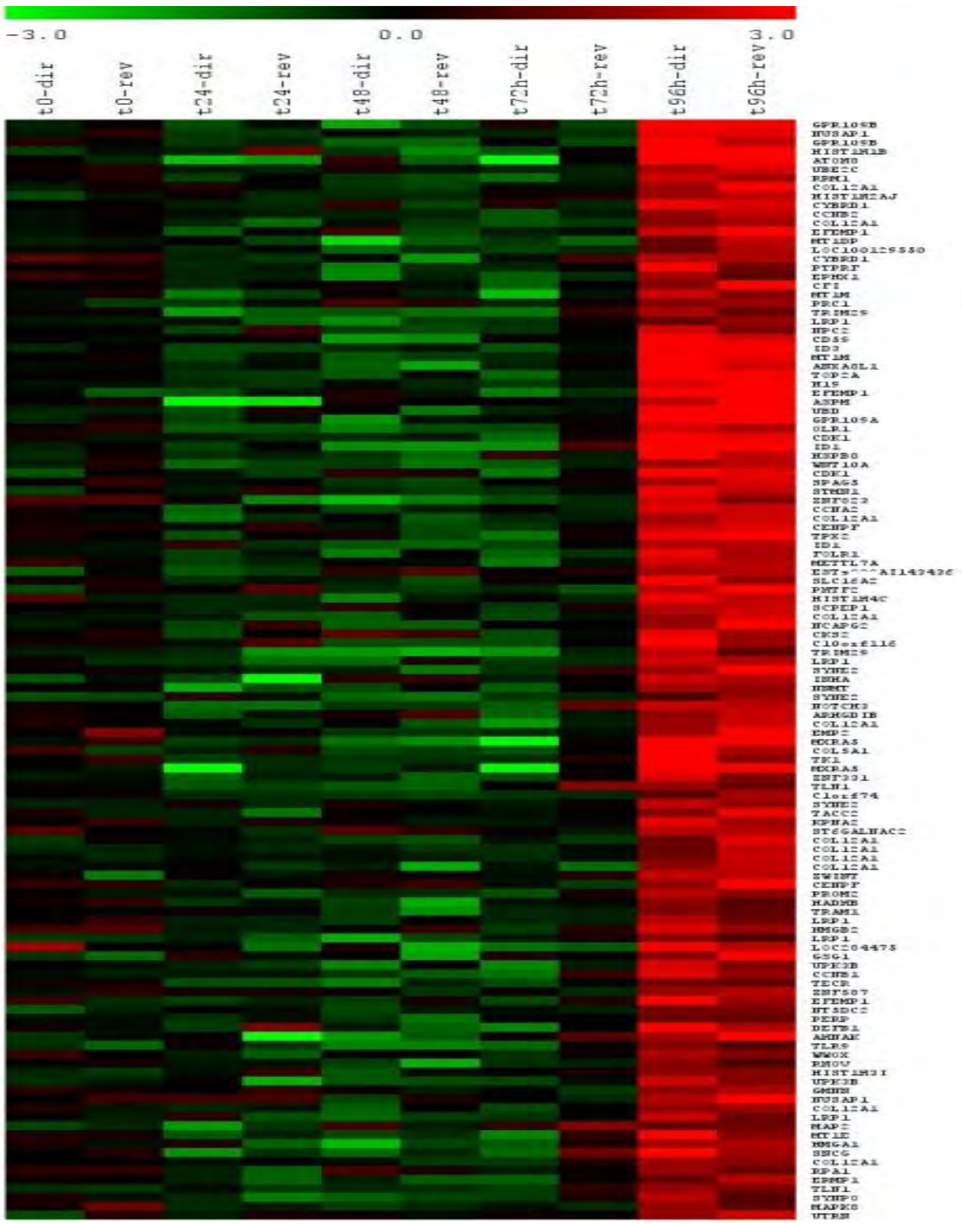
In vitro RSV infection



Activación inicial e inhibición tardía

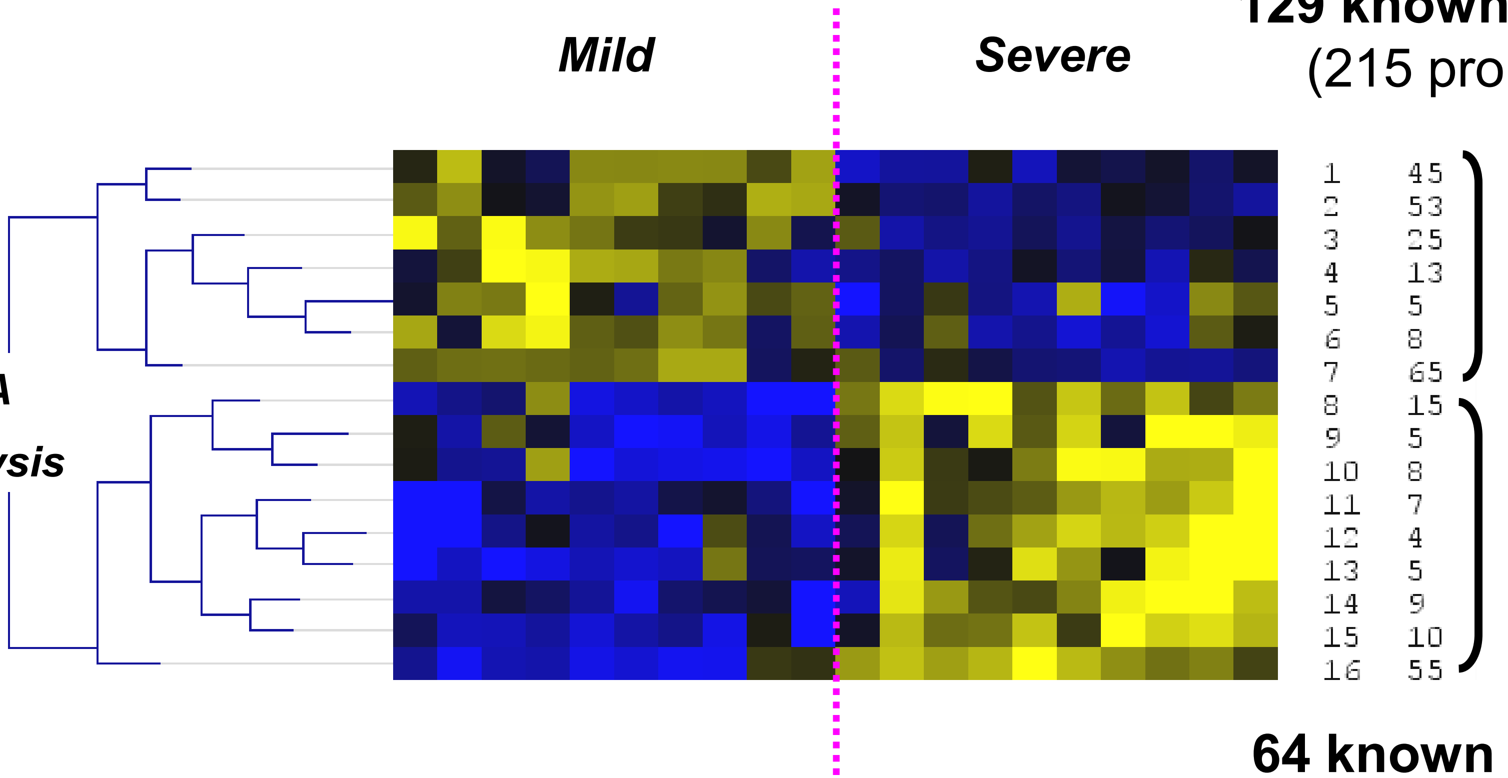
Celulas NCI-H292 epiteliales mucoepidermoides

In vitro RSV infection



Inhibición inicial y activación tardía

Severe and mild infection in RSV affected infants - Blood



129 known genes
(215 probes)

SOTA analysis

1	45
2	53
3	25
4	13
5	5
6	8
7	65
8	15
9	5
10	8
11	7
12	4
13	5
14	9
15	10
16	55

64 known genes
(121 probes)

Whole blood RNA → RNA amplification

↓

HEEBO 48k oligo-microarrays → Significant genes p<0.001

Functional profile of genes in severe and mild RSV infection

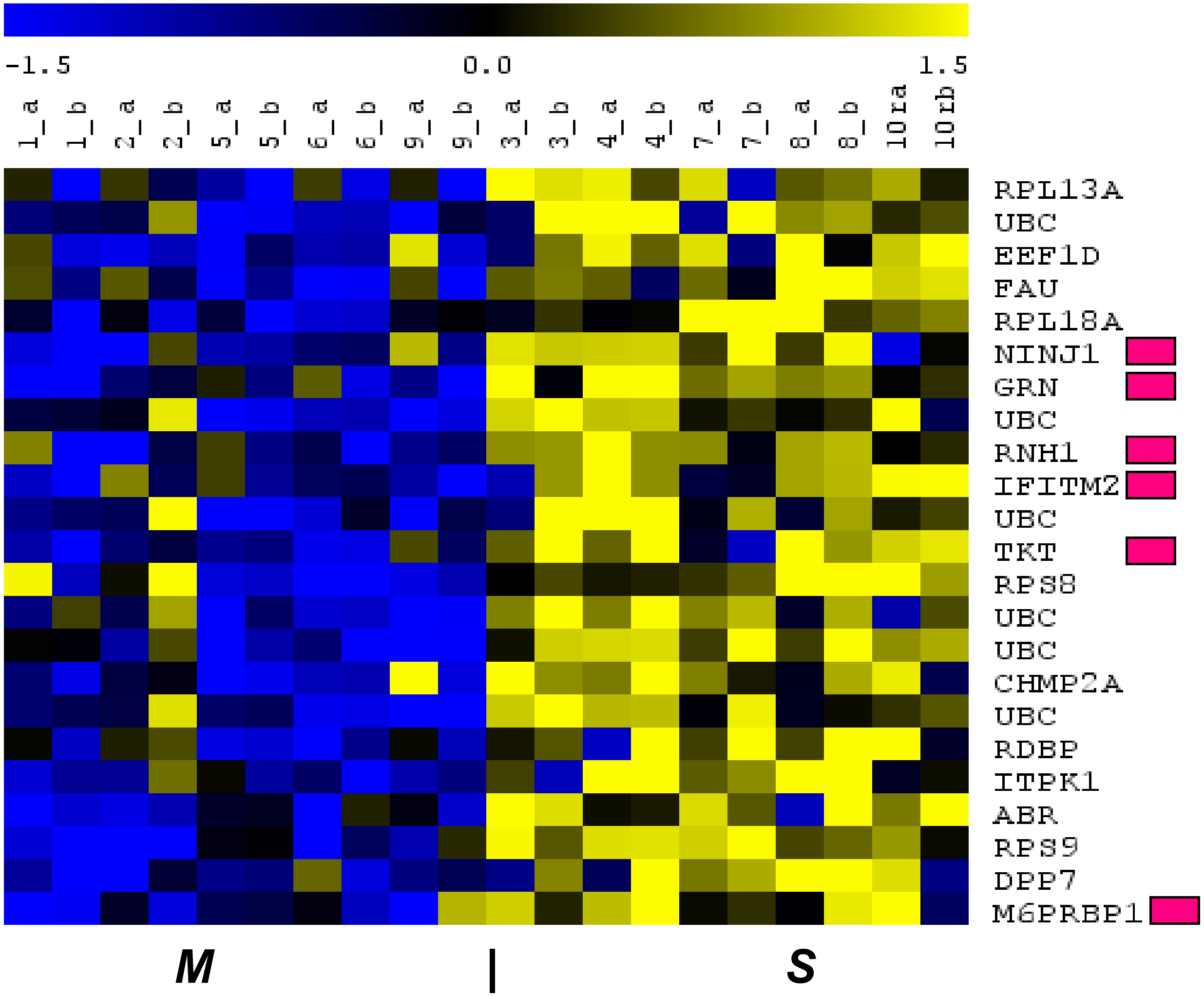
129 genes ⬇

GO term	*Genes*	*P*
Cell cycle	ANAPC2, BIN1, CCNG2, CCPG1, CDC27, CDC42, CDKN1C, GAS7, PIM2, TGFB1, RBL2, RASAL3, RASS5F5, INCENP	1.51e-4
Immune response	FCN2, IL16, IGHG1, PTPRC, TNFRSF14, TCF7, TGFB1, IL1RN, IFI6, TUBB	8.71e-3
Lymphocyte activation	LAT , PTPRC, TGFB1	2.44e-3
G1 phase	CDKN1C, ANAPC2, CDC42	5.85e-4
DAG binding	GMIP, PRKCH, RASS5F5	8.18e-3

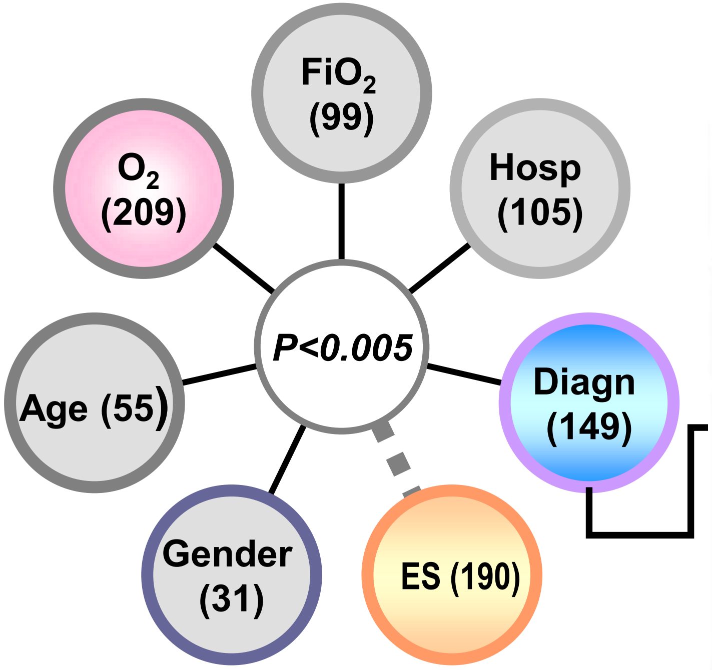
64 genes ⬆

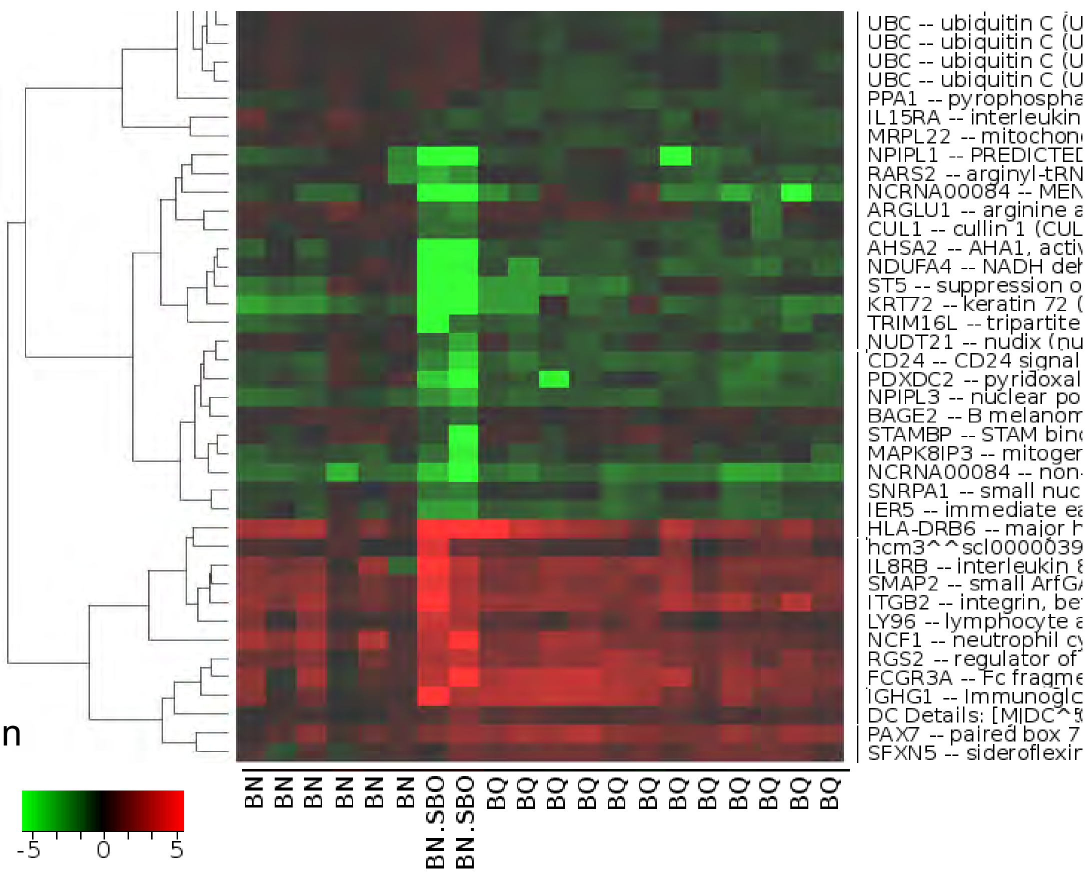
GO term	*Genes*	*P*
Protein synthesis	EEF1D, FAU, MRPS16, RPL13A, RPL18A, RPL35, RPS8, RPS9	4.03e-3
Carbohydrate metabolism	ALDH2, ENO1, GAPDH, TKT, CHID1, GAPDHL18, MANBA	6.52e-3
Notch signaling pathway	APP, DLL1	8.17e-3

Genes overexpressed in infants severely infected by RSV



Blood gene expression - clinical RSV parameters





- O_2 = days under oxygen supplementation
- FiO_2 = fraction of inspired oxygen
- Hosp = days hospitalized
- Diagn = diagnosis (BN, BN+SBO, BQ)
- ES = evolution score (hosp x O_2 x FiO_2)

Functional profile of genes associated to diagnosis

GO term	*Genes*	*P*
Cell proliferation	CAPN1, CKLF, CUL1, FGF18, FRAT2, IL8RB, IL15RA, ISG20, PTEN, STAMBP	5.16e-4
Immune system process	AP2A2, CD14, CD24, CKLF, CORO1A, FCGR3A, IGHG1, IGKC, IL8RB, ITGB2, LY96, MAP3K7, NCF1	2.32e-2
Cell matrix adhesion	ITGB2, PTEN, TNXB	4.10e-3
Leukocyte migration	CKLF, CD24, CORO1A, IL8RB, ITGB2	9.61e-5
Neutrophil chemotaxis	ITGB2, CKLF, IL8RB	5.14e-6
Cellular defense response	NCF1, LY96, IL8RB	5.85e-4
Growth factor activity	FGF18, GMFG, SDCBP, LTBP4	9.79e-3
Cytokine binding	IL8RB, IL15RA, LTBP4	8.88e-3

Ejemplo 3

Wine yeast genomics

Claudio Martinez – CECTA – USACH

Ma Angelica Ganga – CECTA – USACH

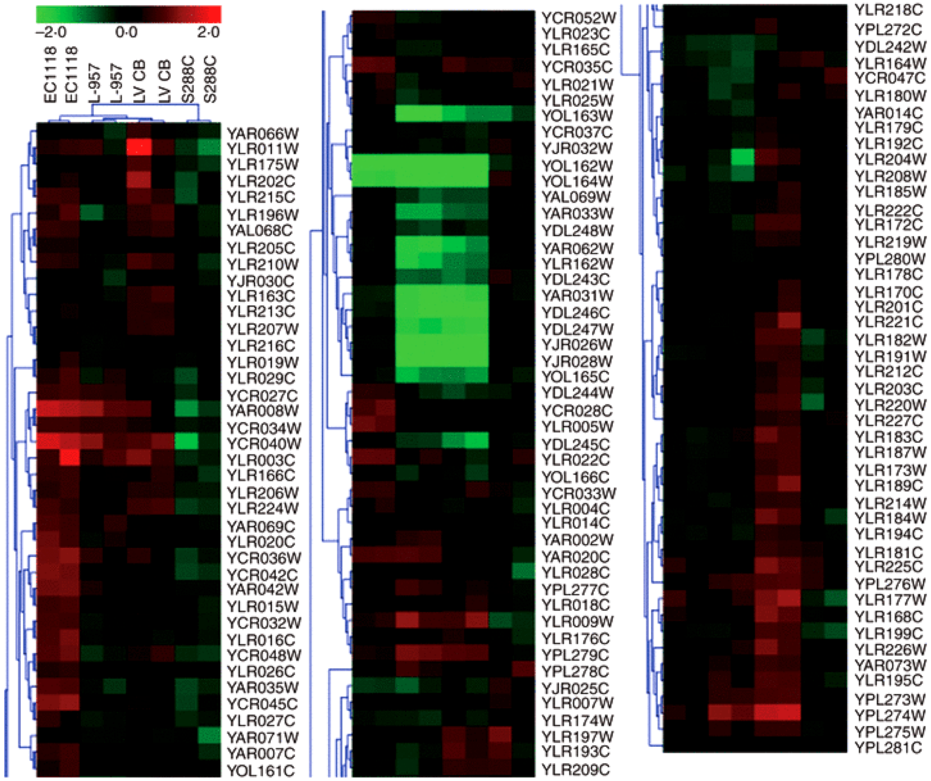
Yeast genome may undergo genomic changes when exposed to the environment

S288C standard lab strain

EC1118 = commercial strain

L-1333 = Casablanca strain

L-957 = Mendoza strain



EC1118	amplif Chr I, III, XII
L-1333	del Chr IV, X, XV
L-957	amplif Chr XVI

Salinas et al; J Appl Microbiol. 2010 May;108(5):1850-8

Yeast genes involved in nitrogen metabolism

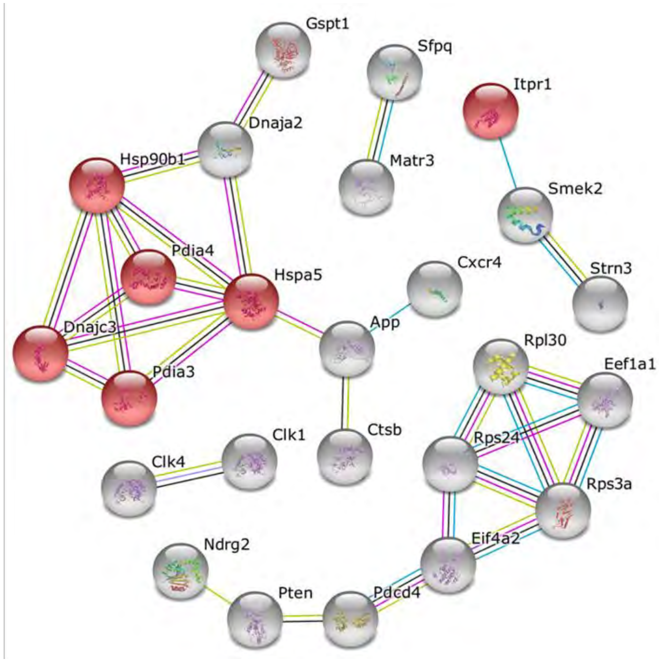
Gene	*ORF*	*Description*	*Highest mRNA level*	*FC (microarray)*	*FC (QPCR)*
ATO2	YNR002C	Putatively involved in ammonium transport	AC19	1.46	2.64
ICY1	YMR195W	Unknown function	AC19	1.5	3.03
ZRT1	YGL255W	Involved in Zn transport	AC19	3.72	4.72
CMP2	YML057W	Isoform of calcineurin A	AC114	1.43	1.66
NHA1	YLR138W	Na+/K+ pump	AC114	2.03	1.25
RDL1	YOR285W	Unknown funtion	AC114	2.52	1.87
VPS8	YAL002W	Vacuolar localization	AC114	3.25	2.6
ARR3	YPR201W	Arsenite transporter	AC115	5.73	3.08
ARR1	YPR199C	Transcriptional factor	AC115	1.57	3.24
RDL1	YOR285W	Unknown funtion	AC115	1.59	3.04
TVP23	YDR084C	Integral membrane protein of Golgi	AC115	9.51	2.02

AC19 vs AC114 – N from NH4

AC19 vs AC115 – N from aa´s

Contreras et al. W J Microbiol Biotechnol. 2012 Mar;28(3):1107-13

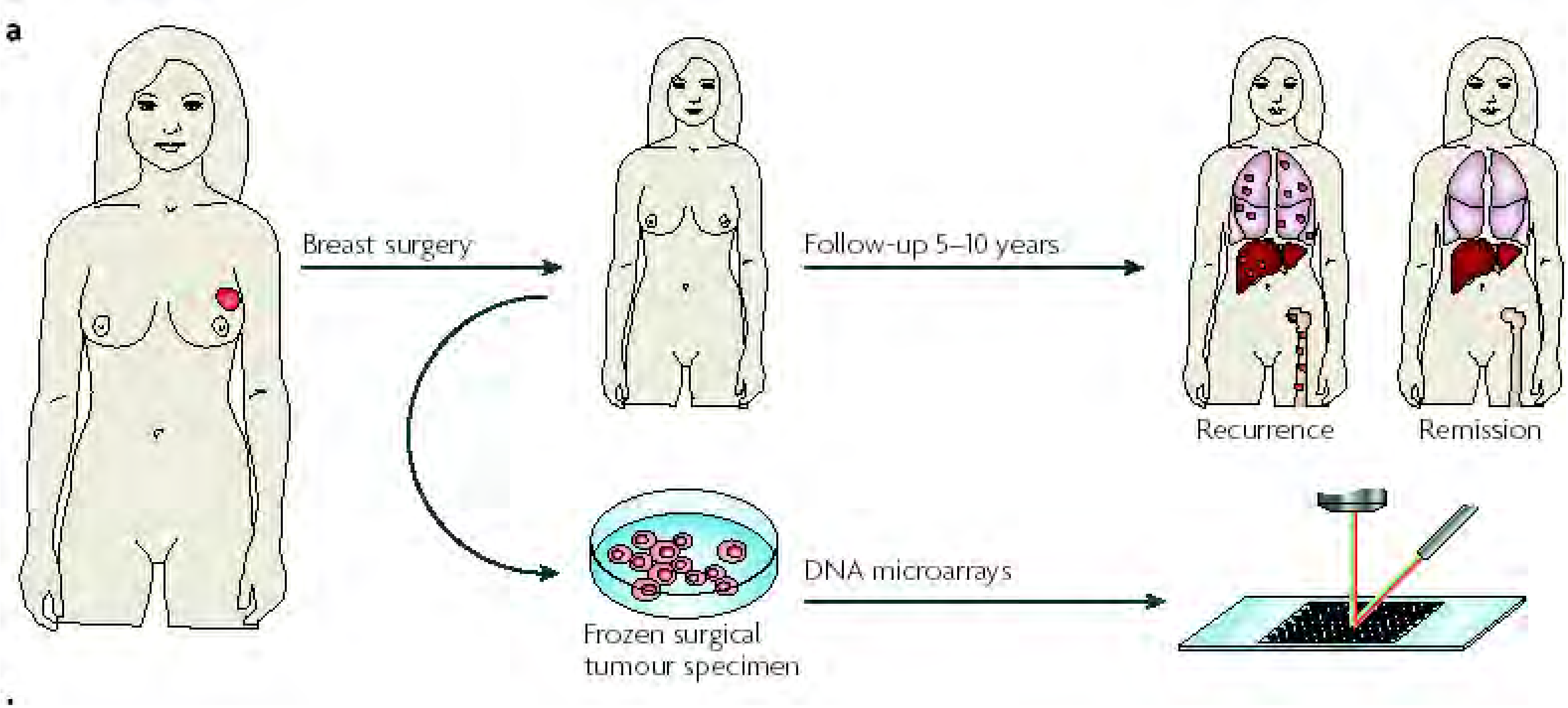
Estudios genómicos en cáncer

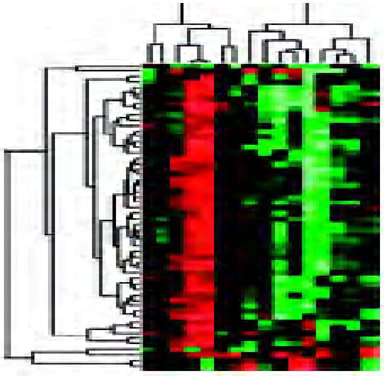


Prof. Ulises Urzúa
DOBC, Facultad de Medicina,
Universidad de Chile

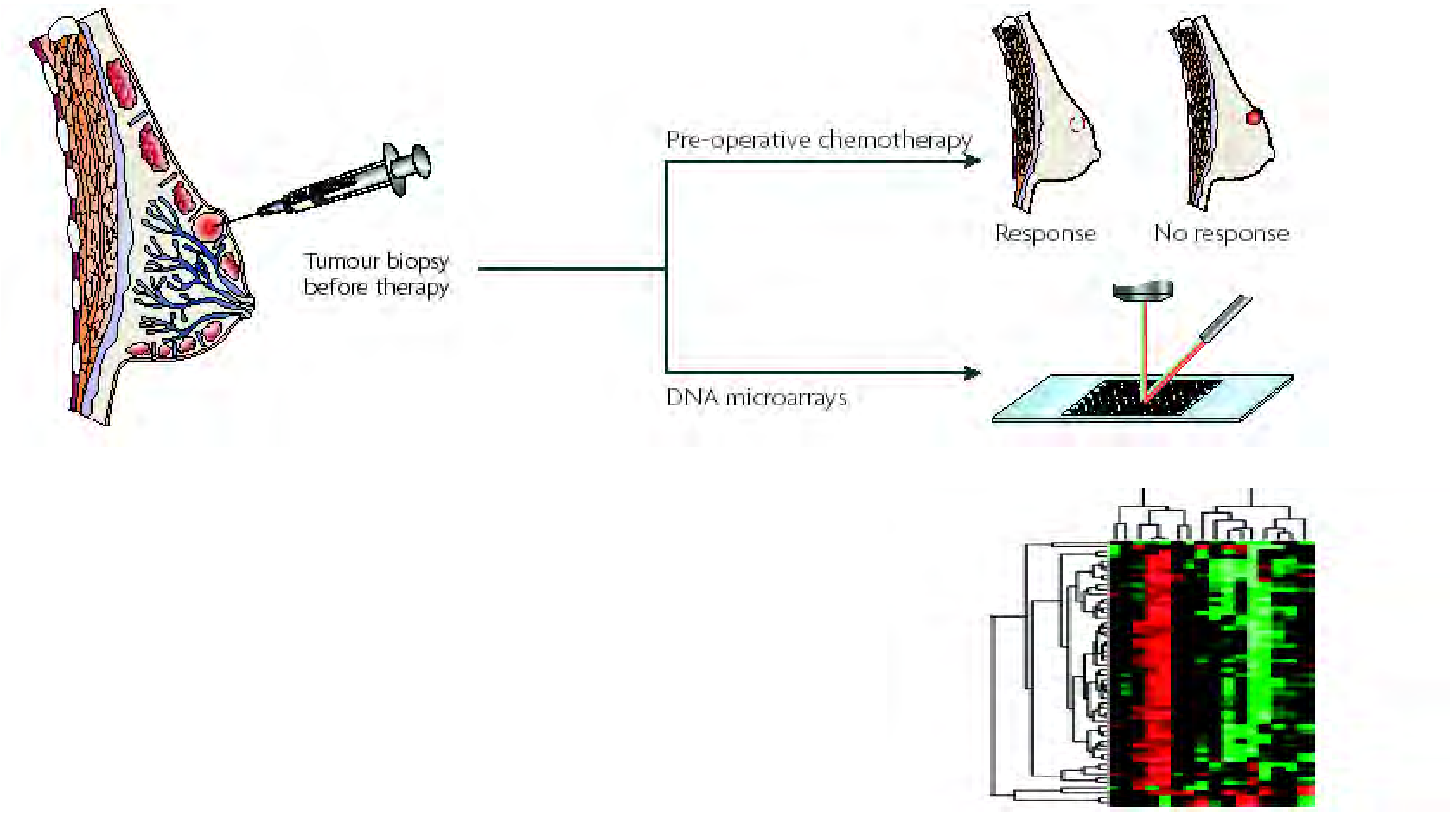
uurzua@med.uchile.cl

- *Gene expression as prognostic classifier*



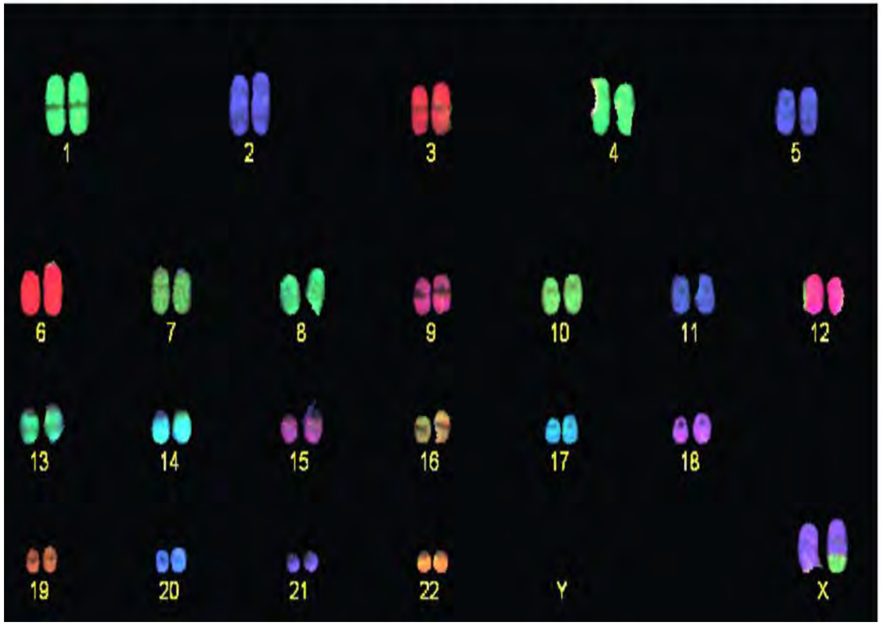


- *Gene expression as predictive classifier*

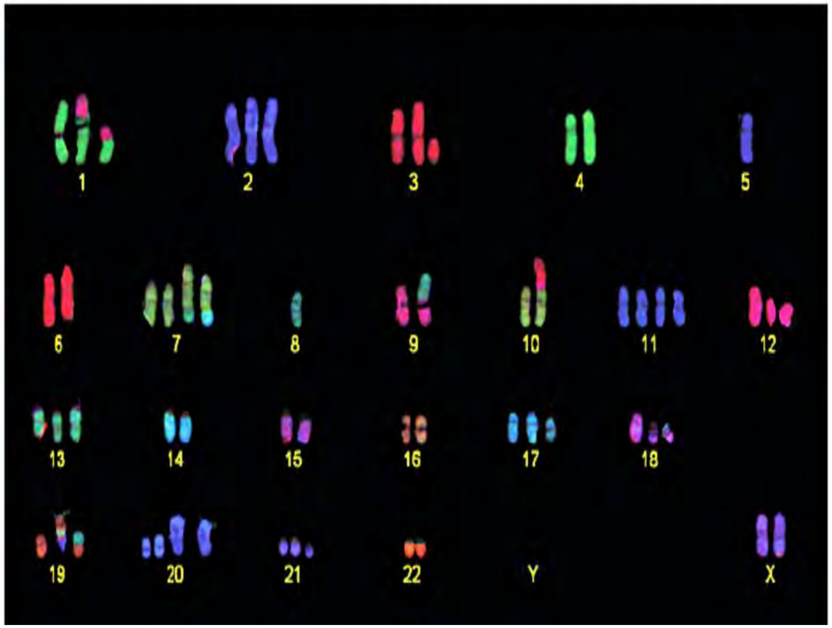


Chromosome aneuploidies are frequent in several solid tumors

46 chromosomes in healthy human cell

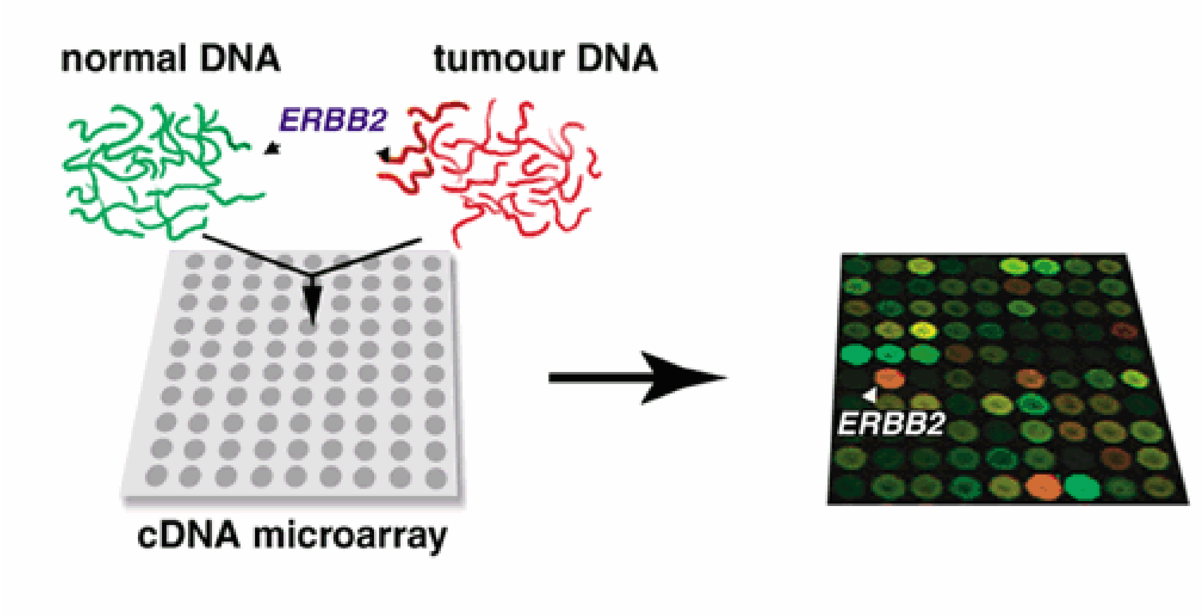


59 chromosomes in colorectal cancer cell

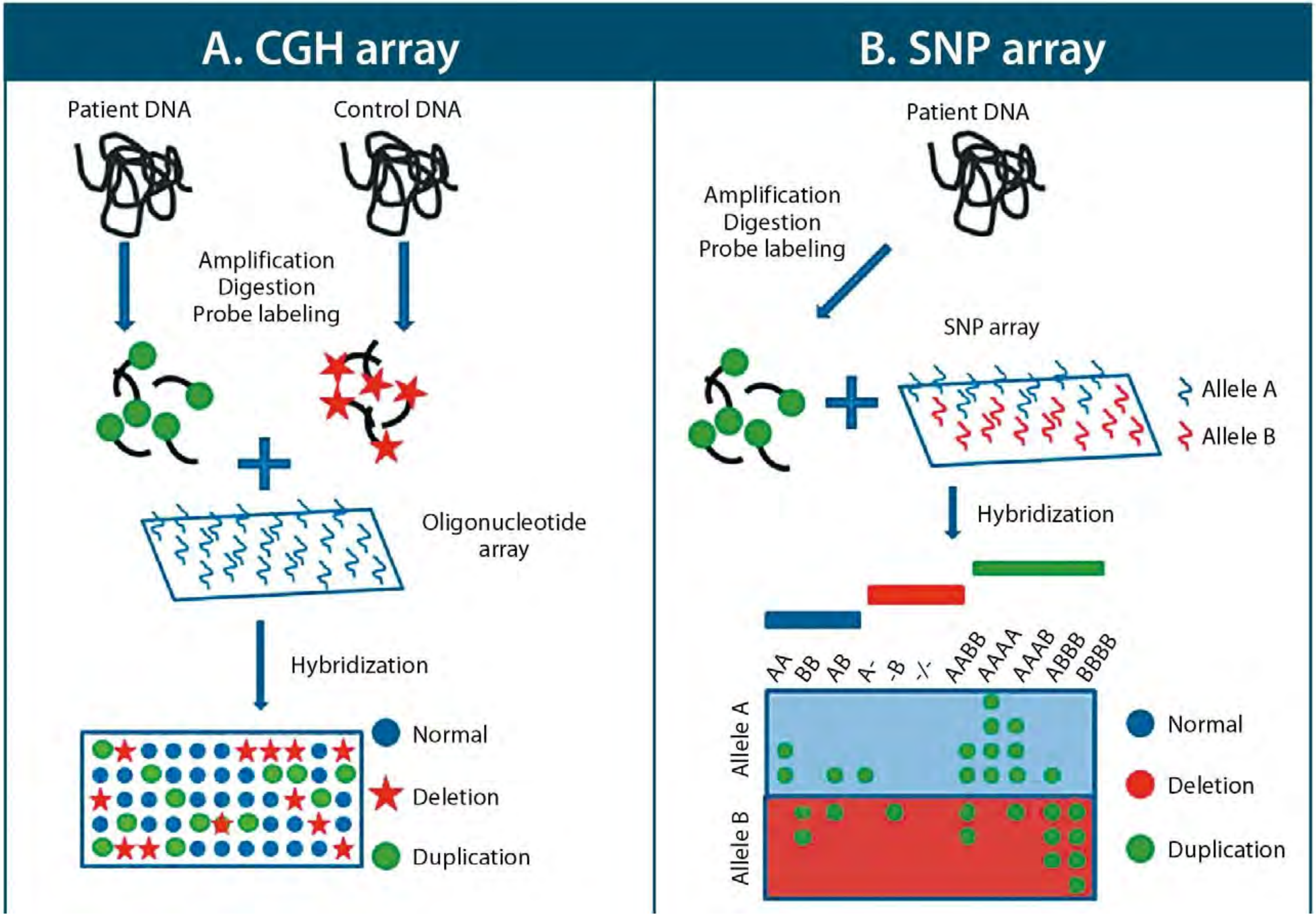


Ejemplo 4

Array-CGH en biomedicina

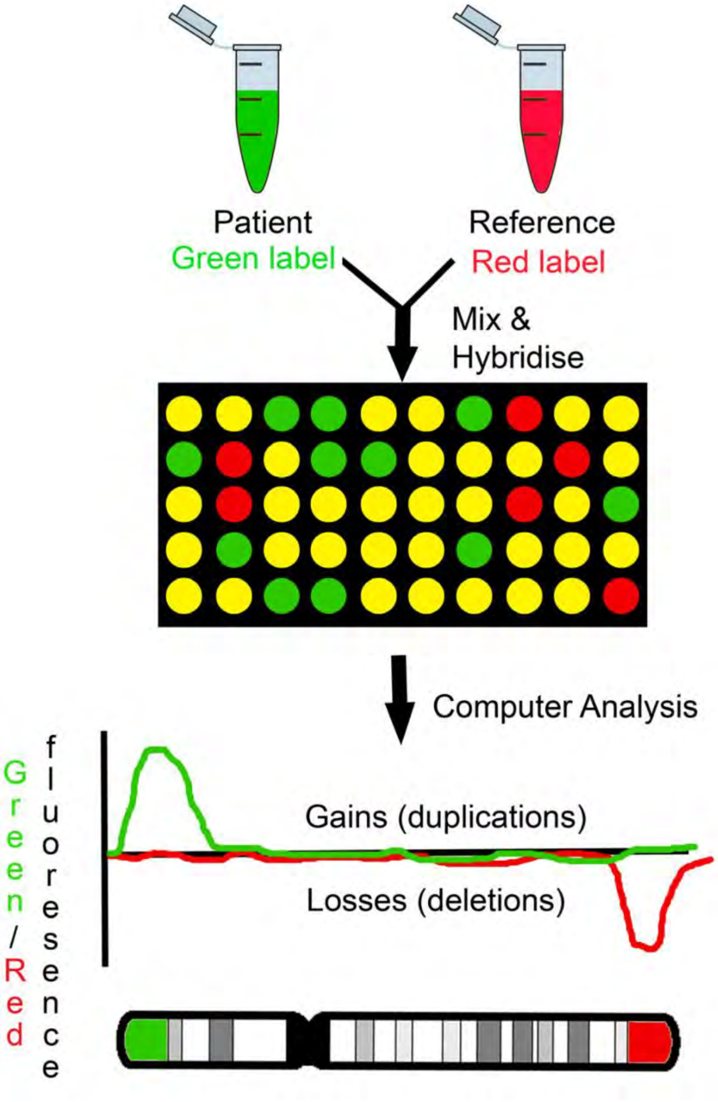


Análisis genómico



http://contemporarypediatrics.modernmedicine.com/

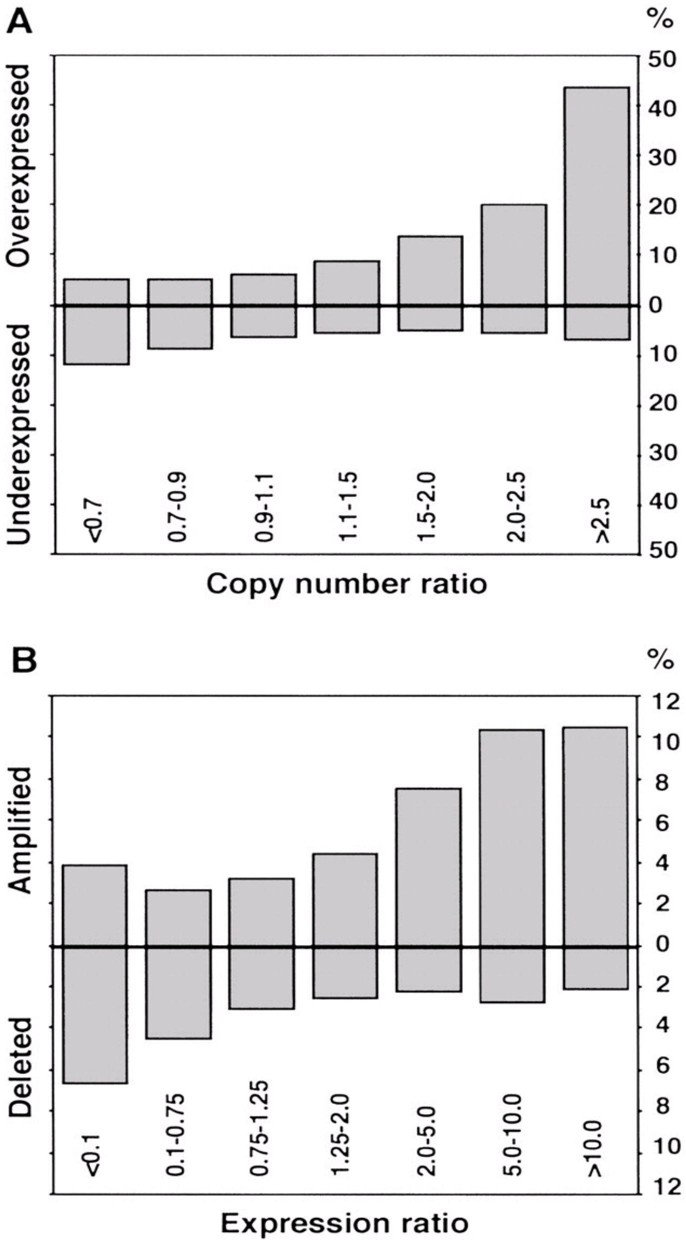
Análisis genómico - array CGH



Spot	Patient	Control	Green : Red
🟡	2 copies	2 copies	1.0 : 1.0
🟢	3 copies	2 copies	1.5 : 1.0
🔴	1 copy	2 copies	0.5 : 1.0

http://www.mdpi.com/2077-0383/3/2/663/htm

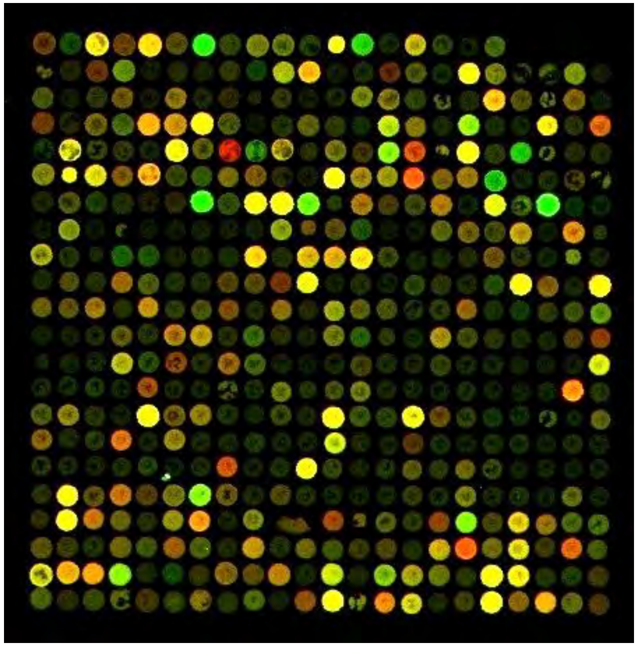
Impact of DNA copy number on gene transcription



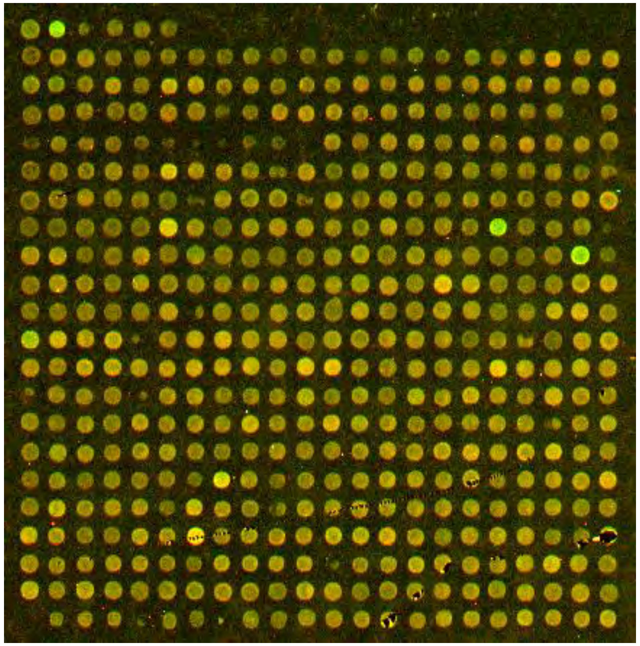
Hyman et al; Cancer Res. 2002 Nov 1;62(21):6240-5.

Microarray-CGH…

RNA (cDNA) sample



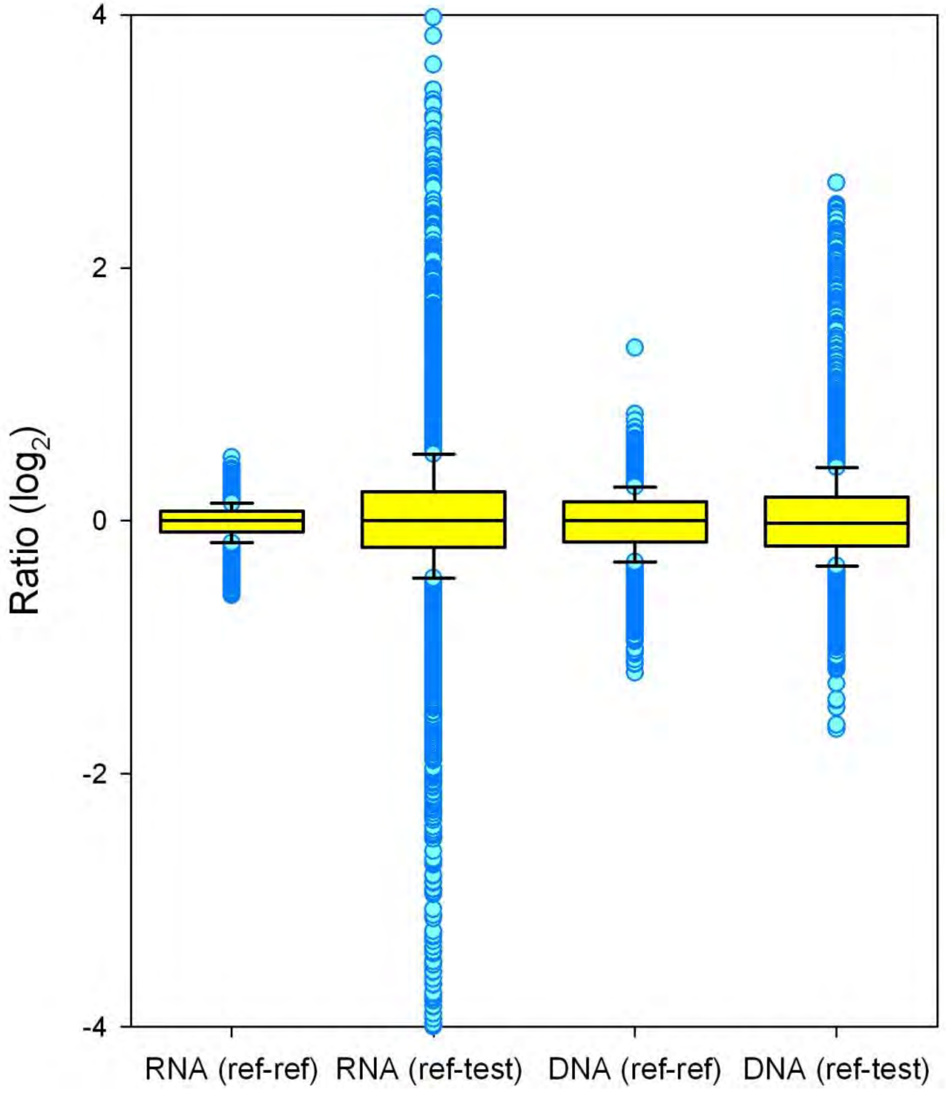
DNA sample



Genome sequence complexity makes a difference.

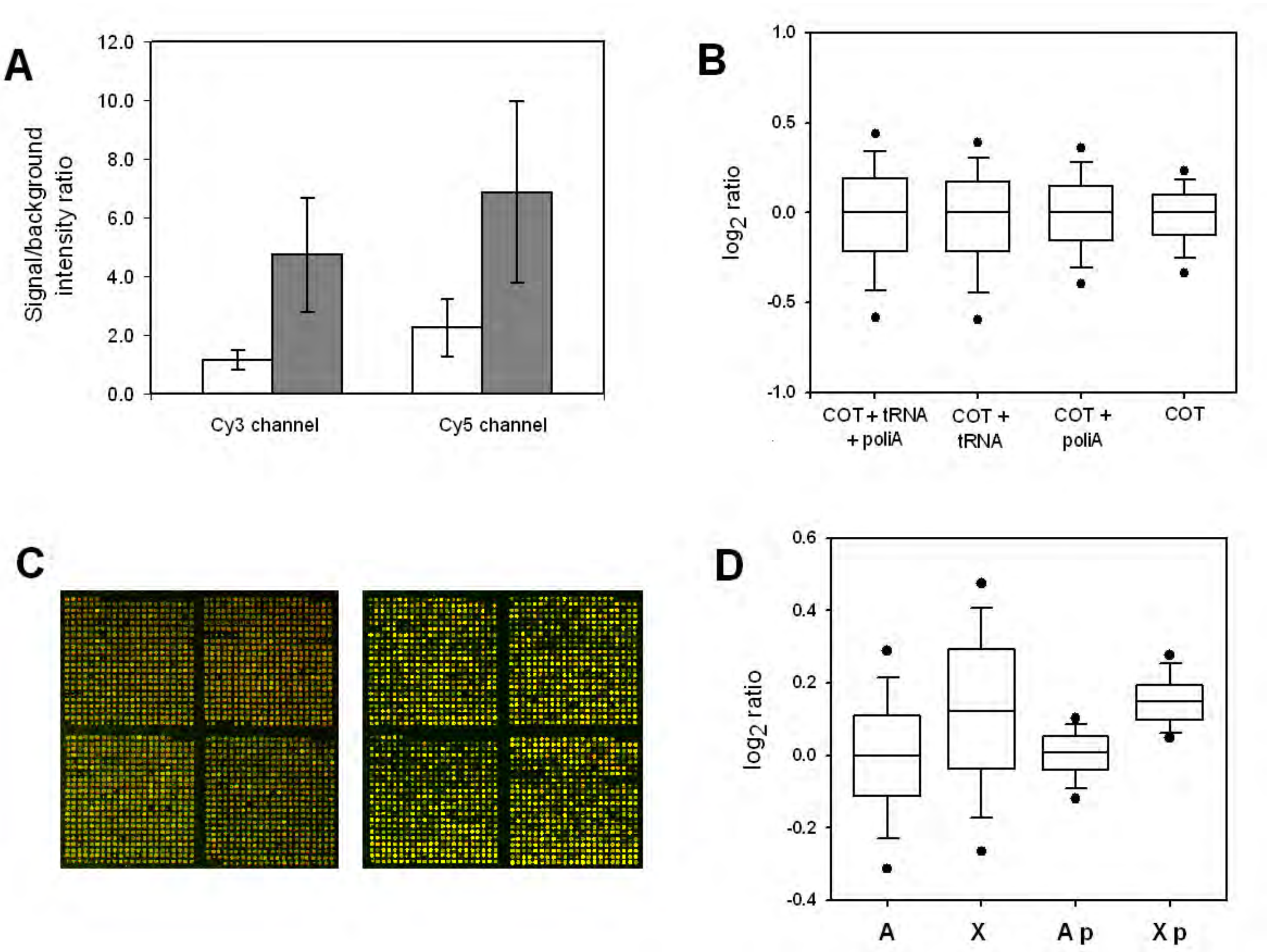
RNA and DNA raw data distribution

- Test RNA and DNA obtained from the same source were hybridized against their respective reference RNA and DNA. Statistical values are shown for 13,417 clones from the NIA-15K cDNA mouse clone set.



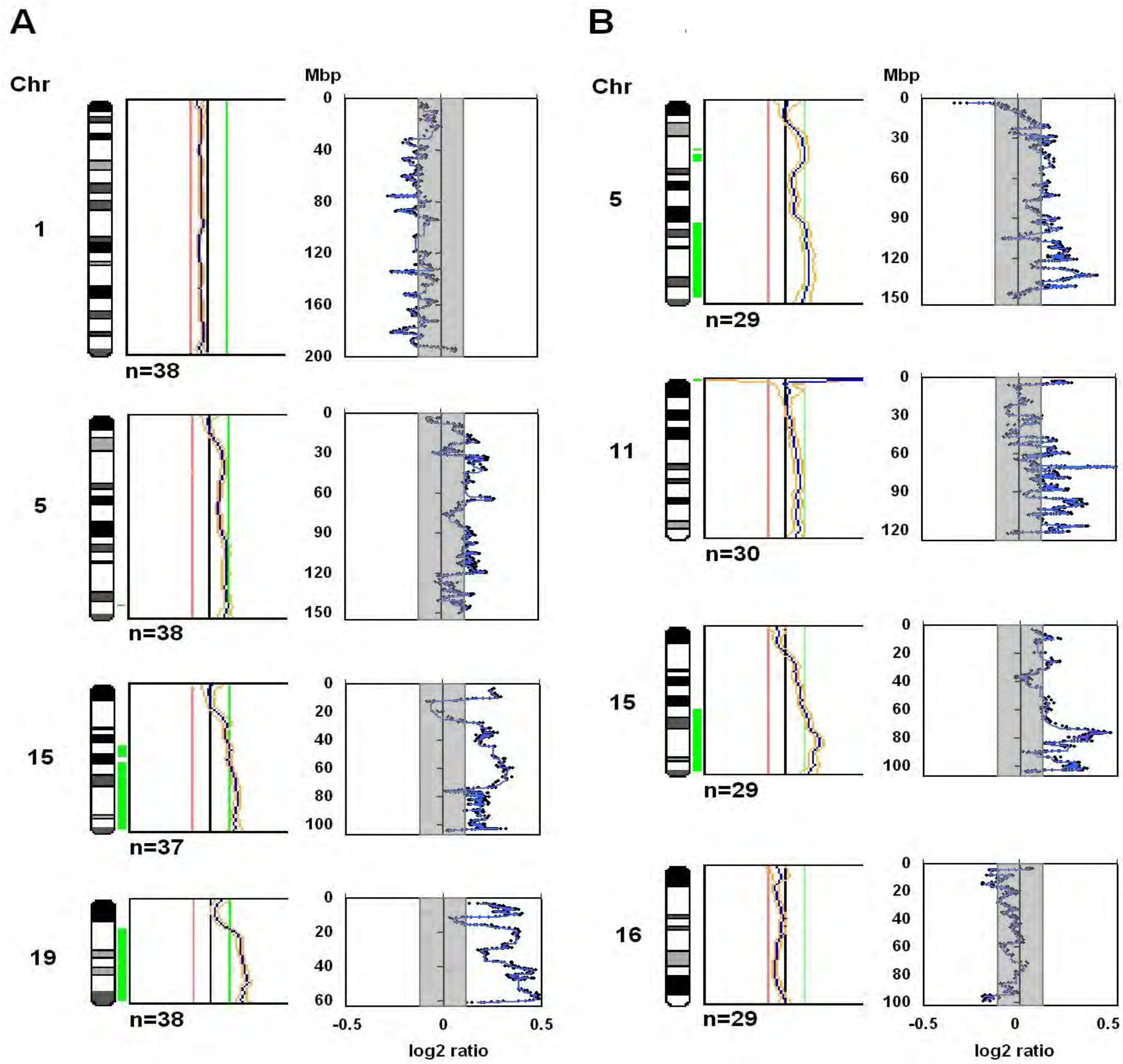
Frankenberger at al; Appl Bioinformatics. 2006;5(2):125-30

Microarray-CGH, experimental optimization



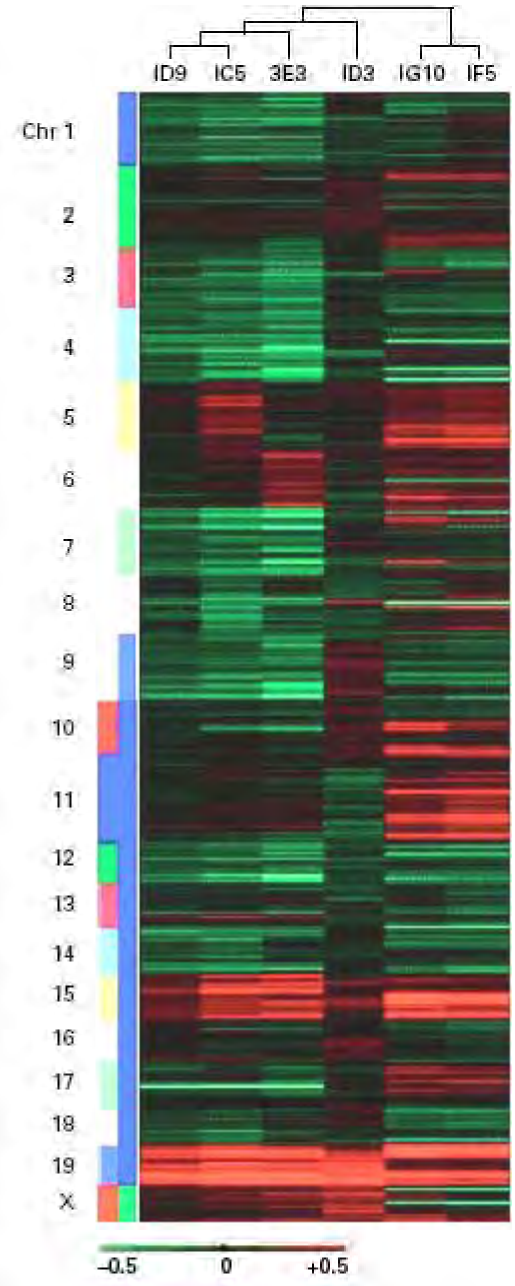
Urzúa et al. (2005) Tumor Biol. 26, 236-44

Conventional-CGH vs microarray-CGH



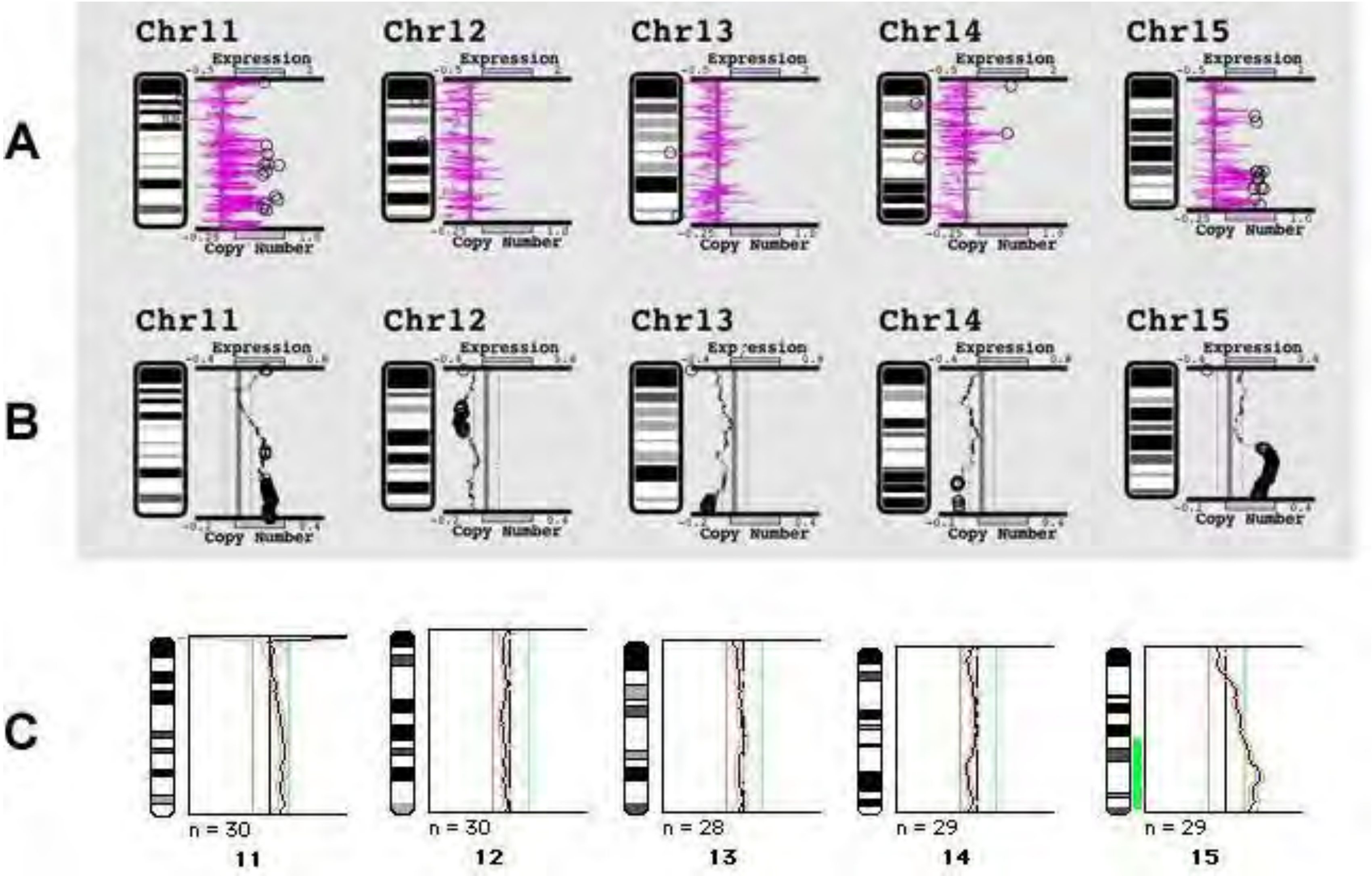
Perfiles de expresión génica

Mouse ovarian surface epithelial (MOSE) cells – an EOC model



Urzúa et al. (2005)
Tumor Biology 26(5):236-44

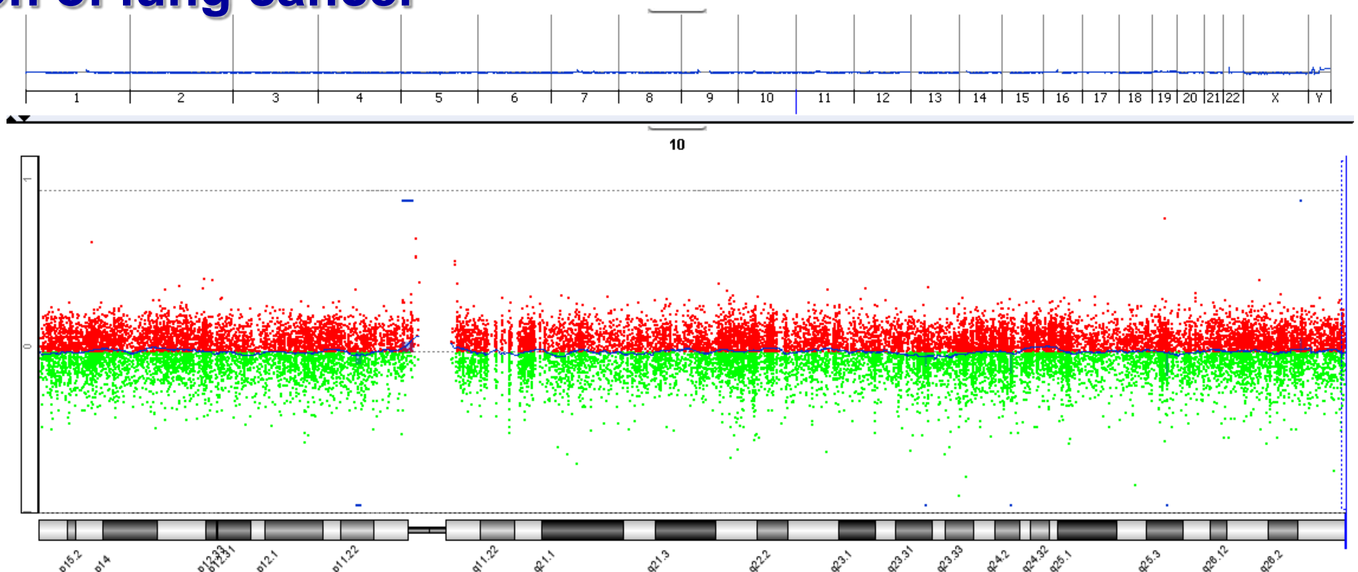
Conventional-CGH vs microarray-CGH



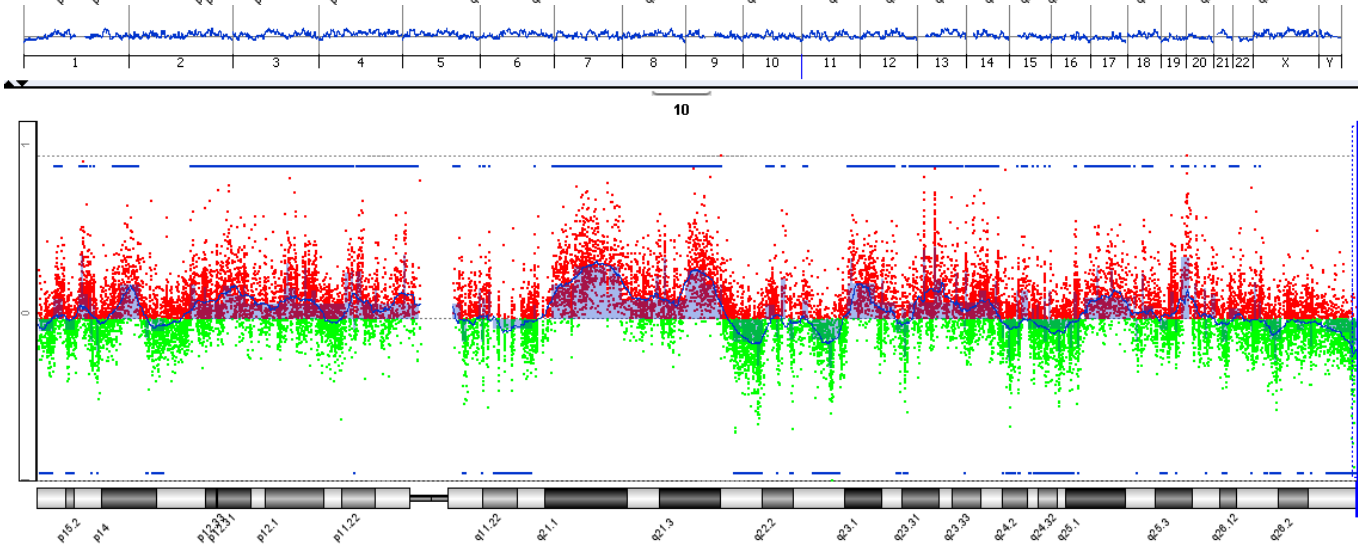
Frankenberger et al. (2006) Appl. Bioinformatics 5, 125-30

Early detection of lung cancer

Voluntario asintomático

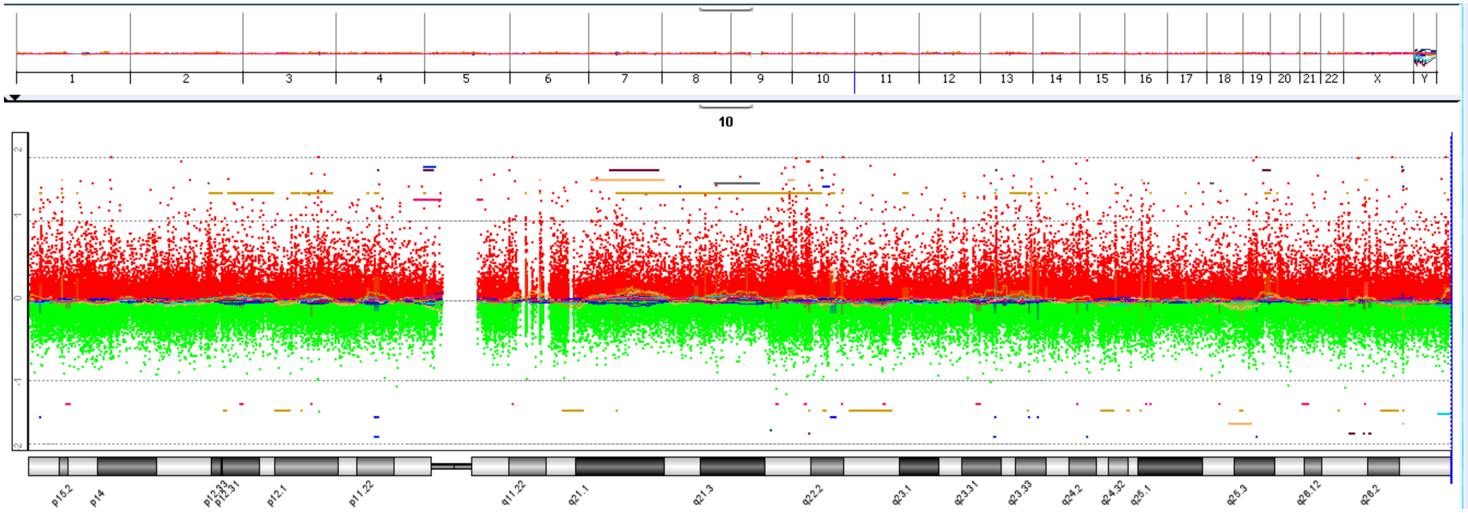


Voluntario cancer pulmonar

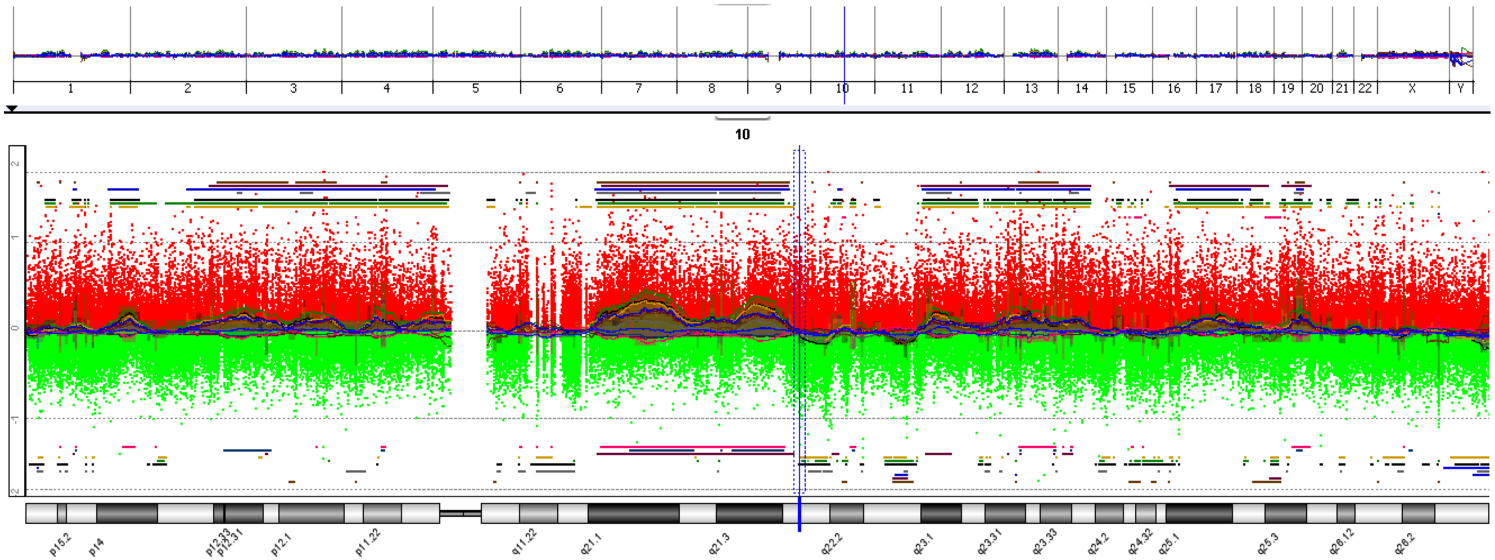


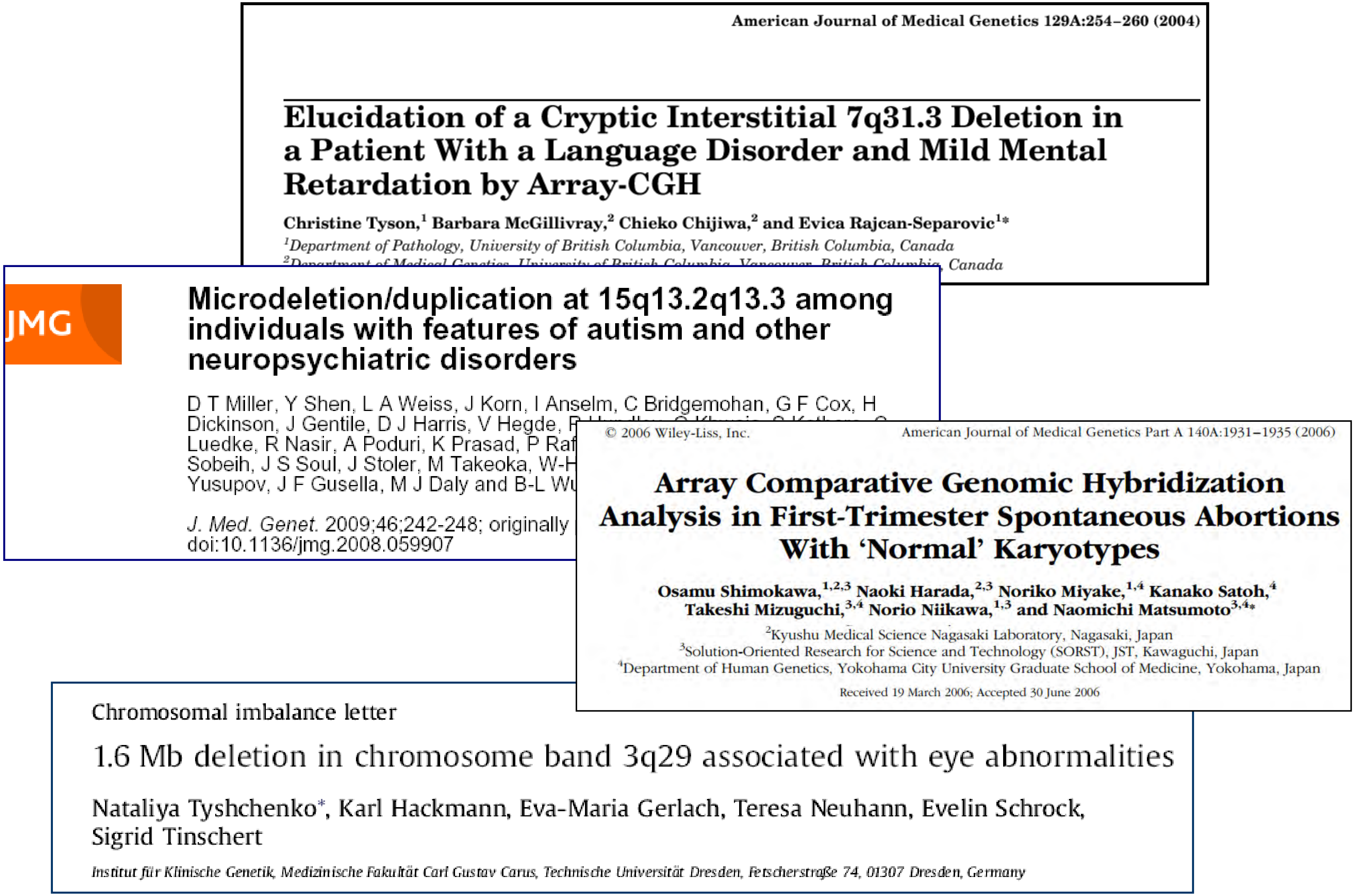
Infinium Human Core Exome-24 beadchip - Illumina

Asintomaticos
(n=11)



Cáncer
pulmonar
(n=11)





American Journal of Medical Genetics 129A:254–260 (2004)

Elucidation of a Cryptic Interstitial 7q31.3 Deletion in a Patient With a Language Disorder and Mild Mental Retardation by Array-CGH

Christine Tyson,[1] Barbara McGillivray,[2] Chieko Chijiwa,[2] and Evica Rajcan-Separovic[1]*

[1]*Department of Pathology, University of British Columbia, Vancouver, British Columbia, Canada*
[2]*Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada*

JMG

Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders

D T Miller, Y Shen, L A Weiss, J Korn, I Anselm, C Bridgemohan, G F Cox, H
Dickinson, J Gentile, D J Harris, V Hegde,
Luedke, R Nasir, A Poduri, K Prasad, P Raf
Sobeih, J S Soul, J Stoler, M Takeoka, W-H
Yusupov, J F Gusella, M J Daly and B-L Wu

J. Med. Genet. 2009;46;242-248; originally
doi:10.1136/jmg.2008.059907

American Journal of Medical Genetics Part A 140A:1931–1935 (2006)

Array Comparative Genomic Hybridization Analysis in First-Trimester Spontaneous Abortions With ‘Normal’ Karyotypes

Osamu Shimokawa,[1,2,3] Naoki Harada,[2,3] Noriko Miyake,[1,4] Kanako Satoh,[4] Takeshi Mizuguchi,[3,4] Norio Niikawa,[1,3] and Naomichi Matsumoto[3,4]*

[2]Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan
[3]Solution-Oriented Research for Science and Technology (SORST), JST, Kawaguchi, Japan
[4]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Received 19 March 2006; Accepted 30 June 2006

Chromosomal imbalance letter

1.6 Mb deletion in chromosome band 3q29 associated with eye abnormalities

Nataliya Tyshchenko*, Karl Hackmann, Eva-Maria Gerlach, Teresa Neuhann, Evelin Schrock,
Sigrid Tinschert

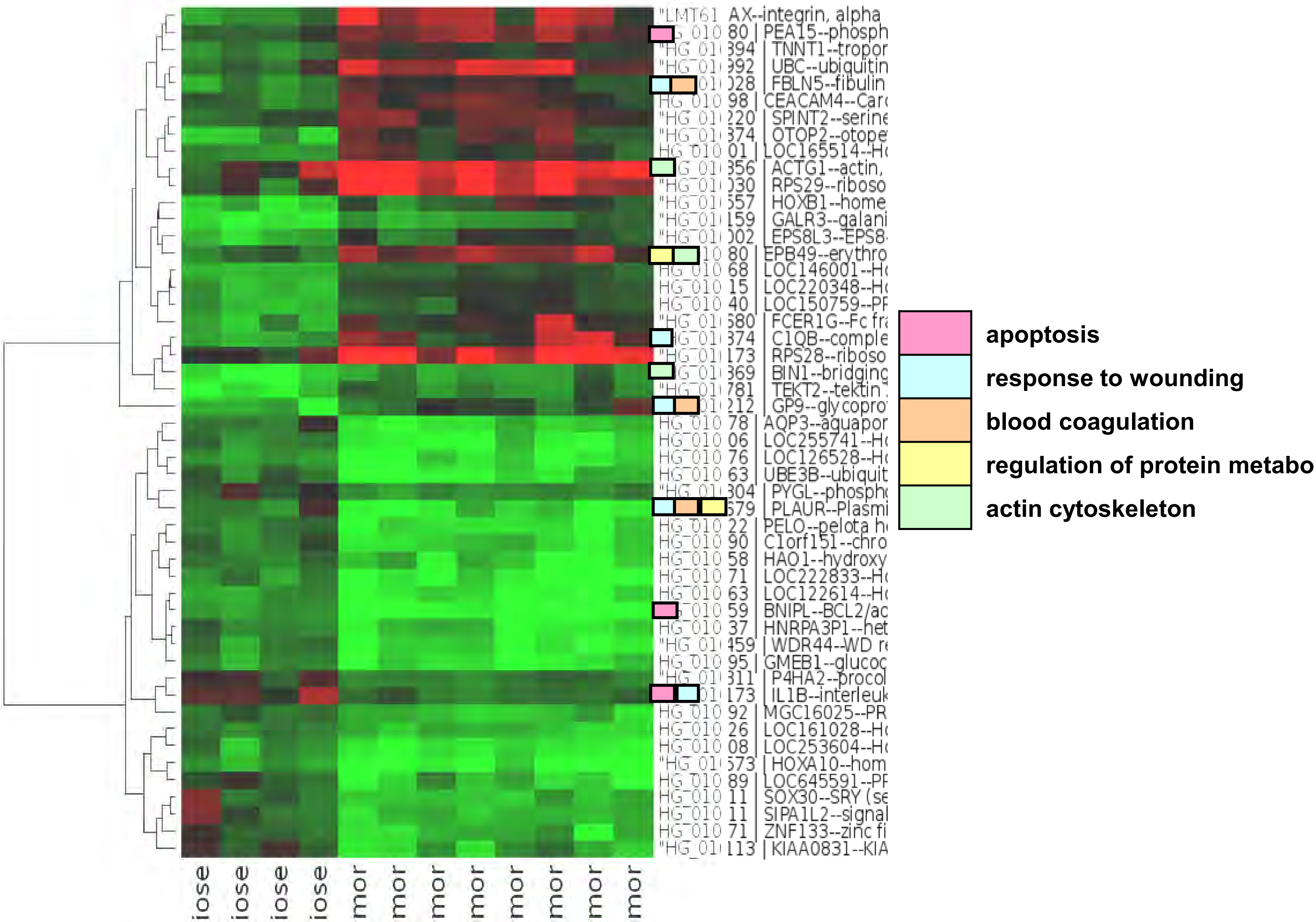
Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany

Caso 1

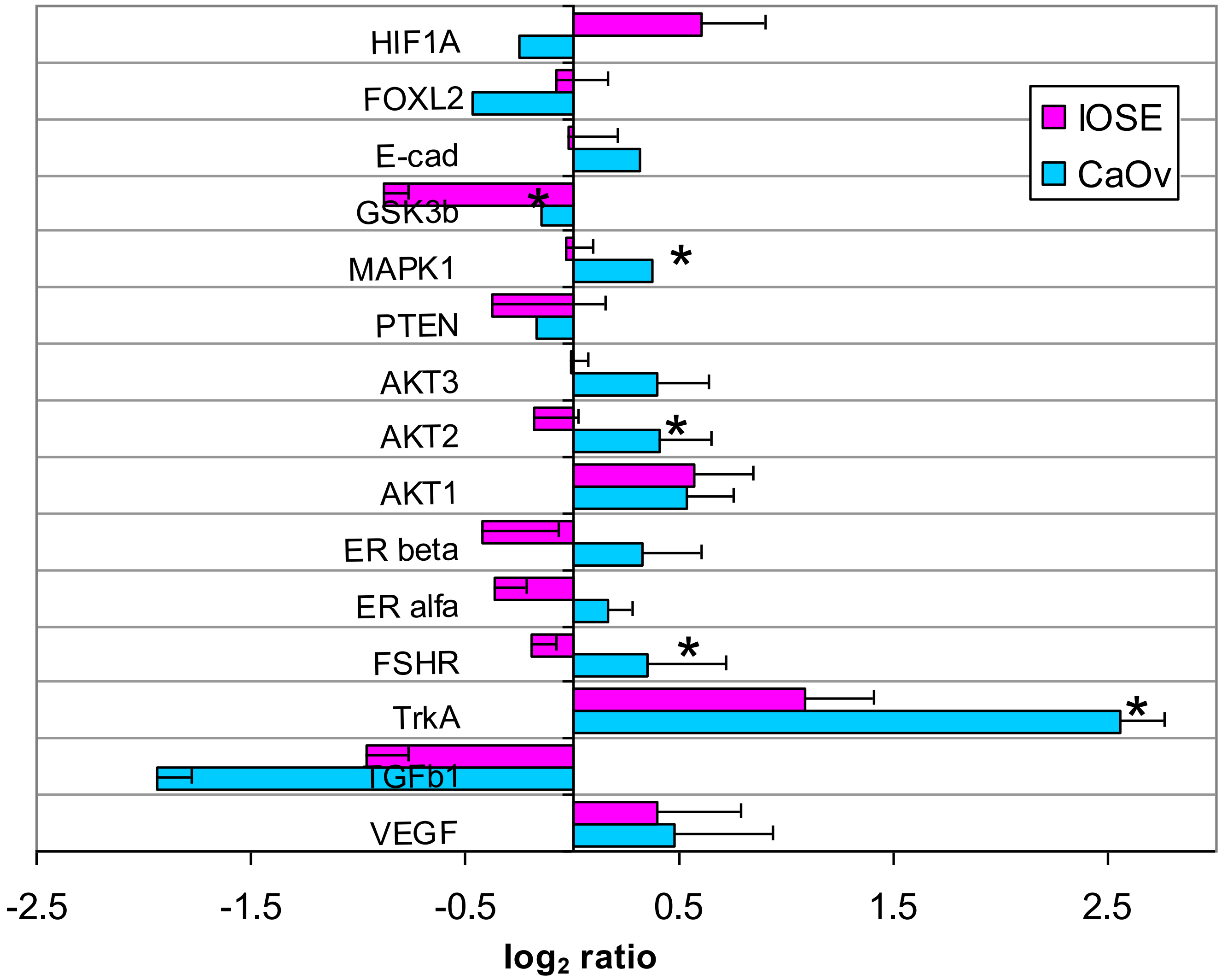
Transcriptomic profiling of human ovarian cancer

Carmen Romero –HCUCH

Gene expression differences - IOSE vs EOC III



NGF signaling pathway and related genes

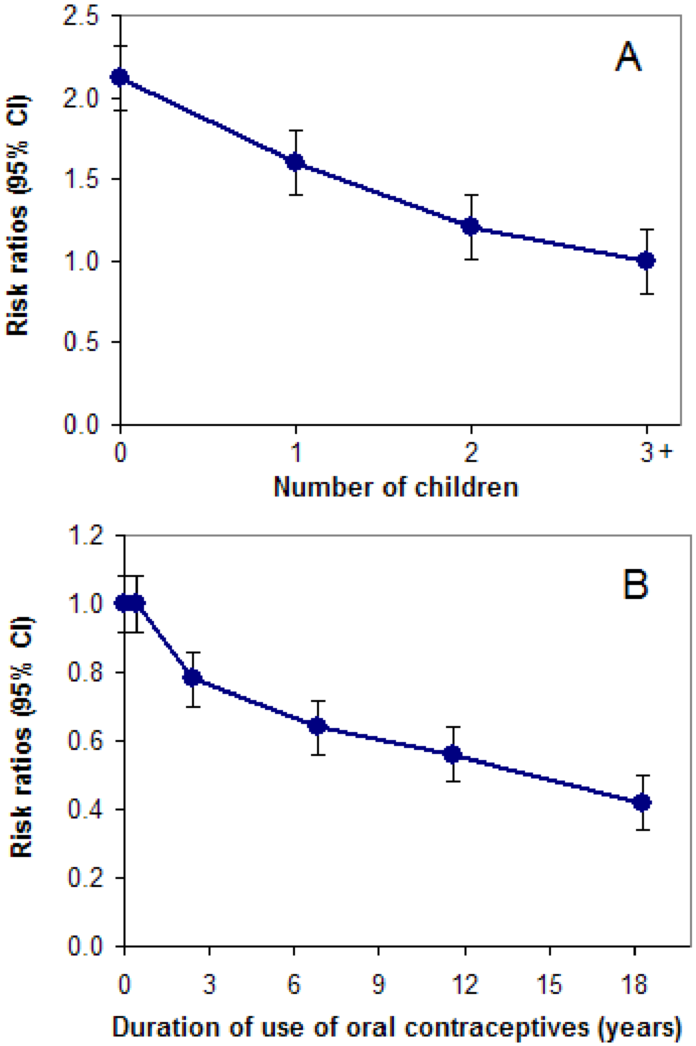


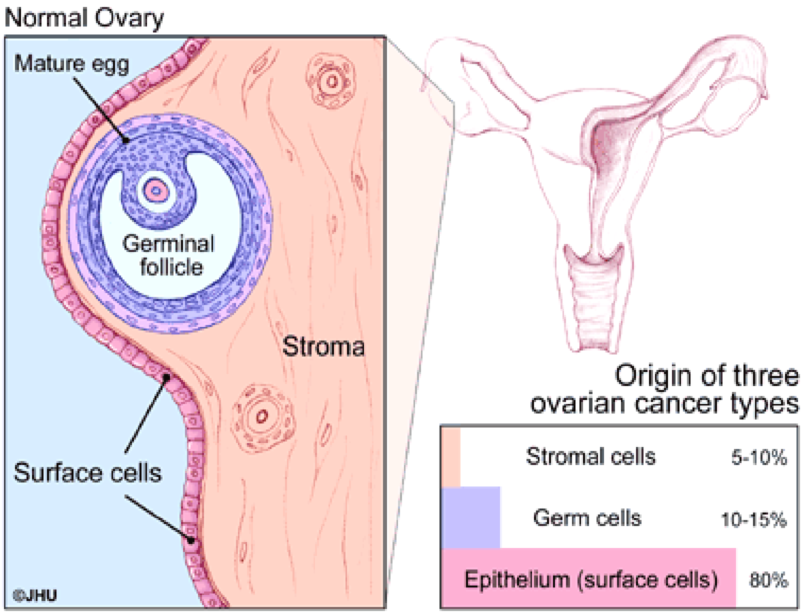
Aplicaciones biomédicas 5

A mouse model of ovarian cancer

DID 2006-08 UChile

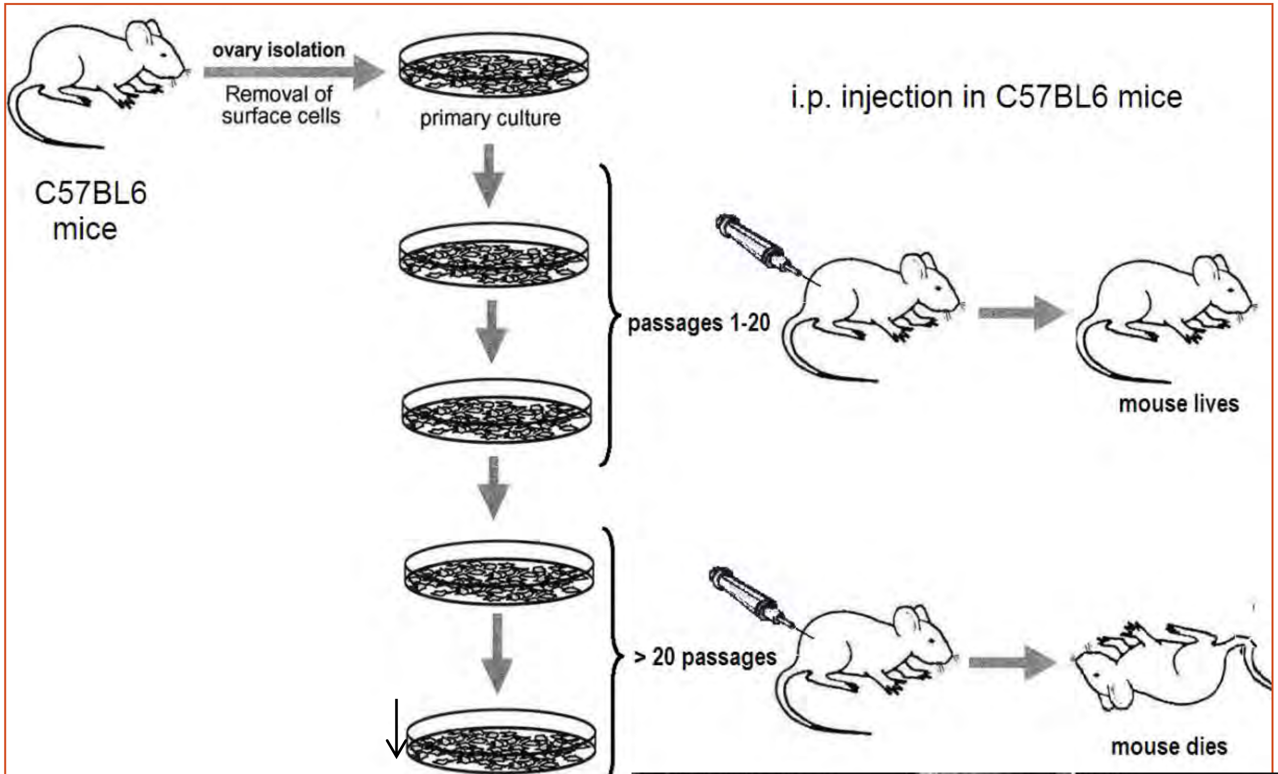
Possible ovarian cancer etiology

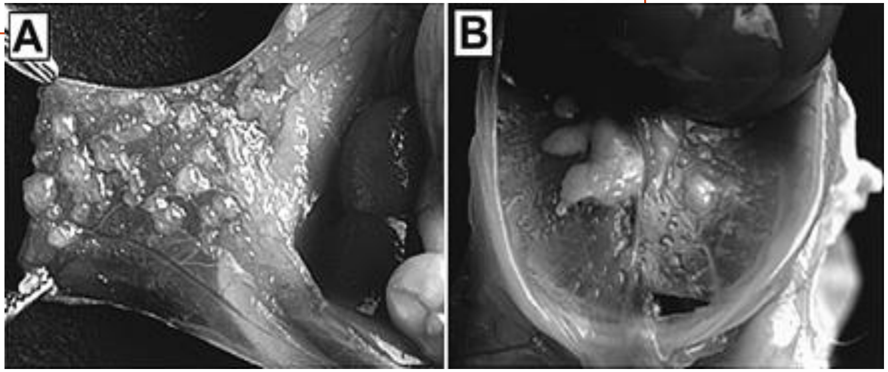




Stromal cells	5-10%
Germ cells	10-15%
Epithelium (surface cells)	80%

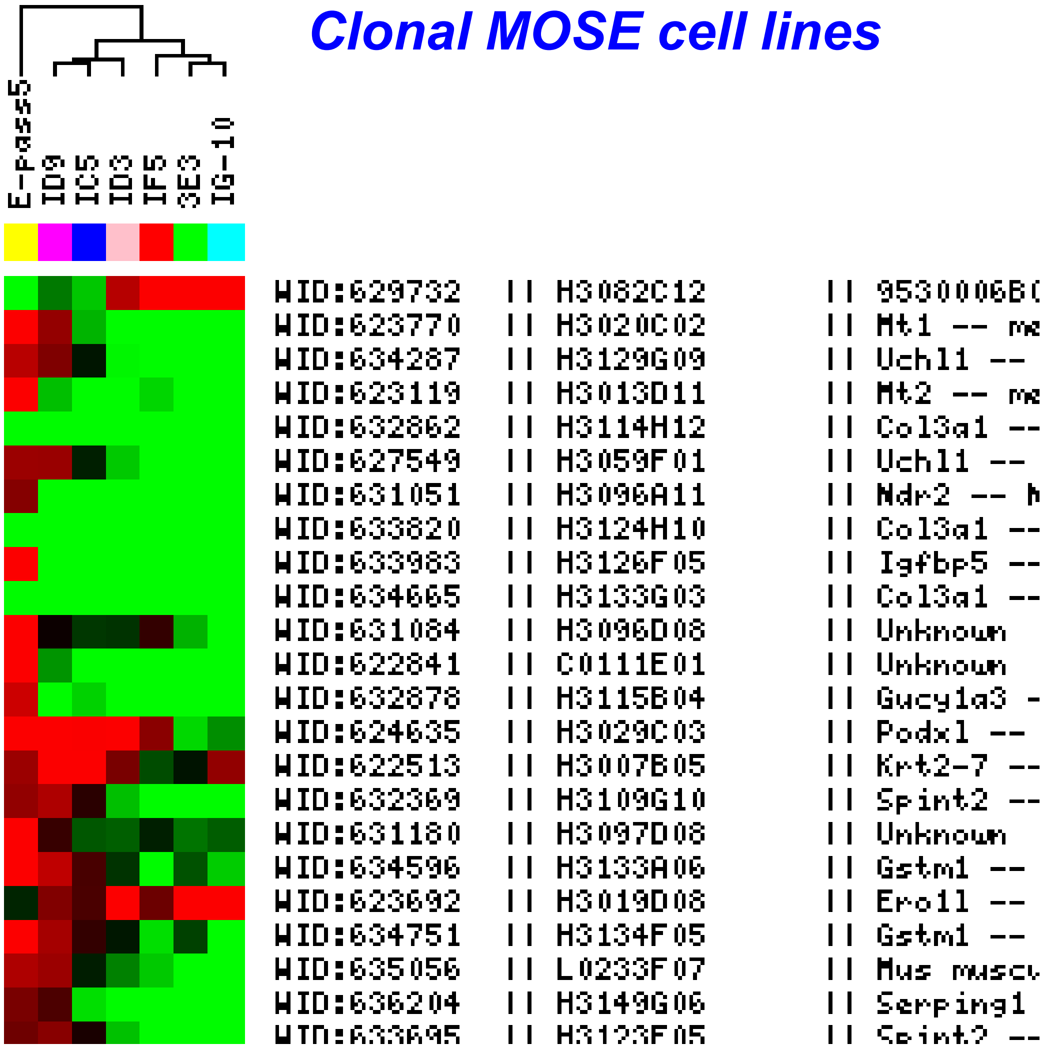
A syngeneic mouse model of ovarian cancer





Roby et al. Carcinogenesis
21, 4, 585-91 (2000)

Clonal MOSE cell lines

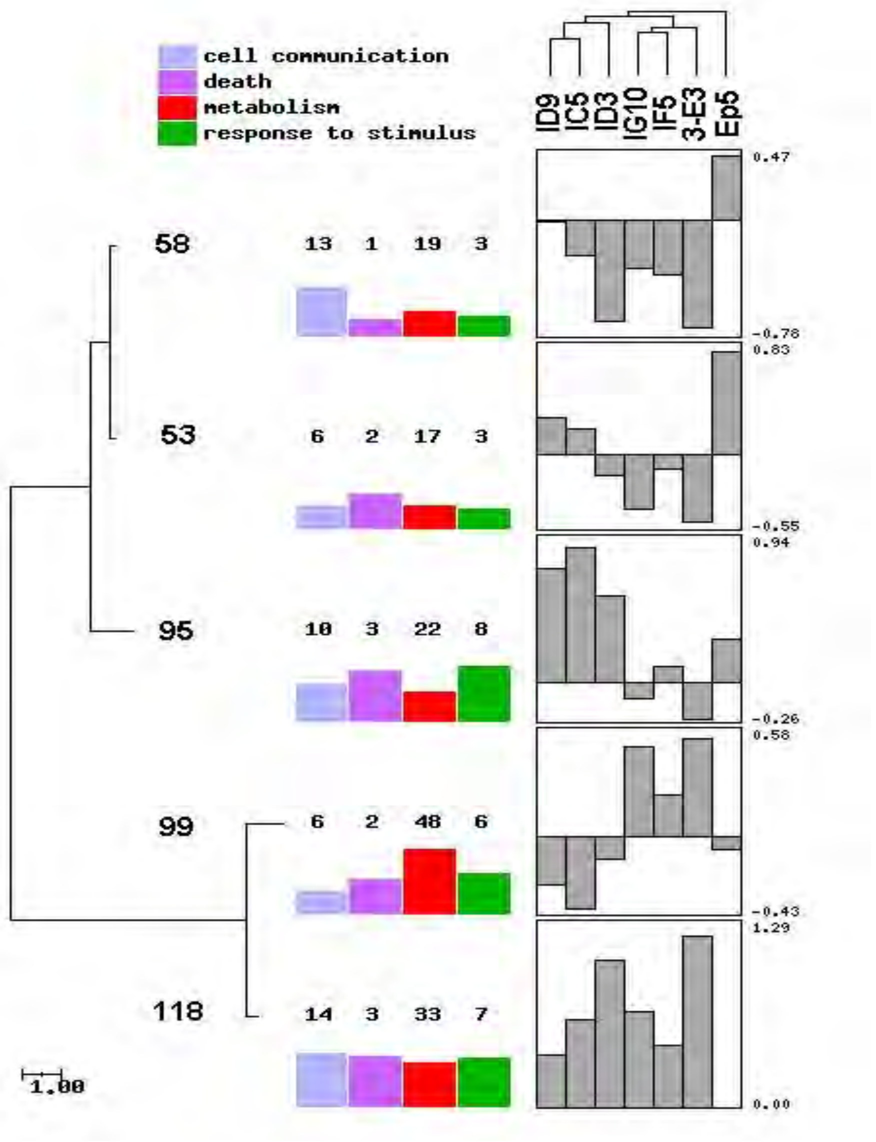


```
WID:629732   || H3082C12        || 9530006B
WID:623770   || H3020C02        || Mt1 -- me
WID:634287   || H3129G09        || Uchl1 --
WID:623119   || H3013D11        || Mt2 -- me
WID:632862   || H3114H12        || Col3a1 --
WID:627549   || H3059F01        || Uchl1 --
WID:631051   || H3096A11        || Ndr2 -- N
WID:633820   || H3124H10        || Col3a1 --
WID:633983   || H3126F05        || Igfbp5 --
WID:634665   || H3133G03        || Col3a1 --
WID:631084   || H3096D08        || Unknown
WID:622841   || C0111E01        || Unknown
WID:632878   || H3115B04        || Gucy1a3 -
WID:624635   || H3029C03        || Podxl --
WID:622513   || H3007B05        || Krt2-7 --
WID:632369   || H3109G10        || Spint2 --
WID:631180   || H3097D08        || Unknown
WID:634596   || H3133A06        || Gstm1 --
WID:623692   || H3019D08        || Ero1l --
WID:634751   || H3134F05        || Gstm1 --
WID:635056   || L0233F07        || Mus muscu
WID:636204   || H3149G06        || Serping1
WID:633695   || H3123F05        || Spint2 --
```

Urzúa et al. (2006)

J. Cell. Physiol. 206, 594-602

Clonal MOSE cell lines



Urzúa et al. (2006)

J. Cell. Physiol. 206, 594-602

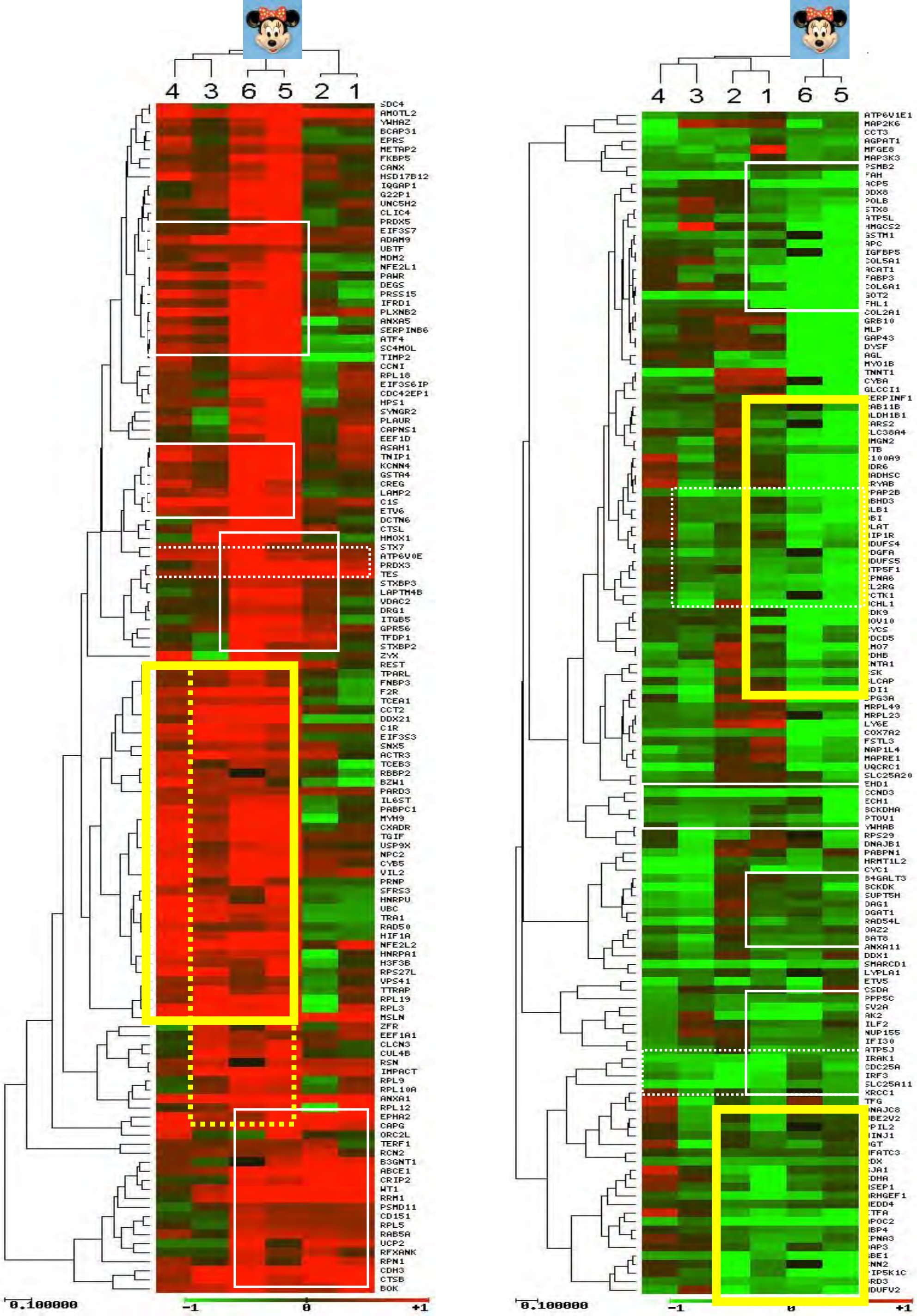
Human-Mouse Comparison

- SOM clustering of IG10 and IF5 cell lines compared to human ovarian tumors based on 872 genes with equivalent biological function. Samples description is as follows:

1.- LMP
2.- Stage III
3.- Serous BOT
4.- Mucinous BOT
5.- Mouse IG-10
6.- Mouse IF-5

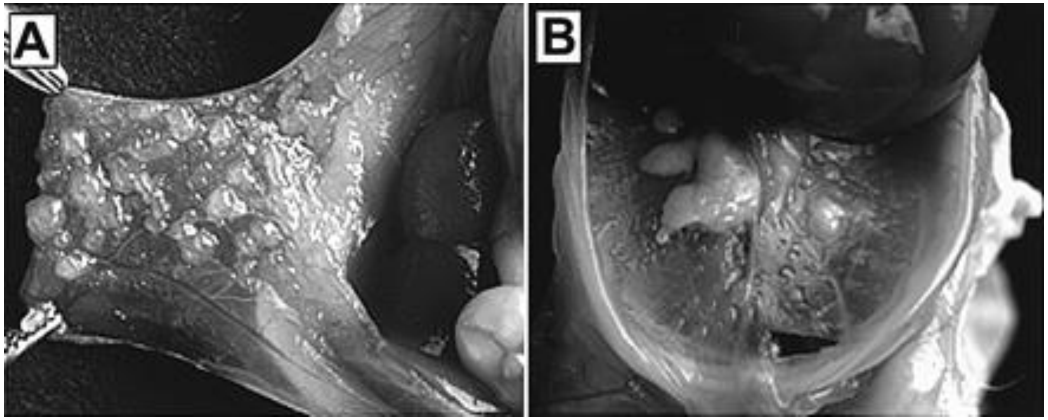
Urzúa et al. (2006)

J. Cell. Physiol. 206, 594-602



A syngeneic mouse model of ovarian cancer

Roby et al. Carcinogenesis
21, 4, 585-91 (2000)



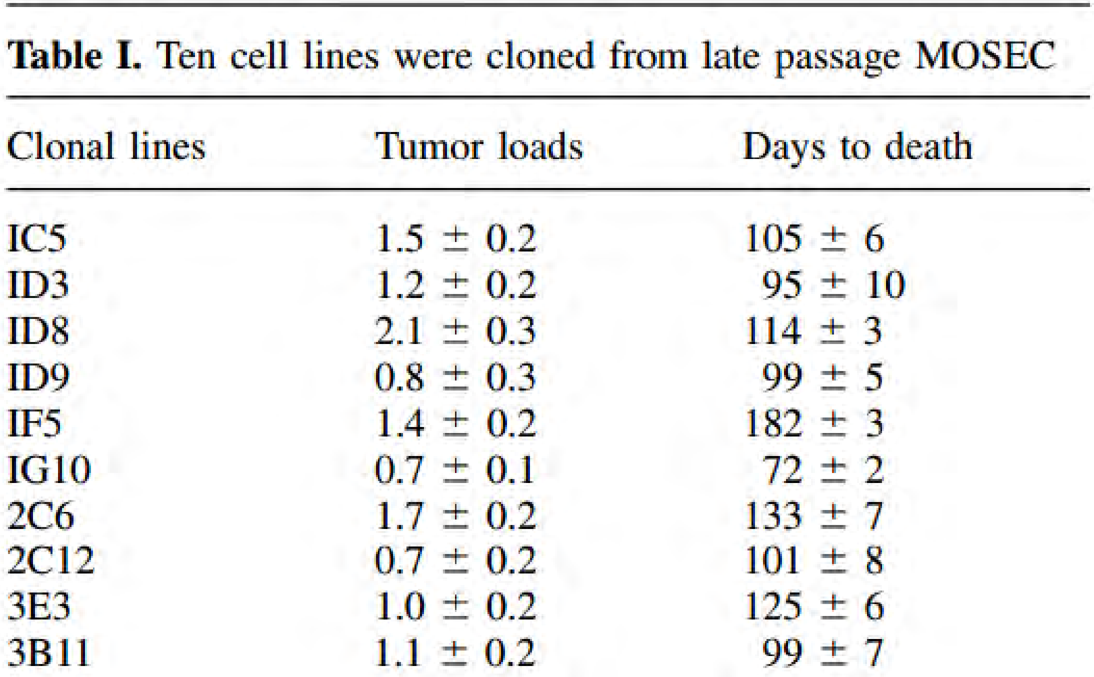
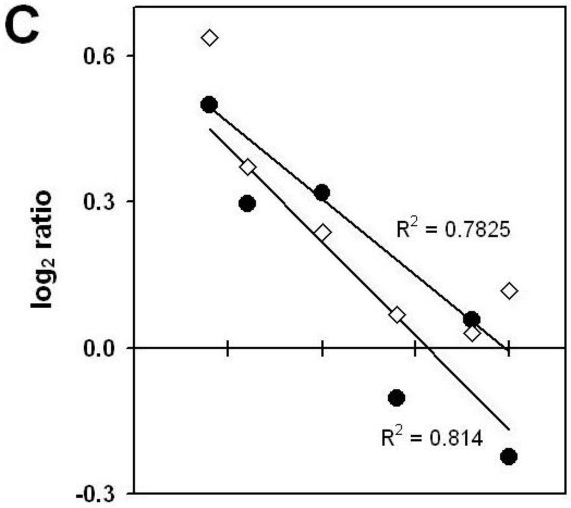


Table I. Ten cell lines were cloned from late passage MOSEC

Clonal lines	Tumor loads	Days to death
IC5	1.5 ± 0.2	105 ± 6
ID3	1.2 ± 0.2	95 ± 10
ID8	2.1 ± 0.3	114 ± 3
ID9	0.8 ± 0.3	99 ± 5
IF5	1.4 ± 0.2	182 ± 3
IG10	0.7 ± 0.1	72 ± 2
2C6	1.7 ± 0.2	133 ± 7
2C12	0.7 ± 0.2	101 ± 8
3E3	1.0 ± 0.2	125 ± 6
3B11	1.1 ± 0.2	99 ± 7

Genes correlated to tumor loads

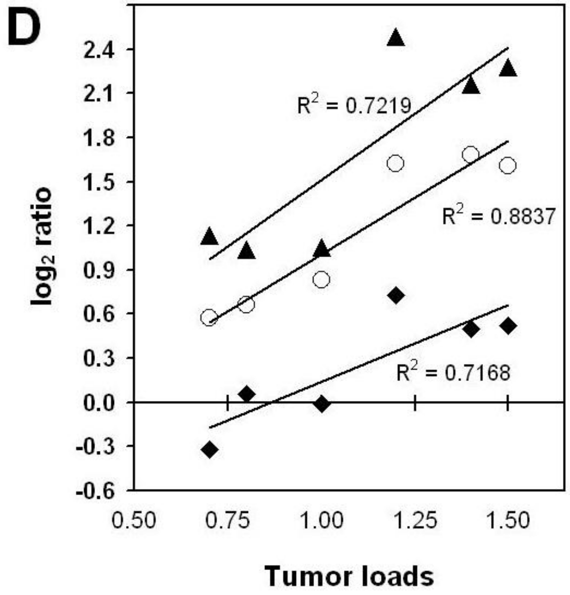


Map4k4, mitogen-activated protein kinase 4

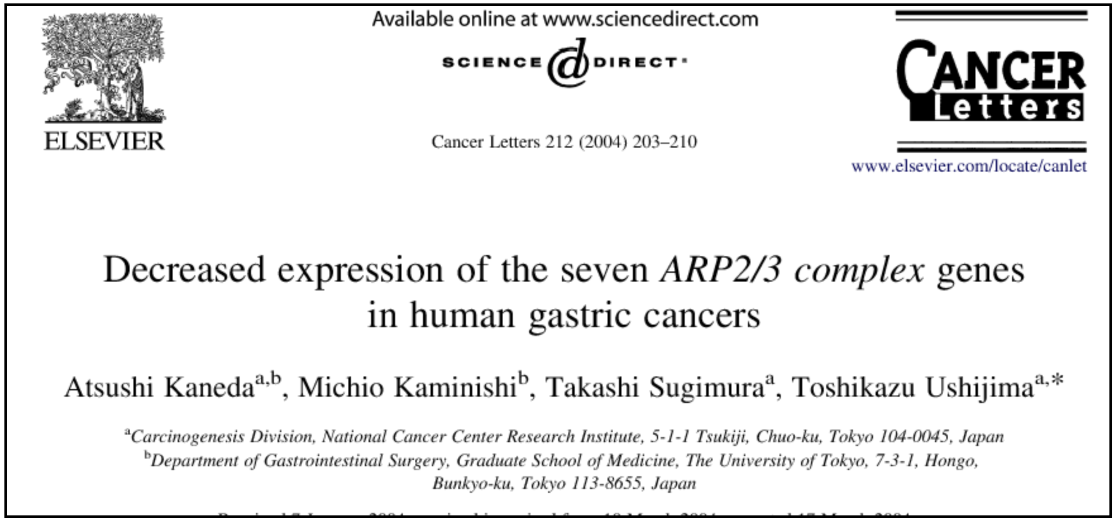

Molecular and Cellular Biology, Mar. 2003, p. 2068–2082 Vol. 23, No. 6
0270-7306/03/$08.00+0 DOI: 10.1128/MCB.23.6.2068–2082.2003

The STE20 Kinase HGK Is Broadly Expressed in Human Tumor Cells and Can Modulate Cellular Transformation, Invasion, and Adhesion

Jocelyn H. Wright,* Xueyan Wang, Gerard Manning, Brandon J. LaMere, Phuong Le, Shirley Zhu, Deepak Khatry, Peter M. Flanagan, Sharon D. Buckley, David B. Whyte, Anthony R. Howlett, James R. Bischoff, Kenneth E. Lipson, and Bahija Jallal
Sugen, Inc., South San Francisco, California 94080



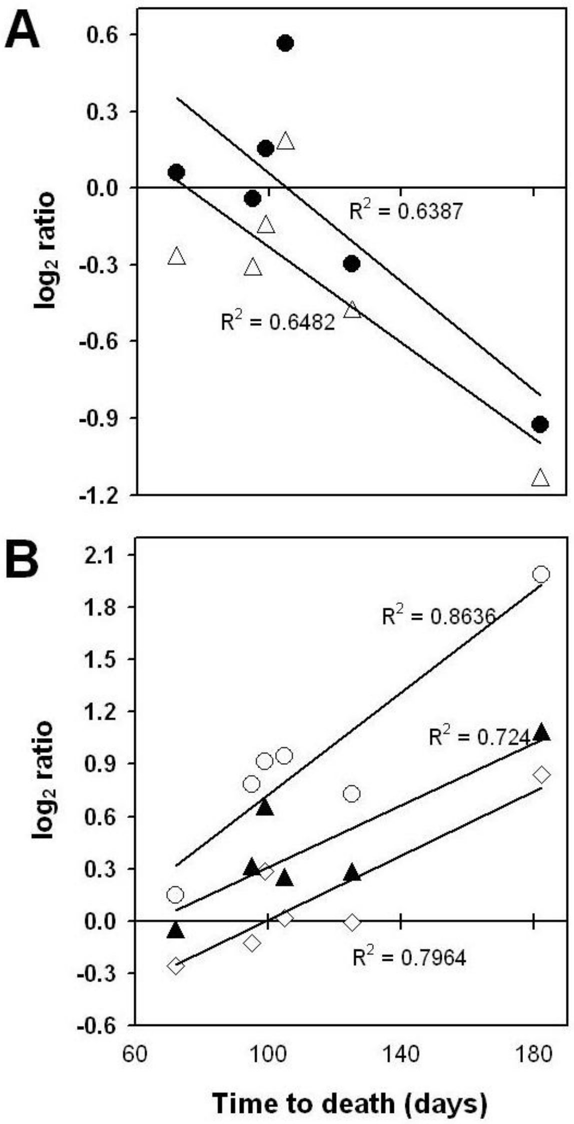
Arpc1b, actin related protein 2/3 complex, subunit 1B


Available online at www.sciencedirect.com
SCIENCE DIRECT·
ELSEVIER
Cancer Letters 212 (2004) 203–210
CANCER Letters
www.elsevier.com/locate/canlet

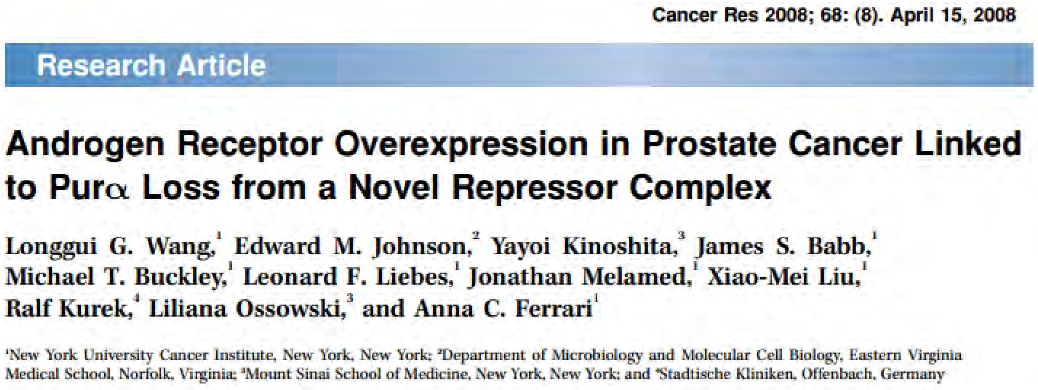
Decreased expression of the seven *ARP2/3 complex* genes in human gastric cancers

Atsushi Kaneda[a,b], Michio Kaminishi[b], Takashi Sugimura[a], Toshikazu Ushijima[a,*]
[a]*Carcinogenesis Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[b]*Department of Gastrointestinal Surgery, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*

Genes correlated to survival



Pura, purine rich element binding protein A



Cancer Res 2008; 68: (8). April 15, 2008

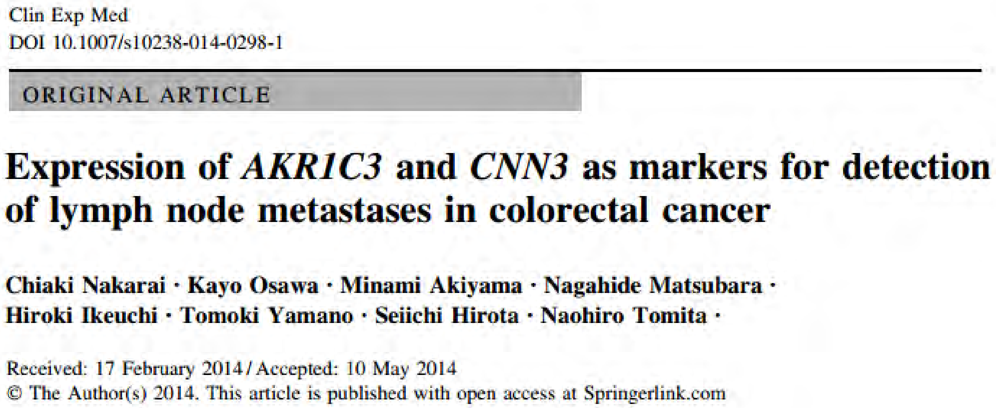
Research Article

Androgen Receptor Overexpression in Prostate Cancer Linked to Purα Loss from a Novel Repressor Complex

Longgui G. Wang,[1] Edward M. Johnson,[2] Yayoi Kinoshita,[3] James S. Babb,[1]
Michael T. Buckley,[1] Leonard F. Liebes,[1] Jonathan Melamed,[1] Xiao-Mei Liu,[1]
Ralf Kurek,[4] Liliana Ossowski,[3] and Anna C. Ferrari[1]

[1]New York University Cancer Institute, New York, New York; [2]Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, Virginia; [3]Mount Sinai School of Medicine, New York, New York; and [4]Stadtische Kliniken, Offenbach, Germany

Cnn3, calponin 3



Clin Exp Med
DOI 10.1007/s10238-014-0298-1

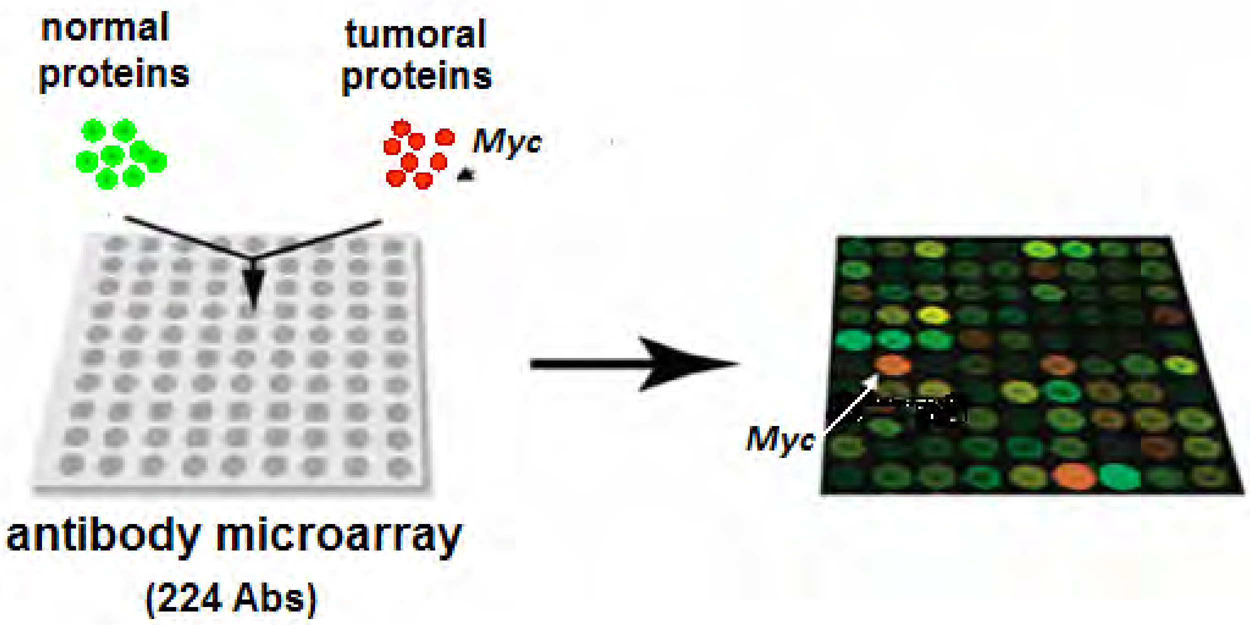
ORIGINAL ARTICLE

Expression of *AKR1C3* and *CNN3* as markers for detection of lymph node metastases in colorectal cancer

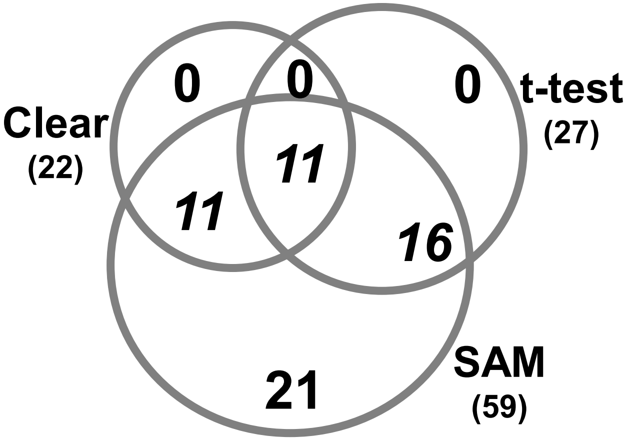
Chiaki Nakarai · Kayo Osawa · Minami Akiyama · Nagahide Matsubara ·
Hiroki Ikeuchi · Tomoki Yamano · Seiichi Hirota · Naohiro Tomita ·

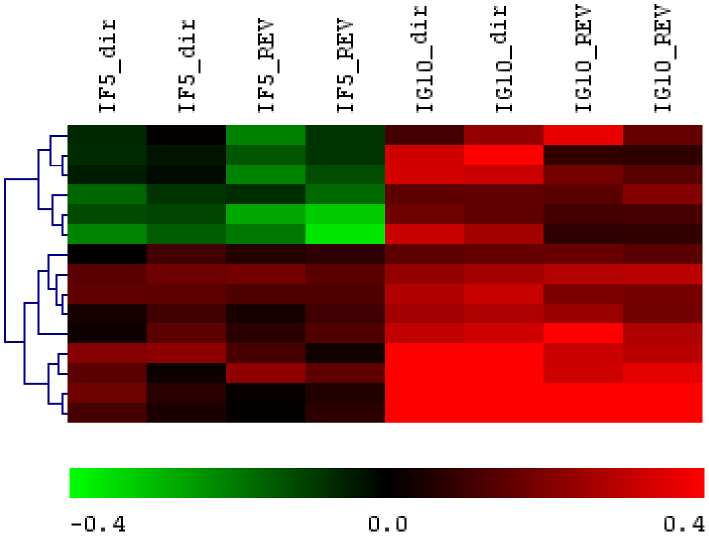
Received: 17 February 2014 / Accepted: 10 May 2014

Proteomics with antibody microarrays



Proteomic comparison between MOSE cells of extreme tumor inducing capacity





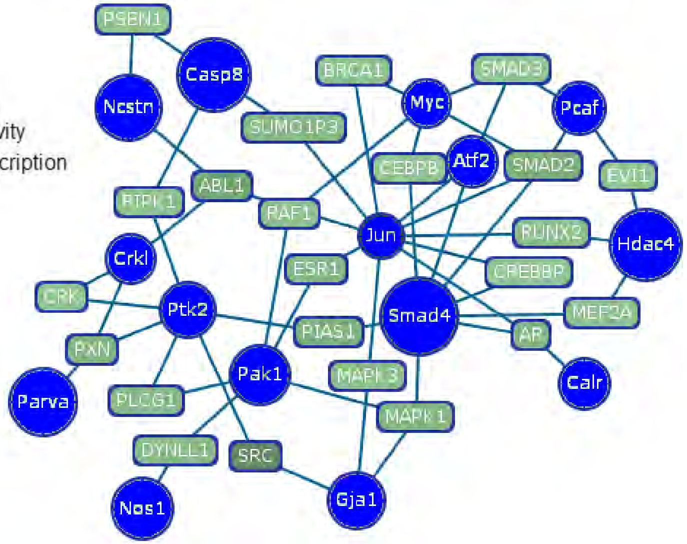
Urzúa et al. (2010) Mol Biosyst 6(12):2521-8

Protein networks in MOSE cells

A

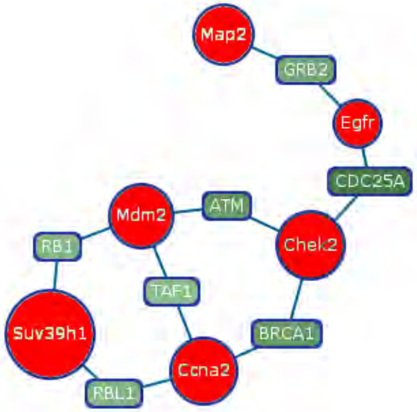
	Myc	Jun	Hdac4	Smad4	App	Atf2	Kat2b	Camk4	Ptk2	Akt1	Nos1	Parva	Pak1	Syt1
transcription regulator activity	🟨	🟨	🟨	🟨	⬛	🟨	🟨	🟨	⬛	⬛	⬛	⬛	⬛	⬛
positive regulation of transcription	🟨	🟨	🟨	🟨	🟨	⬛	🟨	⬛	⬛	⬛	⬛	⬛	⬛	⬛
DNA binding	🟨	🟨	🟨	🟨	🟨	🟨	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
cell projection	🟨	⬛	⬛	⬛	🟨	⬛	⬛	⬛	🟨	🟨	🟨	🟨	🟨	🟨

	Jun	Akt1	Crkl	Ptk2	Myc	Fn1	Parva	Atf2
Focal adhesion	🟨	🟨	🟨	🟨	⬛	🟨	🟨	⬛
ErbB signaling pathway	🟨	🟨	🟨	🟨	🟨	⬛	⬛	⬛
MAPK signaling pathway	🟨	🟨	🟨	⬛	🟨	⬛	⬛	🟨
Renal cell carcinoma	🟨	🟨	🟨	⬛	⬛	⬛	⬛	⬛
T cell receptor signaling pathway	🟨	🟨	⬛	⬛	⬛	⬛	⬛	⬛



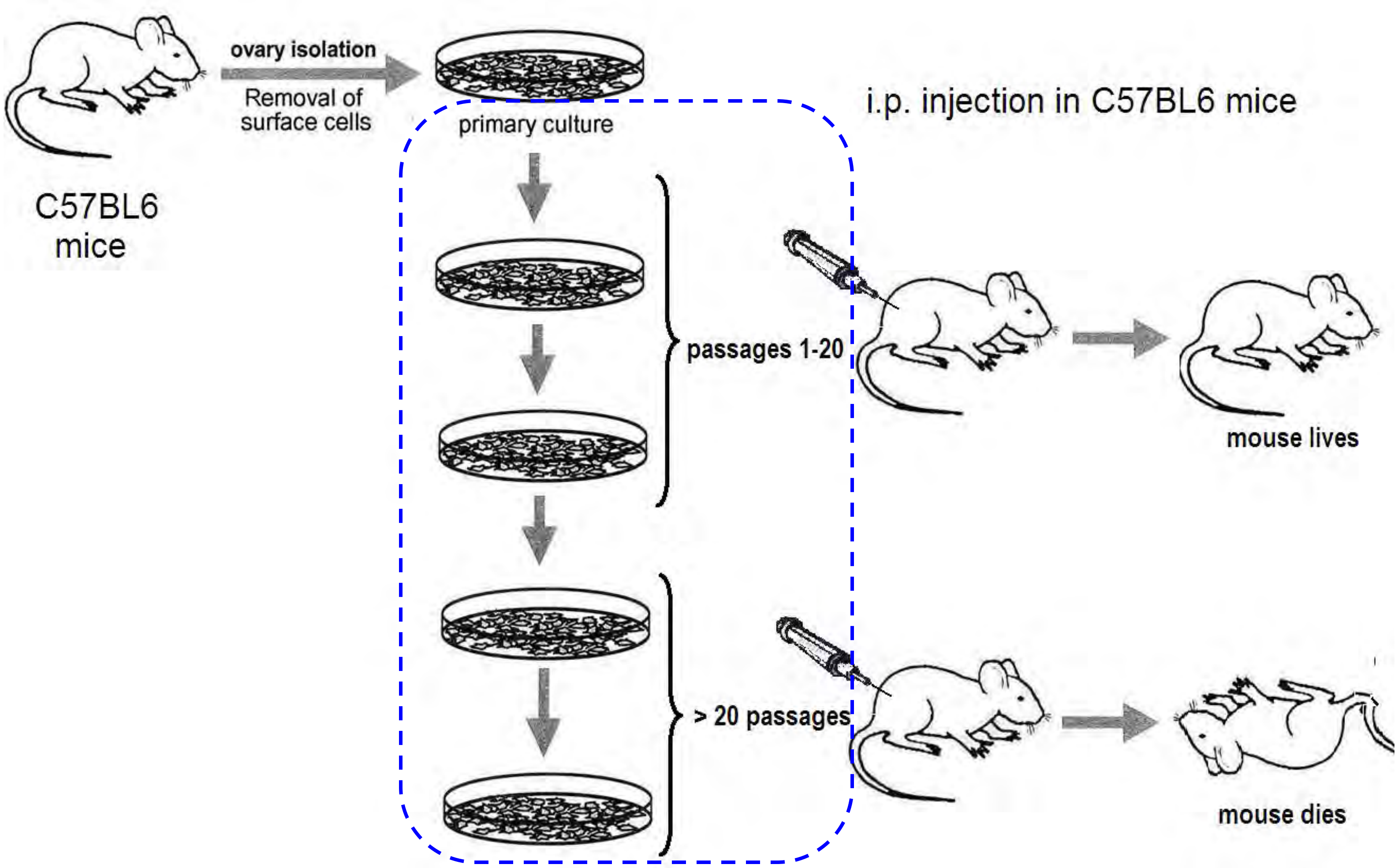
B

	Suv39h1	H3f3a	Ccna2	Sept4	Chek2	Mdm2	Egfr	Mtap2
cell cycle	🟦	⬛	🟦	🟦	🟦	🟦	⬛	⬛
organelle organization	🟦	🟦	🟦	🟦	⬛	⬛	⬛	🟦
nuclear chromatin	🟦	🟦	⬛	⬛	⬛	⬛	⬛	⬛
DNA packaging	🟦	🟦	⬛	⬛	⬛	⬛	⬛	⬛
p53 transcription	⬛	🟦	⬛	⬛	⬛	🟦	🟦	⬛



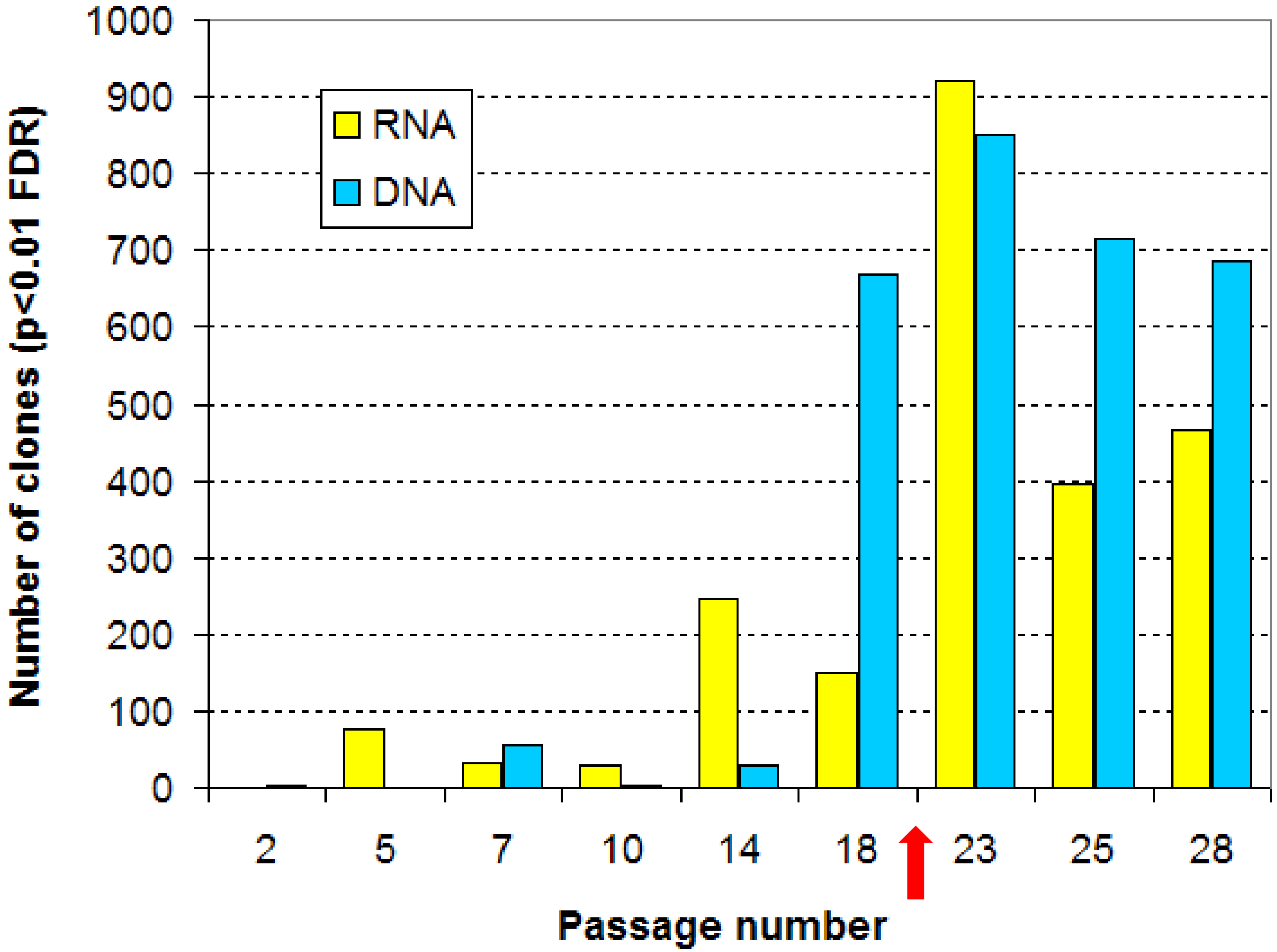
Urzúa et al. (2010) Mol Biosyst 6(12):2521-8

A syngeneic mouse model of ovarian cancer



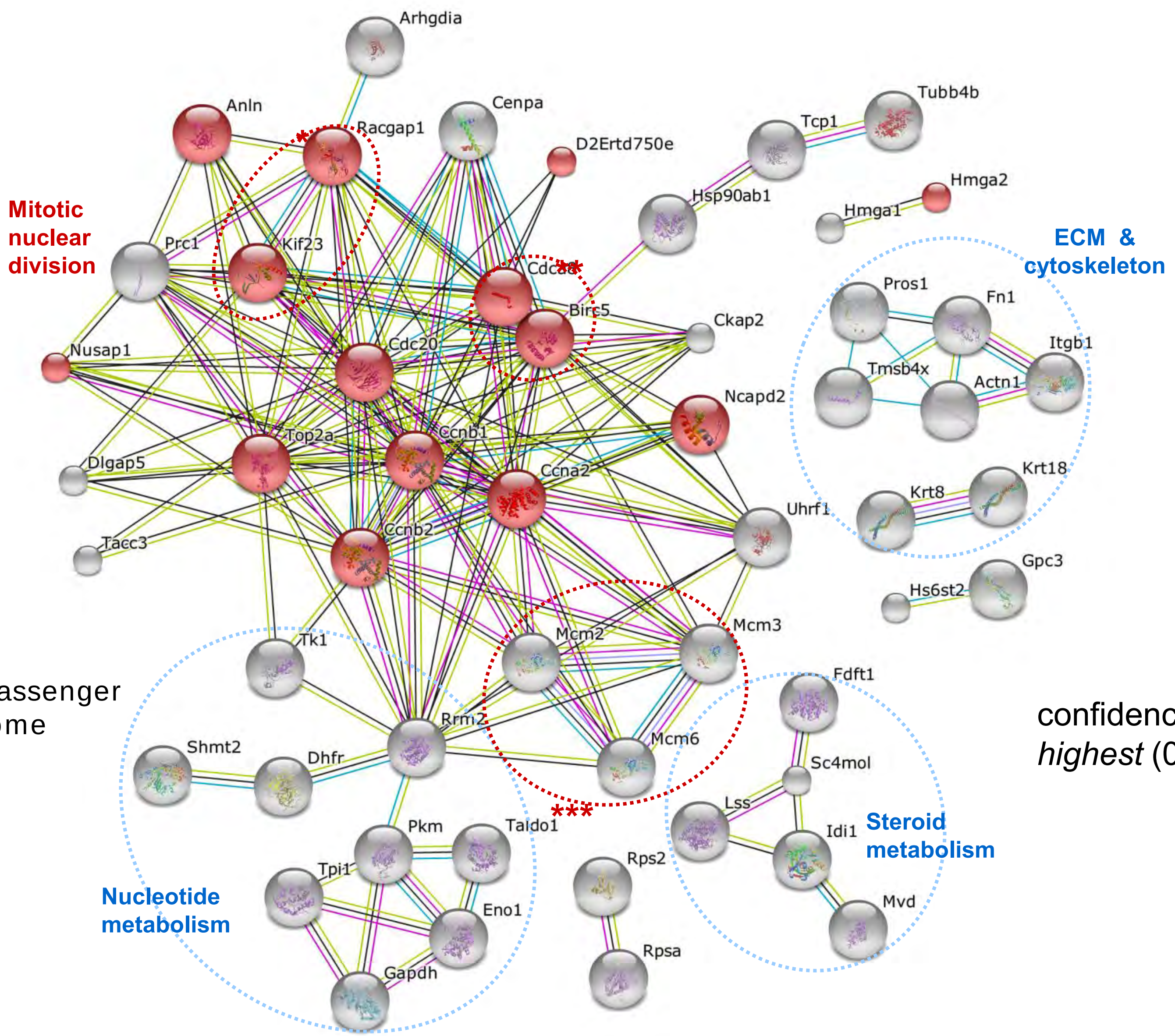
Roby et al. (2000)

Summary of transcriptional and genomic changes



Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

Protein-protein interaction network at passage 14 - Upregulated

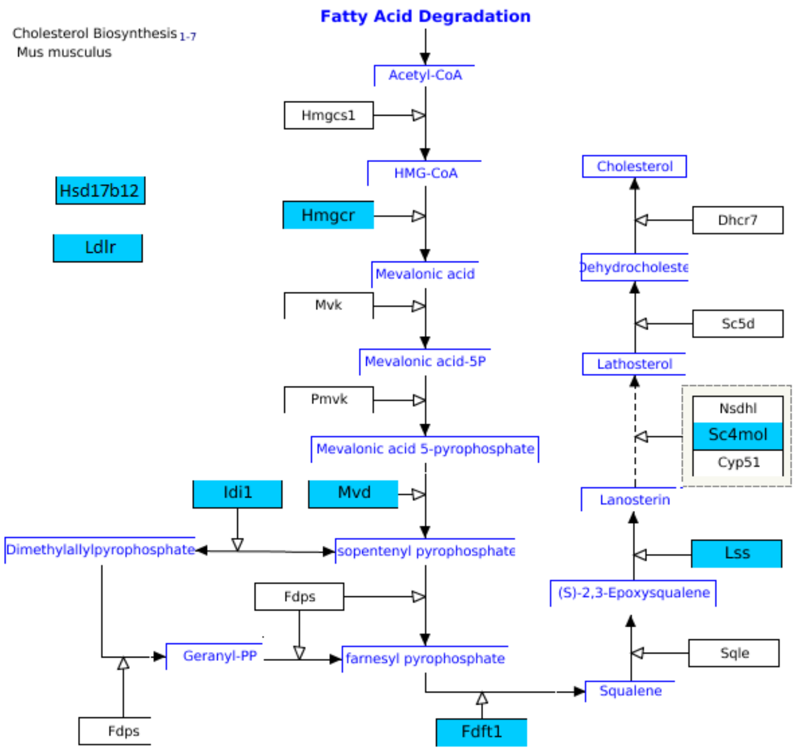


(*) centralspindlin,
(**) chromosome passenger
(***) minichromosome
-maintenaince

confidence score
highest (0.900)

Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

Pathways altered prior to early aneuploidies (up)



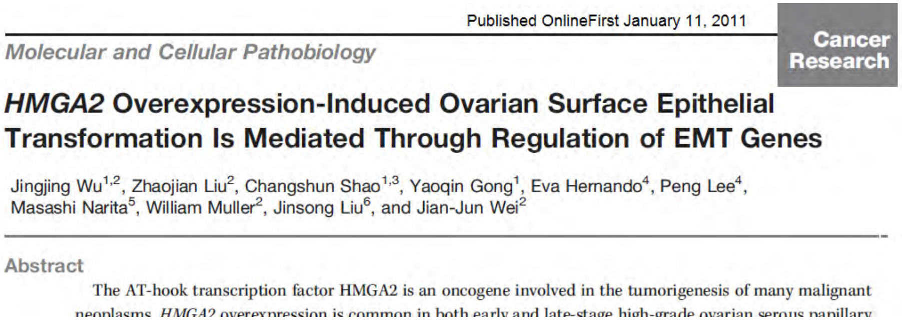
Cytoskeleton (22 genes, adjp 5.6e-7)

Actn1, Anln, Anxa2, Msn, Triobp, Fscn1

Krt18, Krt8, Tmsb4x, Abi2, Tubb2c, Csrp1

Chromosome (14 genes, adjp 2.8e-7)

H2afz, Hist1h2ao, Hist1h4n, Hmga1, Hmga2, Hmgb2, Hmgcr, Hmgn2, Mcm2, Mcm3, Mcm6



Published OnlineFirst January 11, 2011

Molecular and Cellular Pathobiology

Cancer Research

HMGA2 Overexpression-Induced Ovarian Surface Epithelial Transformation Is Mediated Through Regulation of EMT Genes

Jingjing Wu[1,2], Zhaojian Liu[2], Changshun Shao[1,3], Yaoqin Gong[1], Eva Hernando[4], Peng Lee[4], Masashi Narita[5], William Muller[2], Jinsong Liu[6], and Jian-Jun Wei[2]

Abstract

The AT-hook transcription factor HMGA2 is an oncogene involved in the tumorigenesis of many malignant neoplasms. *HMGA2* overexpression is common in both early and late-stage high-grade ovarian serous papillary

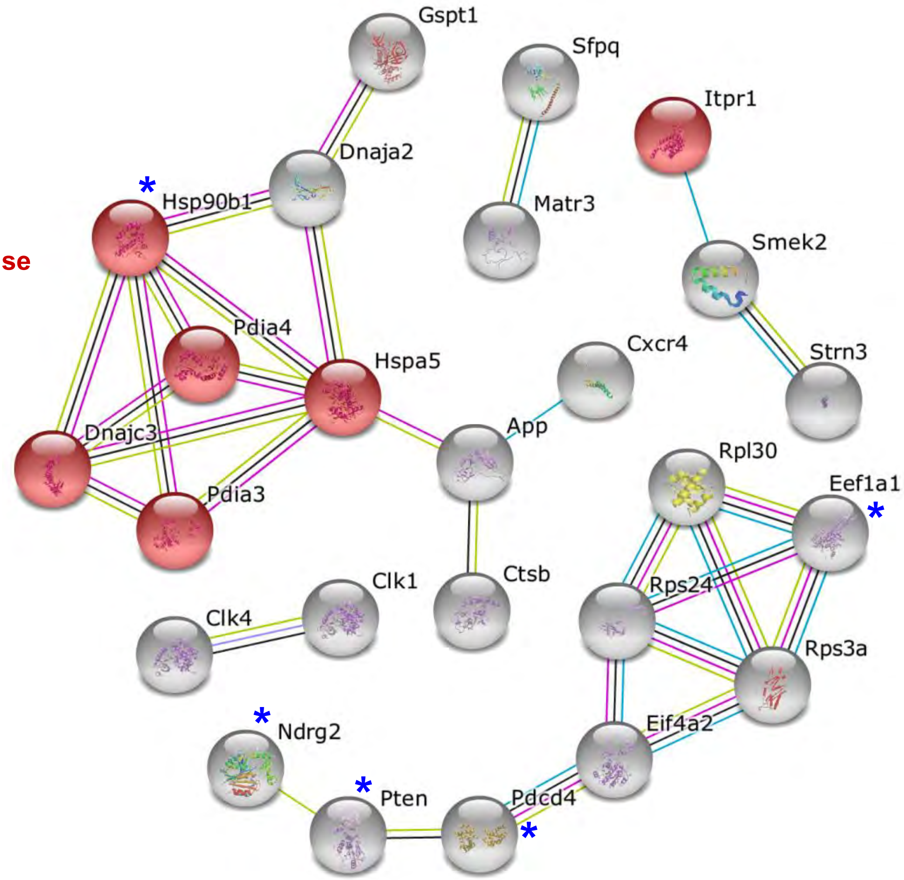
Cenpa, Top2a, Ncapd2, Nusap1, Chaf1b

Cell cycle (22 genes, adjp 4.2e-9)

Ccna2, Ccnb1, Ccnb2, Cdc20, Cdc5l, Cdca8,

Prc1, Birc5, Ncapd2, Racgap1, Dlgap5, Tacc3, Ckap2, Kif23

Protein-protein interaction network at passage 14 - Downregulated

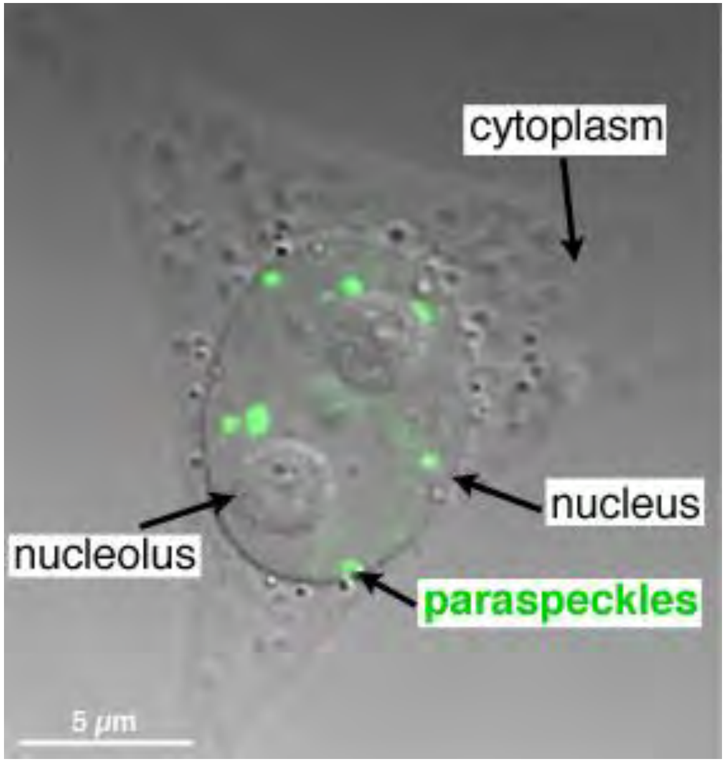


Response to ER stress

(*) putative tumor suppressor genes

confidence score
high (0.700)

Pathways altered prior to early aneuploidies (down)



mRNA processing (11 genes, adjp 4.8e-9)

Ddx3x, Ttc14, Clk4, Rps24, Eif4a2, Sfpq, Matr3, Clk1, Rbms1, Srsf11, Zfml

Nuclear bodies (5 genes, adjp 3.5e-3)

Malat1, Neat1, Sfpq, Atrx, Crebbp

Response to ER stress (6 genes, adjp 7.5e-3)

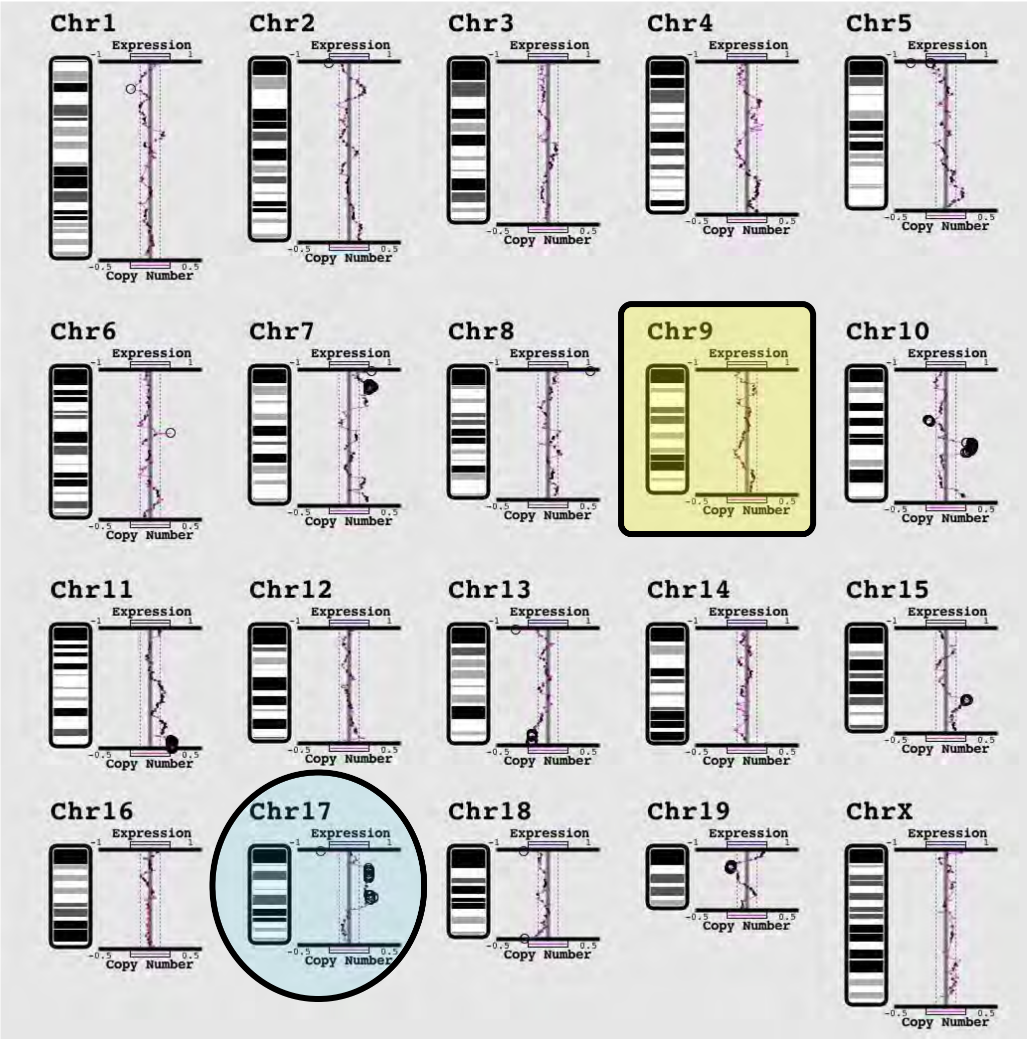
Hspa5, Pdia3, Pdia4, Itpr1, Dnajc3, Hsp90b1

Tumor suppression (9 genes, adjp 3.5e-3)

Crebbp, Ddx3x, Eef1a1, Pten, Hsp90b1, Arhgef12, Nupr1, Pdcd4, Ndrg2

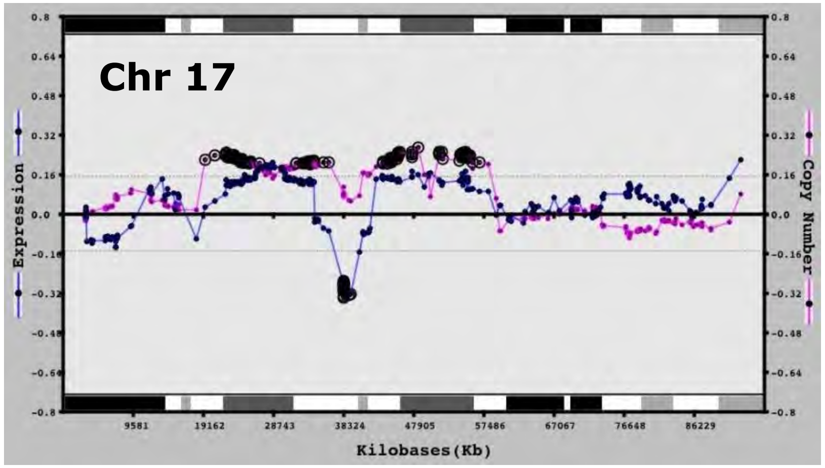
Urzua et al (2016) BMC Genomics - accepted

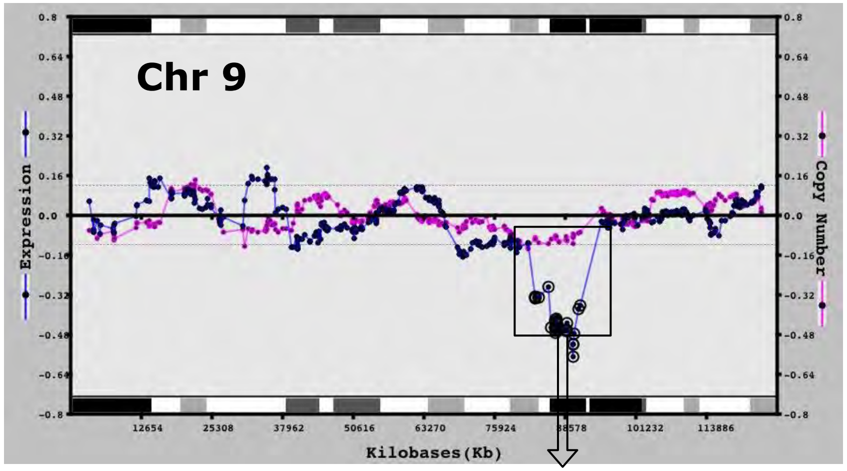
Microarray-CGH of MOSE cells at passage 18



Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

DNA and RNA data correlation at passage 18





Protein kinases

Pkmyt1, Pdpk1, Mapk8ip3, Pacsin1, Mapk13, Mapk14

Proteases (15 genes)

Mmp25, Prss21-22-27-28-29-32-33-34-41,Tmprss3-8

tRNAs (9 genes), Mesothelin, Telo2

Cell cycle (11 genes)

Ccnd3, Cdc5l, Cul7, Cul9, Dazl, Ubr2, Zfp318

Nuclear chromosome

Satb1, Sgol1, Klhdc3, Pot1b

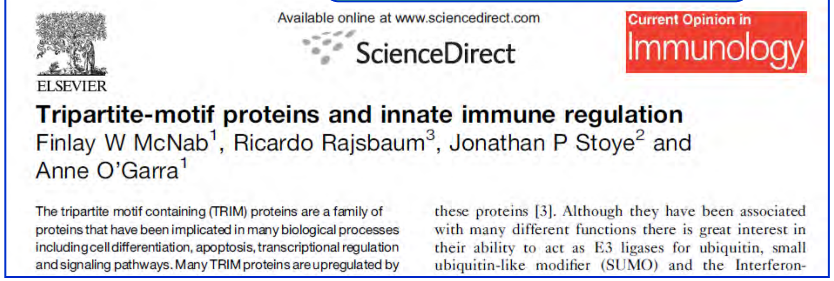
Vegfa, Runx2, Foxp4, Rab5a, Mir693

BCL-related proteins

Bcl2a1a, Bcl2a1b, Bcl2a1d

Tripartite motif

Trim43a, Trim43b, Trim43c



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ScienceDirect

Current Opinion in Immunology

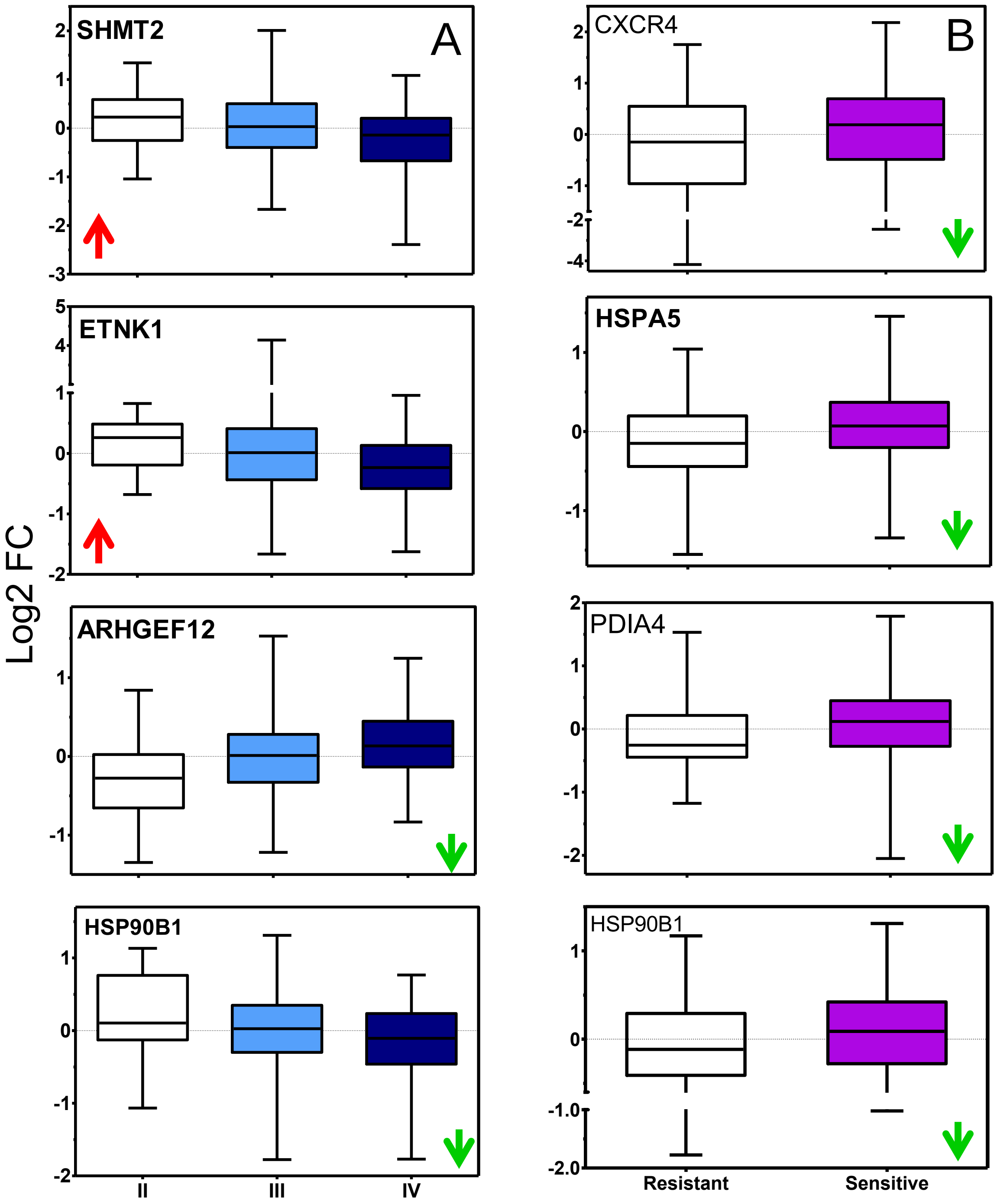
Tripartite-motif proteins and innate immune regulation

Finlay W McNab[1], Ricardo Rajsbaum[3], Jonathan P Stoye[2] and Anne O'Garra[1]

The tripartite motif containing (TRIM) proteins are a family of proteins that have been implicated in many biological processes including cell differentiation, apoptosis, transcriptional regulation and signaling pathways. Many TRIM proteins are upregulated by these proteins [3]. Although they have been associated with many different functions there is great interest in their ability to act as E3 ligases for ubiquitin, small ubiquitin-like modifier (SUMO) and the Interferon-

Urzua et al (2016) BMC Genomics - accepted

Results 7: Datamining of mouse genes in ovarian cancer TCGA data.



Urzua et al - BMC Genomics 2016 Oct 25;17(Suppl 8):728

Ovarian tumor & reproductive parameters

BALB/c

C57BL6

SWR

FVB

Spontaneous ovarian tumors and reproductive phenotypes

Strain	***Tumor frequency****	***Litter size***	***Number of litters***	***Productive matings (%)***	***Relative fecundity ¶***
BALB/c	3.80	4.9	3.6	55.6	9.80
C57BL/6	1.60	6.6	3.8	87.4	21.9
FVB	7.00	9.5	4.8	90.0	41.0
SWR	57.0	7.5	2.3	58.3	10.1

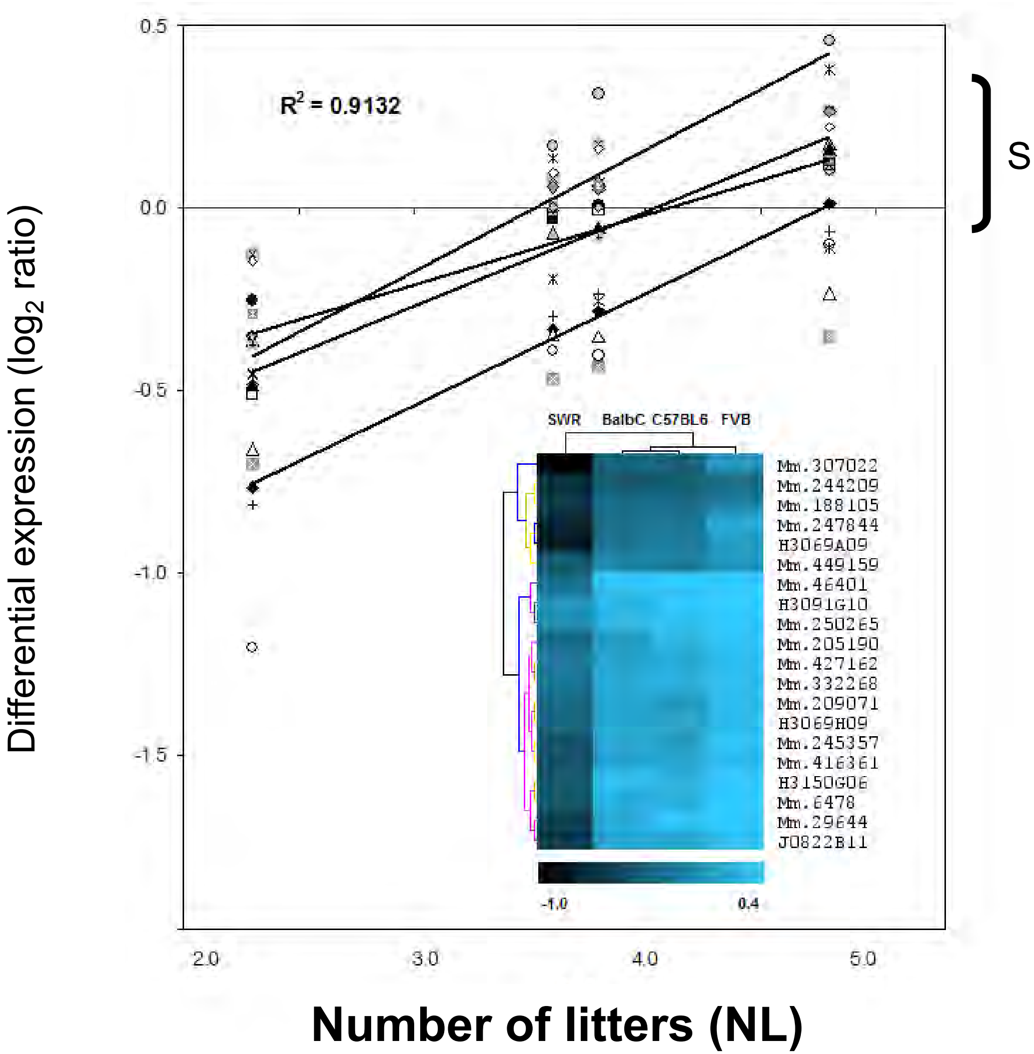
*** Spontaneous tumors**

¶ Derived from LS, NL and PM

MTB
Mouse Tumor Biology Database

MPD
MOUSE PHENOME DATABASE

Correlation – an example

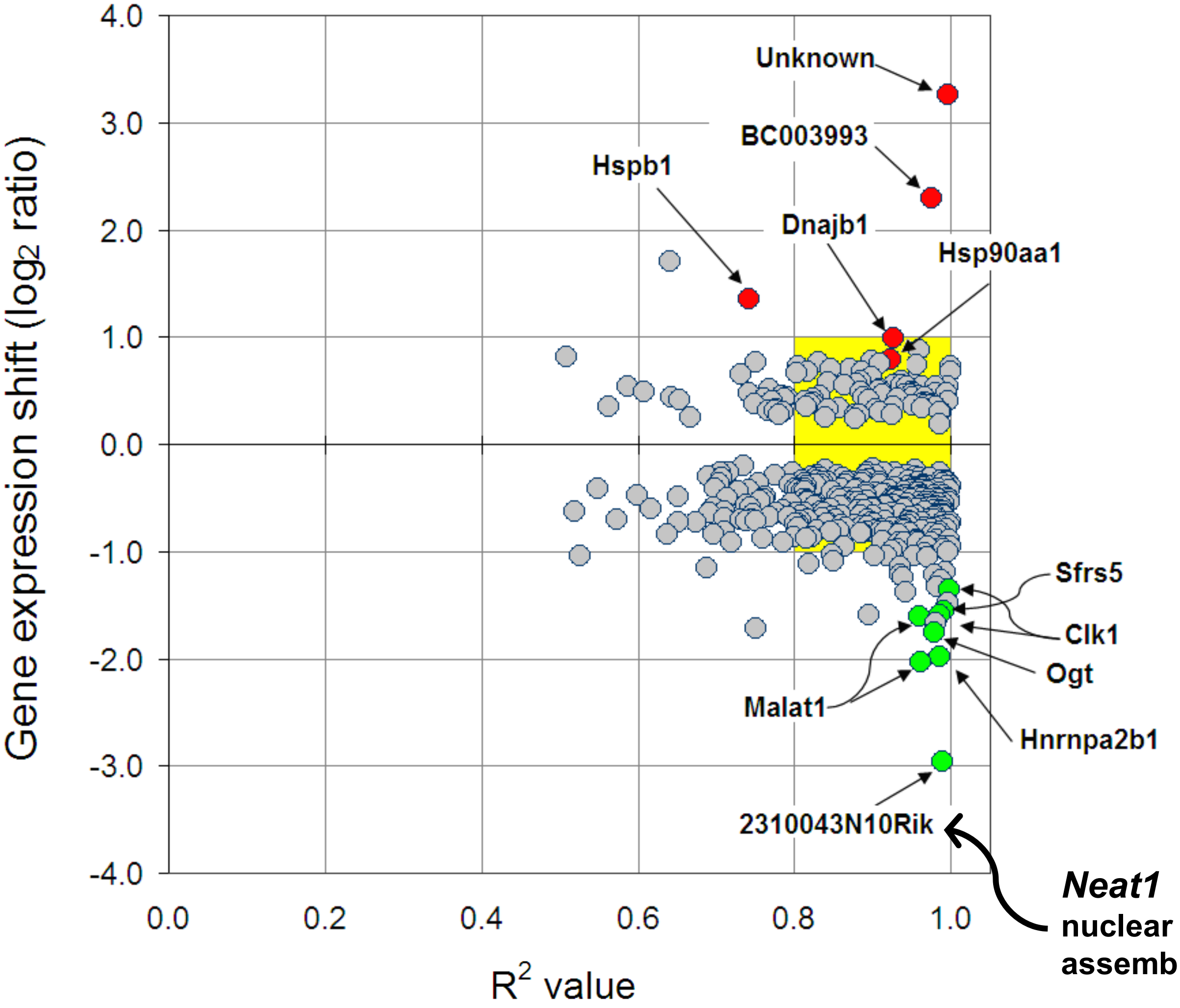


Shift gene A

Shift gene B

Urzúa et al - BMC Genomics. 2010
Dec 22;11 Suppl 5:S1

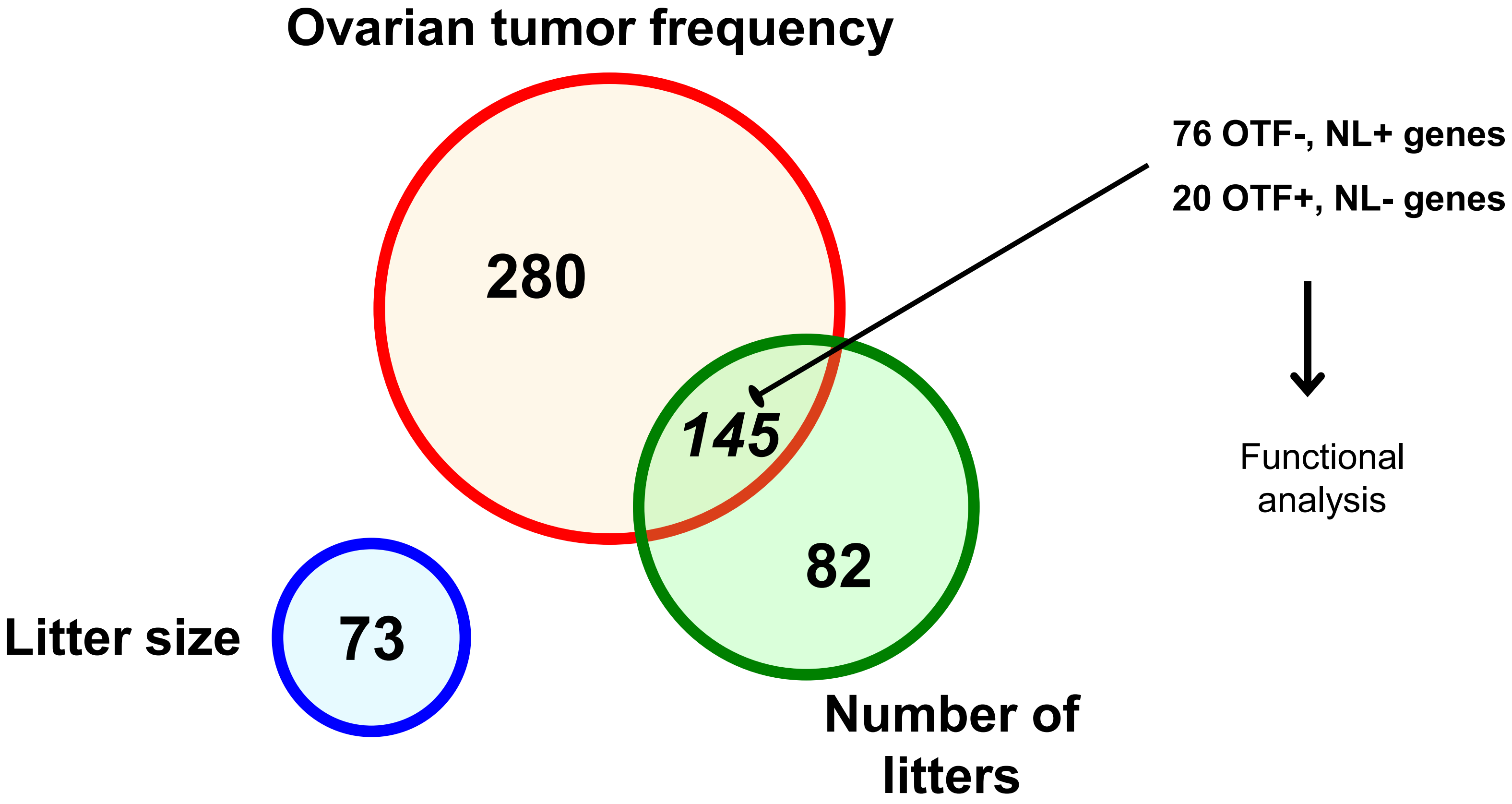
Strength of OTF correlation respective to transcriptional shift



Neat1
nuclear paraspeckle
assembly transcript 1

Urzúa et al - BMC Genomics. 2010
Dec 22;11 Suppl 5:S1

Ovarian expression profiles - phenotypes



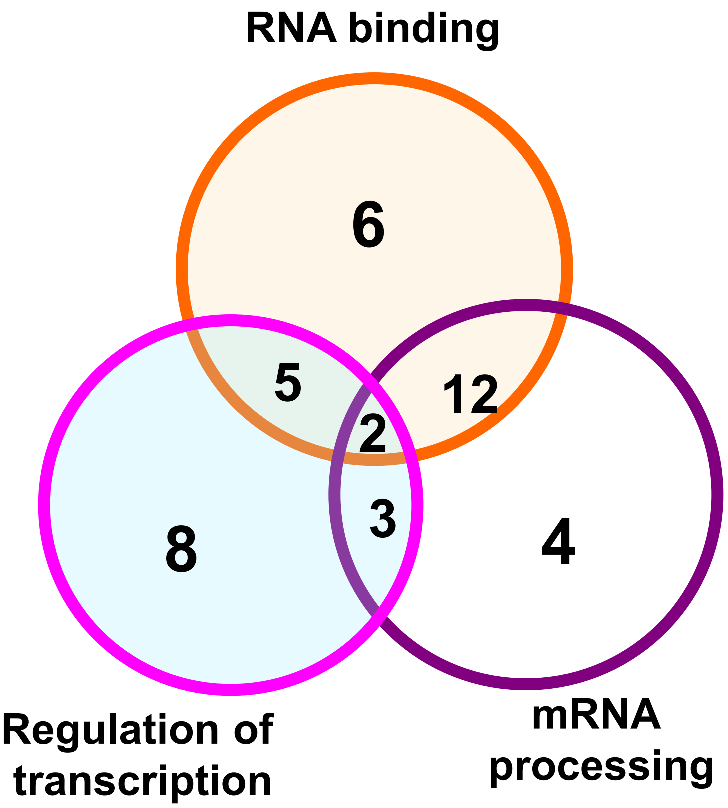
76 OTF-, NL+ genes

20 OTF+, NL- genes

↓

Functional analysis

Functional analysis OTF-, NL+



40 of 76 genes, 6 major funtions

Genes ¶	***Tissue/ System***	***Function/ Disease***
Cpsf6 Ddx17 Arnt Sfrs6 Sfrs2 Fubp1 Rbm39 Rbm25 Hnrpa2b1	Ovary	ovarian cancer ER-alpha transcription C-myc transcription apoptosis Bcl-x splicing antiangiogenic VEGF cisplatin resistance
Rbm26 Son Mll3	Blood	lymphomas leukemogenesis
Sfrs7 Tra2a	Endocrine	GnRH mRNA splicing scavenger recep. splicing
Pabpn1 Mycbp2	Muscle	dystrophy neuromuscular dev

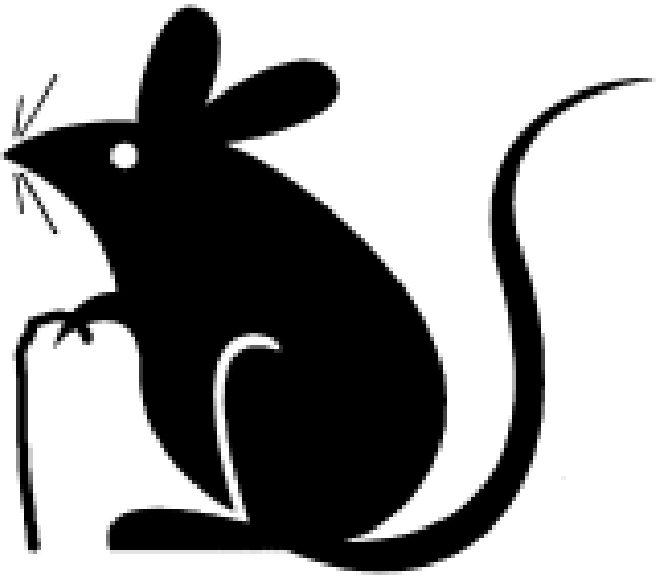
Ovarian parity genes compared to parous breast

CCNL1, FUBP1, HNRPDL, HNRNPA2B1, LUC7L3, MALAT1, MDM4, RBM25, NEAT1, RBM6, OGT ↑

↑ AKAP8, ARNT, CLK2, DMTF1, MLL3, LUC7L2, MYCBP2, NFAT5, ZC3H11A, PTEN, RBM39, RBM4, SRRM1, SON RPS6KB1, USP15, VPS37A, XRCC3, ACCSL, DDX3X, IL20RB, IRF3, PHIP MSL1, NXF1, POC5, RHOA, RNF14

↓ FLNA, HSP90AA1, LMO1, P4HB, GSS SALL4, SLC44A1, SPIN1, ATP1A1, DCN, FANCM, KIFC3, LDHB, MSLN

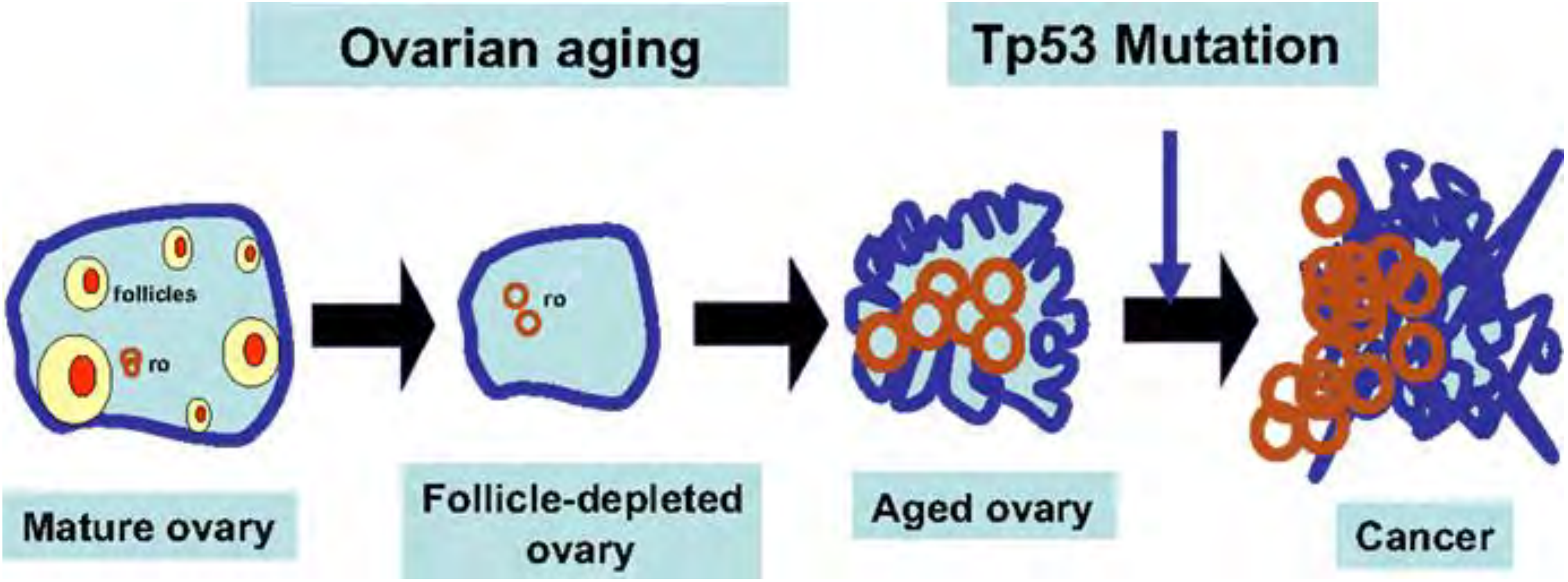
Aplicaciones biomédicas 7



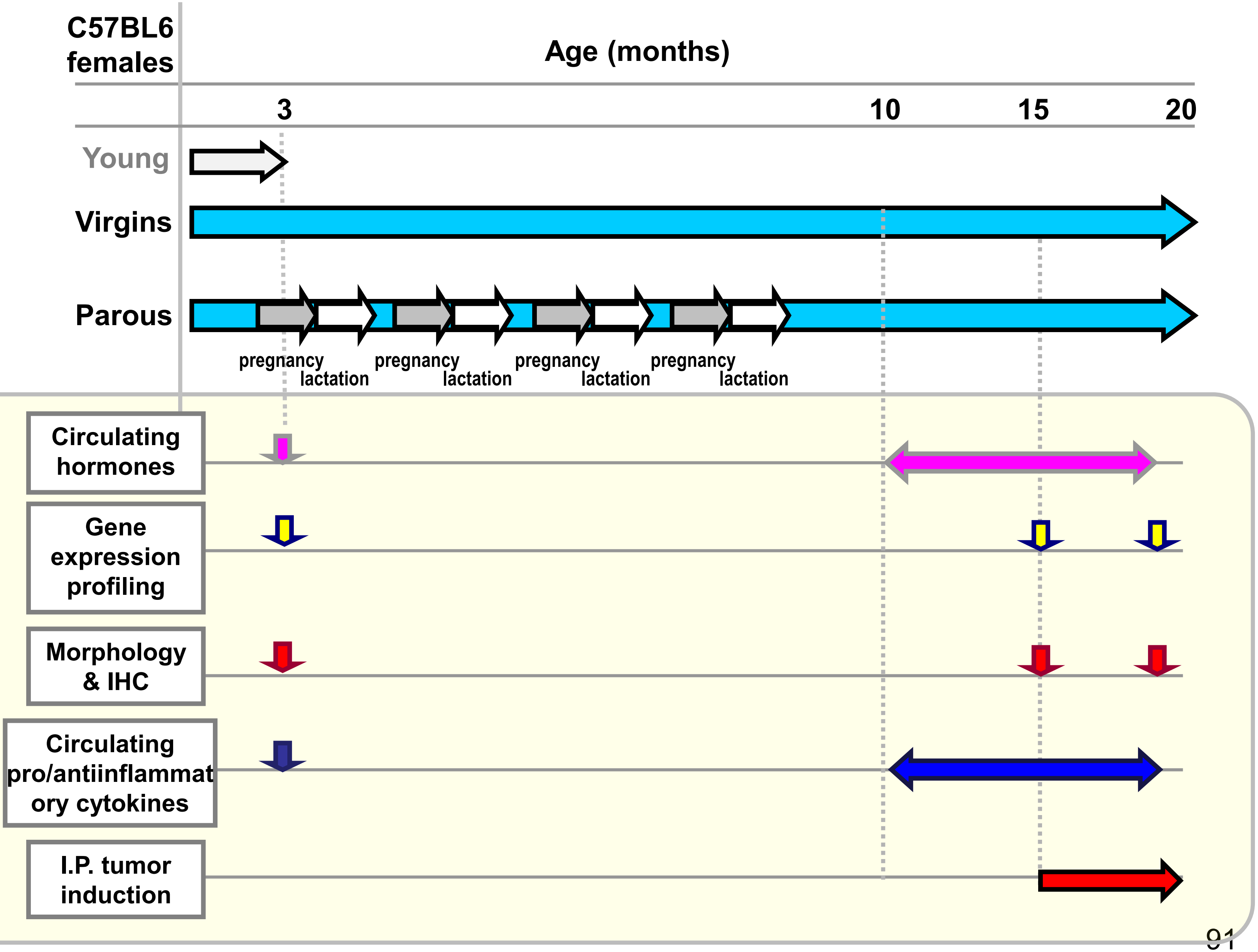
An ovarian cancer mouse model at estropausal age

Fondecyt 1130292

Working model for follicle depletion and ovarian aging in ovarian tumorigenesis



Smith et al. Front Oncol. 2014 Feb 26;4:36.

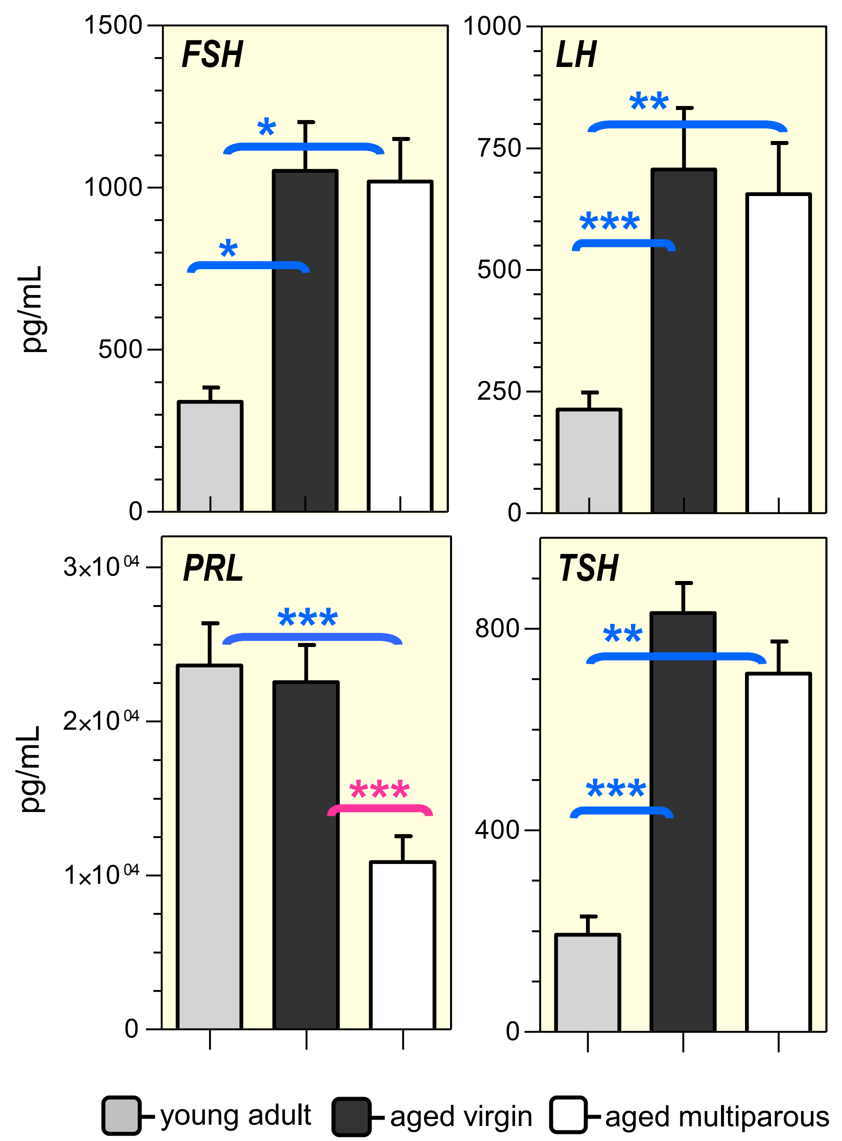

C57BL6 females
Age (months)
3
10
15
20
Young
Virgins
Parous
pregnancy
lactation
pregnancy
lactation
pregnancy
lactation
pregnancy
lactation
Circulating hormones
Gene expression profiling
Morphology & IHC
Circulating pro/antiinflammatory cytokines
I.P. tumor induction

Circulating pituitary hormones

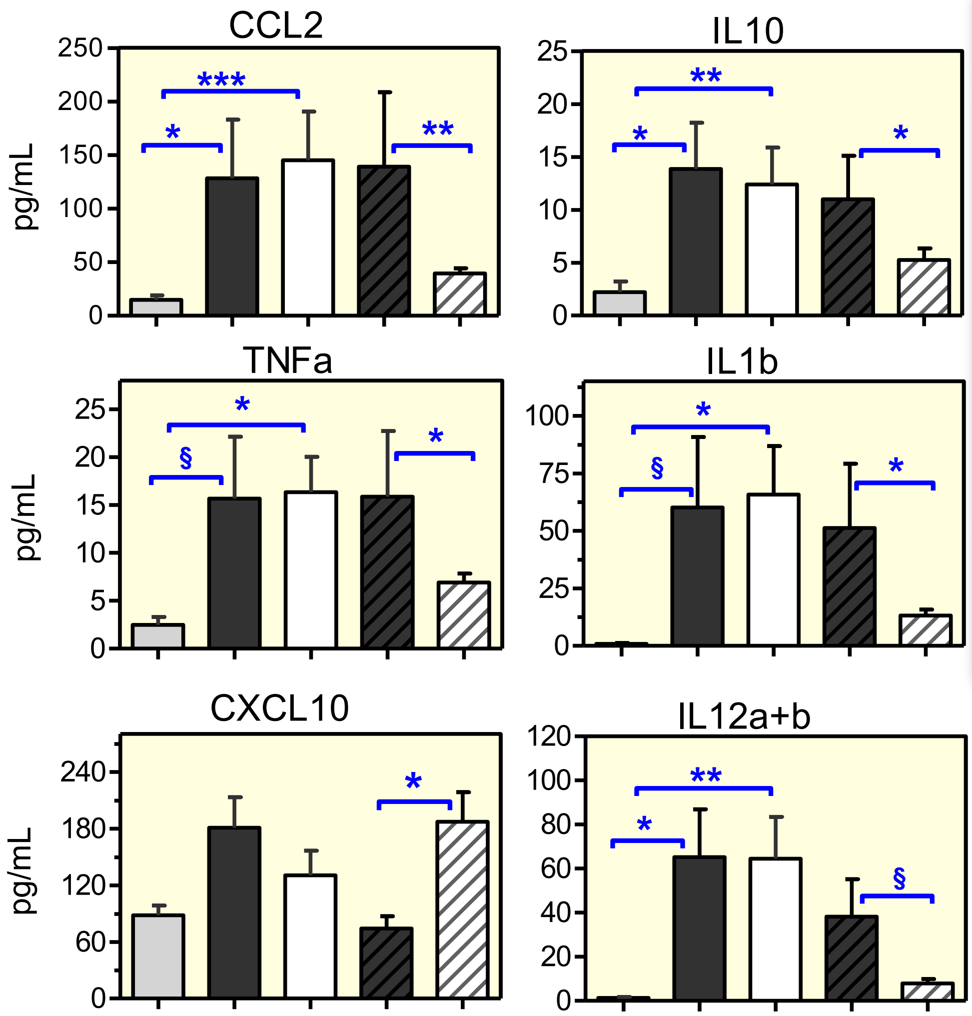
Milliplex magnetic bead assay -

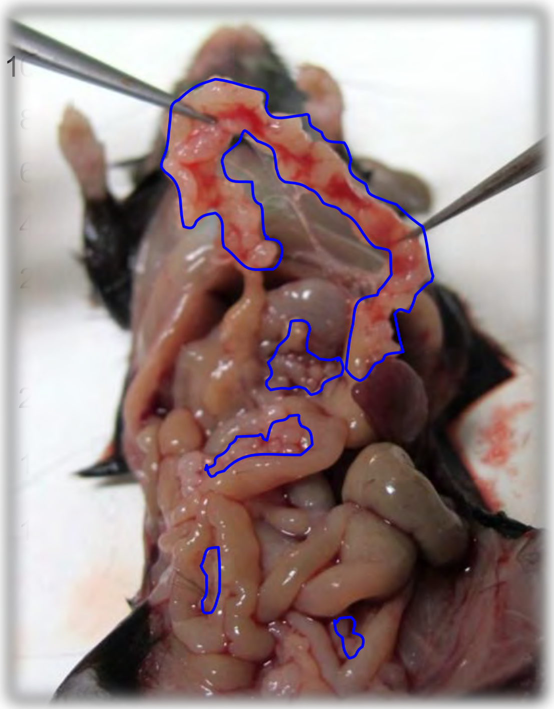
(*) $p < 0.05$

(**) $p < 0.01$



Circulating cytokines, chemokines and interleukins





- ▩ young adult
- ■ aged virgin
- ☐ aged multiparous
- ▨ aged virgin - induced
- ▧ aged multiparous - induced

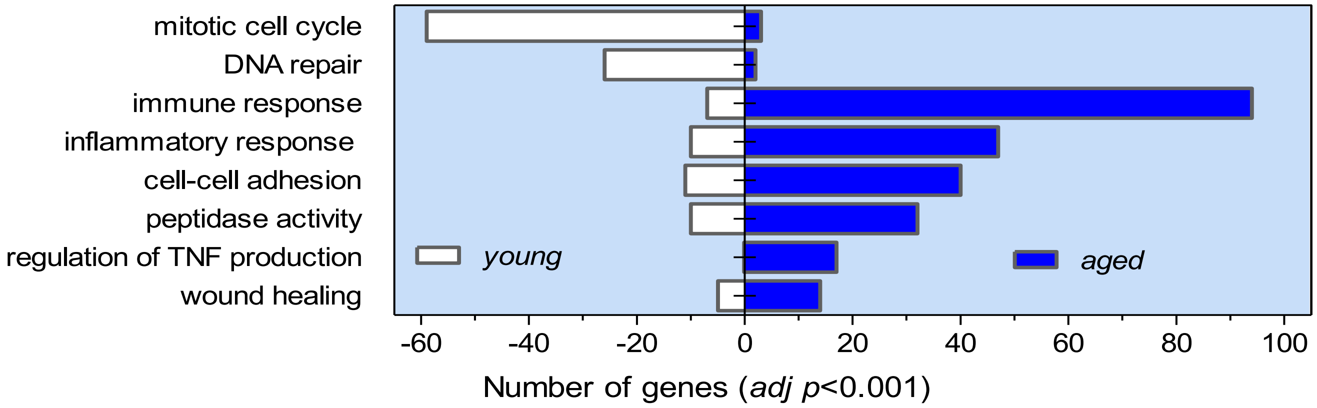
Milliplex magnetic bead assay

(*) $p < 0.05$ (**) $p < 0.01$

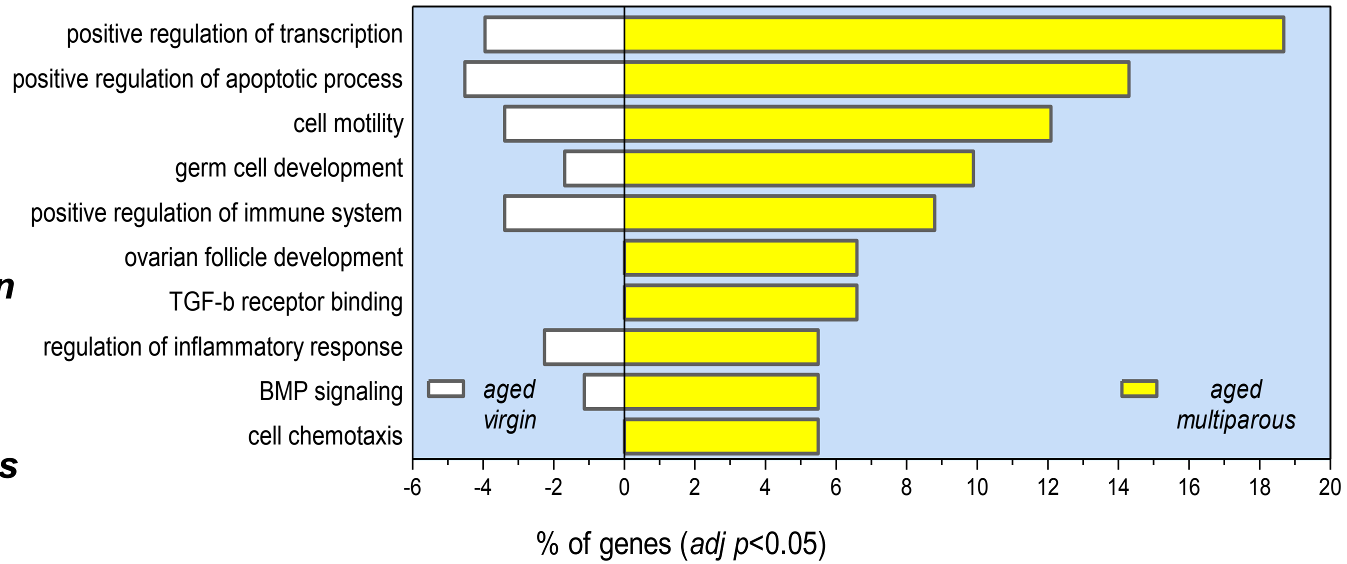
Urzua et al – Aging Dis 2017 Oct 1;8(5):546-557

Gene expression profiling between young and aged ovaries

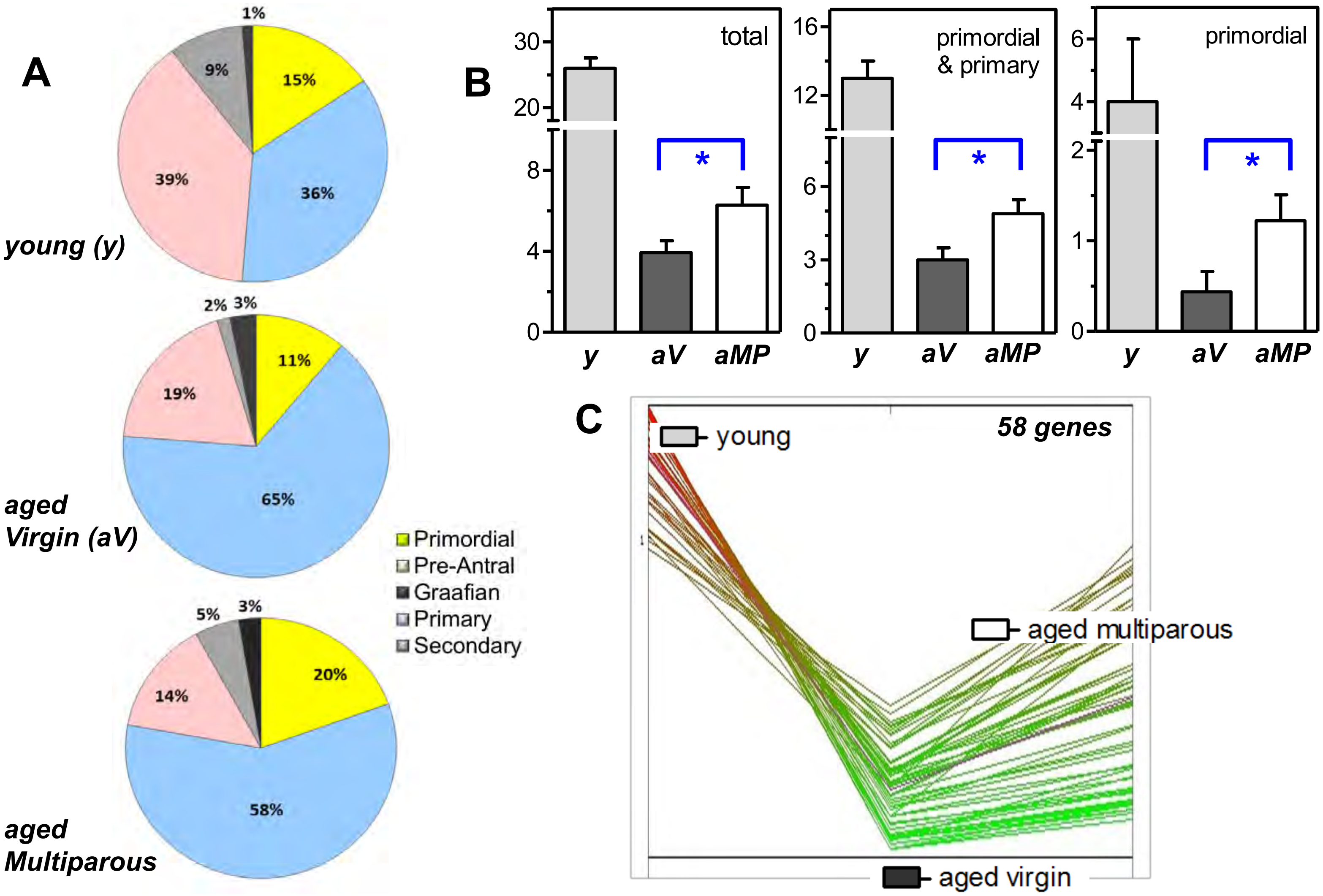
Young vs aged



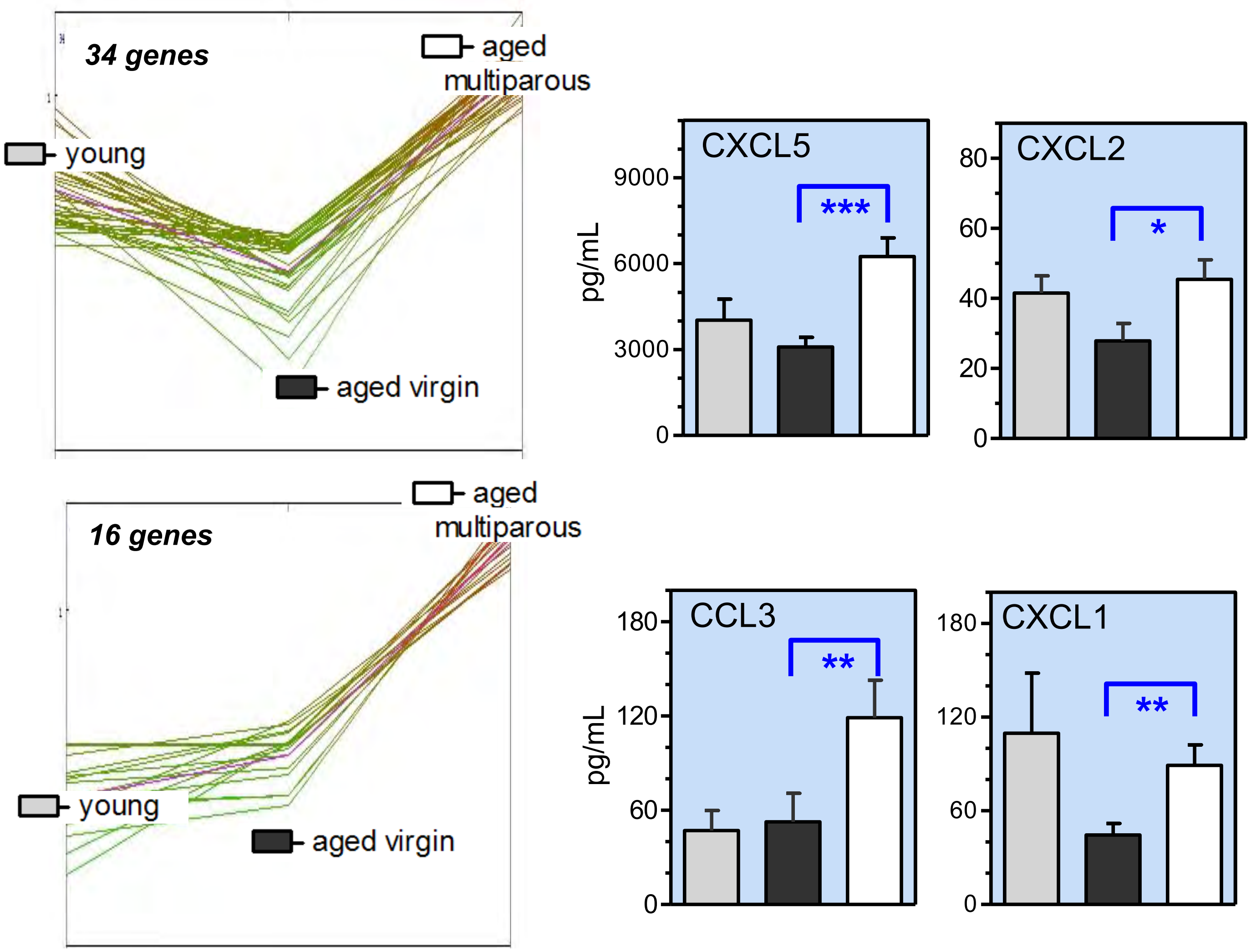
Aged Virgin vs Aged Multiparous



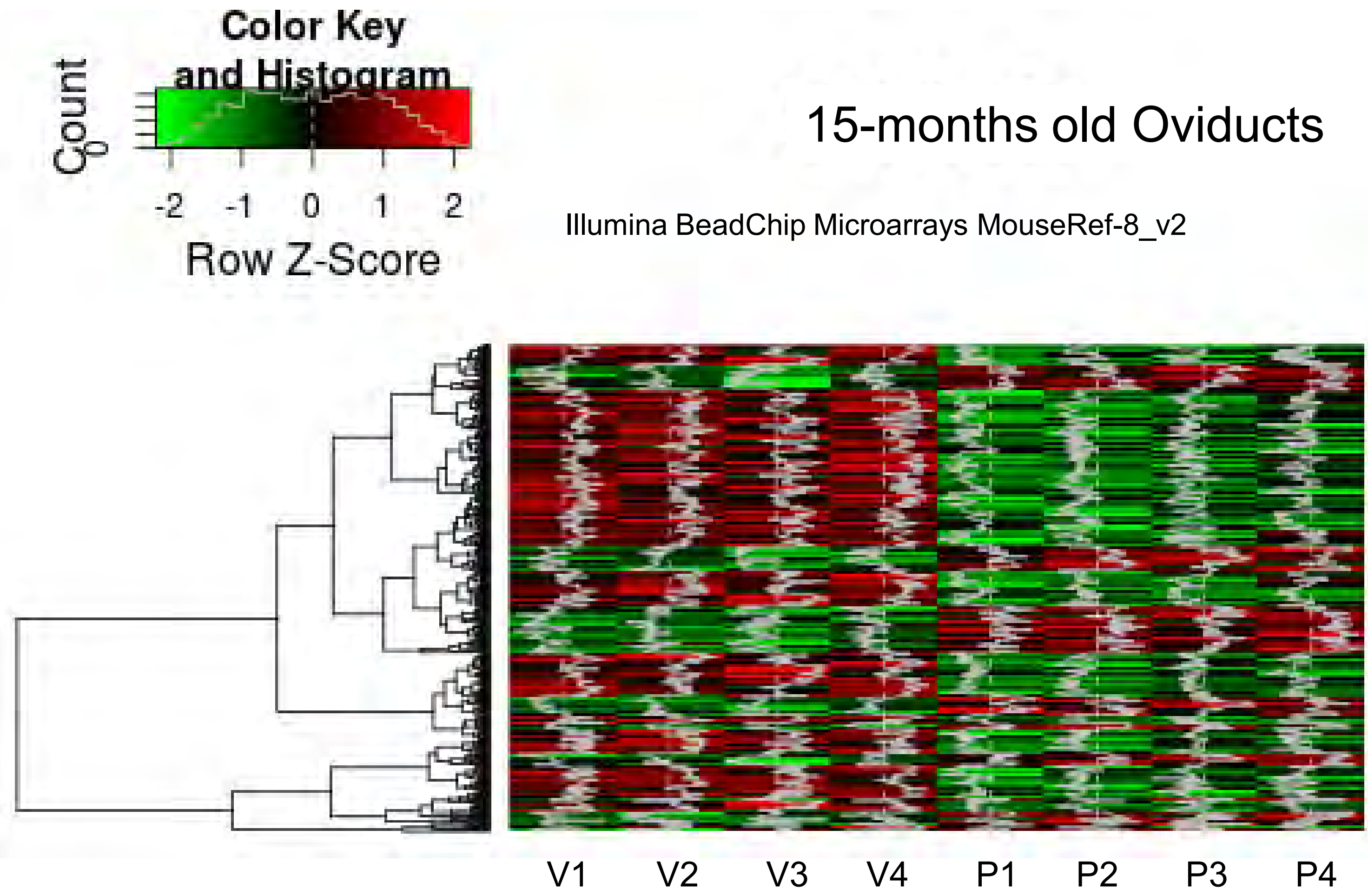
Follicle gene signature in aged ovaries according to parity status



Cell immunity-related gene signature in aged ovaries according to parity status



Gene expression profiling - Oviduct



15-months old Oviducts

Illumina BeadChip Microarrays MouseRef-8_v2

PI3K-Akt signaling pathway (8)

Tight junctions (8)

IFN alpha signaling (4)

T-test (permutations) → 187 DEG p<0,05 FC +/- 1.5