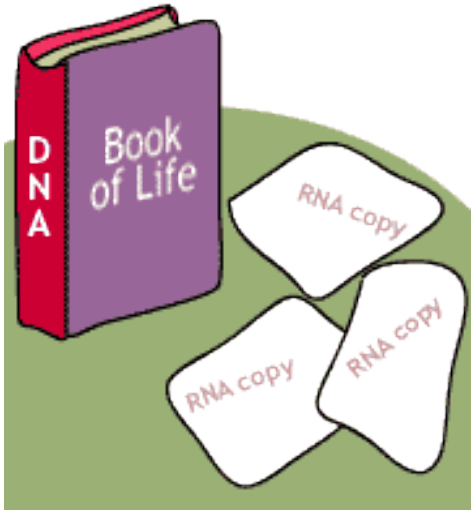
DNA MICROARRAYS: EXPERIMENTOS COMPARATIVOS

JUAN CARLOS OLIVEROS COLLAZOS

BioinfoGP, CNB-CSIC

GENOMAS, GENES Y PROTEÍNAS



nobelprize.org

GENOMA:

CONJUNTO DE TODOS LOS GENES DE UN ORGANISMO

GEN:

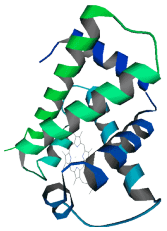
UNIDAD DE ALMACENAMIENTO DE INFORMACIÓN GENÉTICA

CUALQUIER ELEMENTO DEL CROMOSOMA MEDIANTE EL CUAL SE TRANSMITEN LOS CARACTERES DE UN ORGANISMO

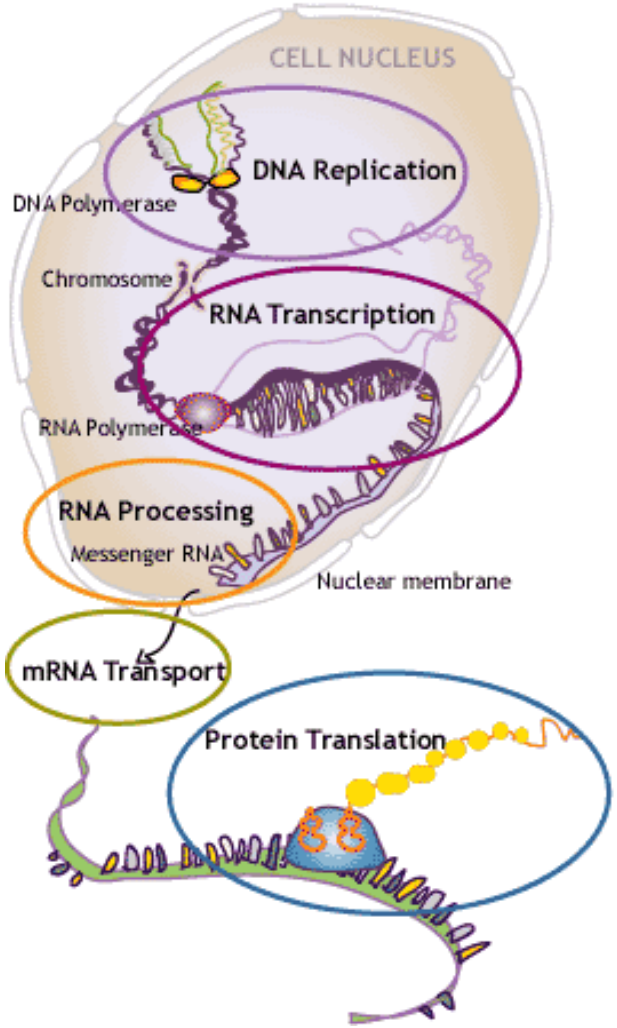
SECUENCIA DE ADN (O ARN), ESENCIAL PARA UNA DETERMINADA FUNCIÓN FISIÓLÓGICA

PROTEÍNA:

SECUENCIAS DE AMINOÁCIDOS QUE ACTUAN COMO ENZIMAS, ELEMENTOS ESTRUCTURALES, HORMONAS, ETC.

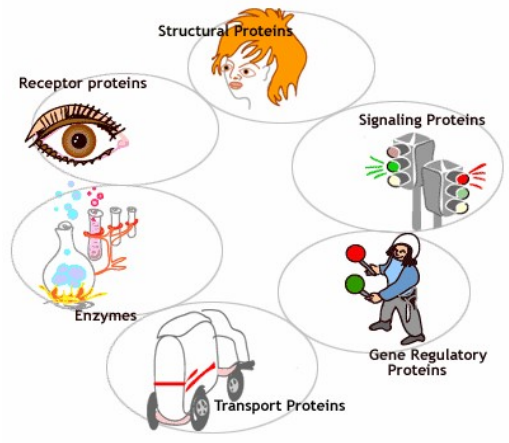


GENOMAS, GENES Y PROTEÍNAS



DEL GENOMA
- **ESTÁTICO, ÚNICO**

AL PROTEOMA
- **DINÁMICO, MÚLTIPLE**



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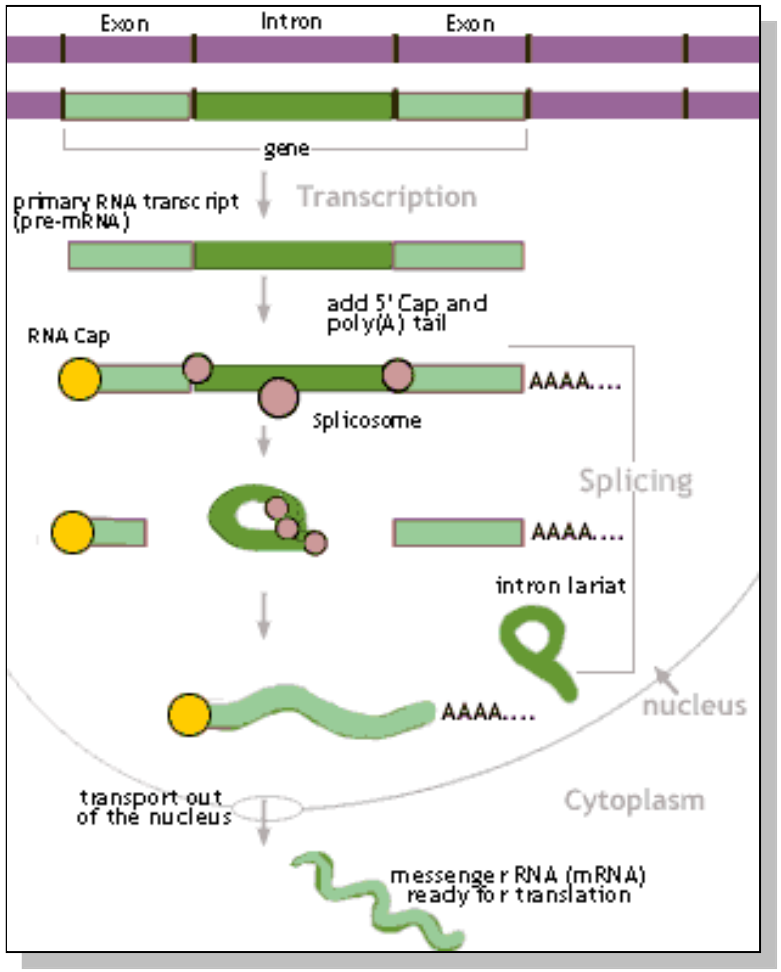
REGULACIÓN DE LA ACTIVIDAD GÉNICA



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VARIOS NIVELES:

- **TRANSCRIPCIÓN**
- **MADURACIÓN**
- **TRANSPORTE AL CITOPLASMA**
- **DEGRADACIÓN**
- **TRADUCCIÓN**
- **POST-TRADUCCIÓN**

REGULACIÓN DE LA ACTIVIDAD GÉNICA

LOS GENES NO ACTÚAN DE FORMA AISLADA

EXISTEN REDES DE INTERACCIÓN

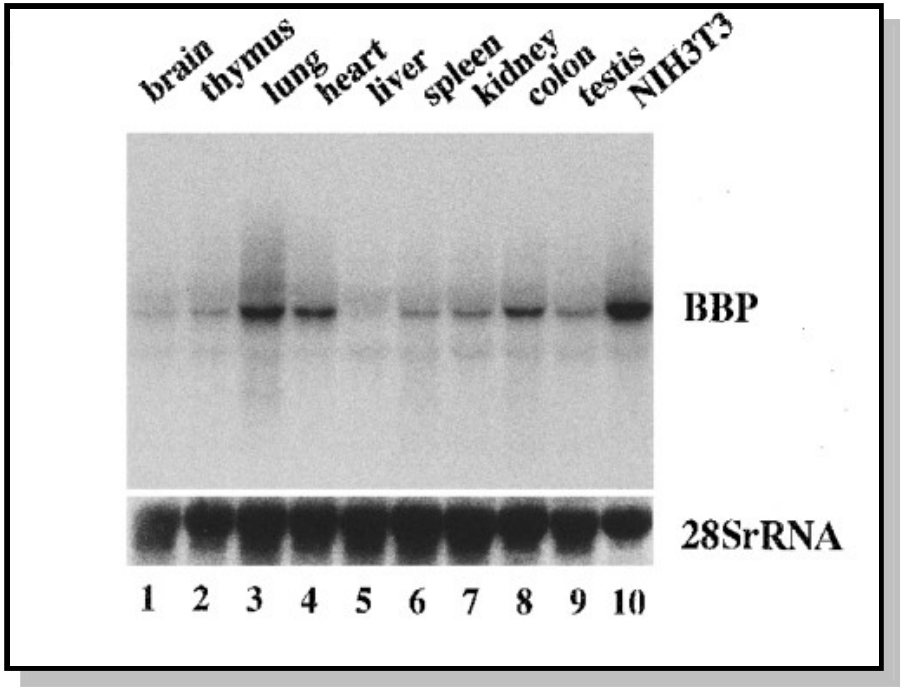
- FÍSICA (DIRECTA O INDIRECTA)
- FUNCIONAL

CIERTA CO-REGULACIÓN ES NECESARIA

LOS *DNA MICROARRAYS* PERMITEN MONITORIZAR LA EXPRESIÓN DE MILES DE GENES A LA VEZ, EN CIENTOS DE CONDICIONES FISIOLÓGICAS

BASES DE LA TECNOLOGÍA DE *DNA MICROARRAYS*

HIBRIDACIONES TIPO *NORTHERN* PARA LA MEDICIÓN DEL NIVEL DE TRANSCRIPCIÓN DE UN GEN EN DISTINTOS TEJIDOS

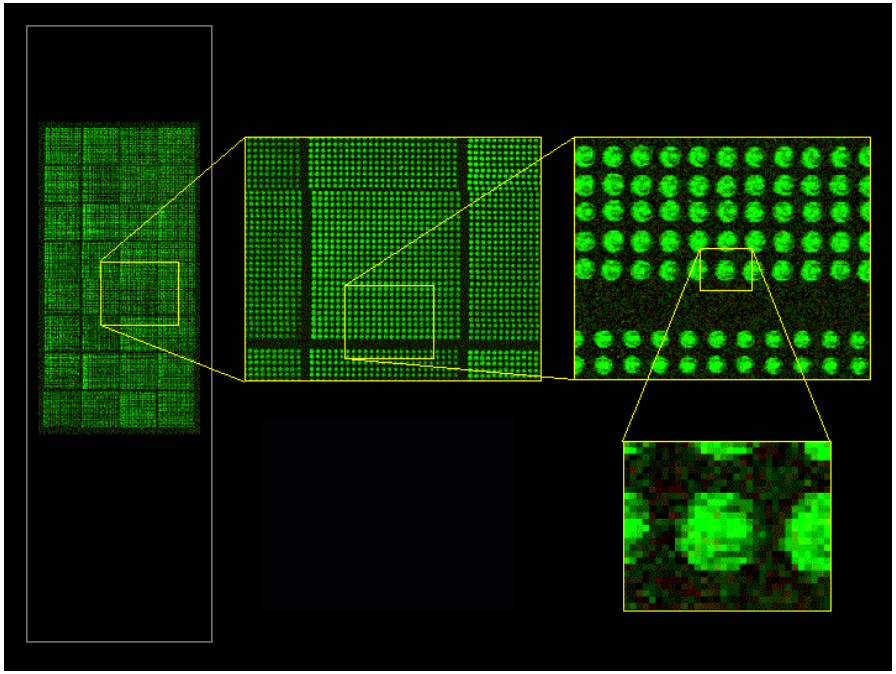


- **FASE SÓLIDA: EXTRACTO CELULAR (RNA)**
- **FASE LÍQUIDA: SONDA MARCADA**

Hasegawa y col. (2000)

BASES DE LA TECNOLOGÍA DE *DNA MICROARRAYS*

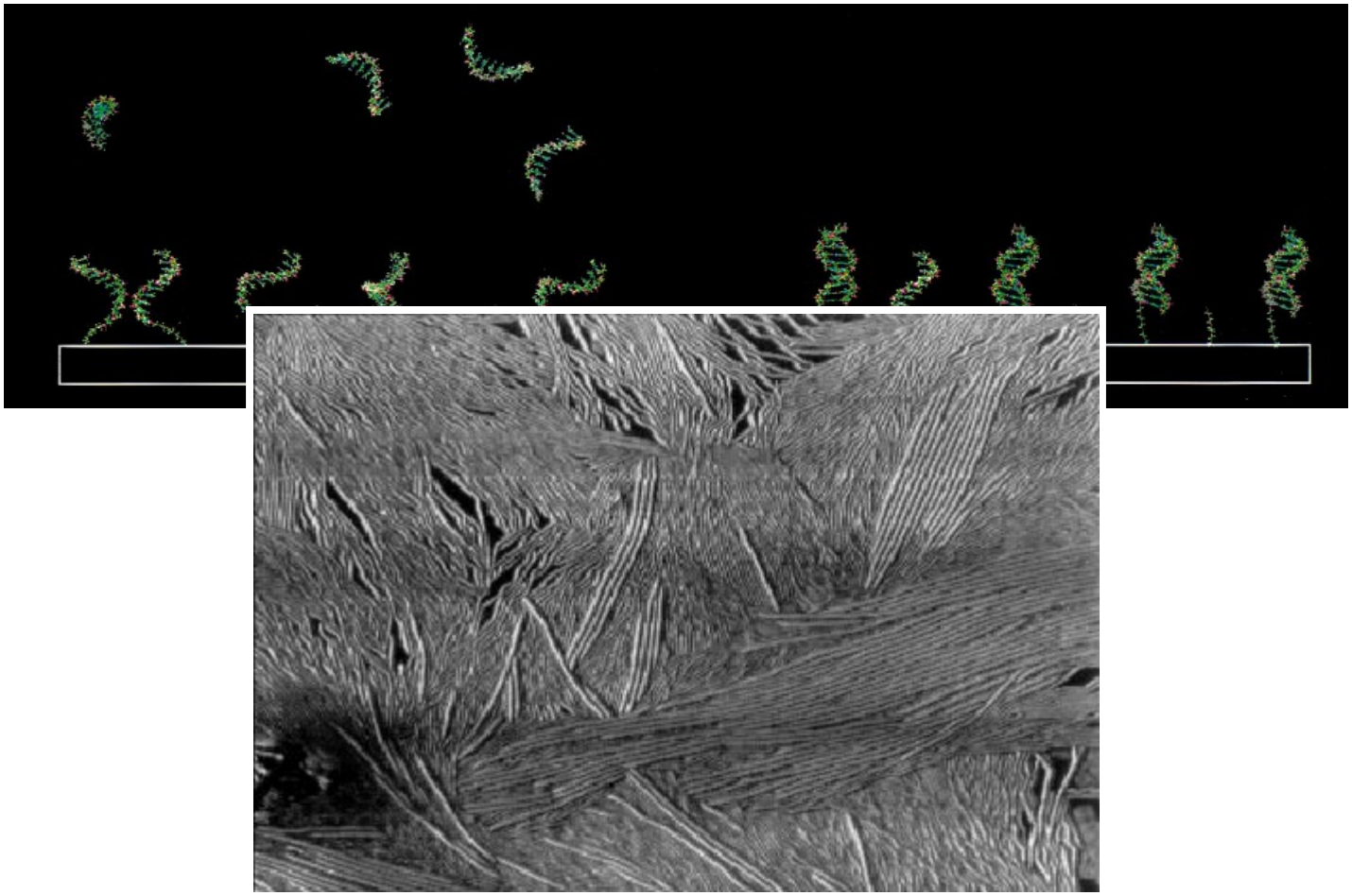
UN *DNA MICROARRAY* CONSISTE EN UNA COLECCIÓN DE SONDAS GÉNICAS ORDENADAS EN UN SOPORTE SÓLIDO



- **FASE SÓLIDA: SONDA**
- **FASE LÍQUIDA: EXTRACTO CELULAR (cDNA MARCADO)**

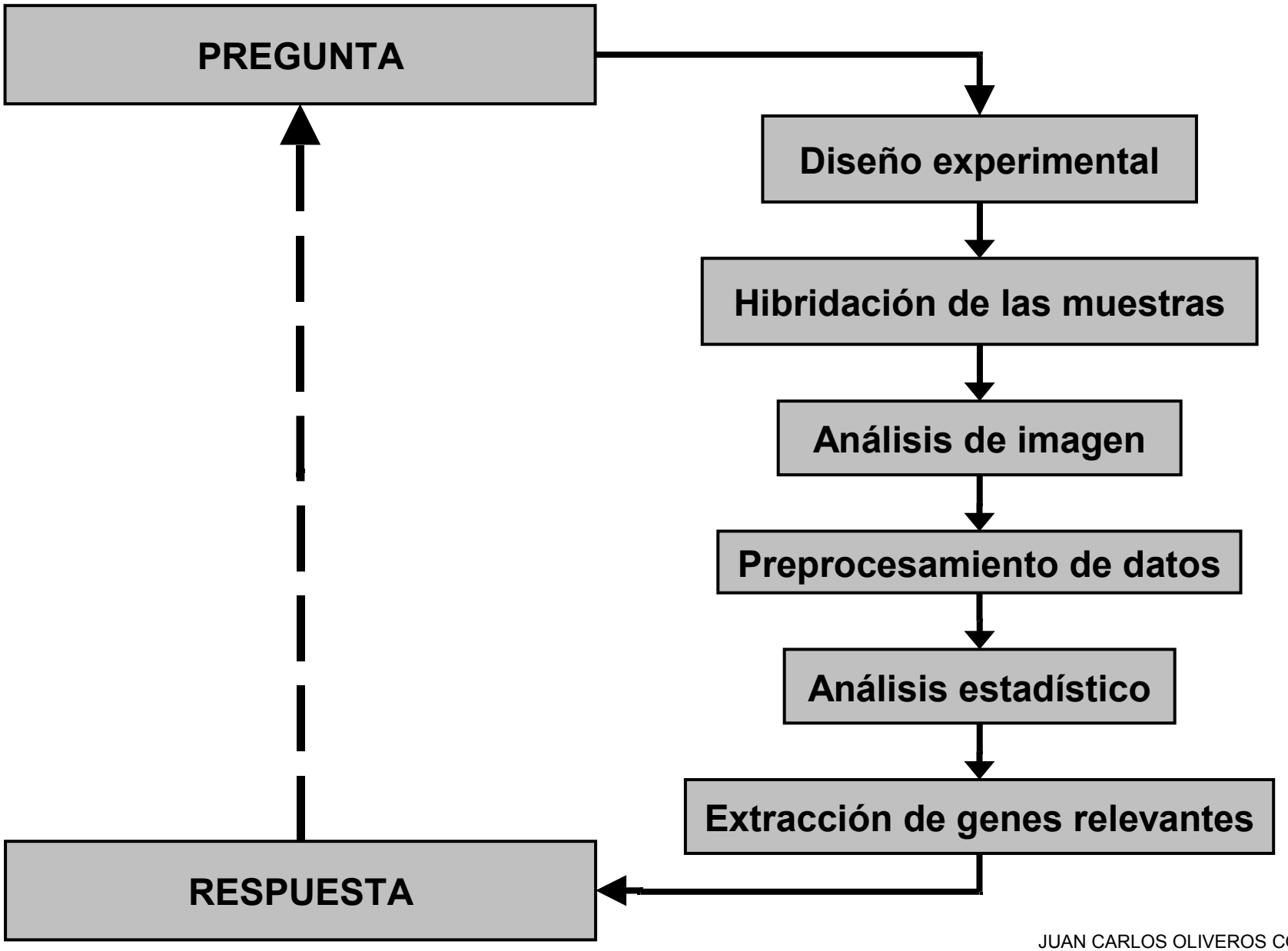
BASES DE LA TECNOLOGÍA DE *DNA MICROARRAYS*

CADA SONDA ESTÁ DISEÑADA PARA UNIRSE A UN GEN DE FORMA ESPECÍFICA



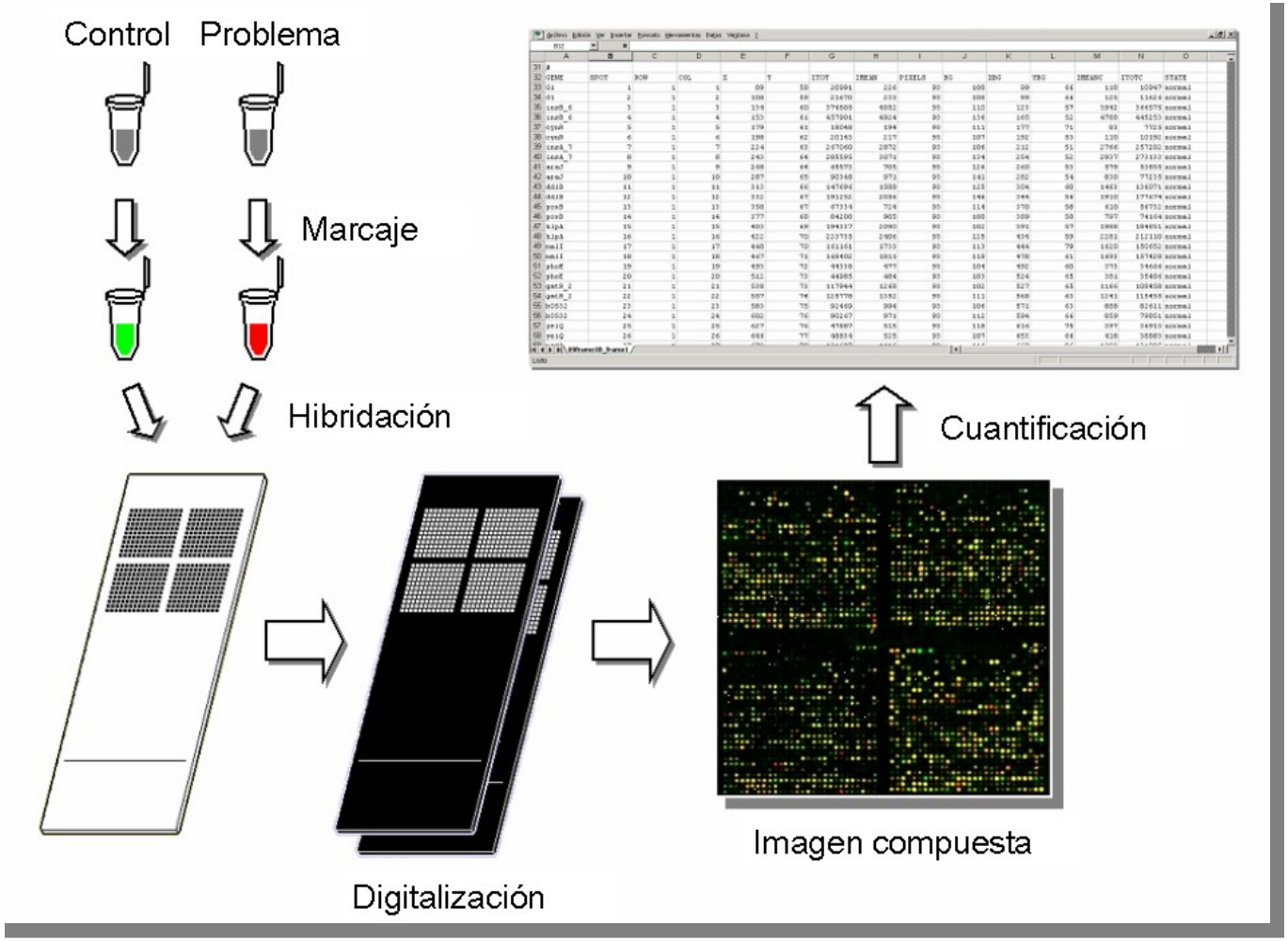
Duggan y col. (1999)

BIOLOGÍA, ESTADÍSTICA E INFORMÁTICA



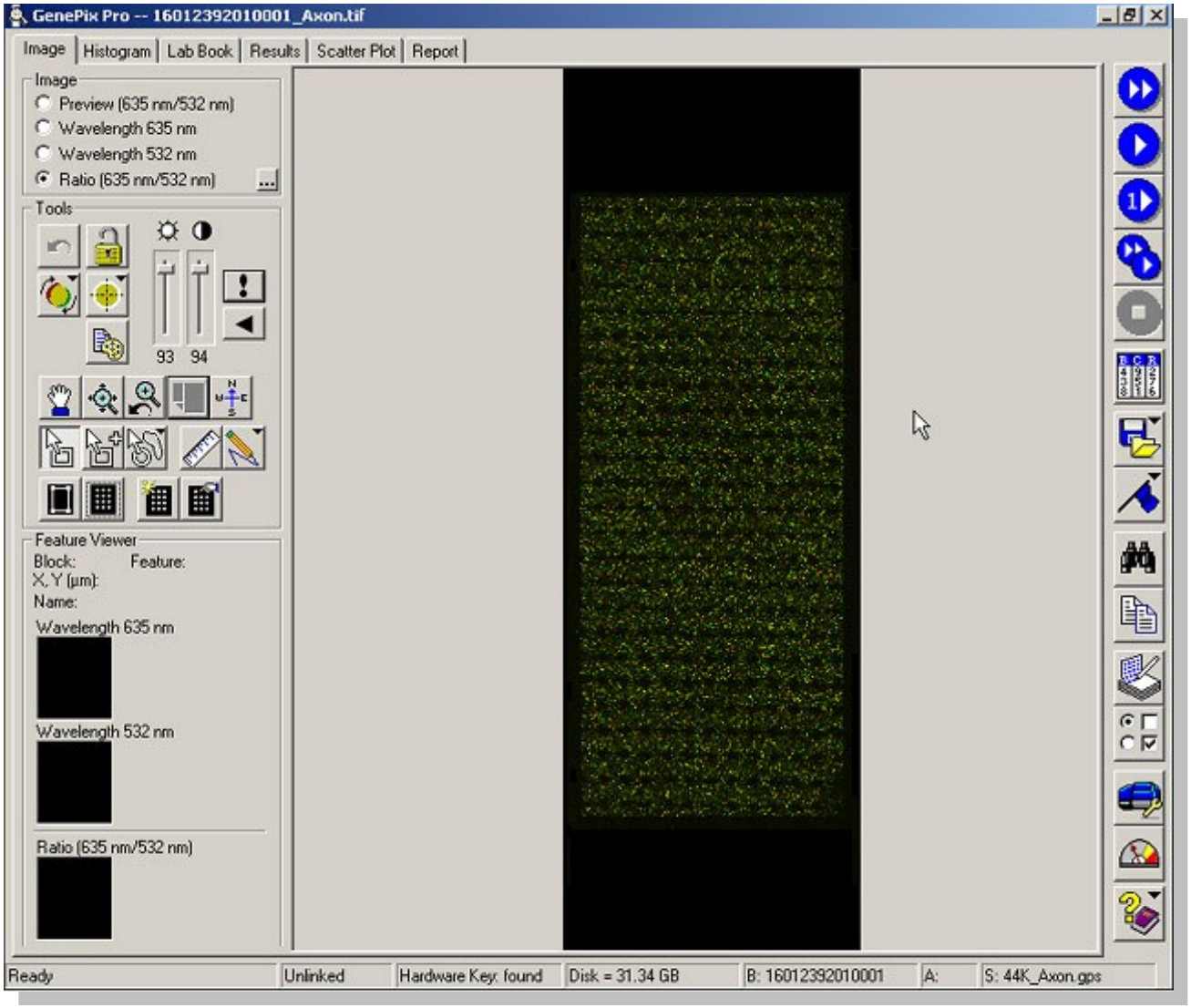
BIOLOGÍA, ESTADÍSTICA E INFORMÁTICA

EXPERIMENTO BÁSICO



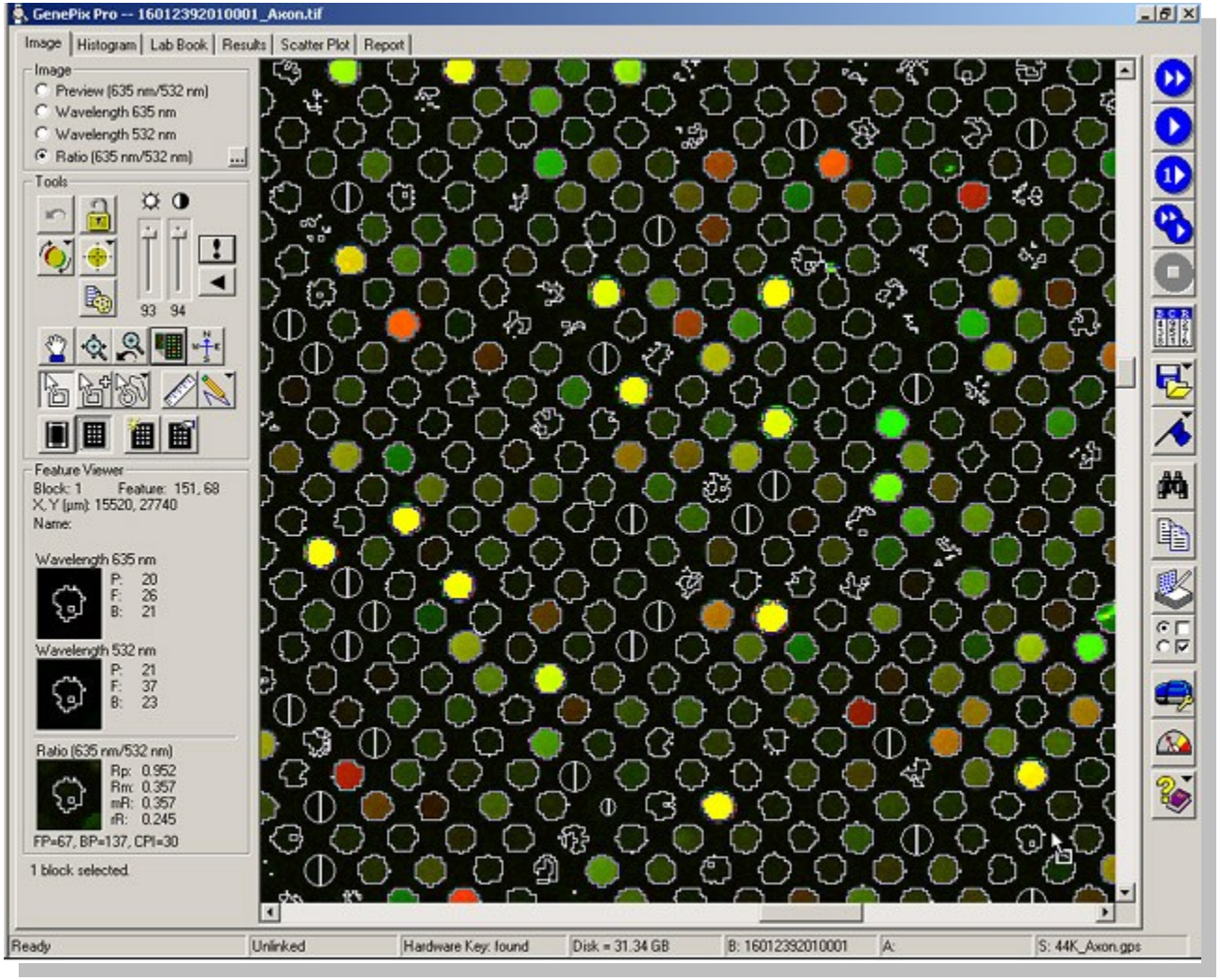
ESTA NUEVA FORMA DE EXPERIMENTAR REQUIERE EL DESARROLLO DE NUEVAS HERRAMIENTAS DE ANÁLISIS Y DE VISUALIZACIÓN DE RESULTADOS

ANÁLISIS DE IMÁGENES



www.moleculardevices.com

ANÁLISIS DE IMÁGENES



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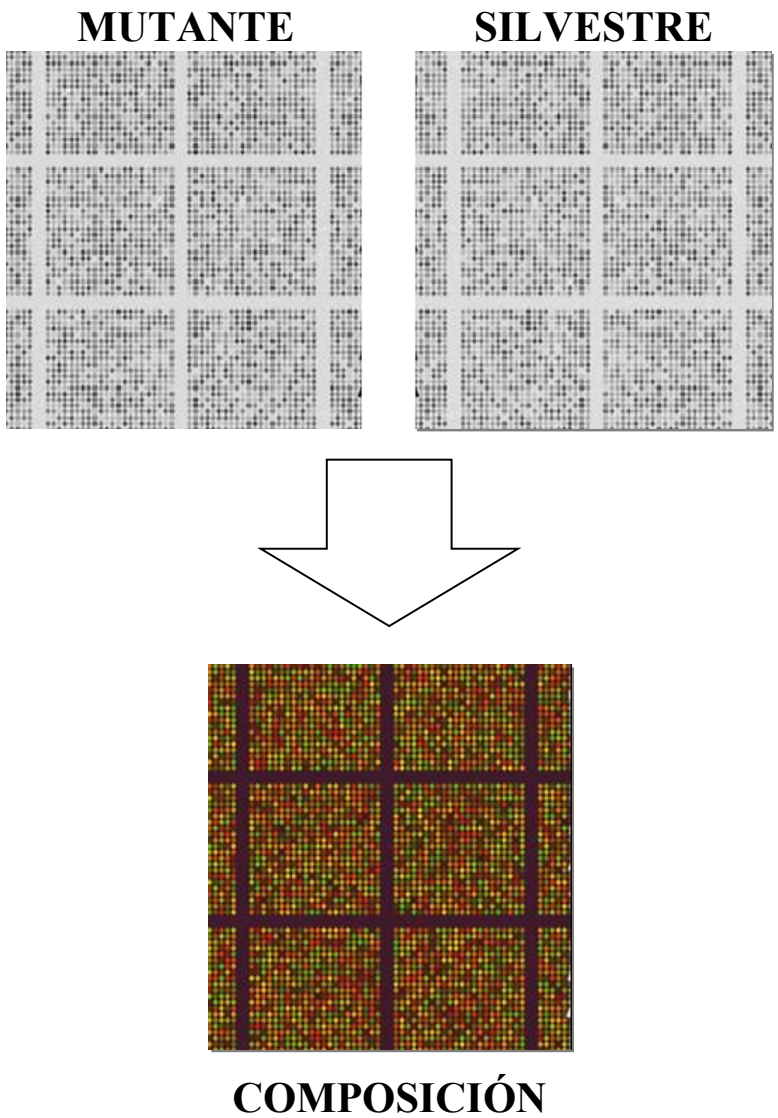
ANÁLISIS DE IMÁGENES

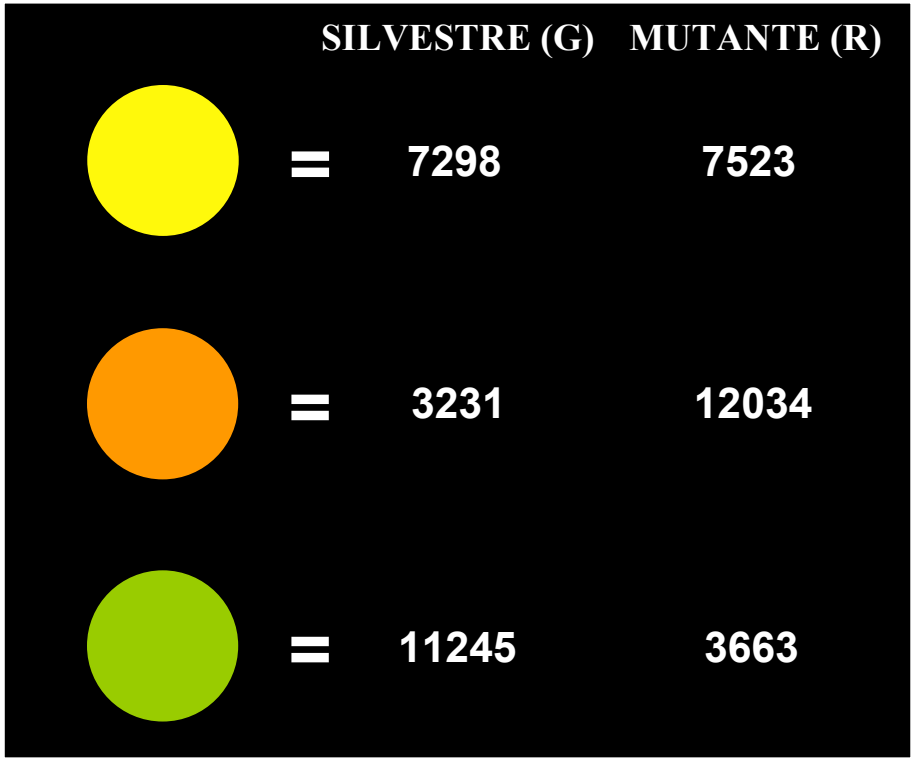
DE LA ESCALA DE GRISES AL COLOR



JUAN CARLOS OLIVEROS COLLAZOS

ANÁLISIS DE IMÁGENES

COLORES Y VALORES DE INTENSIDADES

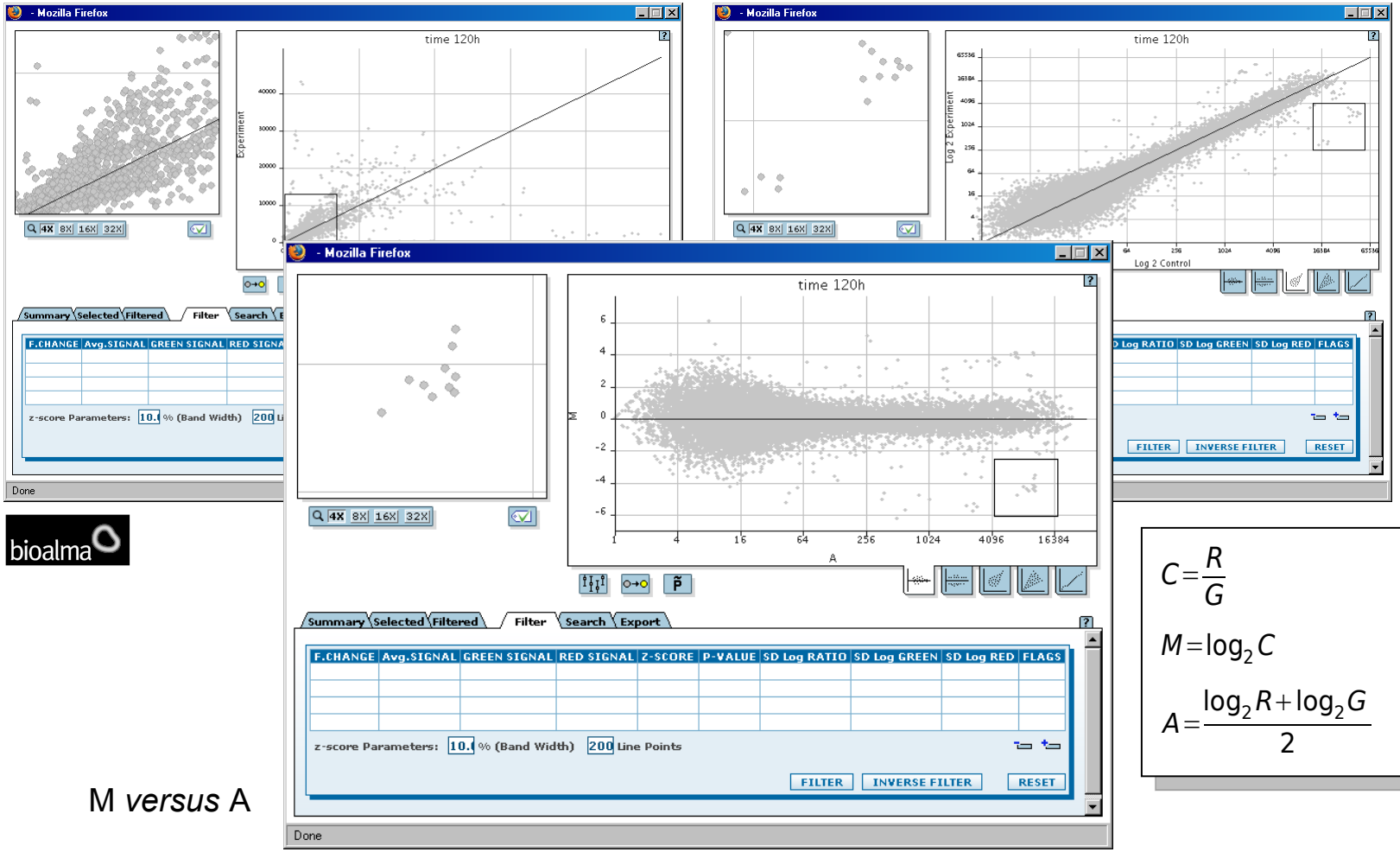


PROCESAMIENTO DE DATOS INICIALES

REPRESENTACIONES GRÁFICAS

R *versus* G

$\log_2 R$ *versus* $\log_2 G$



M *versus* A

$$C = \frac{R}{G}$$

$$M = \log_2 C$$

$$A = \frac{\log_2 R + \log_2 G}{2}$$

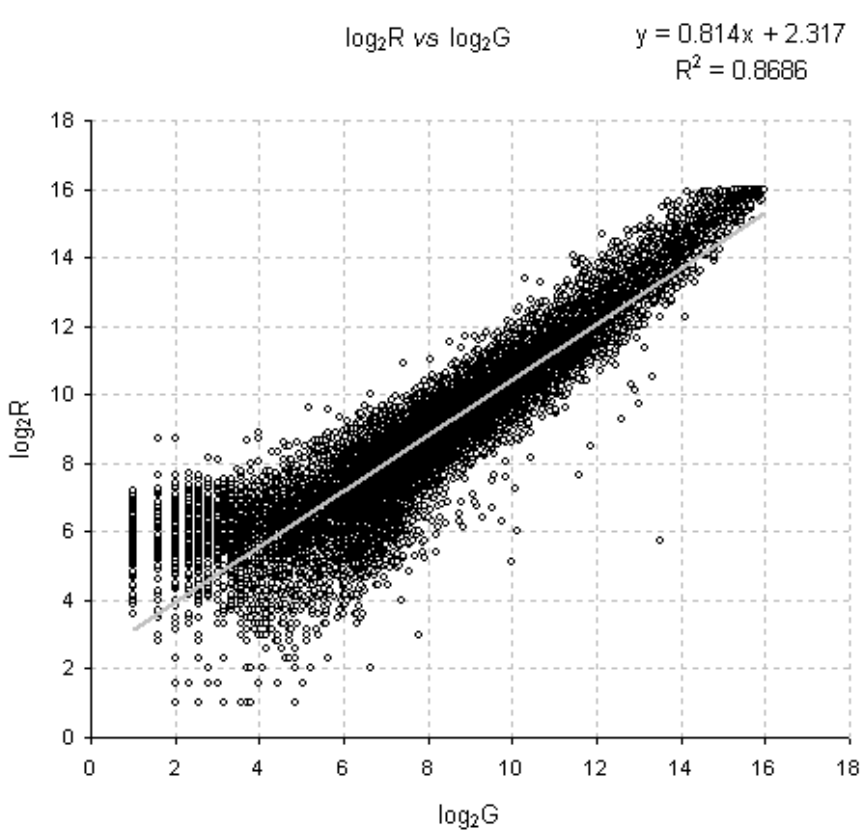
PROCESAMIENTO DE DATOS INICIALES

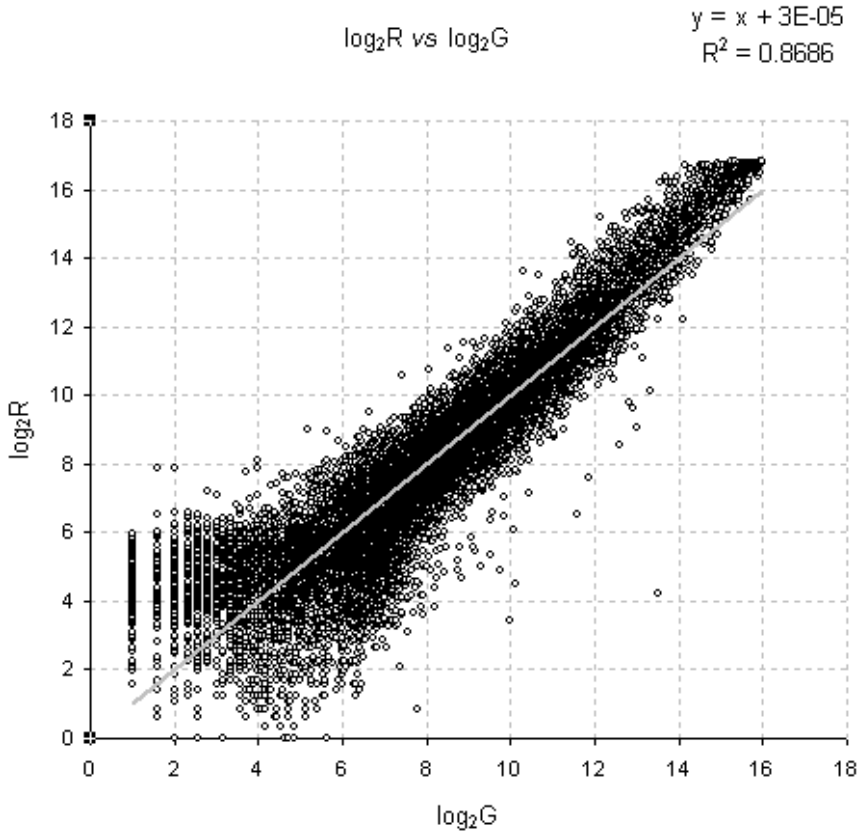
NORMALIZACIÓN DE VALORES

- CADA VALOR DE INTENSIDAD PROVIENE DE UNA IMAGEN (CANAL) INDEPENDIENTE
- ES NECESARIO HACER QUE ESTOS VALORES SEAN COMPARABLES
- AJUSTE BÁSICO: IGUALAR LA INTENSIDAD MEDIA DE AMBAS IMÁGENES

PROCESAMIENTO DE DATOS INICIALES

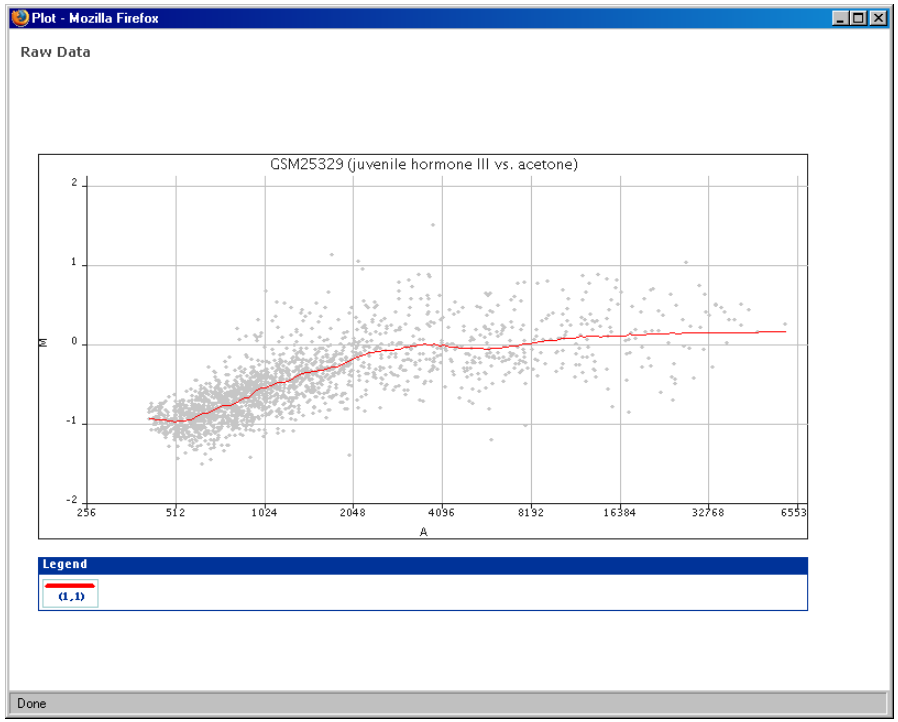
AJUSTE POR REGRESIÓN LINEAL

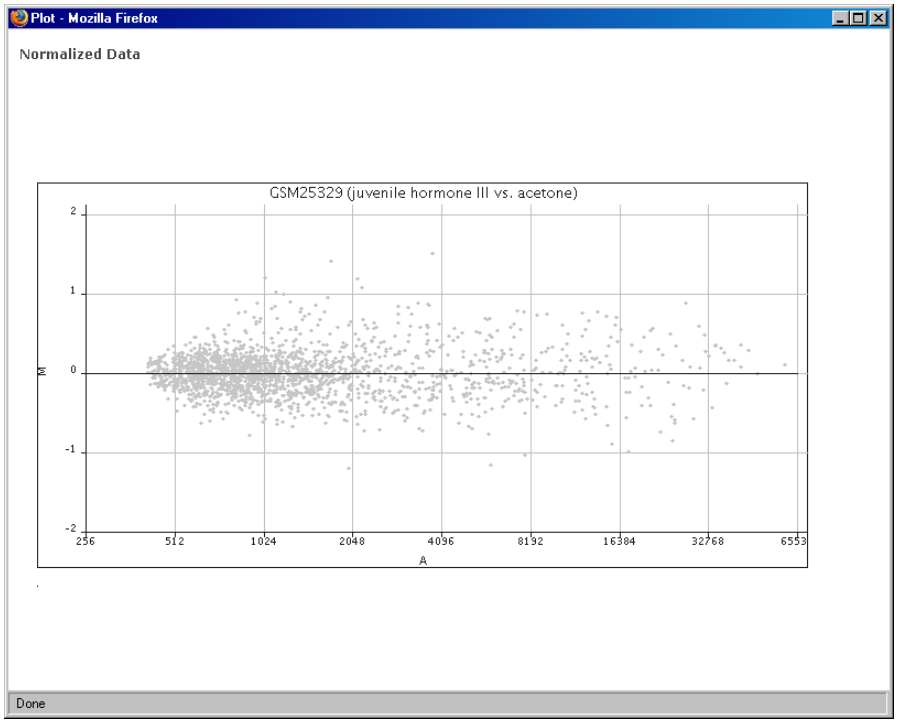




PROCESAMIENTO DE DATOS INICIALES

AJUSTE POR *LOWESS*





bioalma

ESTADÍSTICA CON POCAS MUESTRAS

DE TODOS LOS GENES PRESENTE EN EL *MICROARRAY:*

¿CUALES SE INDUCEN?

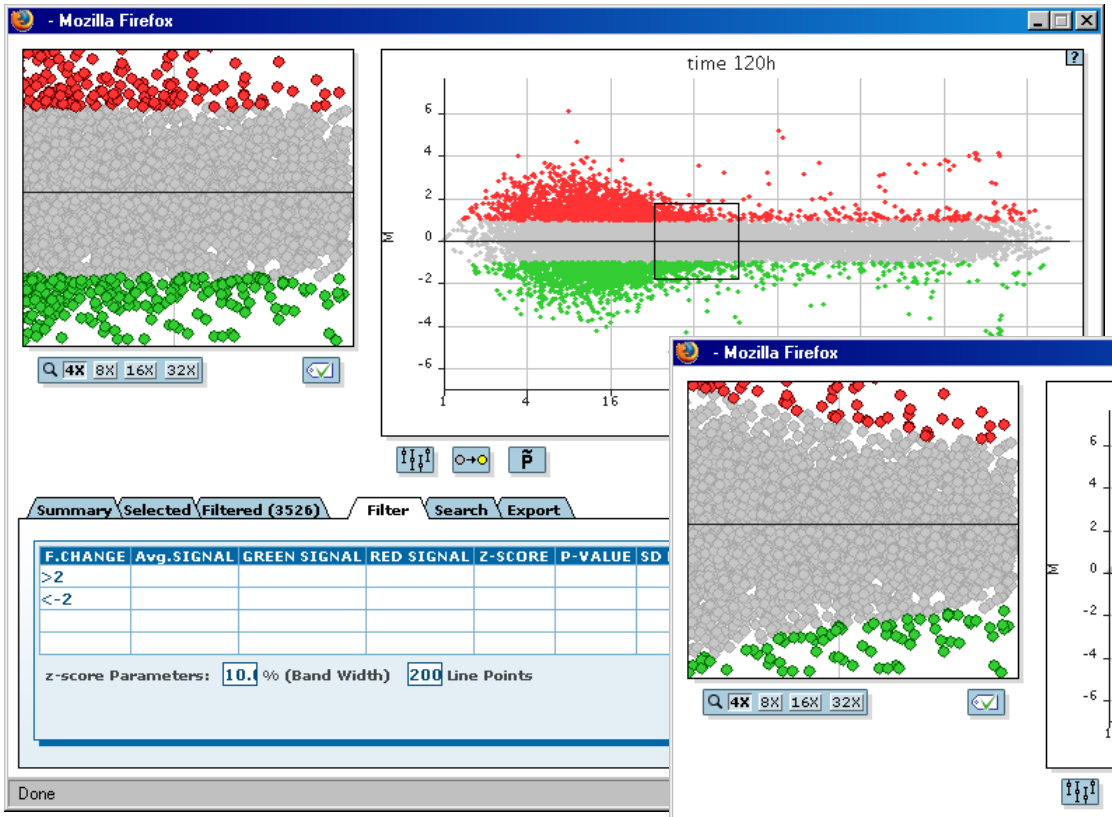
¿CUALES SE REPRIMEN?

UMBRALES *MÁGICOS*:

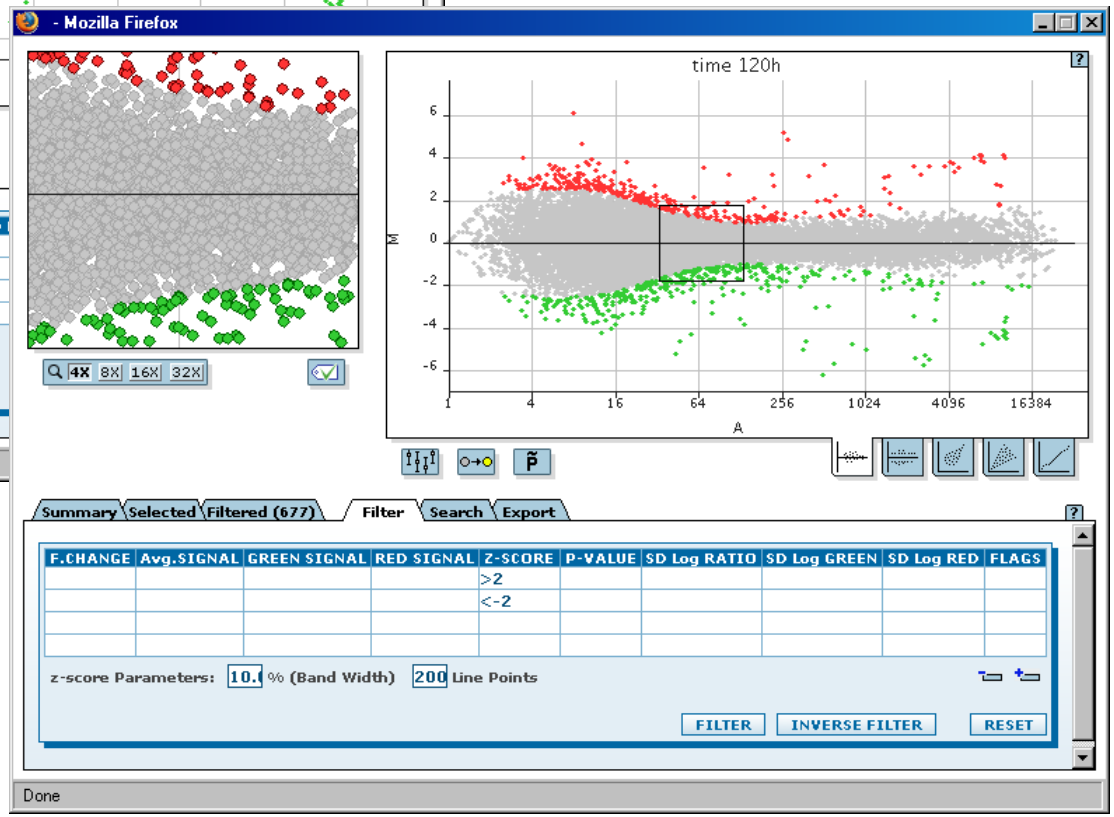
- **TASA DE CAMBIO >2 (INDUCIDOS)**
- **TASA DE CAMBIO < -2 (REPRIMIDOS)**

ESTADÍSTICA CON POCAS MUESTRAS

TASA DE CAMBIO +/- 2



VALOR Z +/- 2



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MÉTODO PROPUESTO POR JOHN QUACKENBUSH

JUAN CARLOS OLIVEROS COLLAZOS

ESTADÍSTICA CON POCAS MUESTRAS

EN CUALQUIER EXPERIMENTO BIOLÓGICO ES ESENCIAL CONOCER EL GRADO DE REPRODUCIBILIDAD DE LAS MEDIDAS

REPETIR EXPERIMENTOS DE *MICROARRAYS* ES COSTOSO

LA CANTIDAD DE MUESTRA BIOLÓGICA PUEDE SER EL FACTOR LIMITANTE

ESTADÍSTICA CON POCAS MUESTRAS

RÉPLICAS TÉCNICAS vs RÉPLICAS BIOLÓGICAS:

- LAS RÉPLICAS TÉCNICAS SUPONEN UN MENOR COSTE
- LA VARIABILIDAD DE LAS RÉPLICAS TÉCNICAS ES MUCHO MENOR
- LAS RÉPLICAS TÉCNICAS NO DAN INFORMACIÓN ÚTIL SOBRE LA REPRODUCIBILIDAD DE LOS VALORES DE EXPRESIÓN GÉNICA

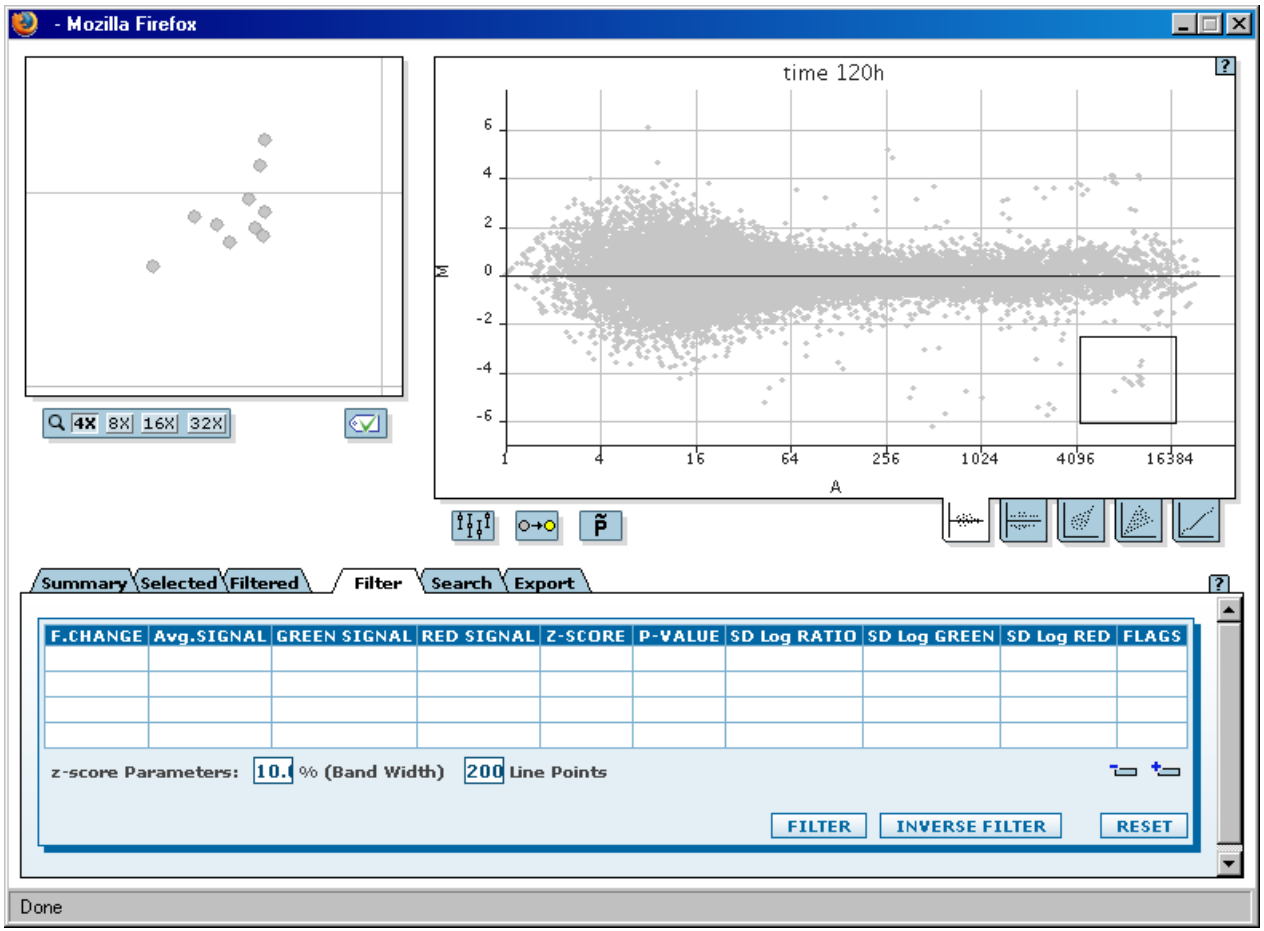
ESTADÍSTICA CON POCAS MUESTRAS

T-TEST

W. S. GOSSETT
(REINO UNIDO, 1876-1937)

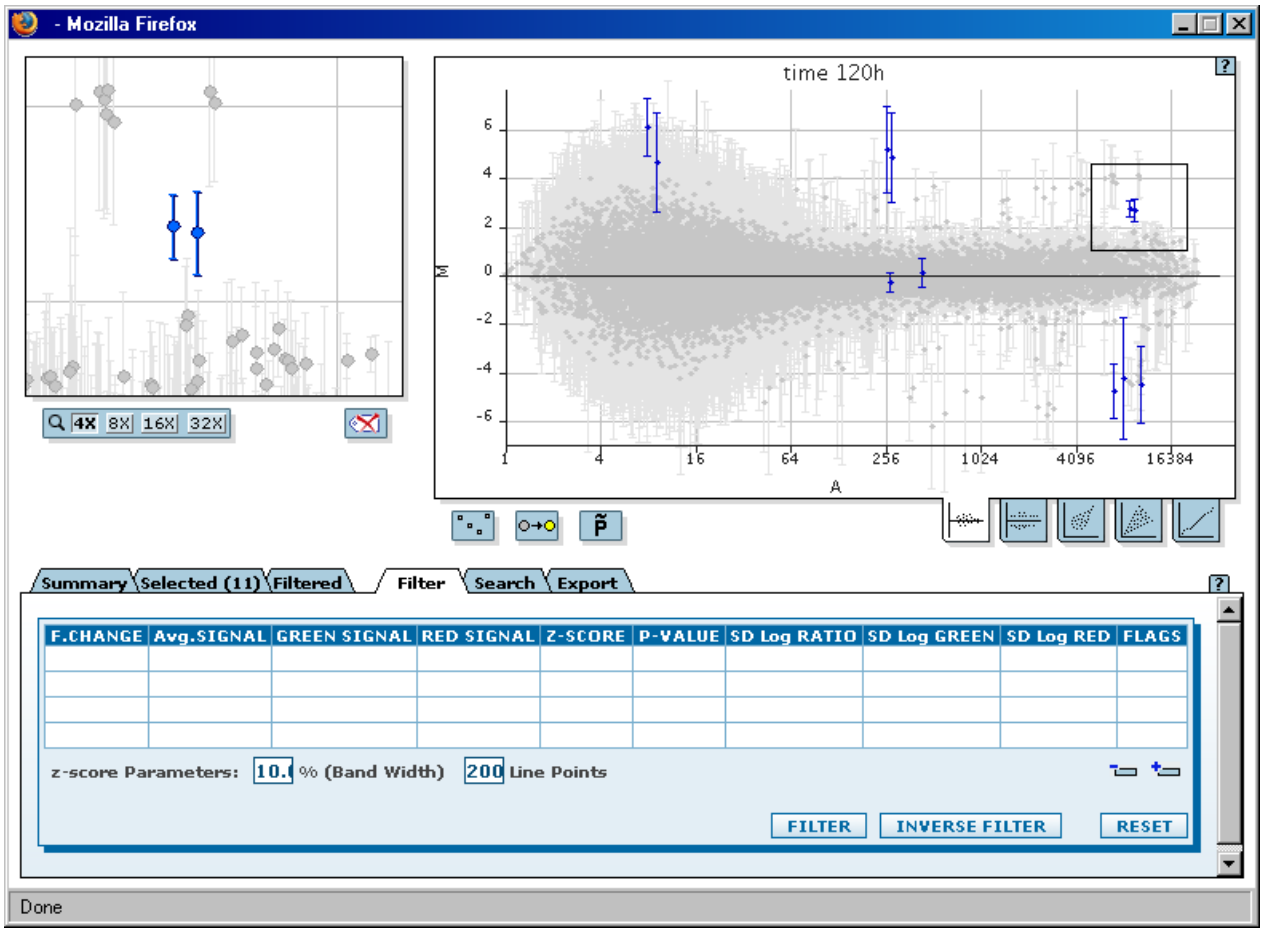
ESTADÍSTICA CON POCAS MUESTRAS

DATOS PROCEDENTES DE UN EXPERIMENTO SIN RÉPLICAS



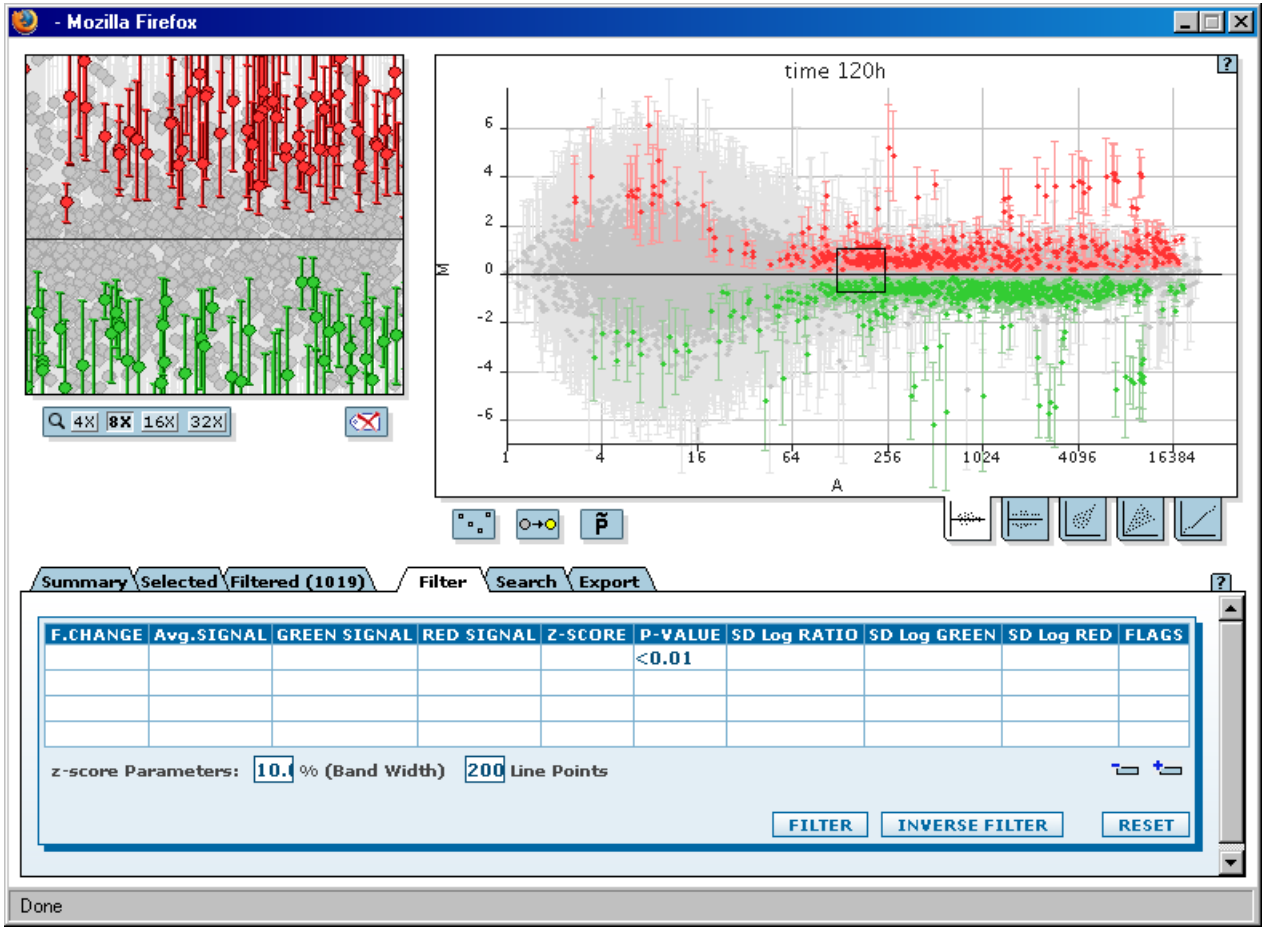
ESTADÍSTICA CON POCAS MUESTRAS

DATOS PROCEDENTES DE UN EXPERIMENTO CON VARIAS RÉPLICAS



ESTADÍSTICA CON POCAS MUESTRAS

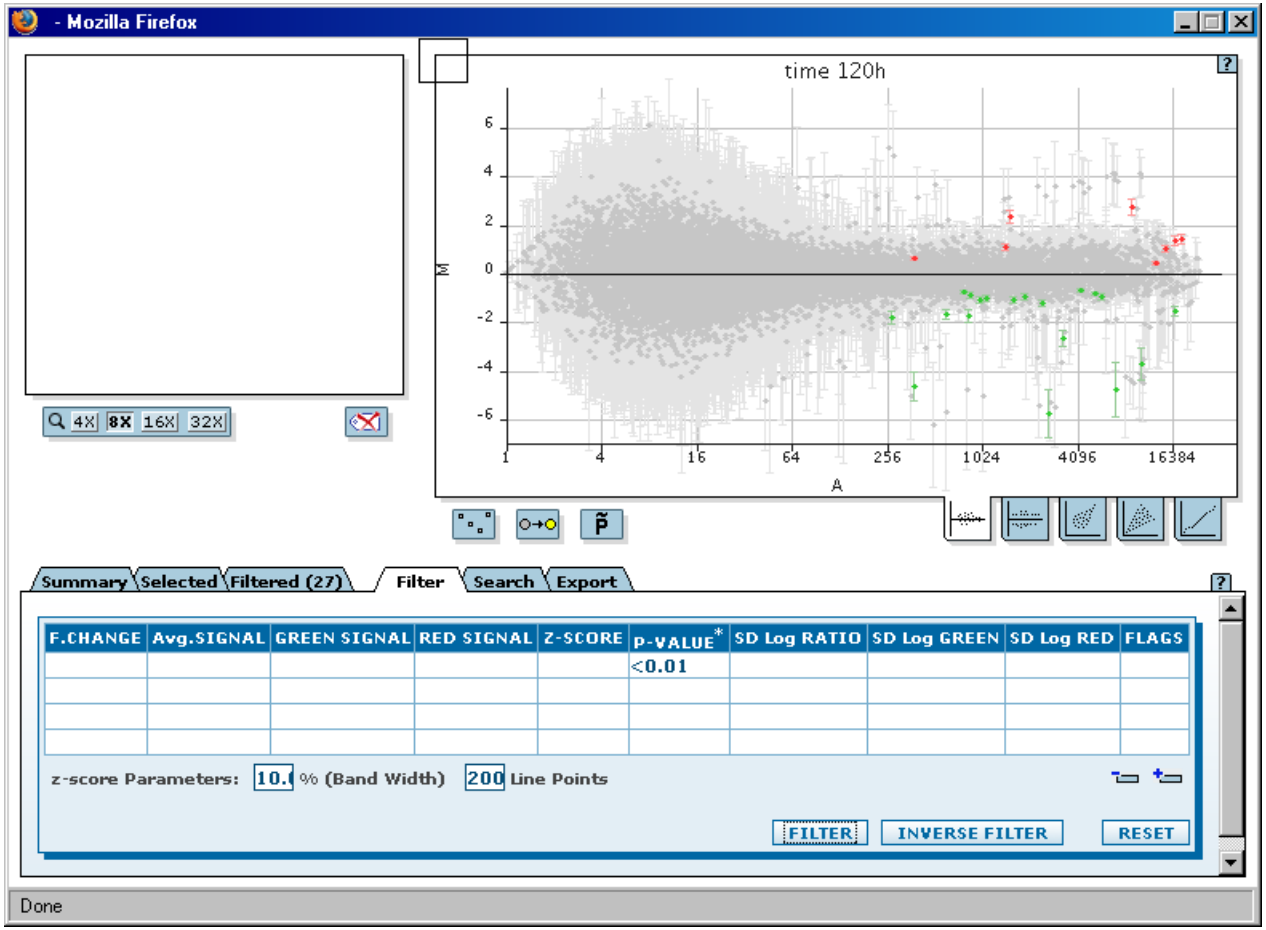
ANÁLISIS ESTADÍSTICO: VALORES P



VALOR P < 0.01 **1019 GENES!**

ESTADÍSTICA CON POCAS MUESTRAS

ANÁLISIS ESTADÍSTICO: TASA DE FALSOS POSITIVOS (FDR)



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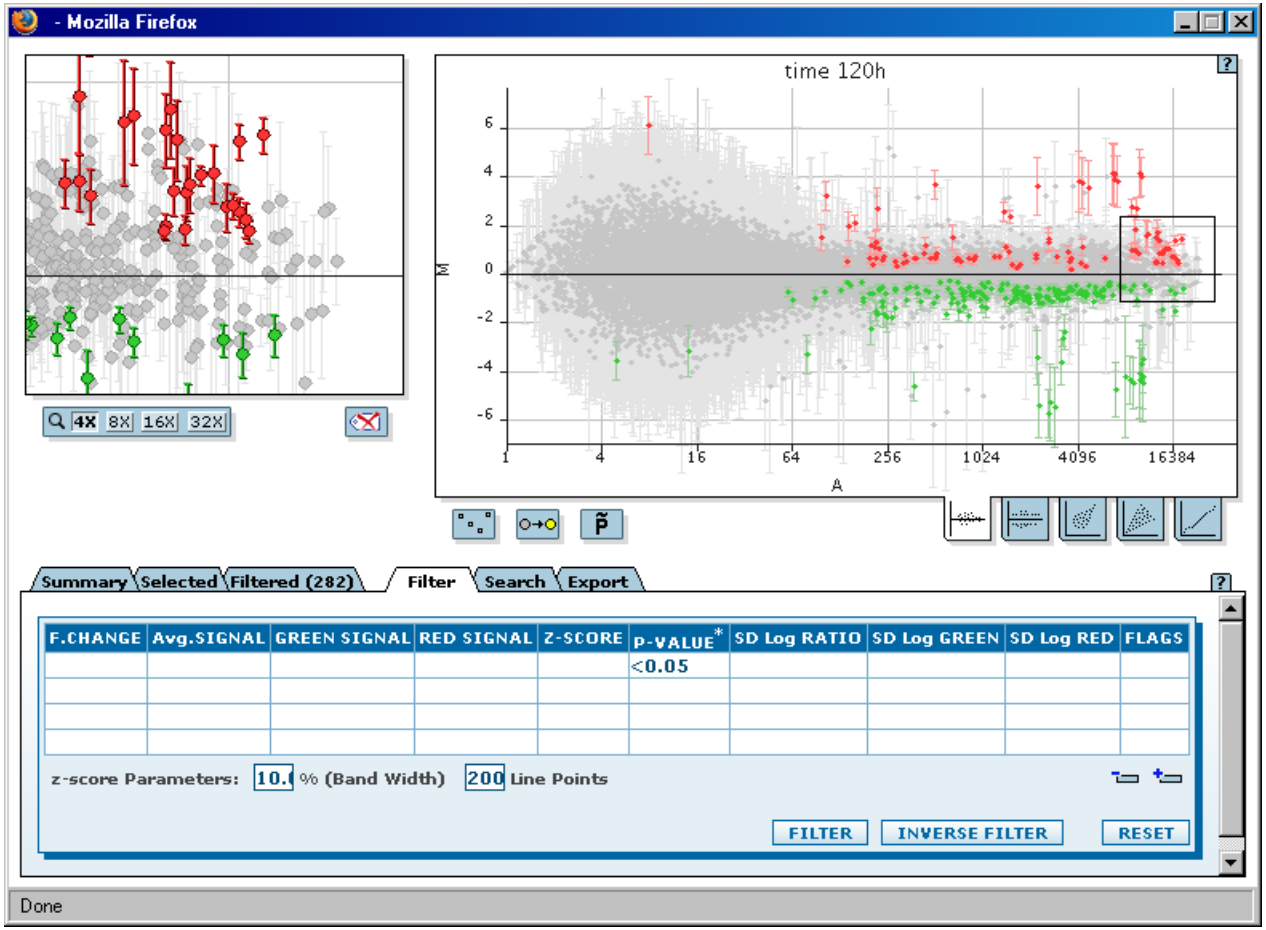
VALOR P (AJUSTADO) < 0.01 **27 GENES**

ESTADÍSTICA CON POCAS MUESTRAS

ANÁLISIS ESTADÍSTICO: TASA DE FALSOS POSITIVOS (FDR)



bioalma

VALOR P (AJUSTADO) < 0.05 **282 GENES**

APLICACIÓN:

(MODIFICACIÓN DE LA EXPRESIÓN GÉNICA ASOCIADA AL EJERCICIO FÍSICO)

The FASEB Journal express article 10.1096/fj.04-3149fje. Published online June 28, 2005.

Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise

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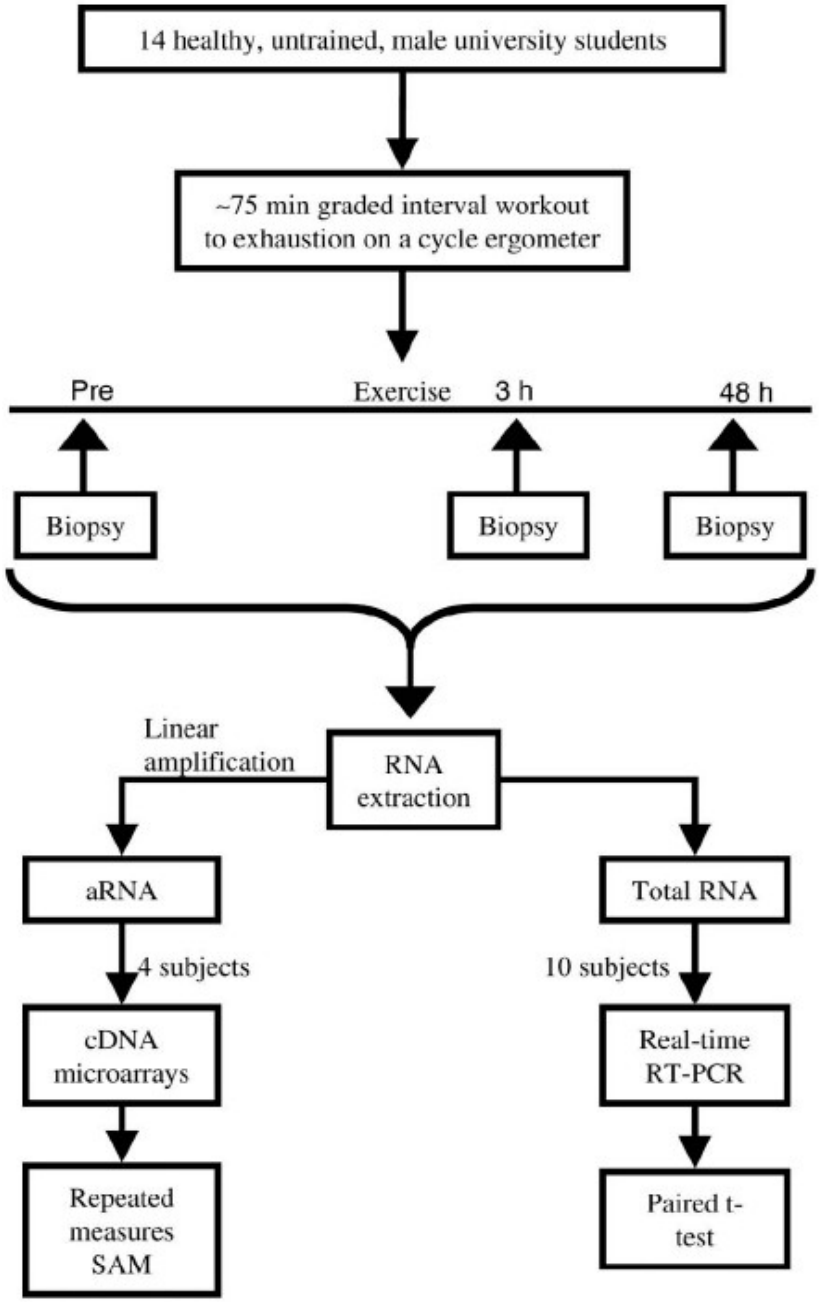
Department of *Medical Sciences, †Kinesiology, ‡Pediatrics and Medicine, McMaster University, Hamilton, Ontario, Canada; and §Buck Institute for Age Research, Novato, California 94945



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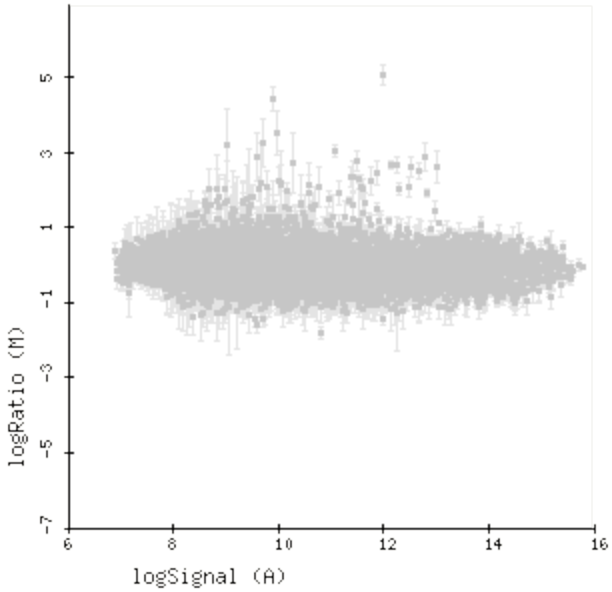

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Differentially increased gene expression after exercise

Gene Name	Accession Number	3 H	48 H	Potential Relevant Function
Metabolism and mitochondria				
Forkhead transcription factor O1A	AA134749	5.2±2.6	1.1±0.4	• activates PDK4 and PGC1α
*Pyruvate dehydrogenase kinase 4	AA169469	3.5±1.2	1.3±0.5	• negatively regulates pyruvate dehydrogenase
Mitochondrial ribosomal protein L2	N94366	3.4±1.4	5.5±2.8	• involved in mitochondrial translation
IL-6 receptor	T52330	3.1±0.5	2.3±0.7	• subunit for IL-6 receptor complex
Ras-related associated with Diabetes	W84445	2.9±0.8	2.6±2.1	• a role in glucose metabolism
*PPARγ coactivator 1 α	N89673	2.9±0.8	0.6±0.1	• regulates mitochondrial biogenesis
*PPARγ	AA088517	2.7±0.7	1.4±0.6	• positively regulates fat metabolism
Nuclear receptor binding protein 2	N30573	2.6±0.4	3.8±0.9	• binds to and co-modulates PPARα
Aminolevulinate δ syntetase 2	AA699919	2.3±0.4	1.5±0.1	• catalyzes first step in the heme biosynthesis
Interferon regulatory factor 1	AA478043	2.1±0.3	1.0±0.1	• transcription factor for iNOS expression
IL-6 signal transducer (gp130)	T61343	2.0±0.1	1.5±0.1	• component for IL-6 receptor complex
†PPAR δ	n/a	2.6±0.6	1.1±0.1	• positively regulates fat metabolism
†PPAR α	n/a	1.7±0.1	1.6±0.4	• positively regulates fat metabolism
Oxidant stress and signaling				
Metallothionein 1G	H53340	7.5±0.6	2.4±0.9	• metallothioneins are involved in protection against oxidative stress, metal ion homeostasis and detoxification, cell proliferation and apoptosis; transiently responds to most forms of stress or injury providing cytoprotective action, particularly oxidative injury.
Metallothionein 1H	H77766	7.0±1.0	2.0±0.5	
Metallothionein 1F	N55459	6.6±1.8	2.3±1.0	
Metallothionein 3	AI362950	6.1±0.9	1.6±0.8	
Metallothionein 1B	H72722	5.3±0.8	2.0±0.6	
Metallothionein 2A	AA872383	4.7±0.5	2.2±1.0	
Metallothionein 1L	AI289110	4.2±0.4	2.2±0.5	
Tyrosyl-DNA phosphodiesterase 1	AI215965	6.7±4.1	8.0±4.0	• repairs free-radical DNA double-strand breaks
*JunB	N94468	4.9±0.4	n/a	• part of AP-1 complex
Interferon regulatory factor 1	AA478043	2.1±0.3	1.0±0.1	• O_2^- mediated trascription factor
Electrolyte transport				
NMDA receptor	R88267	n/a	25±16	• role in synaptic plasticity
*Ca^{2+} ATPase (SERCA 3)	AA857542	4.5±0.8	1.6±0.6	• pumps Ca^{2+} into SR
Solute carrier 17 (1)	N73241	3.9±14	2.7±0.3	• Sodium phosphate carrier
Na^+/K^+ ATPase (β3)	AA489275	2.7±0.2	n/a	• regulatory component of Na^+/K^+ ATPase
Chloride channel 4	AA019316	2.4±0.3	0.6±0.2	• regulates cell volume, intracellular pH
Solute carrier 22 (3)	AA460012	2.4±0.5	1.9±0.5	• Mediates transport of organic cations
GABA receptor	R40790	2.2±0.3	2.3±0.3	• chloride channel; inhibitory neurotransmitor

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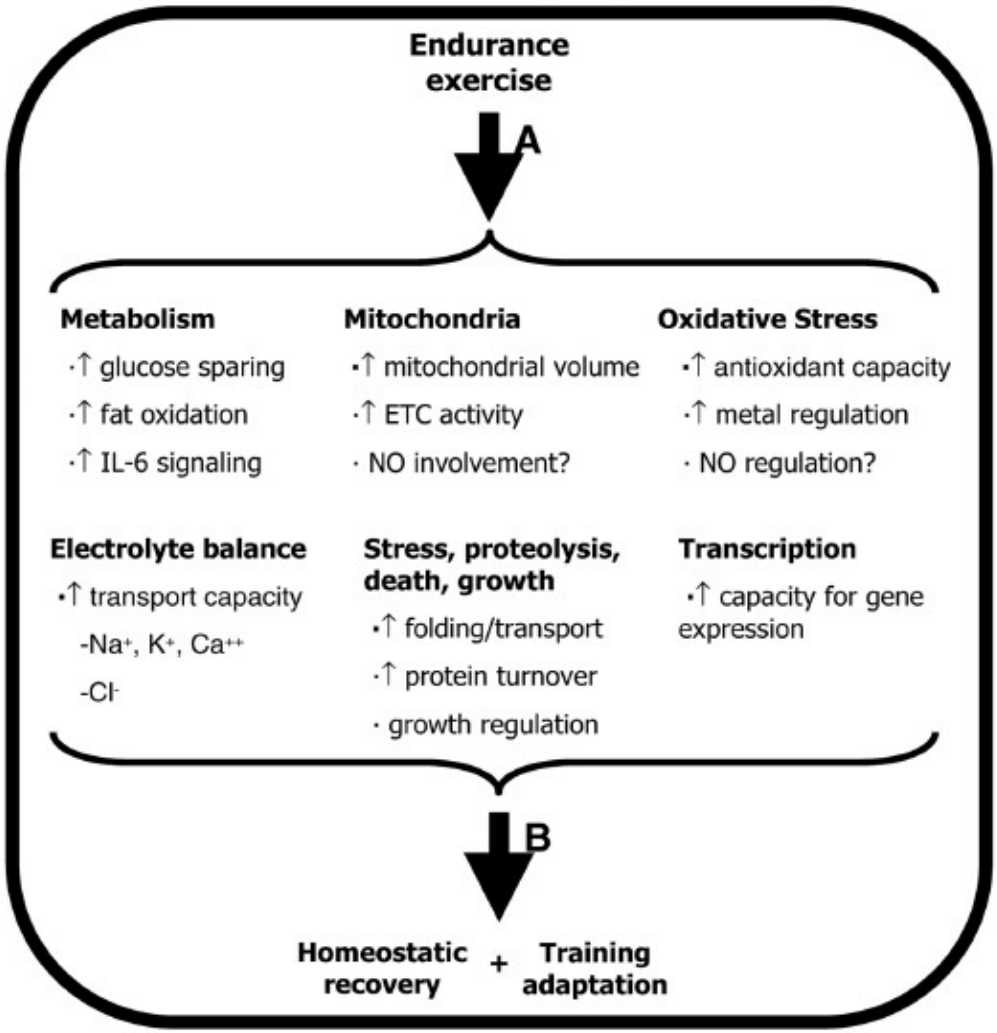

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APLICACIÓN: (MODIFICACIÓN GENÉTICA EN MAMÍFEROS)

MOLECULAR AND CELLULAR BIOLOGY, May 2006, p. 3610–3624 Vol. 26, No. 9
0270-7306/06/$08.00+0 doi:10.1128/MCB.26.9.3610–3624.2006

Disruption of Spermatogenic Cell Adhesion and Male Infertility in Mice Lacking TSLC1/IGSF4, an Immunoglobulin Superfamily Cell Adhesion Molecule†

Daisuke Yamada,[1] Midori Yoshida,[2] Yuko N. Williams,[1] Takeshi Fukami,[1] Shinji Kikuchi,[1] Mari Masuda,[1] Tomoko Maruyama,[1] Tsutomu Ohta,[3] Dai Nakae,[2] Akihiko Maekawa,[2] Tadaichi Kitamura,[4] and Yoshinori Murakami[1]*

Tumor Suppression and Functional Genomics Project[1] and Genetics Division,[3] National Cancer Center Research Institute, Tokyo, Japan; Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo, Japan[2]; and Department of Urology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan[4]

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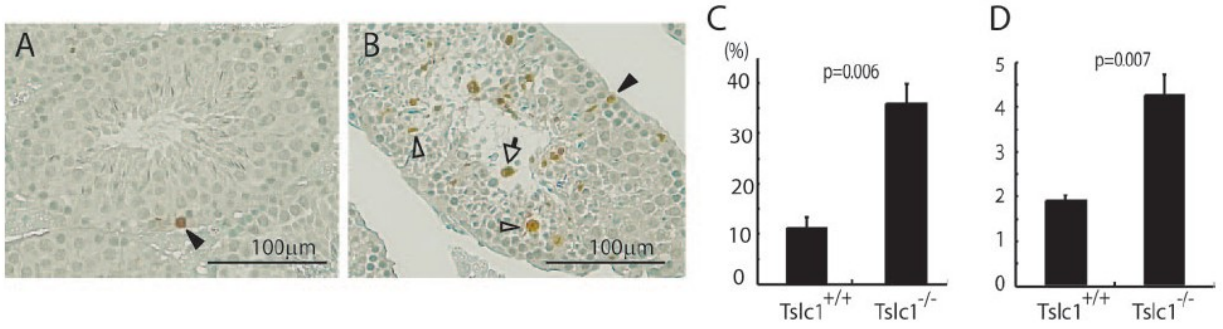

FIG. 6. Detection of apoptosis by TUNEL assays. (A and B) Histochemistry of the testes from *Tslc1*$^{+/+}$ (A) and *Tslc1*$^{-/-}$ (B) mice by TUNEL assay. Cells stained brown are TUNEL-positive cells. Nuclei were counterstained with methyl green (green). Closed arrowheads and open arrowheads indicate spermatocytes and spermatids, respectively. The open arrow indicates the sloughed cell. (C) Ratios of TUNEL-positive tubules to total tubules. (D) Average numbers of TUNEL-positive cells in TUNEL-positive tubules.

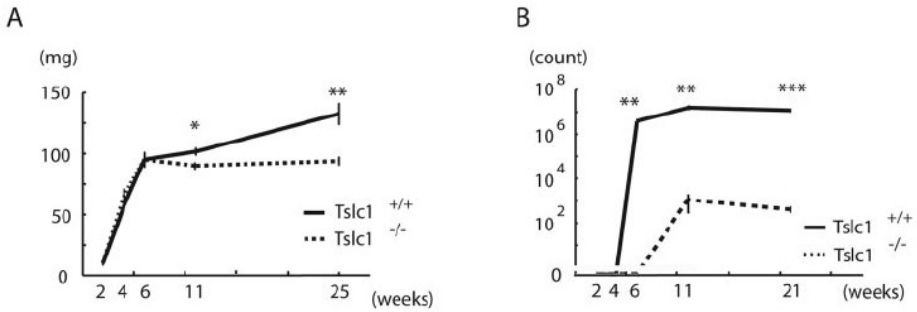

FIG. 8. Weights of testes and numbers of normal sperm during postnatal development of *Tslc1*$^{+/+}$ and *Tslc1*$^{-/-}$ mice. (A) Weights of testes. (B) Numbers of normal sperm. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0001$.

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***AFFYMETRIX MURINE ARRAY* (U74Av2)**

18400 SONDAS

2 EXPERIMENTOS INDEPENDIENTES (RÉPLICAS BIOLÓGICAS)

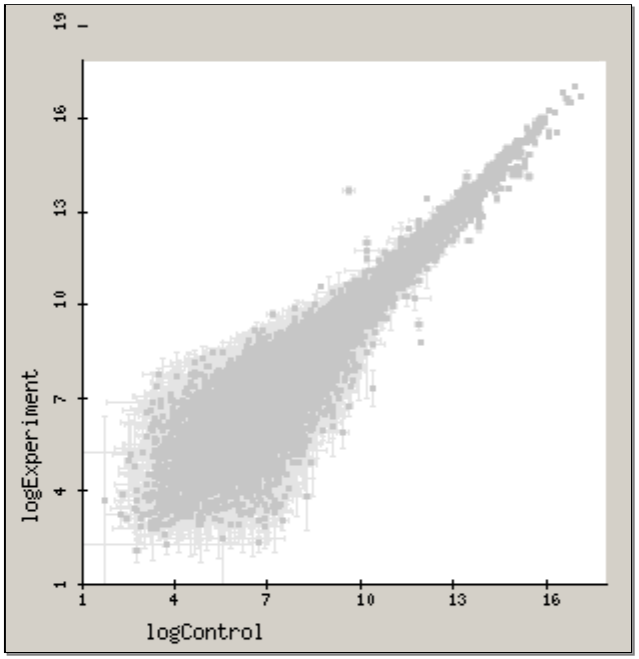

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TABLE 3. Genes up- and down-regulated in testes from *Tslc1*$^{-/-}$ mice

Gene product	Accession no.	Microarray analysis results: Signal intensity[a] *Tslc1*$^{-/-}$	Microarray analysis results: Signal intensity[a] *Tslc1*$^{+/+}$	Microarray analysis results: Ratio of *Tslc1*$^{-/-}$ value/*Tslc1*$^{+/+}$ value	Quantitative RT-PCR analysis results[b]: *Tslc1*$^{-/-}$ value	Quantitative RT-PCR analysis results[b]: *Tslc1*$^{+/+}$ value	*P* value[c]
Up-regulated genes in *Tslc1*$^{-/-}$ testes							
Phospholipase A2, group XIIA (Pla2g12a)	AI845798	13,102	771	17.0	11 ± 2.1	4.1 ± 0.8	0.03
Purine-nucleoside phosphorylase (Pnp)	U35374	1,629	410	4.0	6.1 ± 0.7	2.5 ± 0.6	0.02
Mus musculus cDNA 5′ end clone	AA874329	706	272	2.6			
FUS interacting protein 1 (Fusip1)	AF060490	695	282	2.5			
Hemoglobin, beta adult major chain (Hbb-b1)	J00413	1,446	636	2.3			
DnaJ homolog, subfamily A, member 2 (Dnaja2)	AA763945	1,833	860	2.1	470 ± 160	300 ± 120	NS
Vascular cell adhesion molecule 1 (Vcam1)	U12884	2,321	1,546	1.5	110 ± 5.8	38 ± 2.5	0.0002
Guanylate kinase 1 (Guk1)	U53514	3,225	2,294	1.4	23 ± 12	4.4 ± 0.1	NS
Angiopoietin-like 4 (Angpt14)	AF110520	6,143	4,823	1.3	3.1 ± 0.2	1.2 ± 0.2	0.002
Down-regulated genes in *Tslc1*$^{-/-}$ testes							
Immunoglobulin superfamily 4 (Igsf4/Tslc1)	AF061260	444	3,943	0.11	0 ± 0	100 ± 18	0.001
Immunoglobulin superfamily 4 (Igsf4/Tslc1)	AB021966	690	3,739	0.18			
Platelet/endothelial cell adhesion molecule 1 (Pecam1)	L06039	181	799	0.23	1.6 ± 0.2	1.2 ± 0.3	NS
Mus musculus cDNA 3′ end clone	AW047207	241	895	0.27			
Peroxiredoxin 2 (Prdx2)	U20611	281	892	0.31	16 ± 3.0	11 ± 0.6	NS
Dehydrogenase/reductase X chromosome (Dhrsx)	AI846822	4,509	11,760	0.38	11 ± 1.6	12 ± 2.1	NS
Ornithine decarboxylase antizyme 3 (Oaz3)	AB016275	17,735	44,962	0.39	94 ± 6.8	150 ± 9.2	0.007
Splicing factor, arginine/serine-rich 16 (Sfrs16)	AF042799	430	1,030	0.42			
Protein phosphatase 2, regulatory subunit B, beta isoform (Ppp2r2b)	AW048155	6,134	14,358	0.43	9.2 ± 1.9	23 ± 1.5	0.004
Mouse endogenous murine leukemia virus modified polytopic provirus DNA	M17327	789	1,769	0.45			
Deleted in polyposis 1-like 1 (Dplll)	AA755260	14,224	29,836	0.48	340 ± 30	970 ± 130	0.008
Lysophospholipase 1 (Lypla1)	U89352	6,971	14,600	0.48	7.8 ± 0.4	5.6 ± 1.6	NS
Suppressor of K^+ transport defect 3 (Skd3)	U09874	7,709	15,612	0.49			
S100 calcium binding protein A13 (S100a13)	X99921	773	1,430	0.54			
Protamine 1 (Prm1)	Z47352	18,600	31,700	0.58	12,000 ± 2,200	16,000 ± 1,700	NS
RAN GTPase activating protein 1 (Rangap1)	U20857	4,960	8,480	0.58	72 ± 33	110 ± 47	NS
Growth arrest-specific protein 6 (Gas6)	X59846	1,120	1,820	0.61	80 ± 8.2	170 ± 35	0.04
Bcl2-associated athanogene 1 (Bag1)	AF022223	3,412	5,546	0.61	0.1 ± 0.1	1.1 ± 0.4	NS
Cyclin-dependent kinase inhibitor 1C (Cdkn1c)	U22399	1,090	1,650	0.66	2.3 ± 0.2	1.8 ± 0.1	NS
Mothers against decapentaplegic homolog 6 (Smad6)	AF010133	2,070	2,930	0.70	79 ± 11	94 ± 8.4	NS
Sperm mitochondrion-associated cysteine-rich protein (Smcp)	M88463	29,700	38,900	0.76	430 ± 43	930 ± 81	0.002

[a] Average value of two independent experiments.
[b] The Amount of Igsf4/Tslc1 in the *Tslc1*$^{+/+}$ testis was assigned a value of 100. Data are average values ± SE of three to five independent experiments.
[c] NS, not significant.

APLICACIÓN: (MODIFICACIÓN GENÉTICA EN MAMÍFEROS)

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