Transcriptómica

Ejemplos en Parasitología y Micología

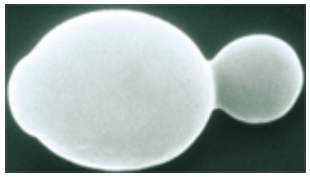
Transcriptómica

- Microrrays:
  - cDNA spoteado
  - Oligonucleótidos sintetizados *in situ*
  - Oligonucleótidos largos spoteados

- RNA Seq
  - 454 pyrosequencing
  - Illumina (Solexa) sequencing
  - SOLiD sequencing
  - Ion Torrent semiconductor sequencing

Transcriptómica

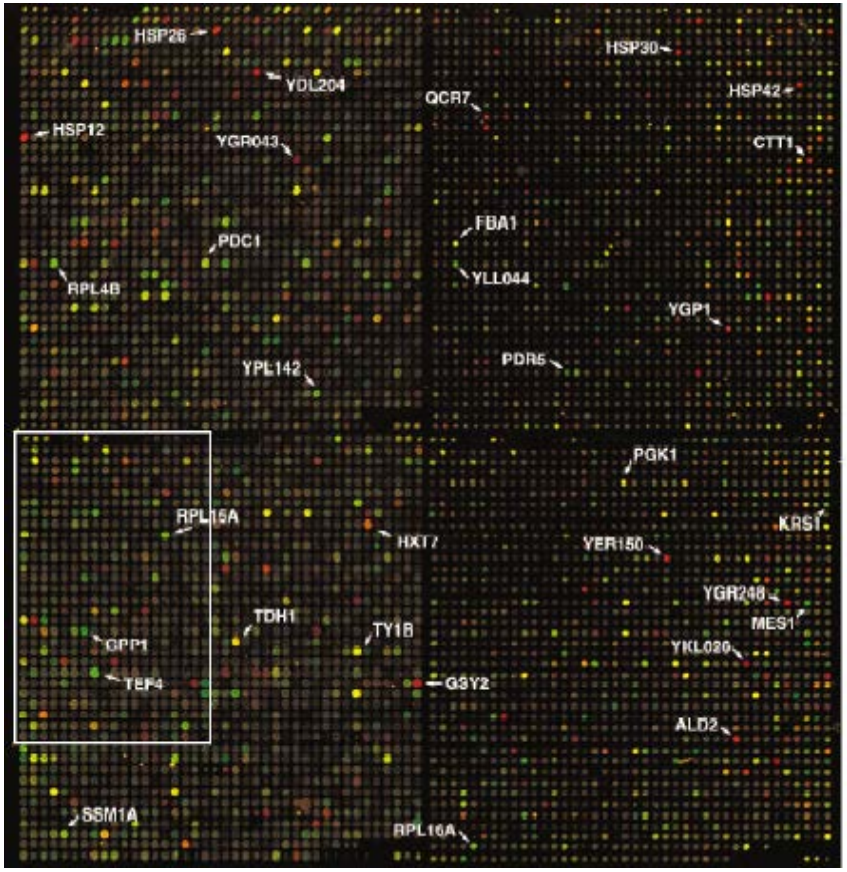
Primer uso de Microarrays de cDNA spoteados para el estudio de una serie temporal en Levaduras



- ***Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale***
  - DeRisi, Iyer, Patrick O. Brown. *Science. 1997 Oct 24;* ***278****(5338): 680-6.*

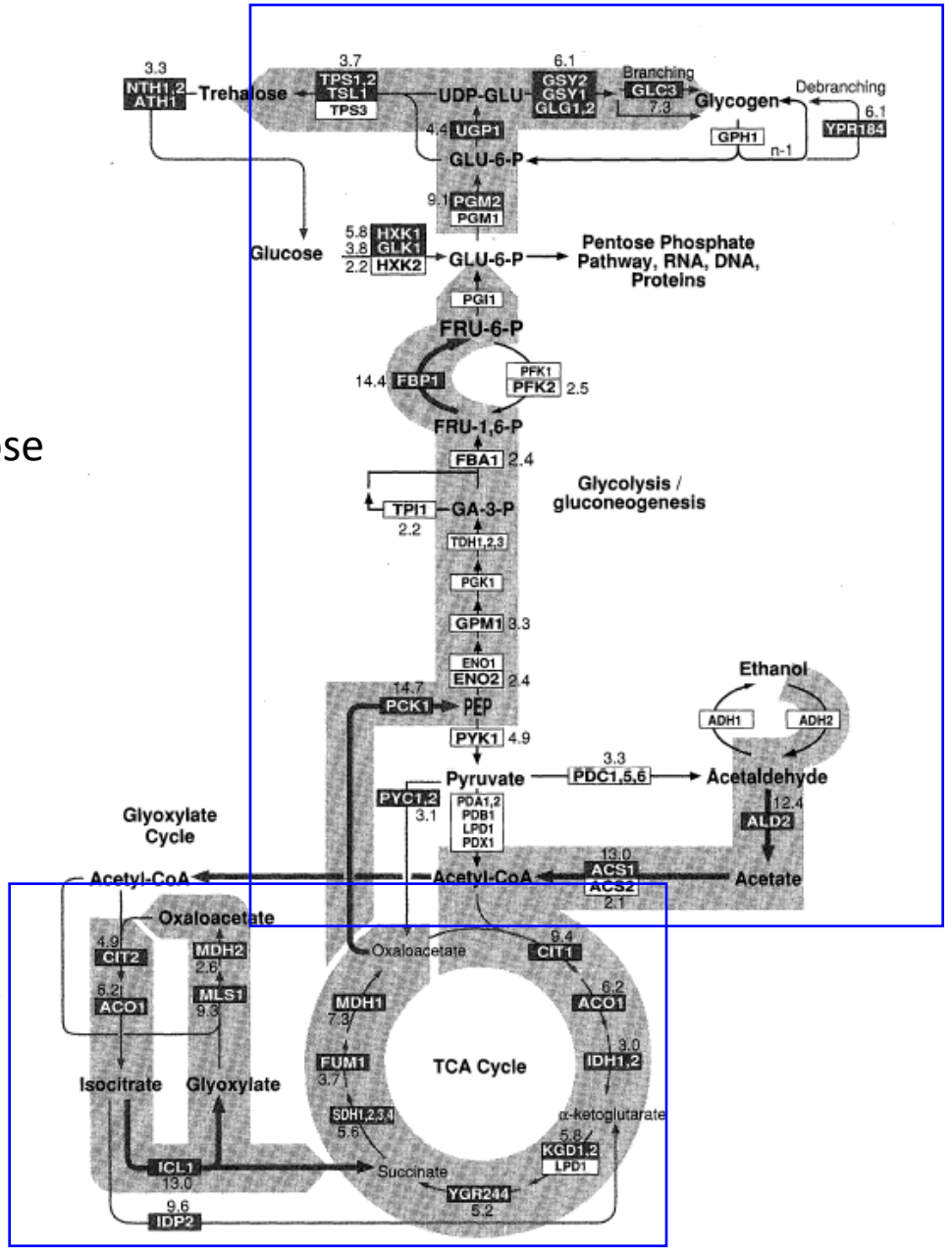
Transcriptómica

Muestras tomadas en un cultivo en transición entre metabolismo anaeróbico (fermentación) y aeróico (respiración)



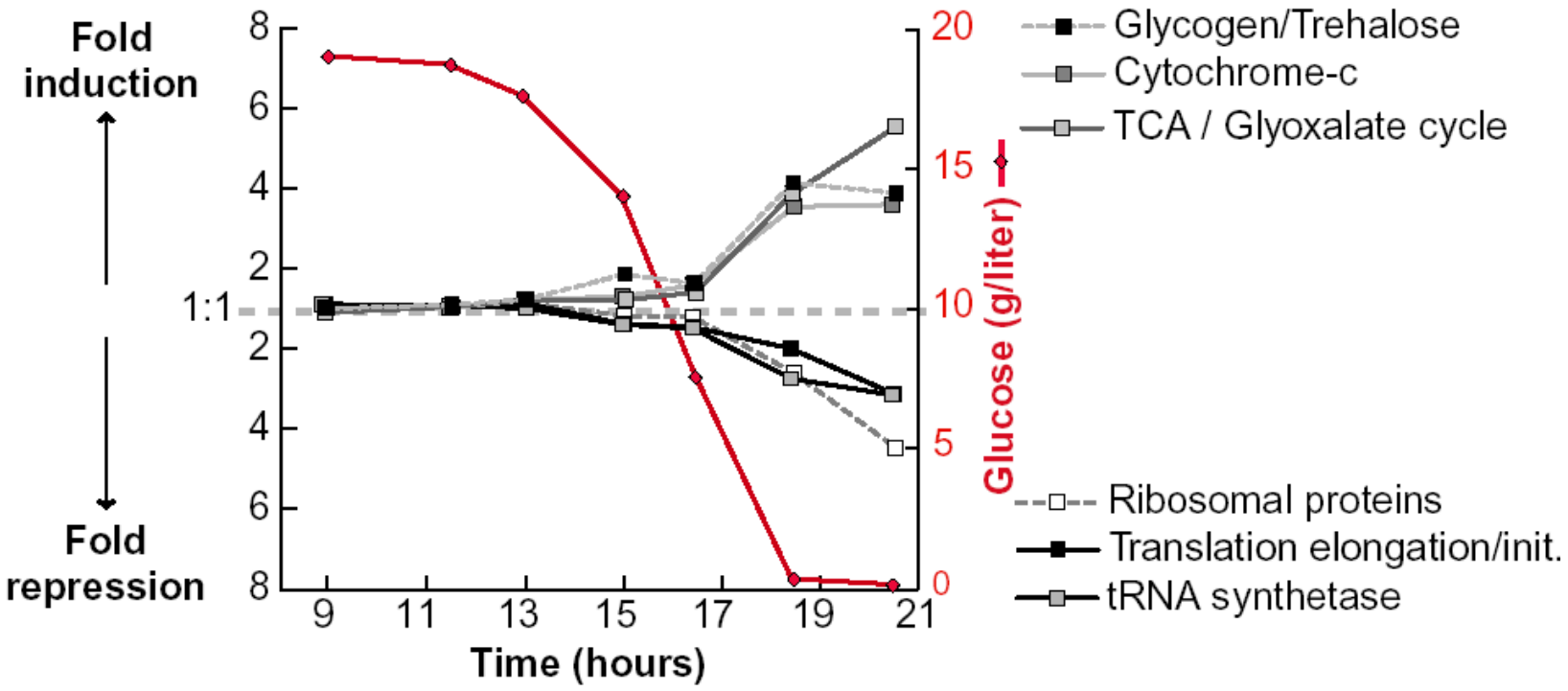
High glucose

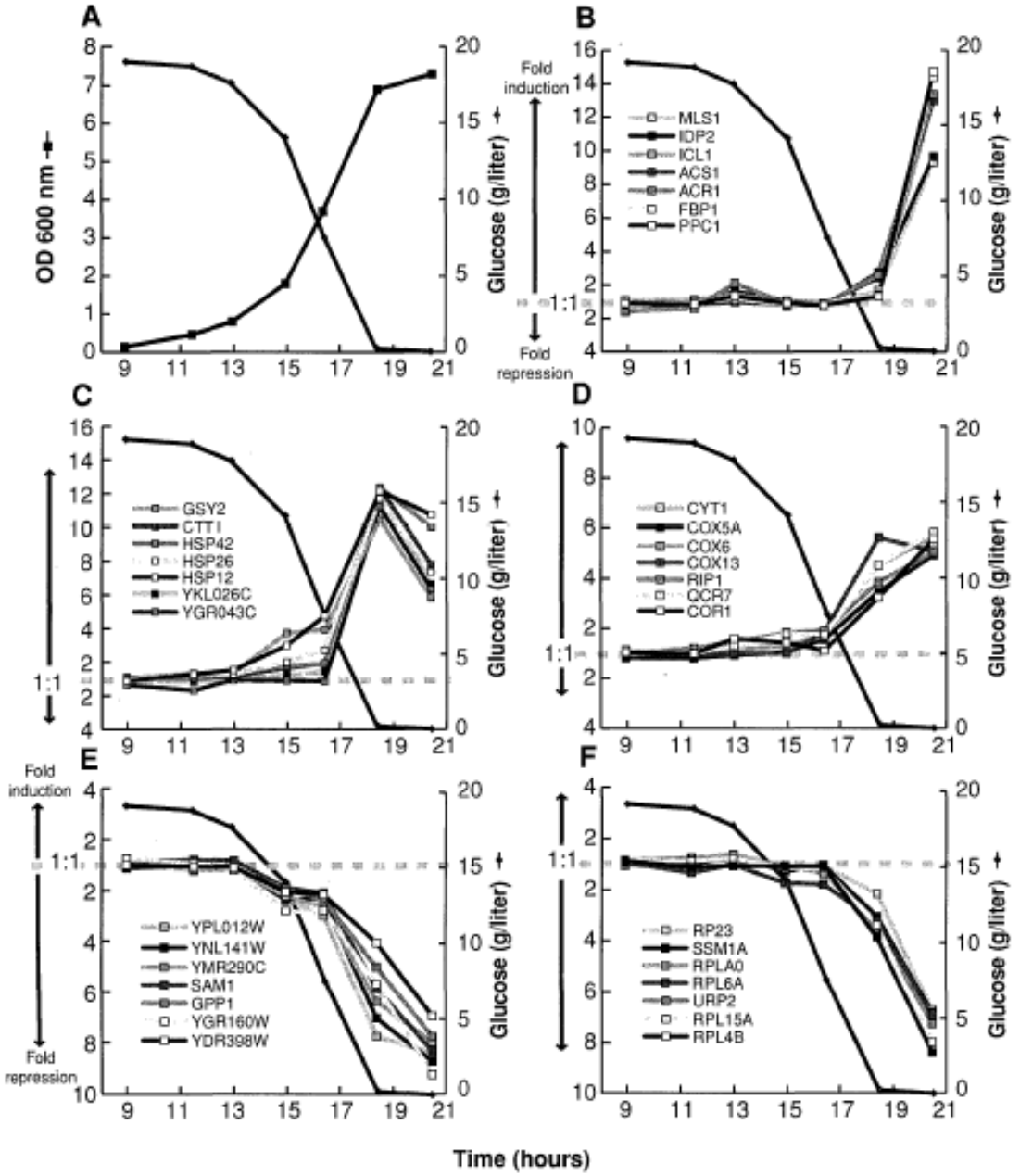
Low glucose



Transcriptómica

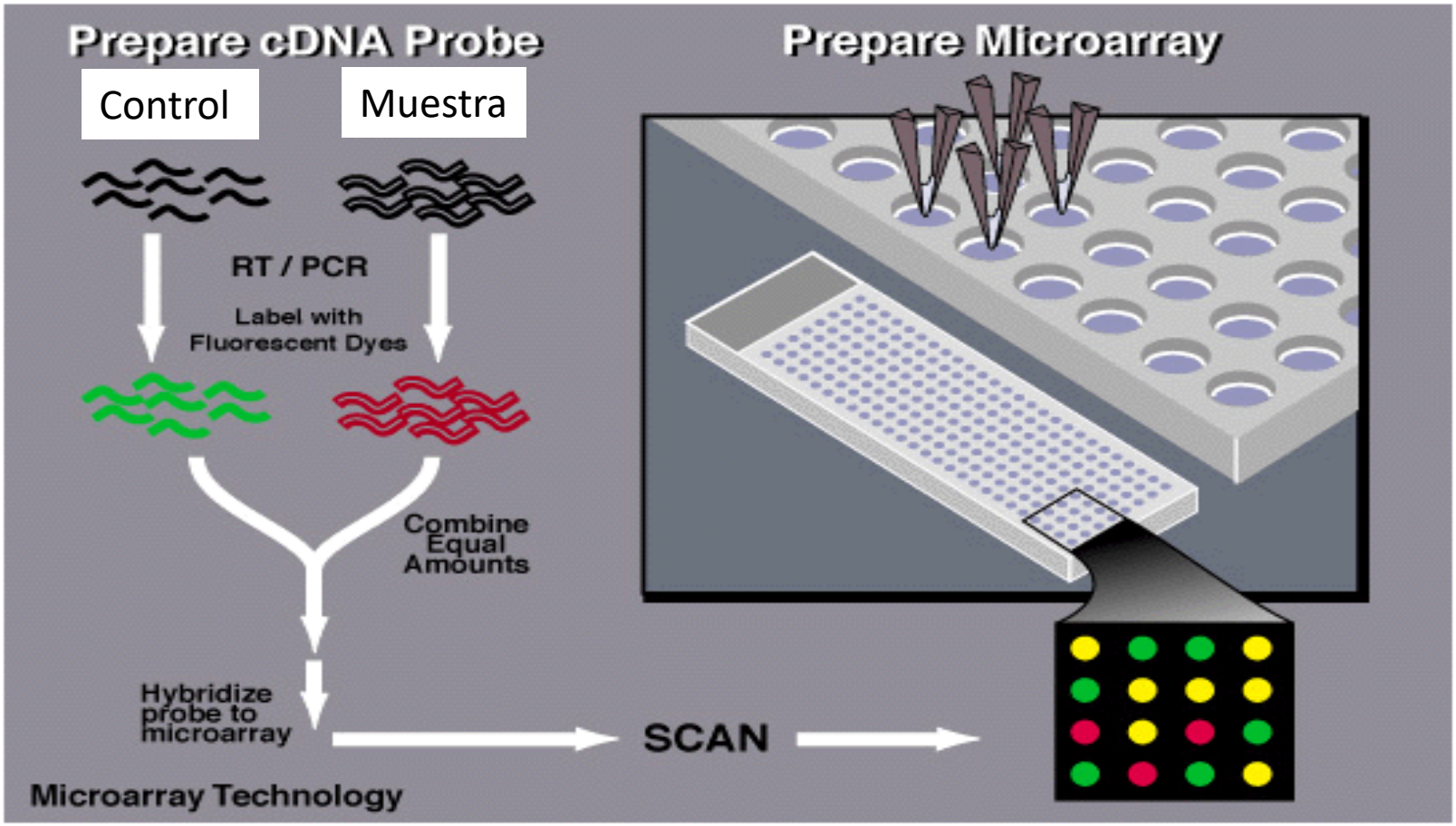
Muestras tomadas en un cultivo en transición entre metabolismo anaeróbico (fermentación) y aeróico (respiración)




A
B
C
D
E
F
OD 600 nm
Glucose (g/liter)
Fold induction
Fold repression
1:1
MLS1
IDP2
ICL1
ACS1
ACR1
FBP1
PPC1
GSY2
CTT1
HSP42
HSP26
HSP12
YKL026C
YGR043C
CYT1
COX5A
COX6
COX13
RIP1
QCR7
COR1
YPL012W
YNL141W
YMR290C
SAM1
GPP1
YGR160W
YDR398W
RP23
SSM1A
RPLA0
RPL6A
URP2
RPL15A
RPL4B
Time (hours)

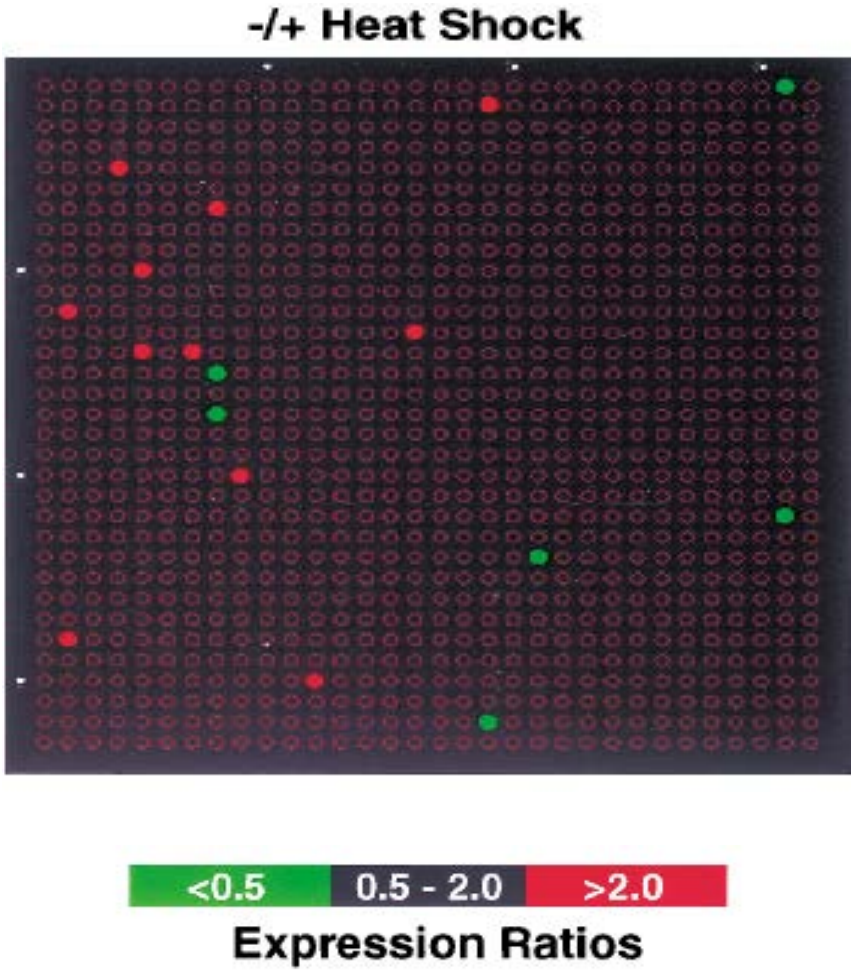
Transcriptómica

cDNA spoteado
Oligonucleótidos largos spoteados



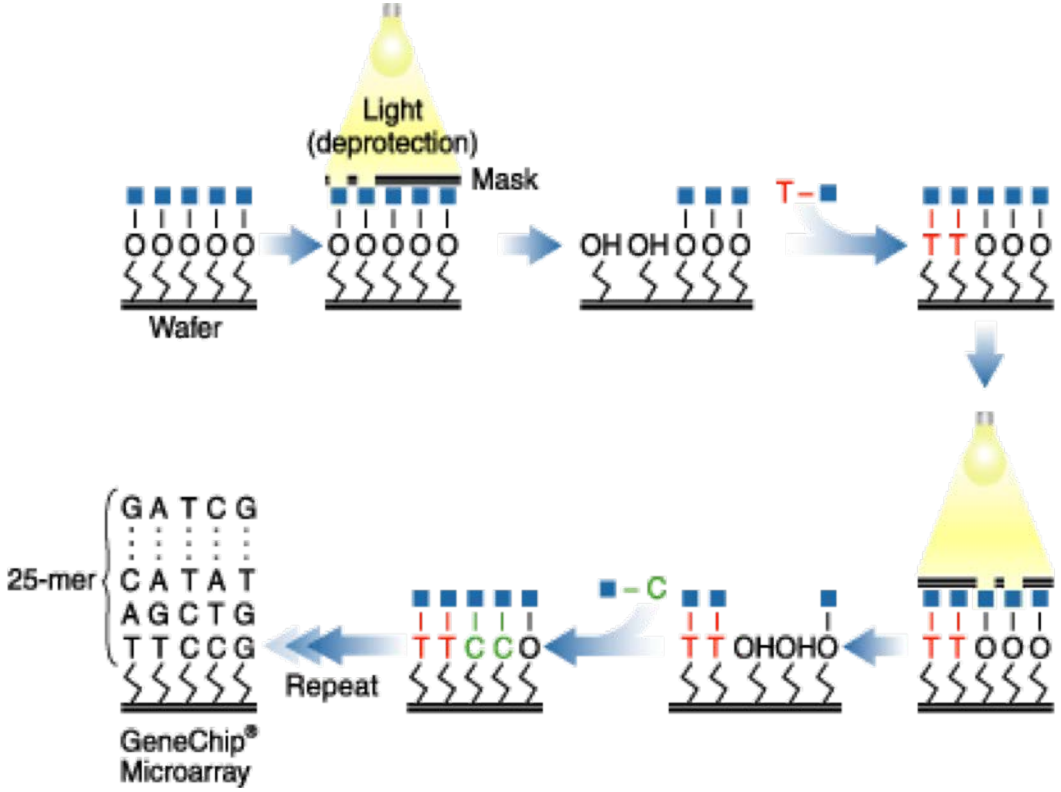
Transcriptómica

- Detección de genes diferencial mente exoresados.
- Se realizan hibridaciones cruzadas y se seleccionan los resultados coincidentes.
- Importante tratamiento estad´ditico de los datos para separar ruido de señal



cDNA spoteado
Oligonucleótidos largos spoteados

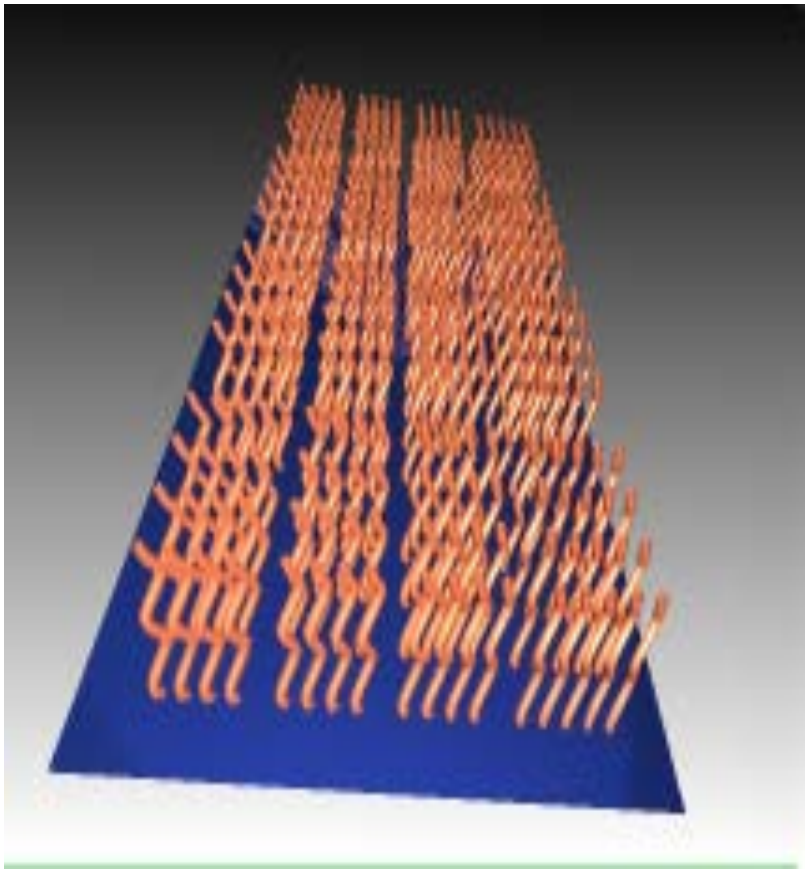
Oligonucleótidos sintetizados *in situ* : Affymetrix



- Light Directed Spatiallly Addressable Parallel Chemical Synthesis. Fodor et al, 1991 Science 251:767-773
  - *We have developed an approach in which sequence information is used, directly to design high-density, two-dimensional arrays of synthetic peptides and oligonucleotides.*

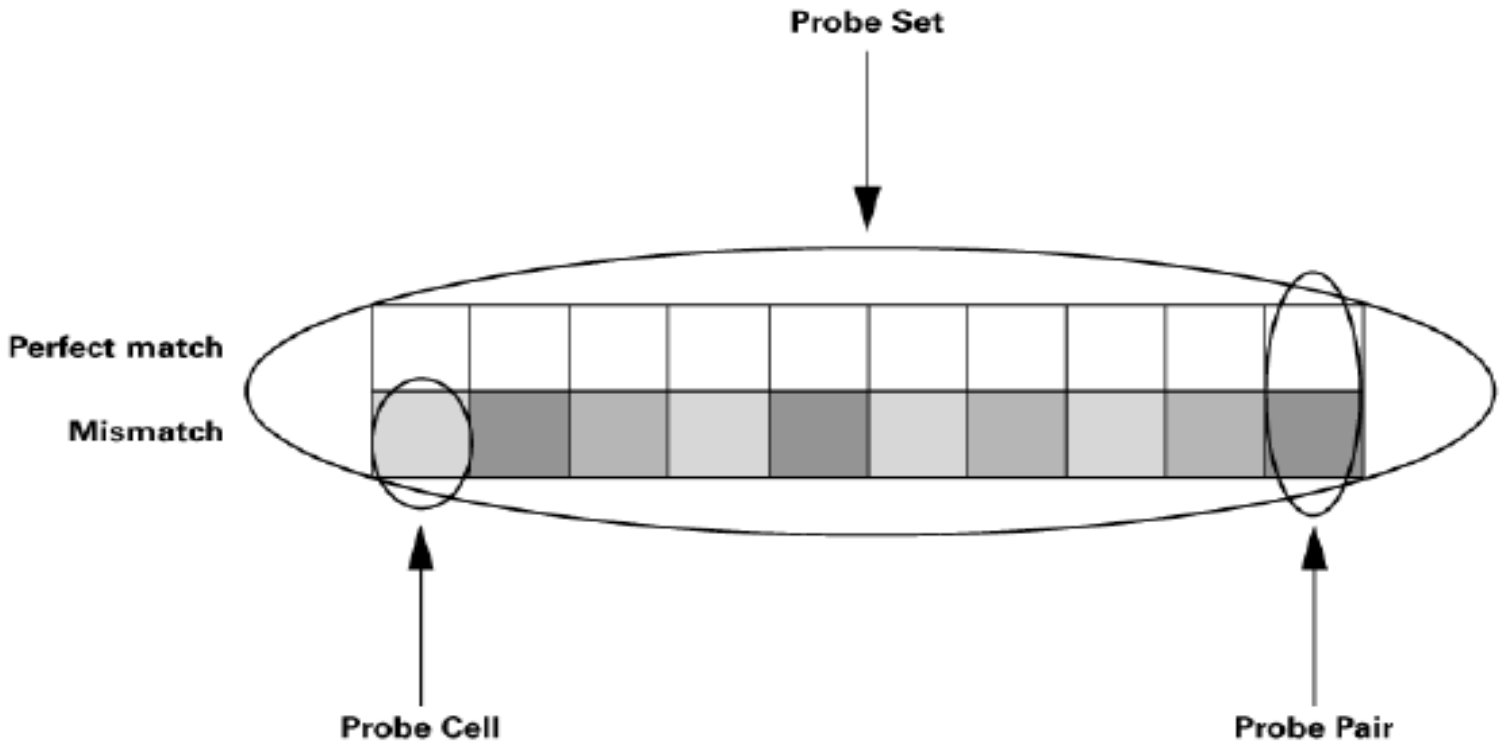
Oligonucleótidos sintetizados *in situ* : Affymetrix

- Oligos cortos
  - ~25 nt
- Un gen
  - ~ 12–25 oligos
- Affymetrix

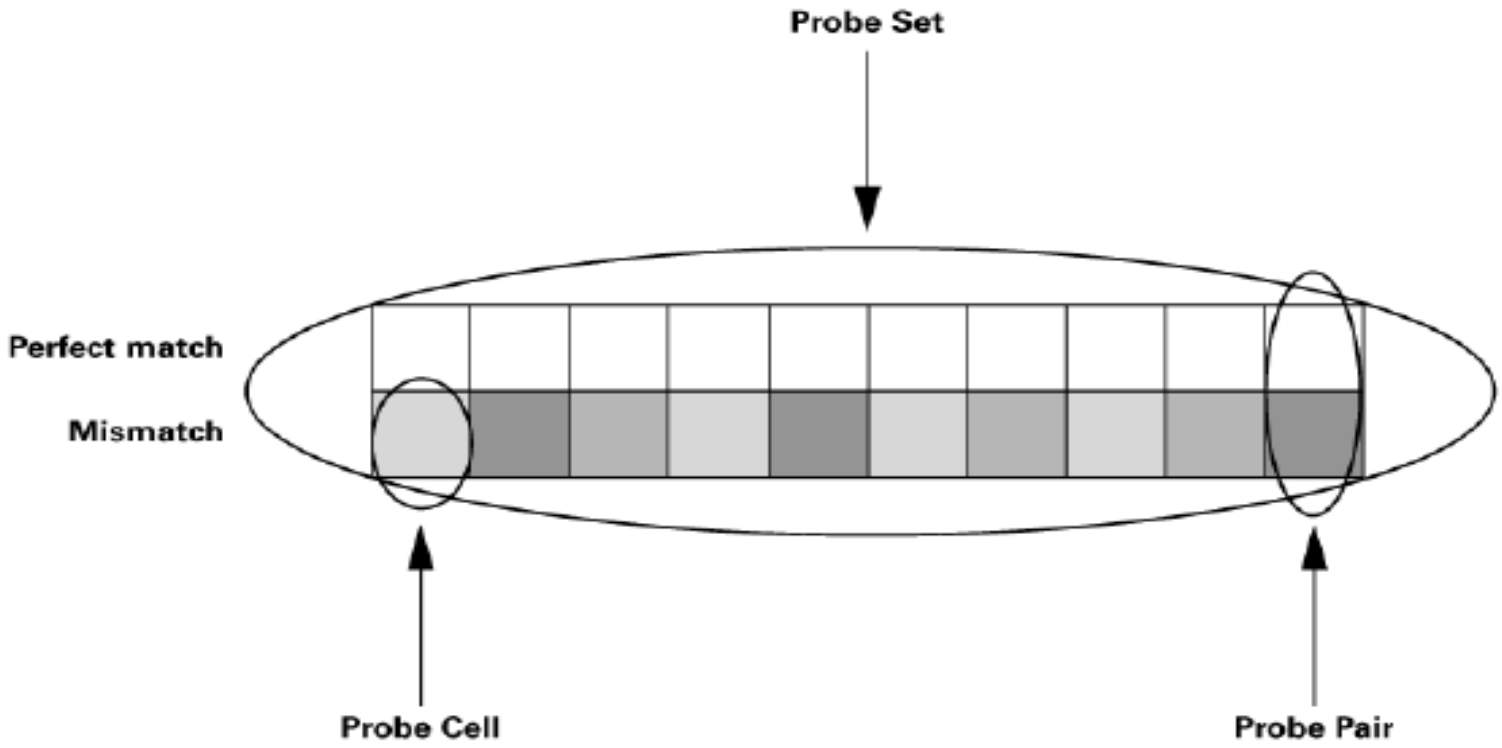


Oligonucleótidos sintetizados *in situ* : Affymetrix

Affymetrix Probe Set



Affymetrix Probe Set

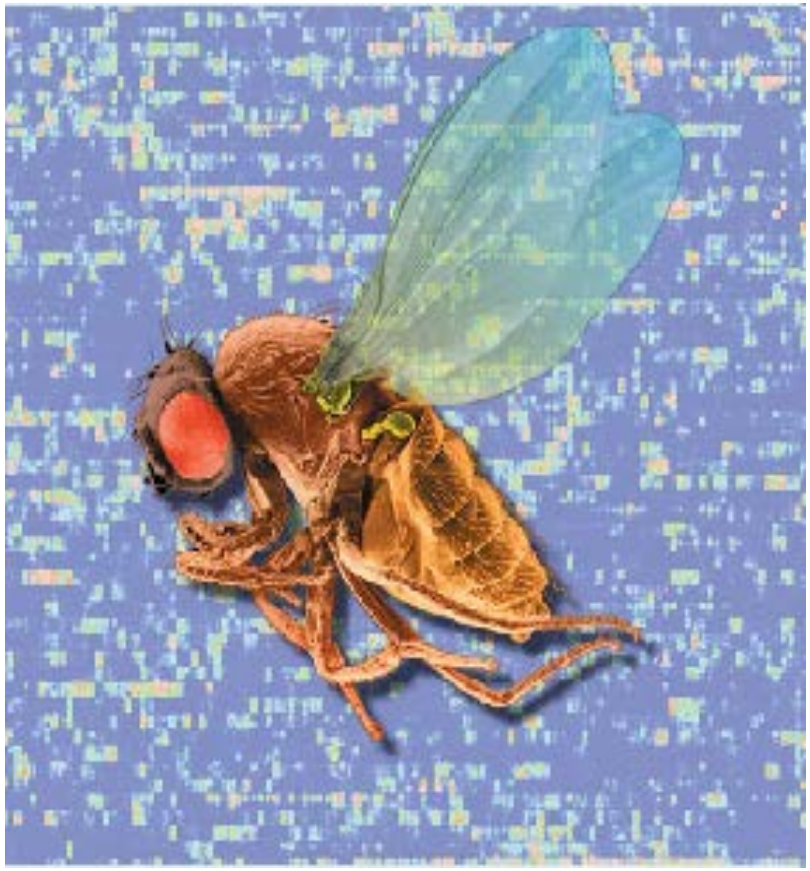


Human Genome U133 chip

- Spots
  - 33000 genes represented
- Gene Expression
  - Tissues & Cell lines
  - Induction
  - Differentiation
  - Tumorigenesis

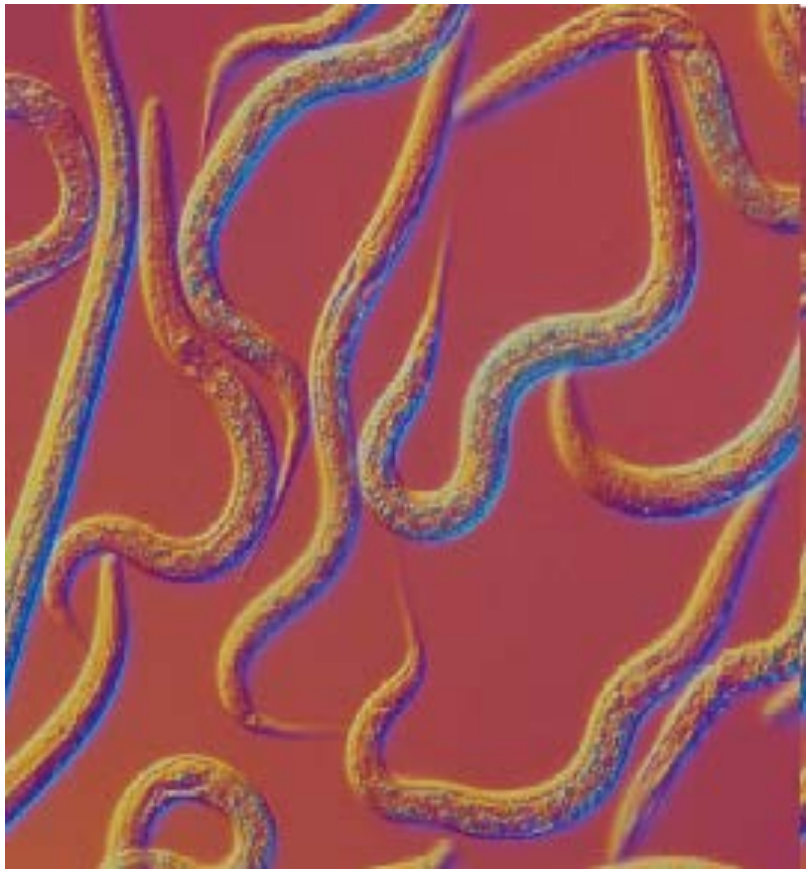
Drosophila Genome Array

- Spots
  - 13500 genes represented
- Gene Expression
  - Development Genes
  - Human Diseases
  - Mutations
  - Agricultural Research



C. Elegans Genome Array

- Spots
  - 22500 genes represented
- Gene Expression
  - Development Genes
  - Human Diseases
  - Mutations
  - Agricultural Research

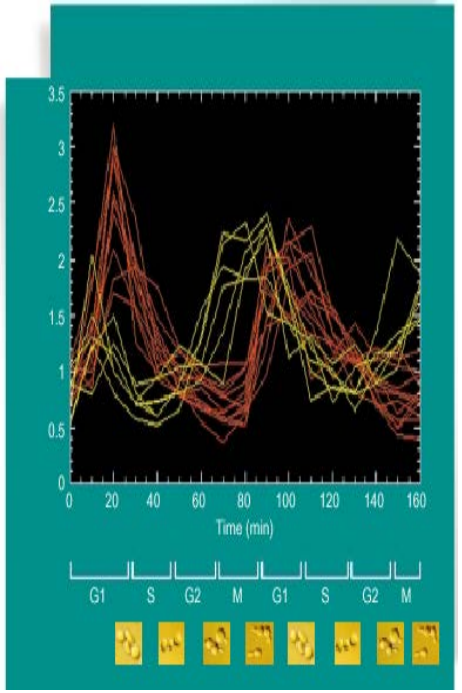


Rat Genome

- Spots
  - 24000 genes & ESTs
- Toxicology
- Neurobiology
- Immunology

Yeast Genome Ar

- Spots
  - 6400 ORFs
- Gene Functions
- Pathways
- Explore uncharacterized ORFs

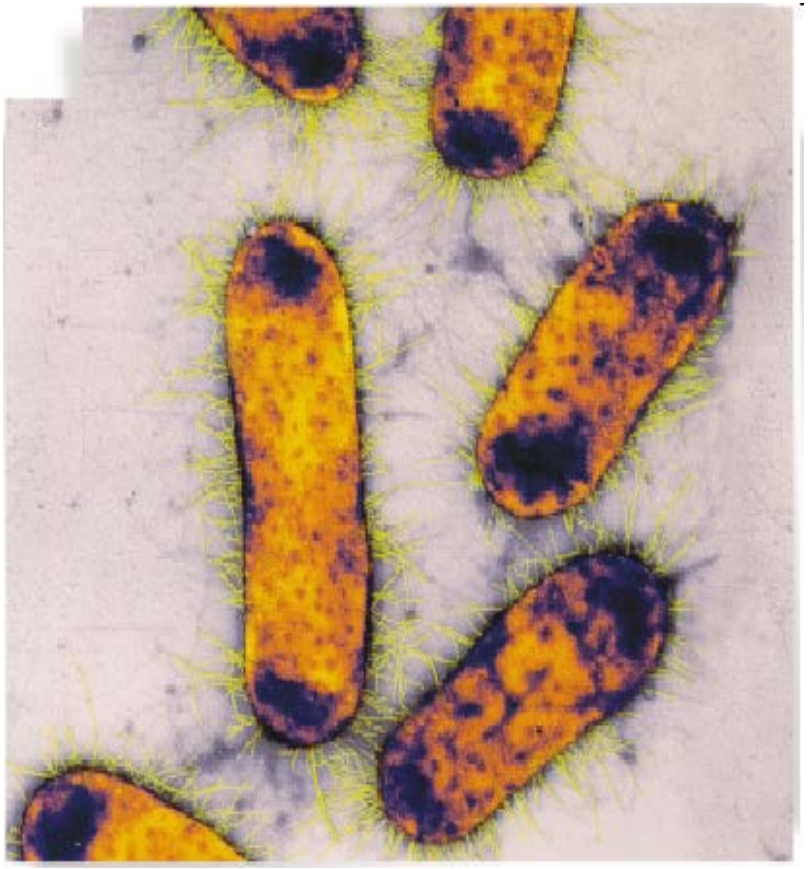


Arabidopsis Thaliana Array

- Spots
  - 24000 genes represented
- Plants Genomics
  - Development Genes
  - Environmental Conditions
  - Mutant Lines

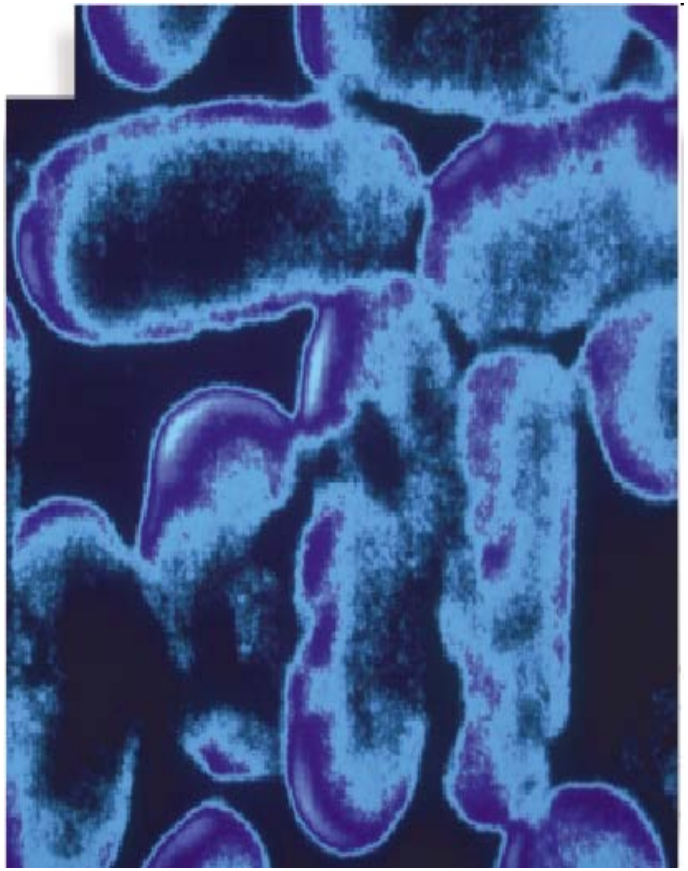
E. Coli Antisense Genome Array

- Spots
  - 4200 ORFs
  - 1200 Intergenic Regions
- Biological Mechanisms
- Optimize Culture



Pseudomonas Aeruginosa Array

- Spots
  - 5500 ORFs
- Hospital Acquired Diseases
  - Virulent on Cystic Fibrosis
- Antibiotic resistance
- Biofilm formation
- Host-pathogens



Transcriptómica: RNA-Seq

Nat Rev Genet. 2009 January ; 10(1): 57–63. doi:10.1038/nrg2484.

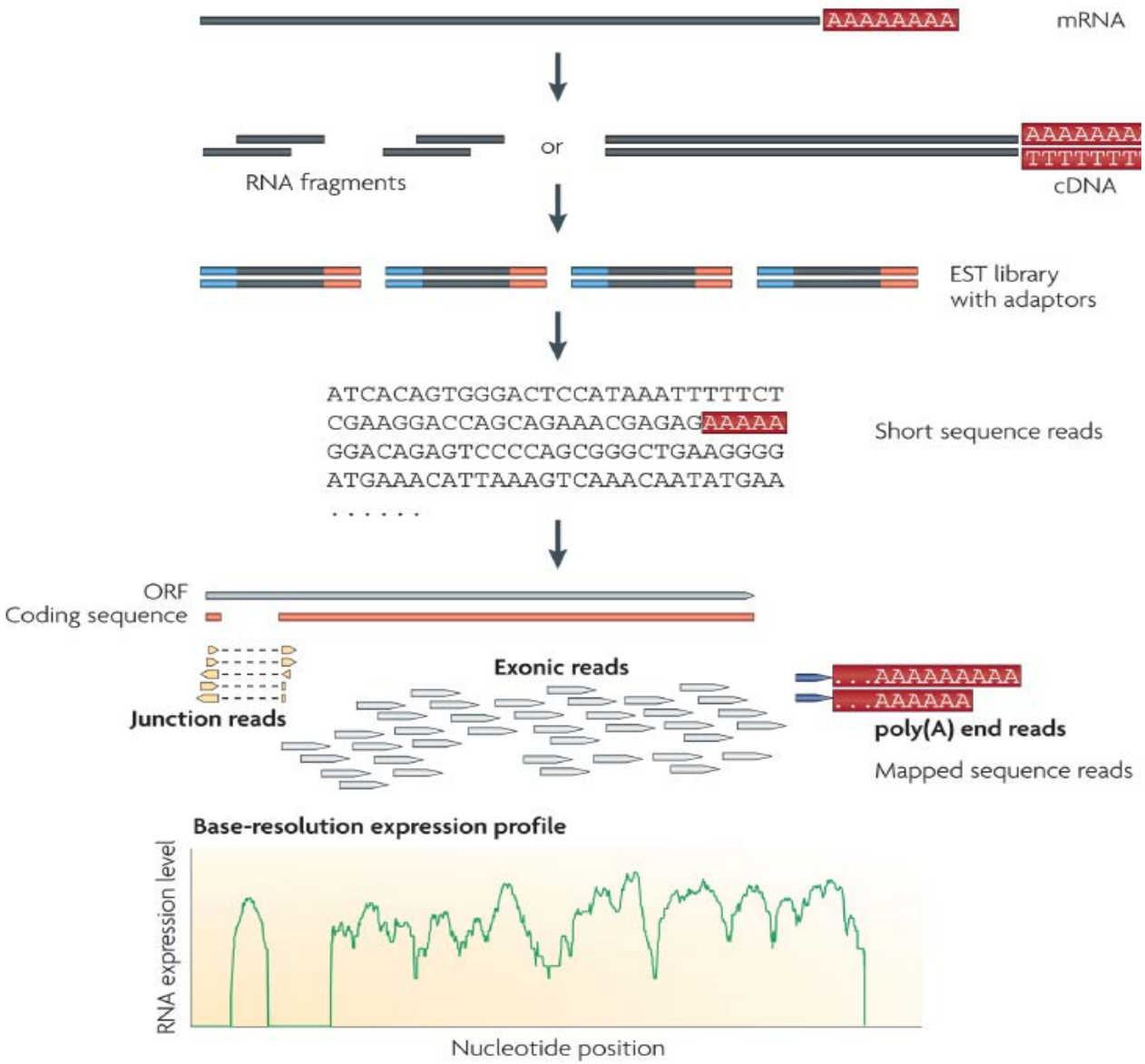
RNA-Seq: a revolutionary tool for transcriptomics

Zhong Wang, **Mark Gerstein**, and **Michael Snyder**
Zhong Wang and Michael Snyder are at the Department of Molecular, Cellular and Developmental Biology, and Mark Gerstein is at the Department of Molecular, Biophysics and Biochemistry, Yale University, 219 Prospect Street, New Haven, Connecticut 06520, USA.

Transcriptómica: RNA-Seq

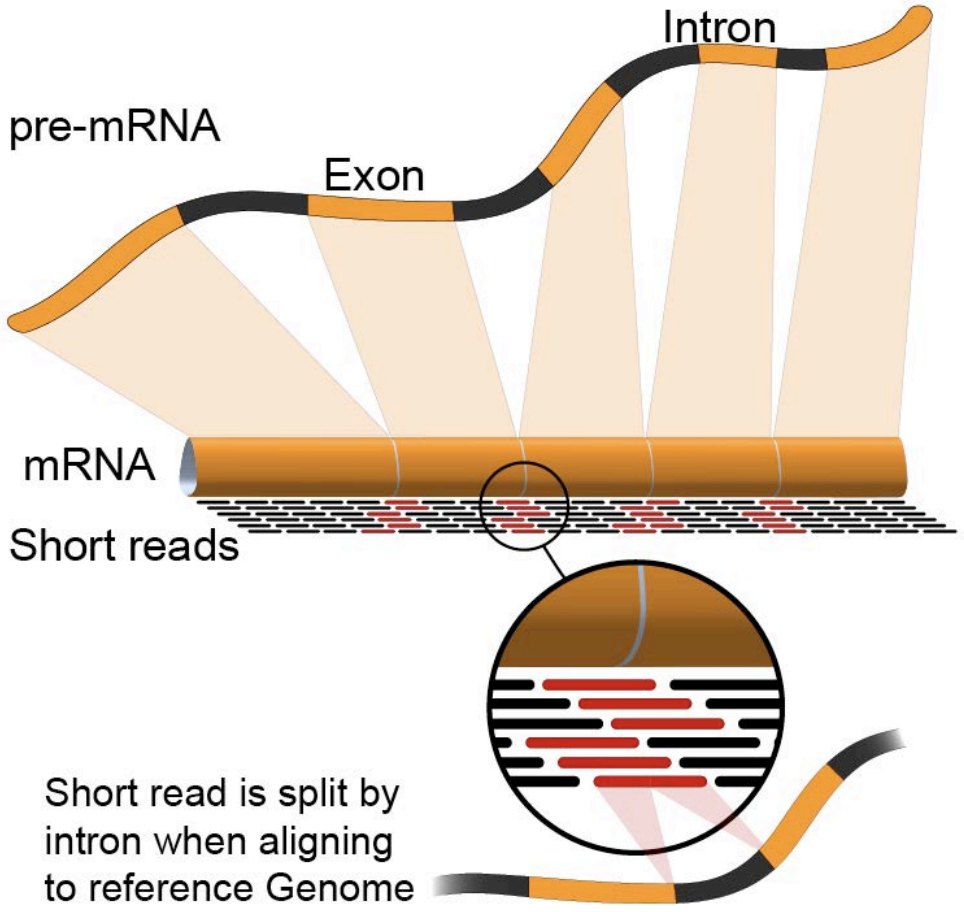
- Whole Transcriptome Shotgun Sequencing
  - Secuenciado de cDNA
  - Mediante “NexGen technology”
- Una herramienta revolucionria para la transcriptómica
  - Mediciones más precisas
  - Permite la realización de experimentos a gran escala con material de partida limitado

Transcriptómica: RNA-Seq



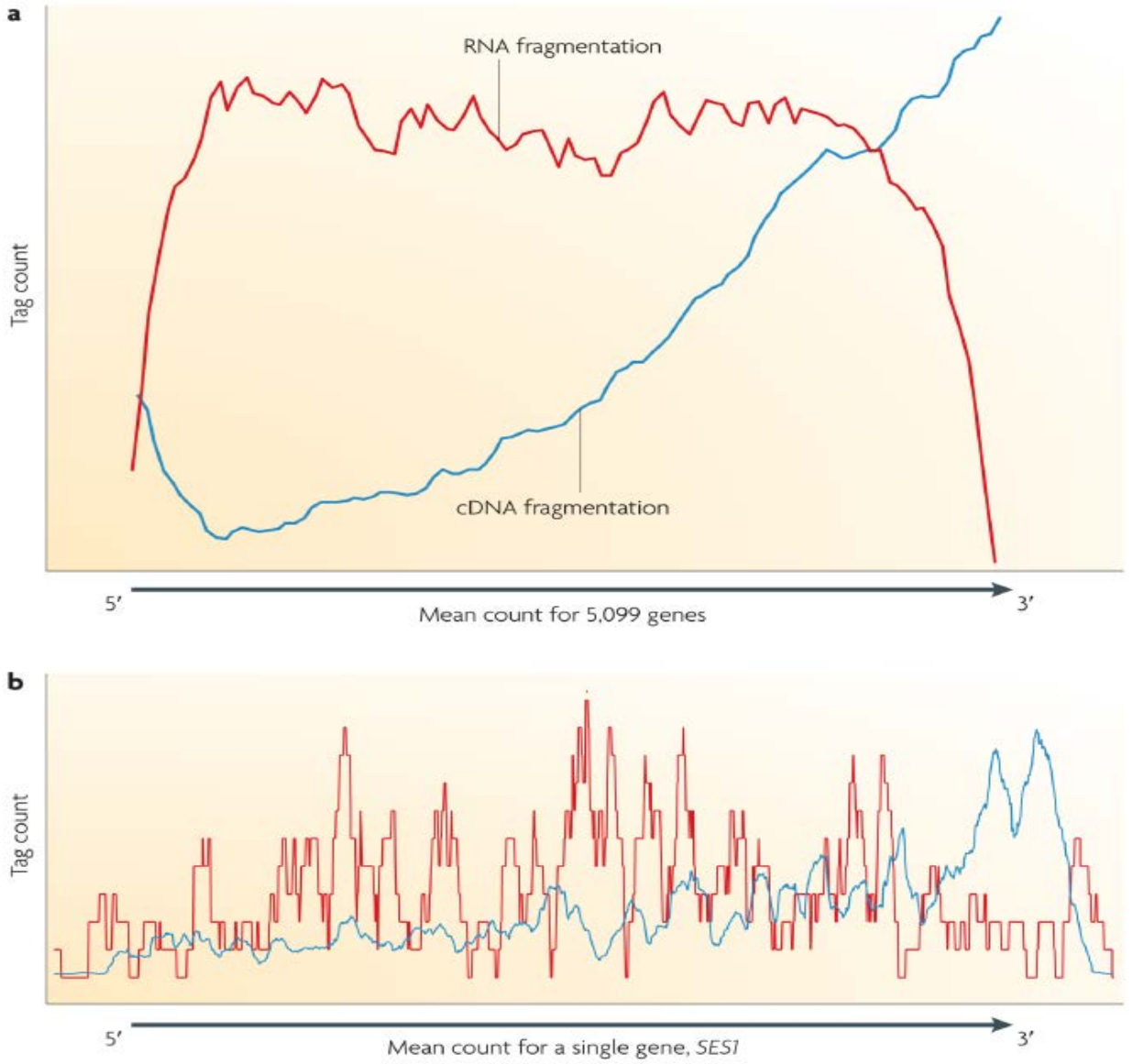
RNA-Seq / Mapeado

Ubica los “reads” sobre el genoma de referencia



http://en.wikipedia.org/

Transcriptómica: RNA-Seq



Transcriptómica: RNA-Seq

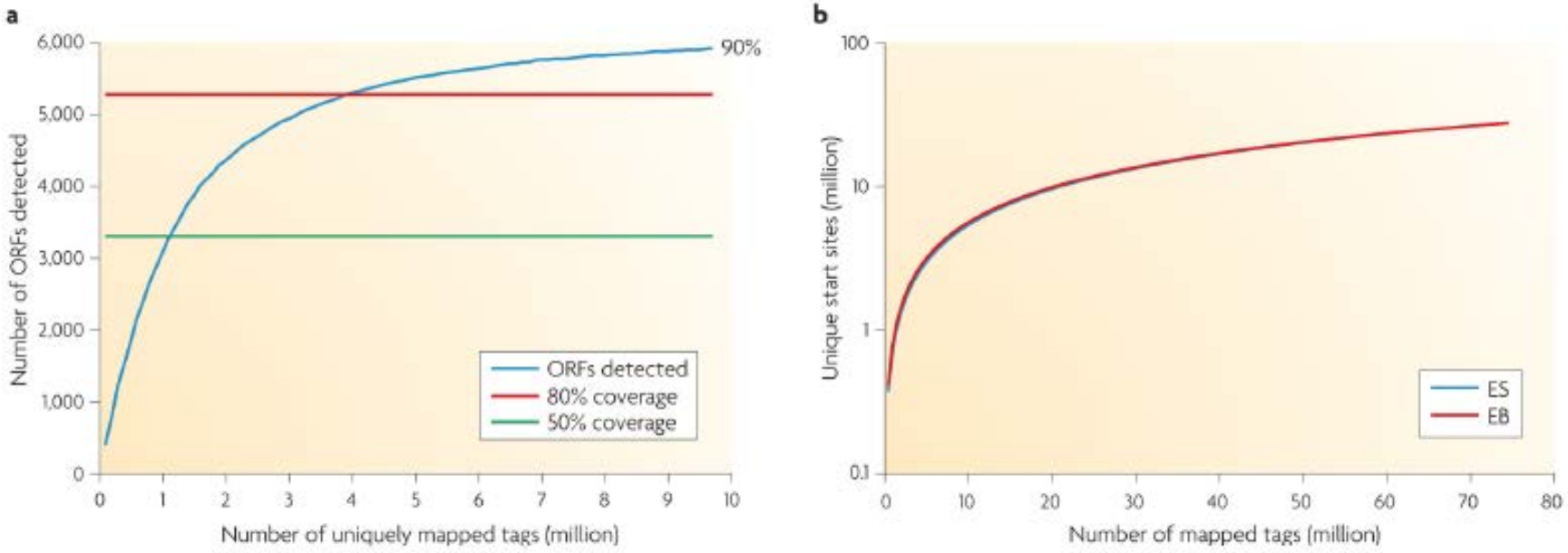
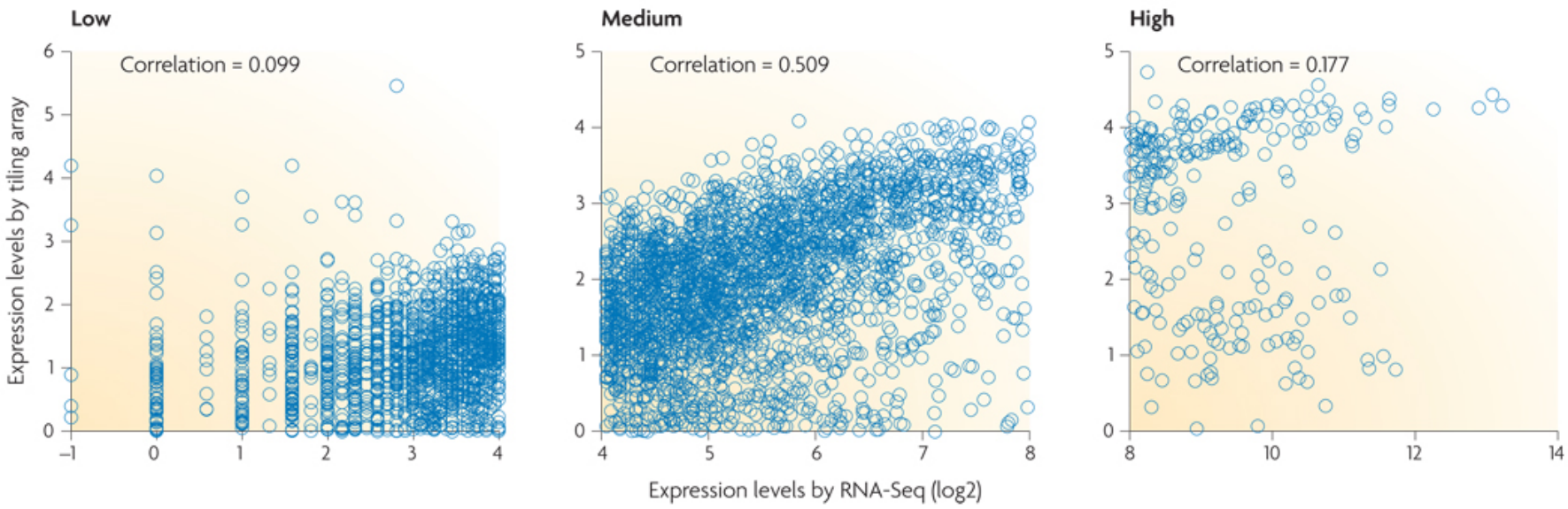


Figure 5. Coverage versus depth

a | 80% of yeast genes were detected at 4 million uniquely mapped RNA-Seq reads, and coverage reaches a plateau afterwards despite the increasing sequencing depth. Expressed genes are defined as having at least four independent reads from a 50-bp window at the 3′ end. Data is taken from REF. [18]. **b** | The number of unique start sites detected starts to reach a plateau when the depth of sequencing reaches 80 million in two mouse transcriptomes. ES, embryonic stem cells; EB, embryonic body. Figure is modified, with permission, from REF. [22]

Transcriptómica: RNA-Seq Vs Microarrays



Quantifying expression levels: RNA-Seq and microarray compared:
Expression levels are shown, as measured by RNA-Seq and tiling arrays, for *Saccharomyces cerevisiae* cells grown in nutrient-rich media. The two methods agree fairly well for genes with medium levels of expression (middle), but correlation is very low for genes with either low or high expression levels. The tiling array data used in this figure is taken from REF. [2], and the RNA-Seq data is taken from REF.

Transcriptómica

Table 1

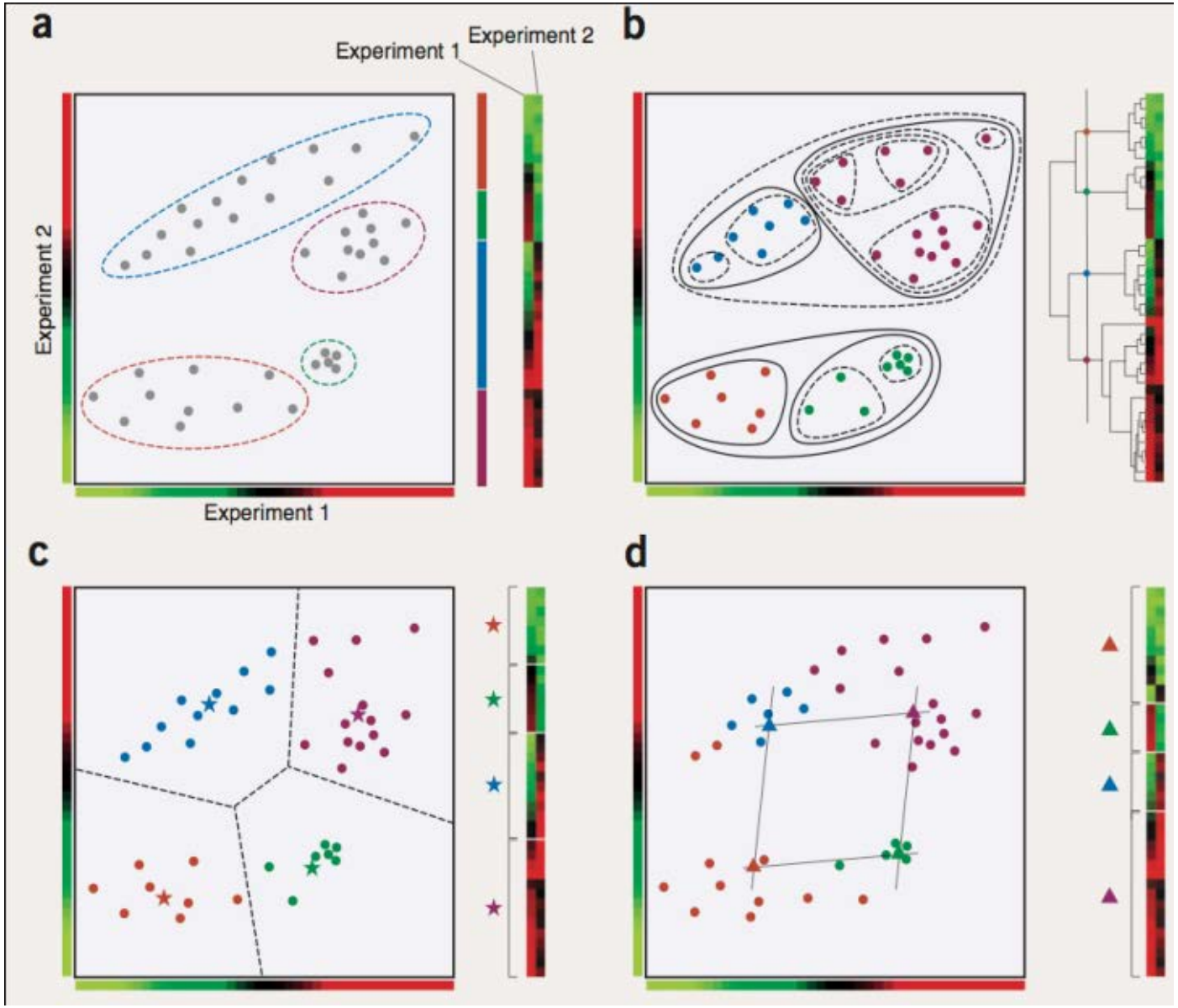
Advantages of RNA-Seq compared with other transcriptomics methods

Technology	Tiling microarray	cDNA or EST sequencing	RNA-seq
Technology specifications			
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to 100 bp	Single base	Single base
Throughput	High	Low	High
Reliance on genomic sequence	Yes	No	In some cases
Background noise	High	Low	Low
Application			
Simultaneously map transcribed regions and gene expression	Yes	Limited for gene expression	Yes
Dynamic range to quantify gene expression level	Up to a few-hundredfold	Not practical	>8,000-fold
Ability to distinguish different isoforms	Limited	Yes	Yes
Ability to distinguish allelic expression	Limited	Yes	Yes
Practical issues			
Required amount of RNA	High	High	Low
cost for mapping transcriptomes of large genomes	High	High	Relatively low

Análisis de los datos: Clusterización

- Agrupa una colección de datos o objetos en subgrupos, de manera tal que losobjetos dentro de un subgrupo sean más relacionados entre ellos que con cualquier objeto asignado a un subgrupo diferente.
- Necesita:
  - Una medida de distancia
  - Un algoritmo de cluterización

Análisis de los datos: Clusterización



Distancia Euclidiana

- No detecta relaciones inversas

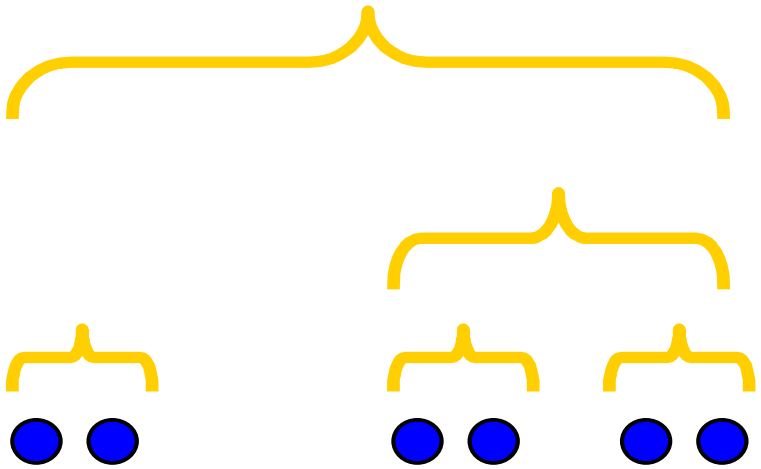
$$\sqrt{\sum_{j=1}^{p}(X[j]-Y[j])^2}$$

Pearson Correlation

- Detecta relaciones inversas (compara perfiles normalizados)

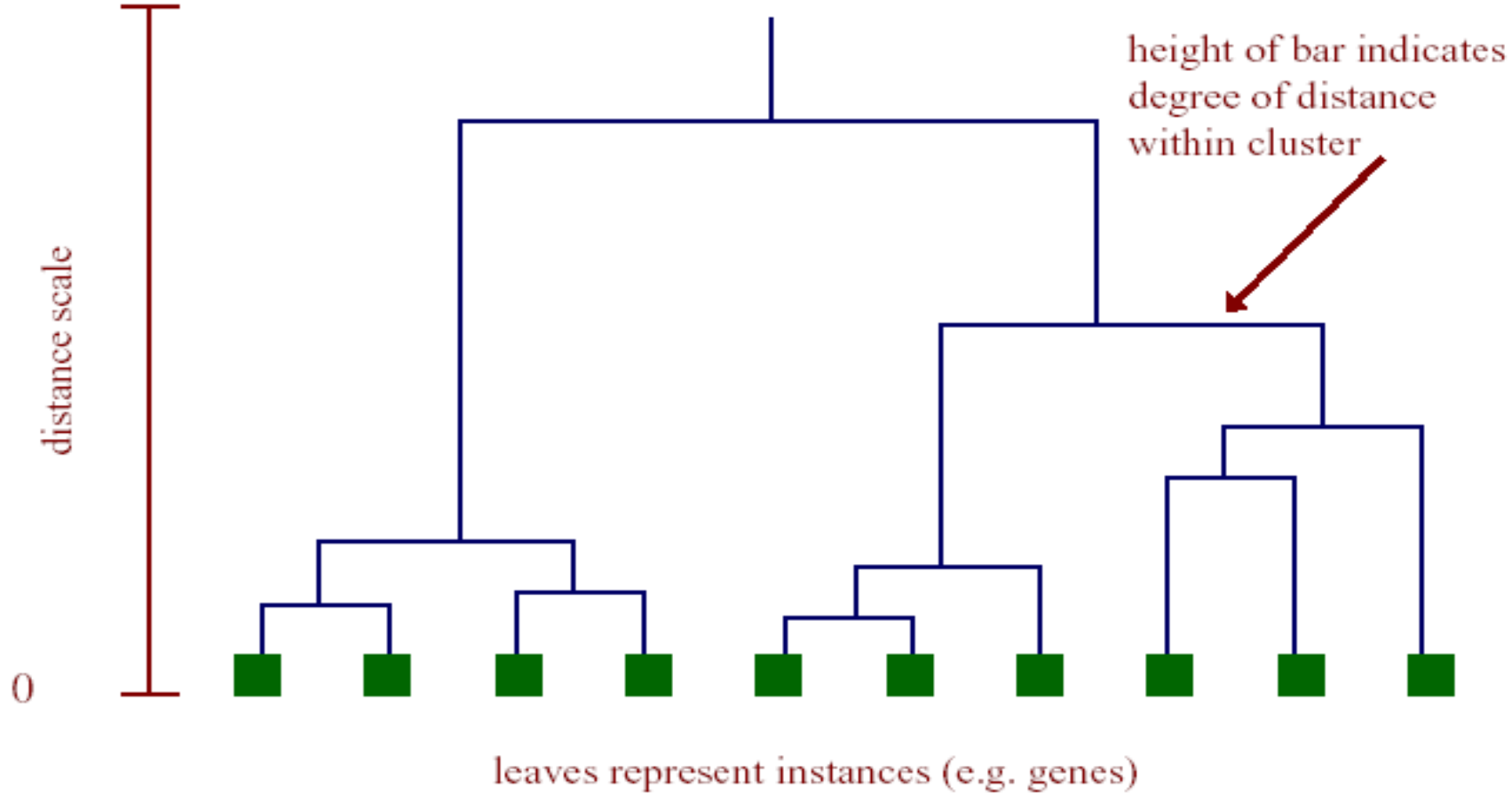
$$r = \frac{1}{p-1} \sum_{i=1}^{p} \left(\frac{x_i - \bar{x}}{s_x} \right) \left(\frac{y_i - \bar{y}}{s_y} \right)$$

Clusterización Gerárquica (Hartigan 1975)



- Agglomerative Bottom-Up
- Dendograma

Dendograma


height of bar indicates
degree of distance
within cluster
distance scale
0
leaves represent instances (e.g. genes)

Gene clustering

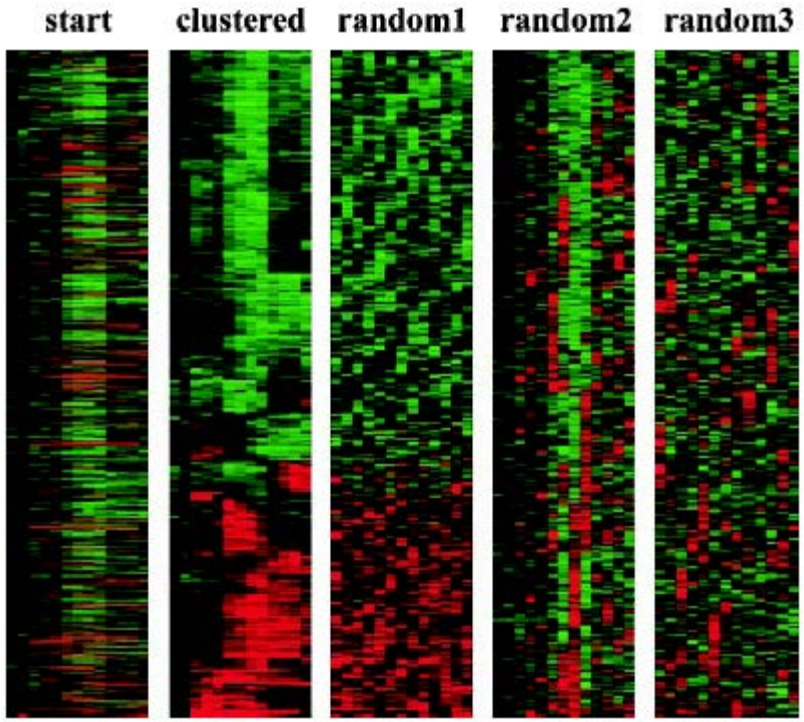
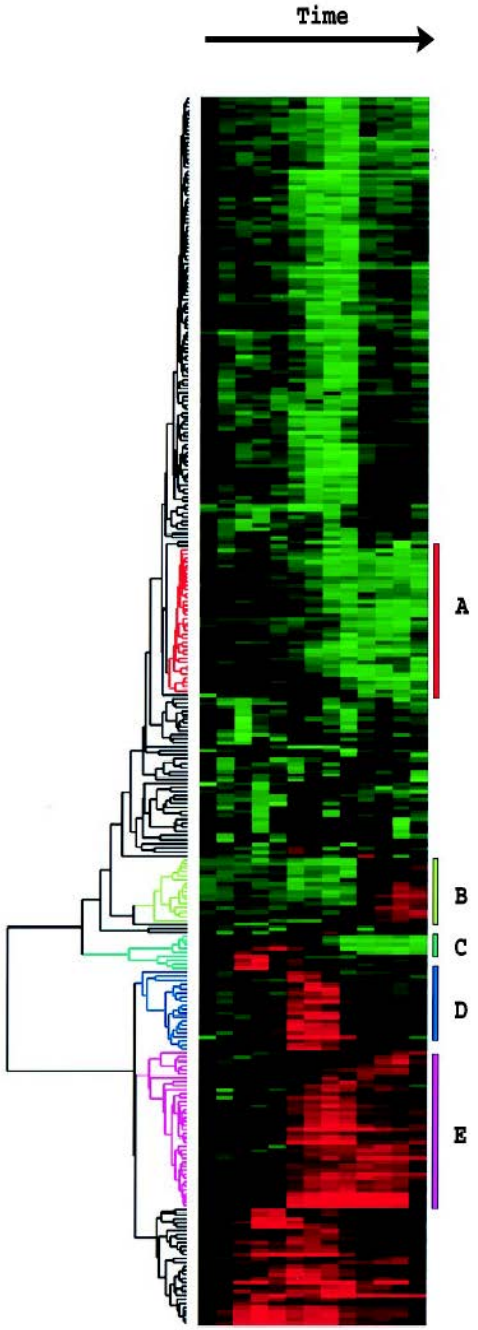
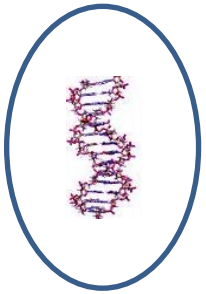


FIG. 3. To demonstrate the biological origins of patterns seen in Figs. 1 and 2, data from Fig. 1 were clustered by using methods described here before and after random permutation within rows (random 1), within columns (random 2), and both (random 3).

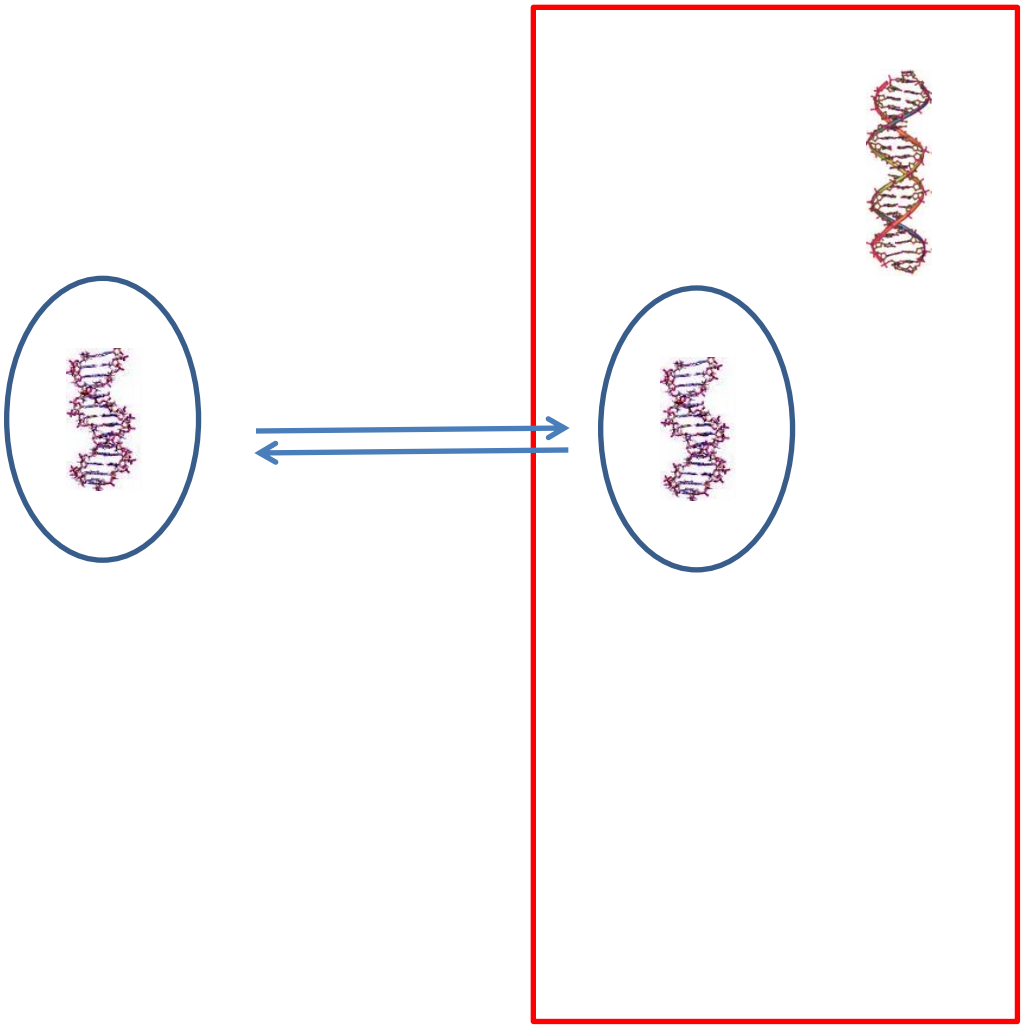


Análisis de transcriptomas en Parásitos

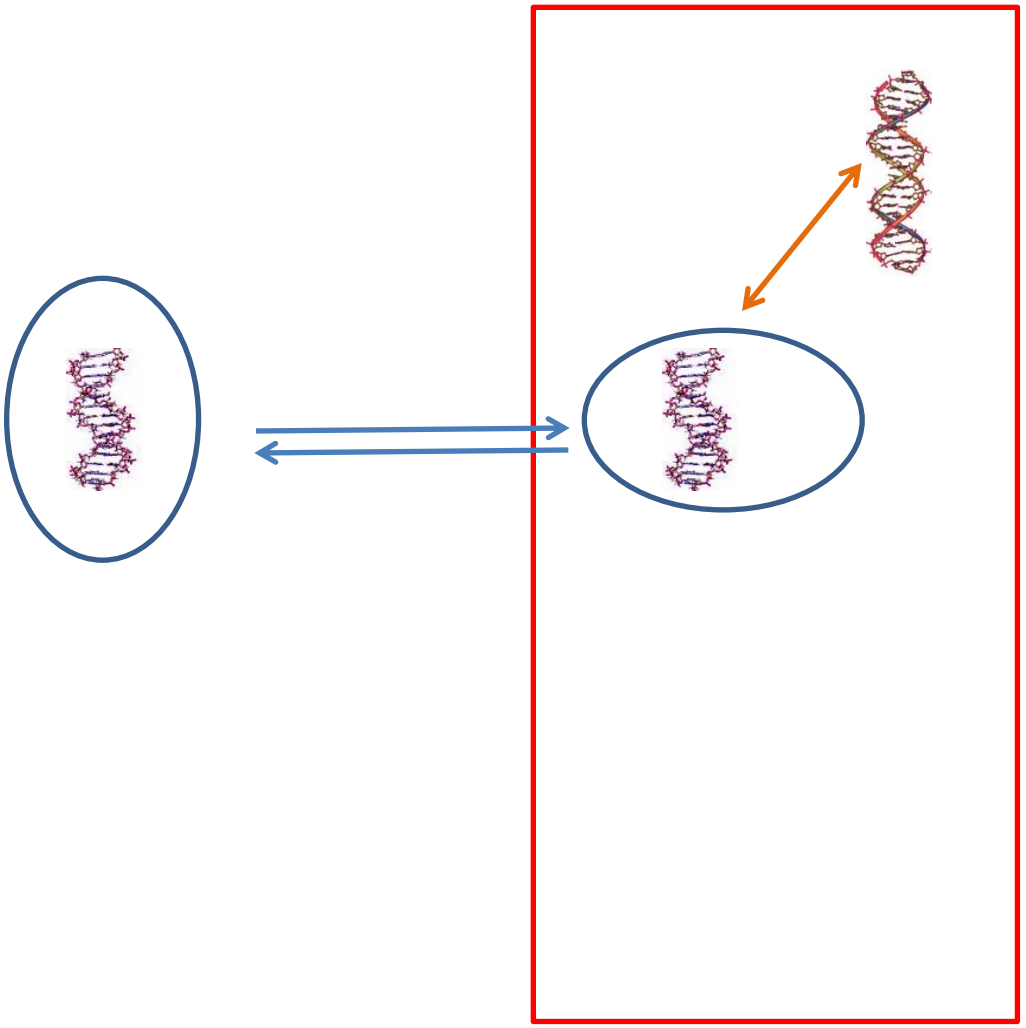
Ciclo de Vida - Relación Parásito Hospedador



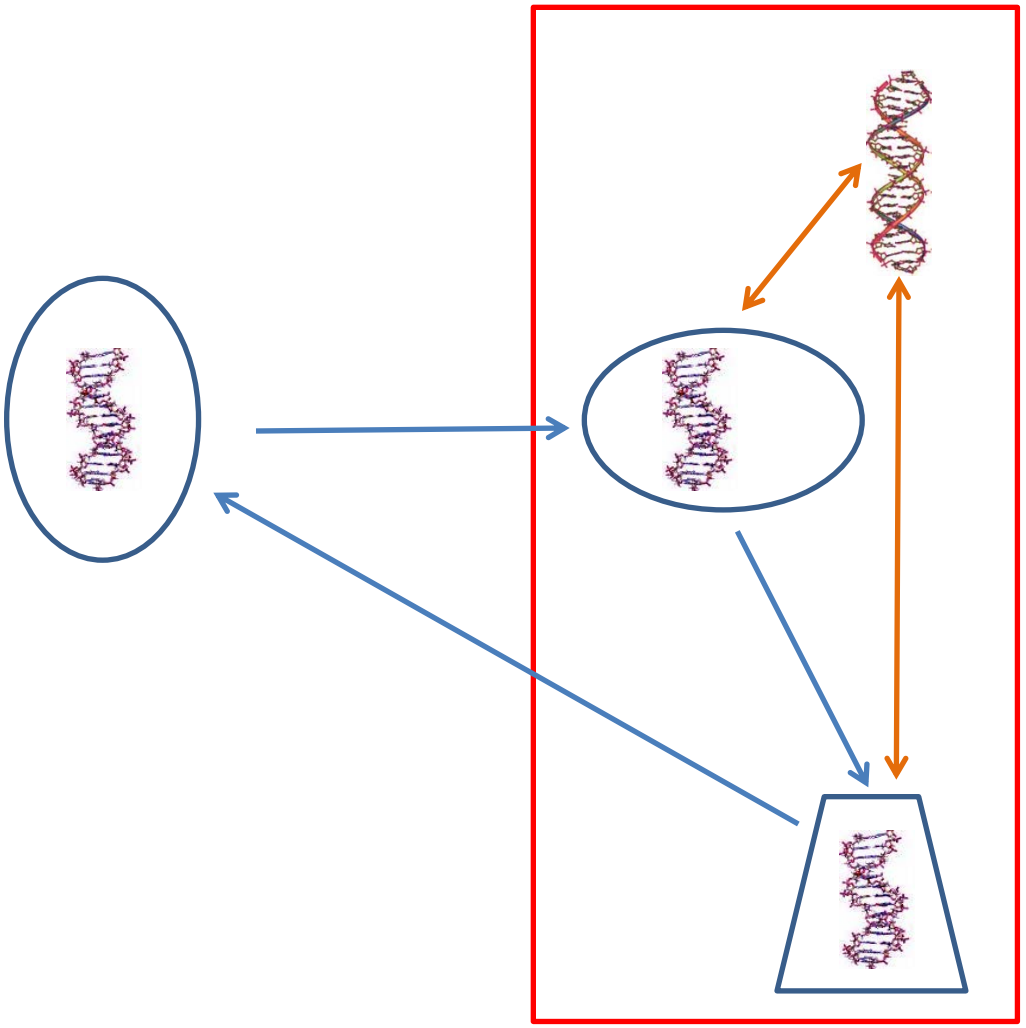
Ciclo de Vida / Relación Parásito - Hospedador



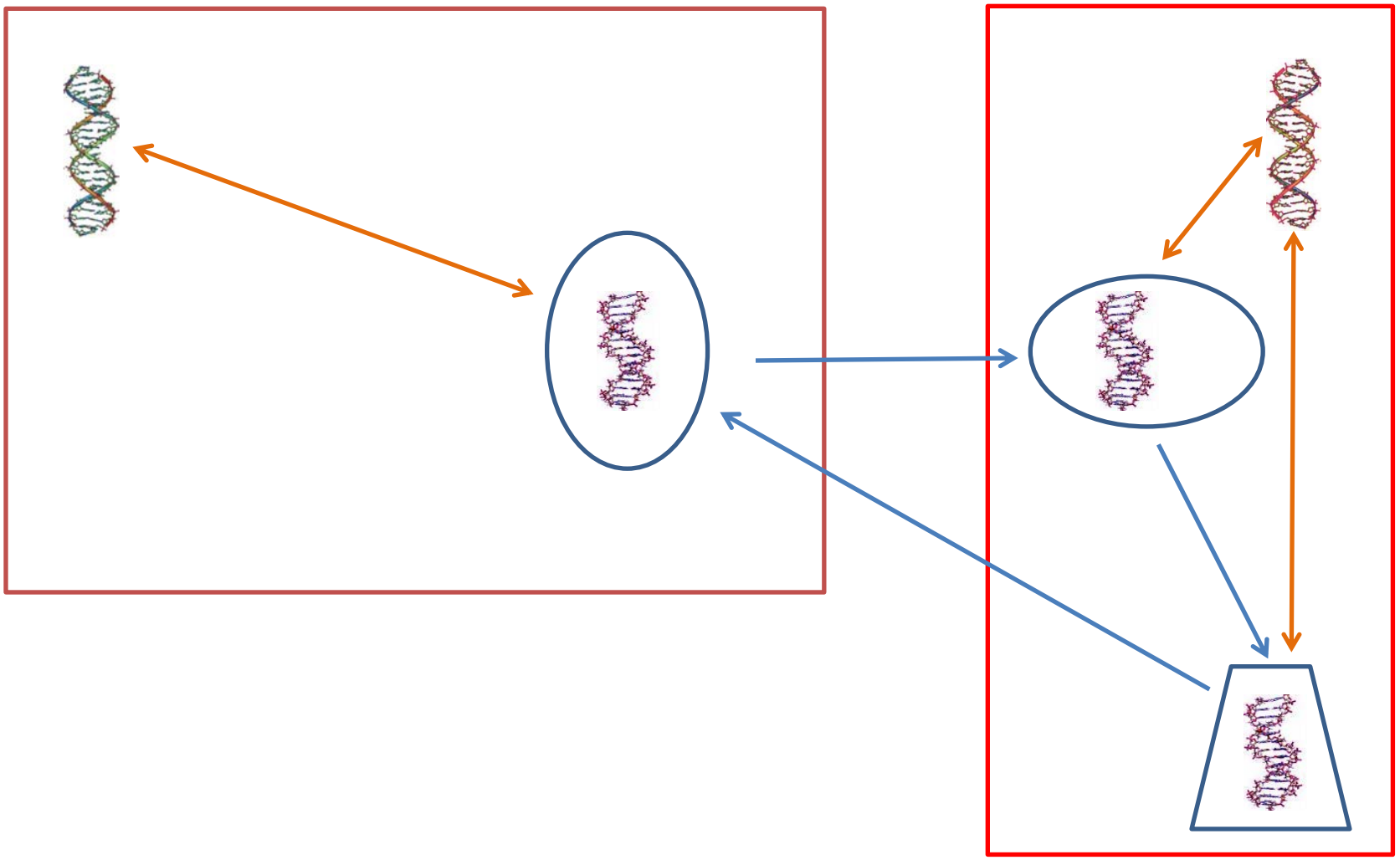
Ciclo de Vida / Relación Parásito - Hospedador



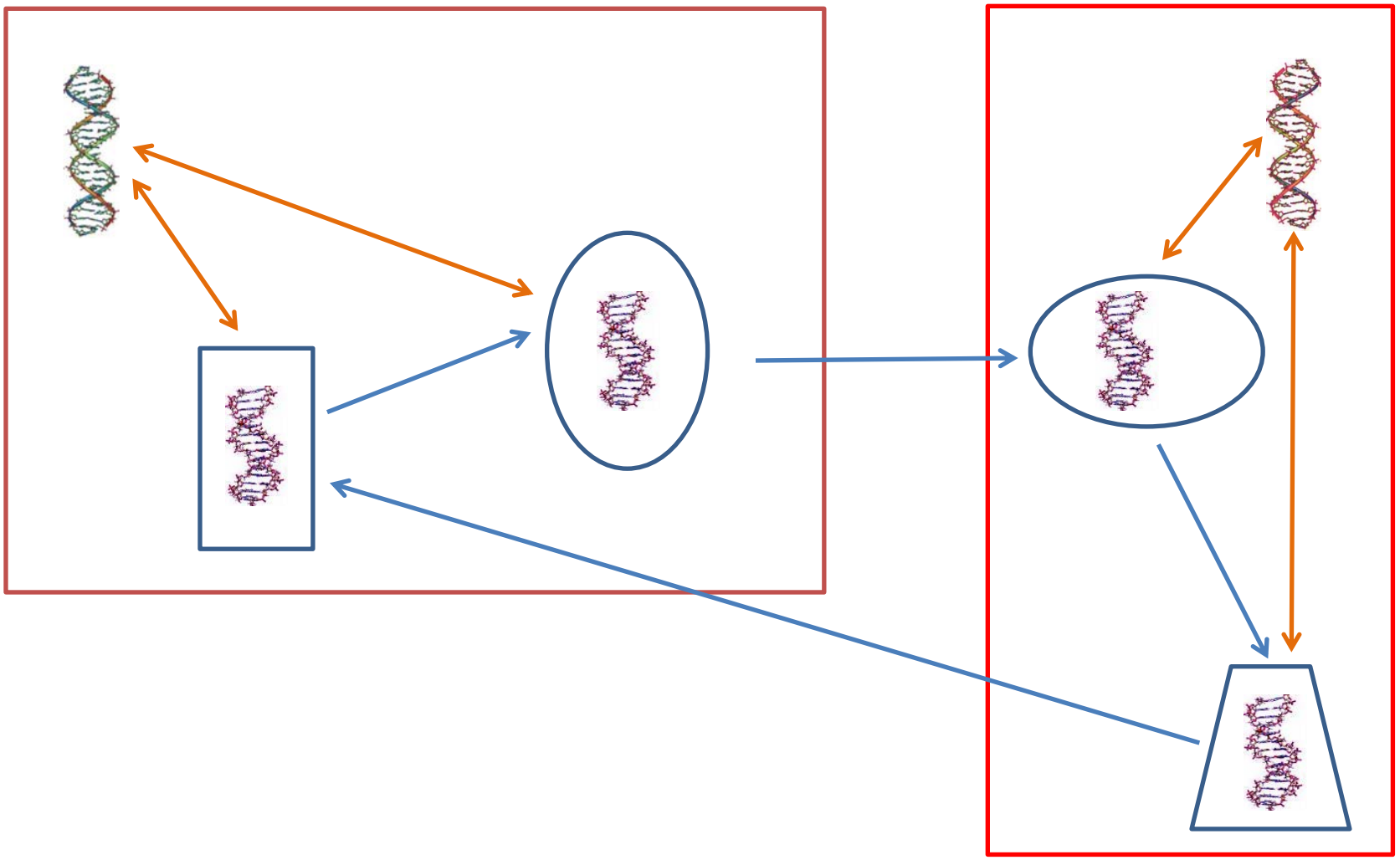
Ciclo de Vida / Relación Parásito - Hospedador



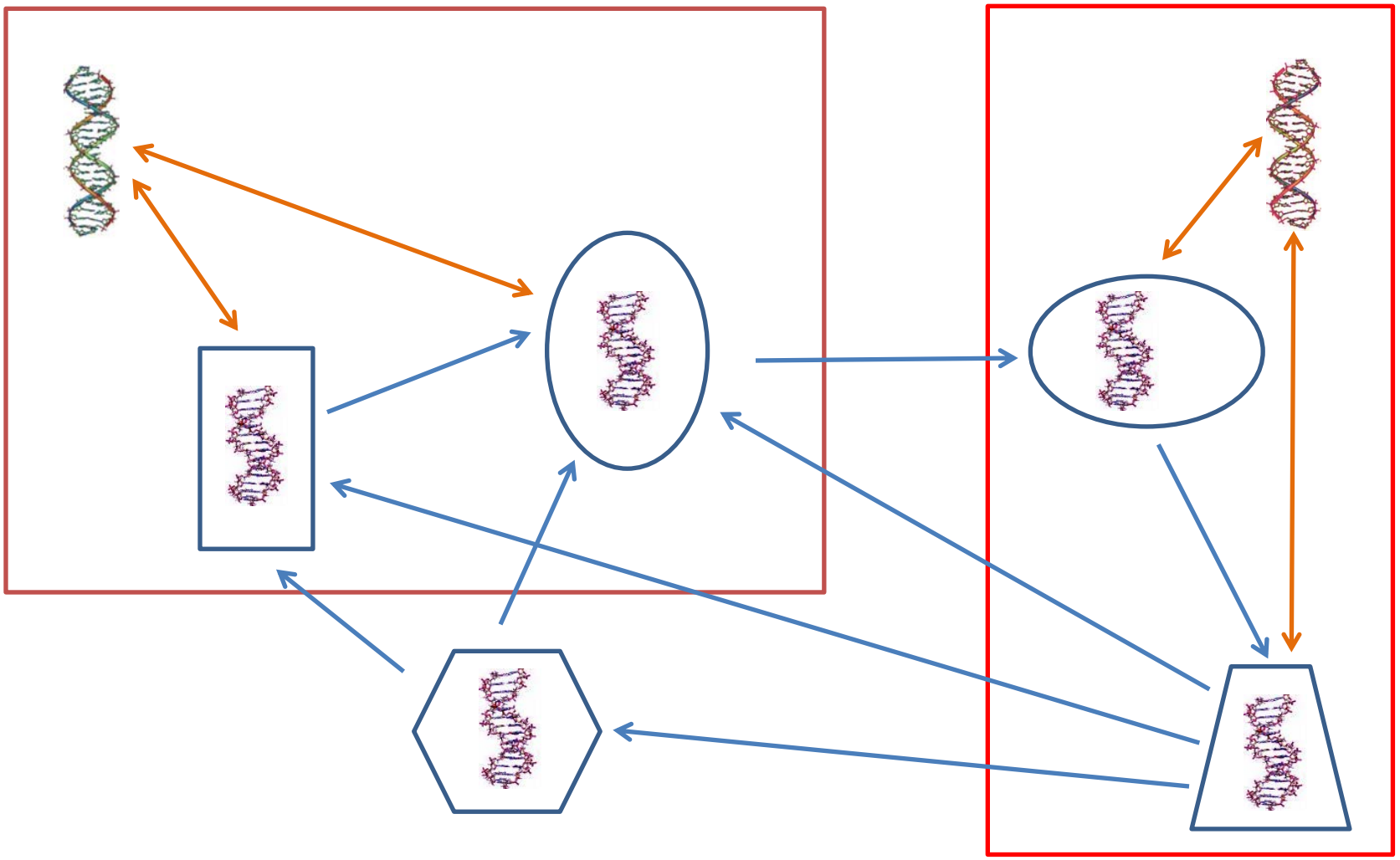
Ciclo de Vida / Relación Parásito - Hospedador



Ciclo de Vida / Relación Parásito - Hospedador

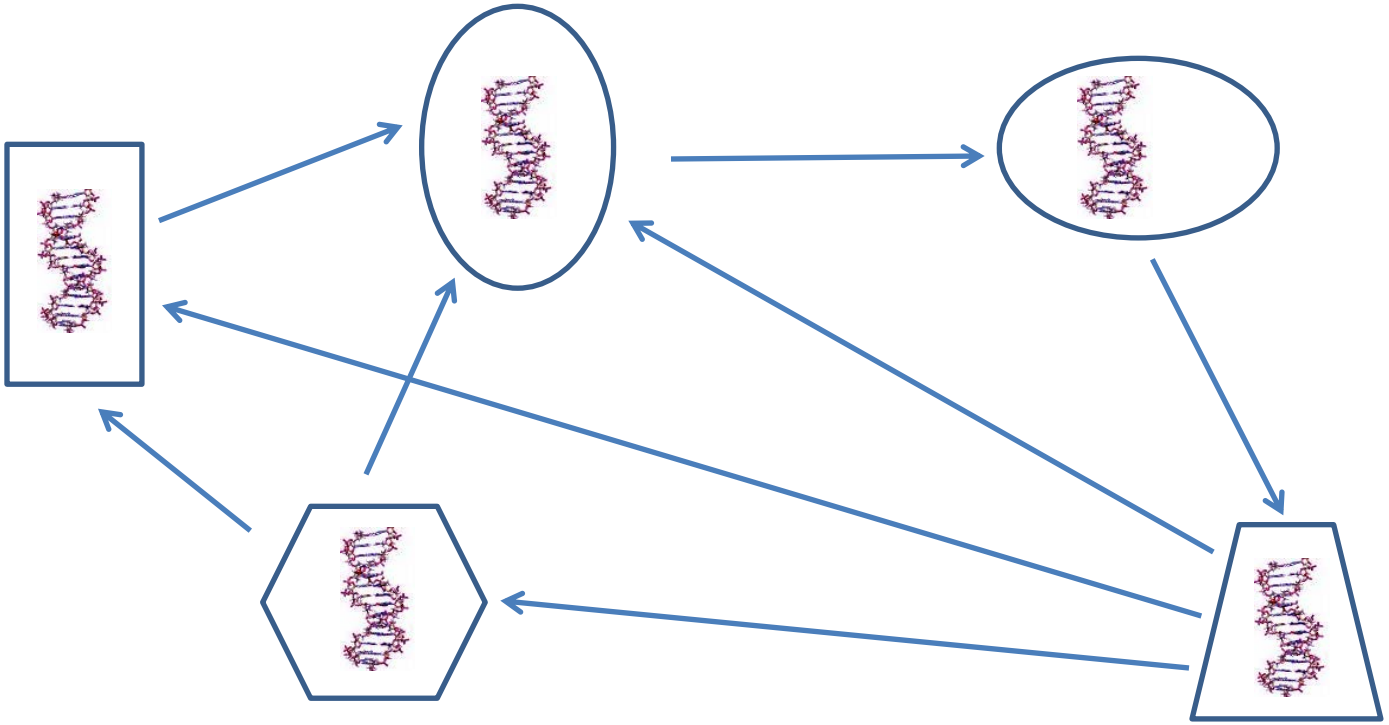


Ciclo de Vida / Relación Parásito - Hospedador



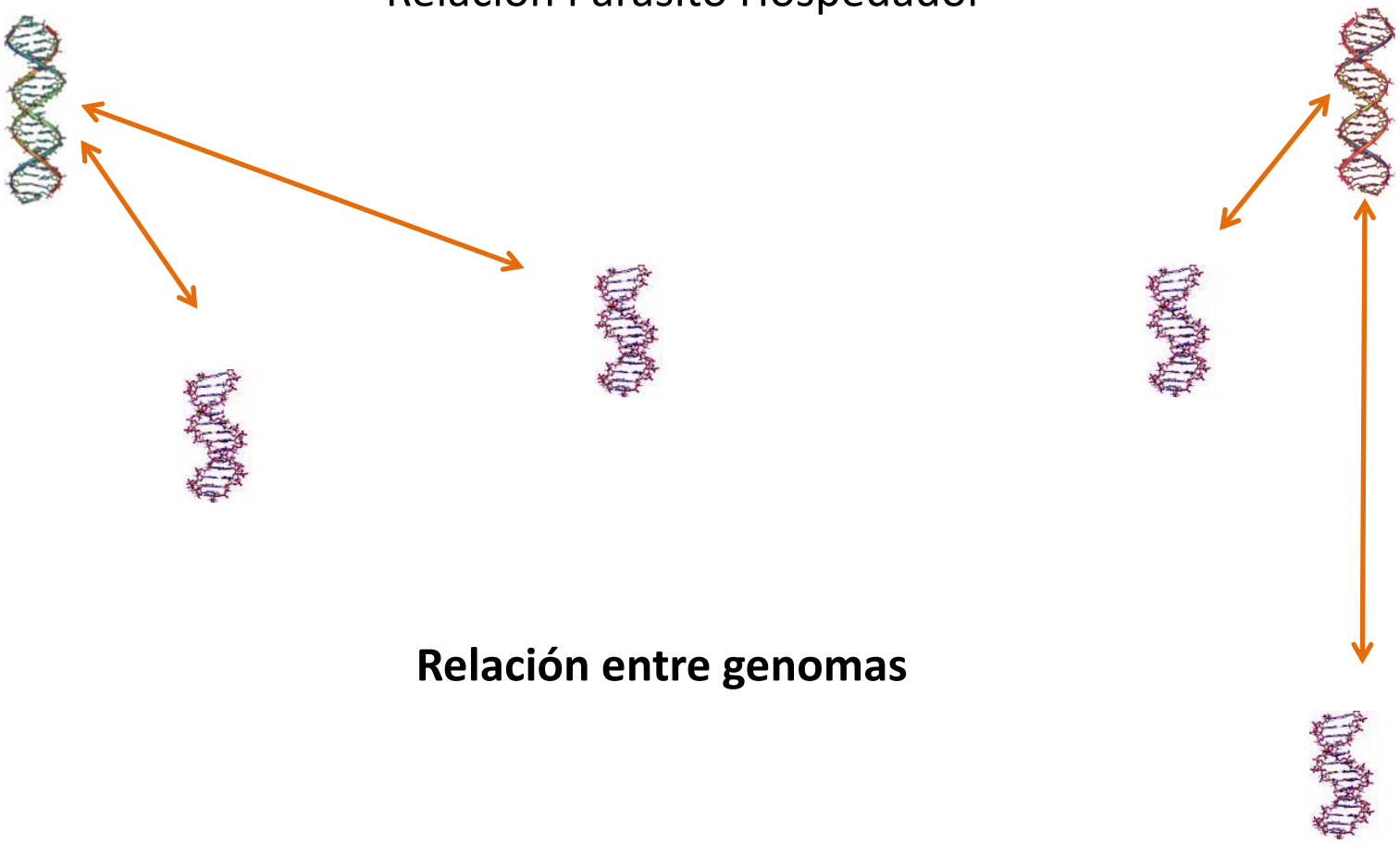
Ciclo de Vida

Expresión diferencial del genoma del parásito



Ciclo de Vida

Relación Parásito Hospedador

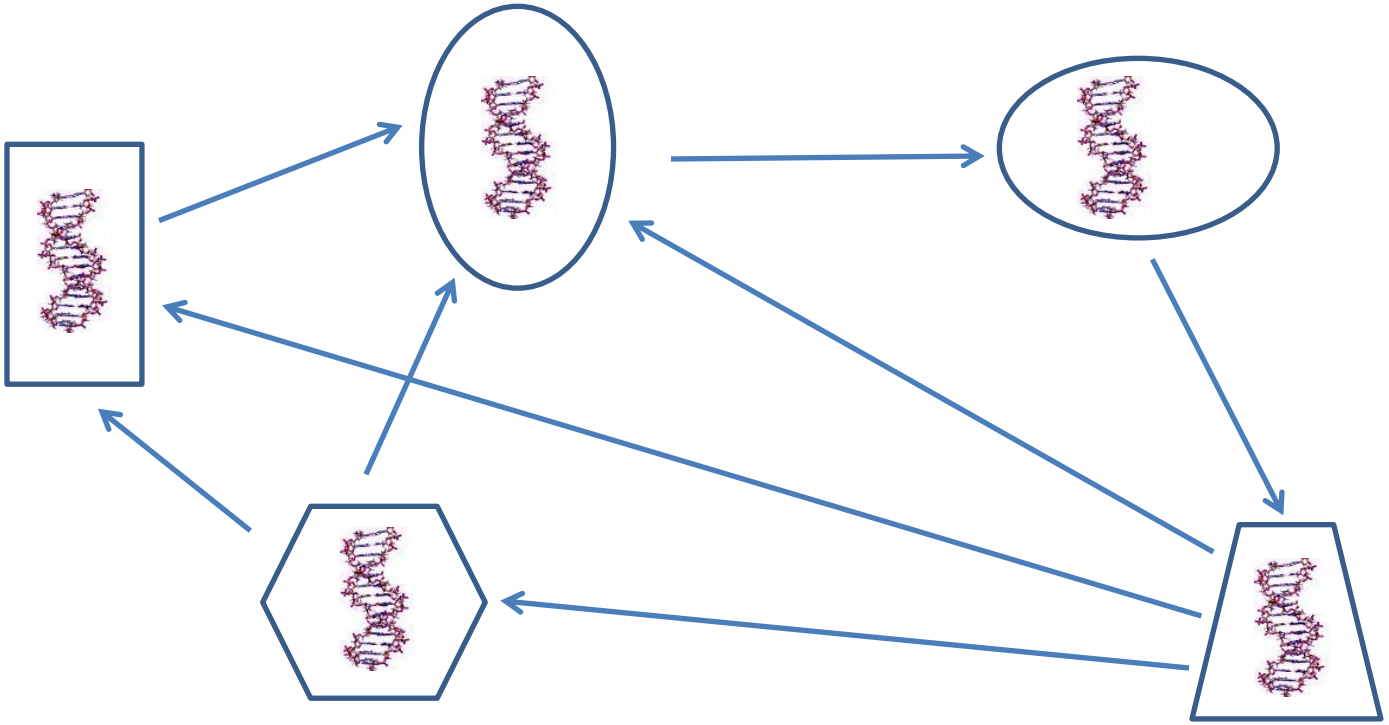


Relación entre genomas

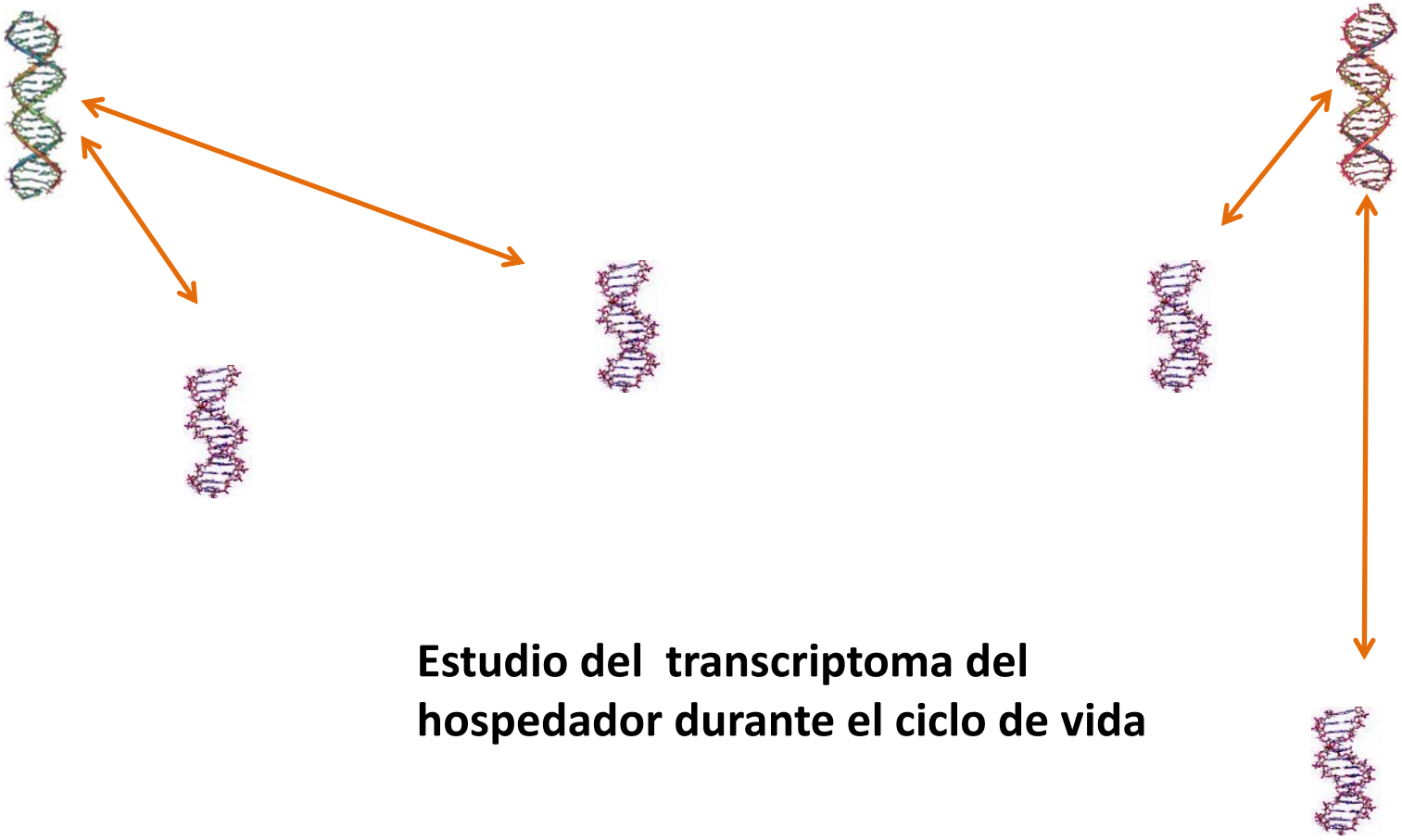
´Transcriptómica en Parasitología

´Transcriptómica en Parasitología

Estudio del transcriptoma del parásito durante el ciclo de vida

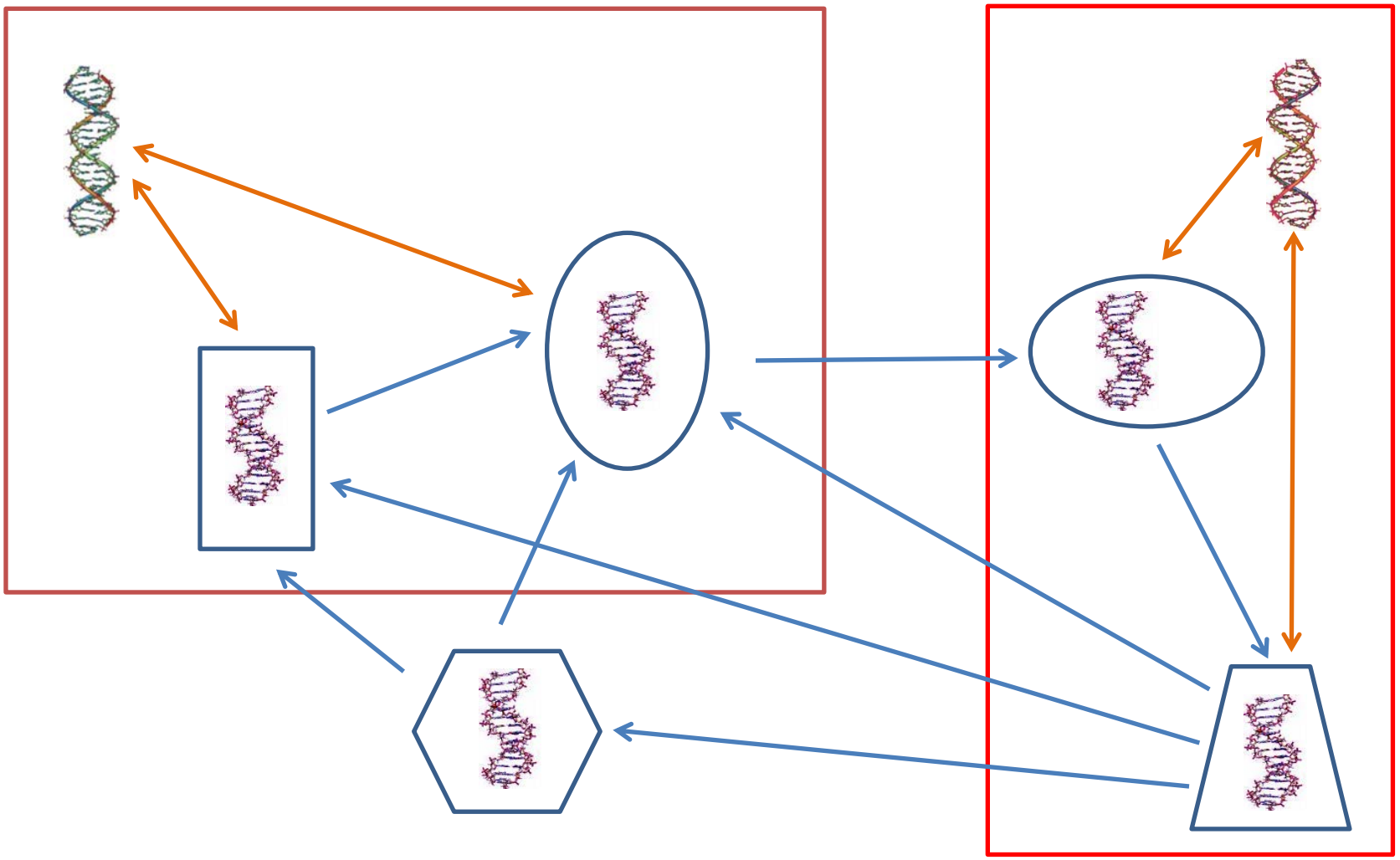


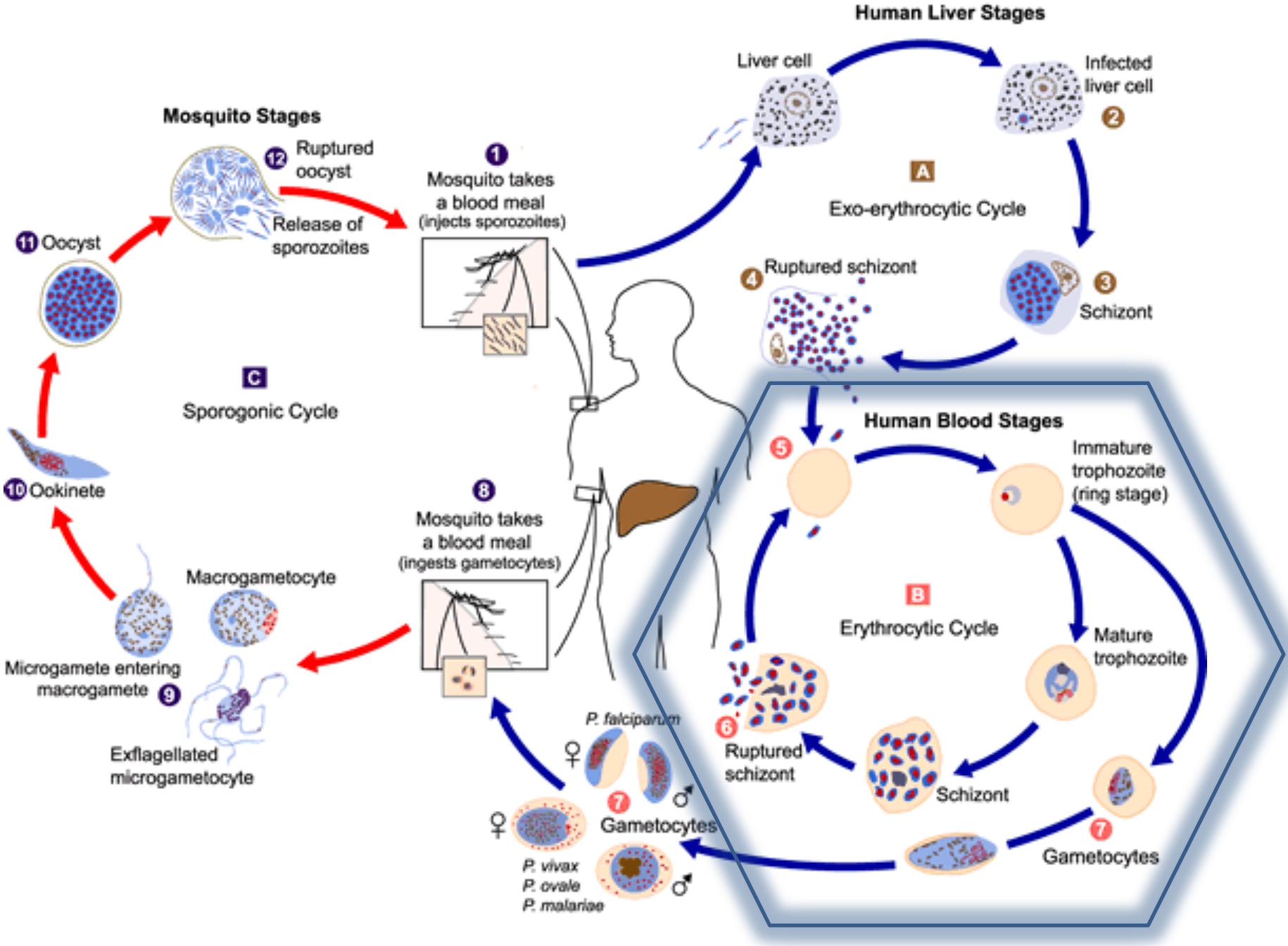
´Transcriptómica en Parasitología



Estudio del transcriptoma del hospedador durante el ciclo de vida

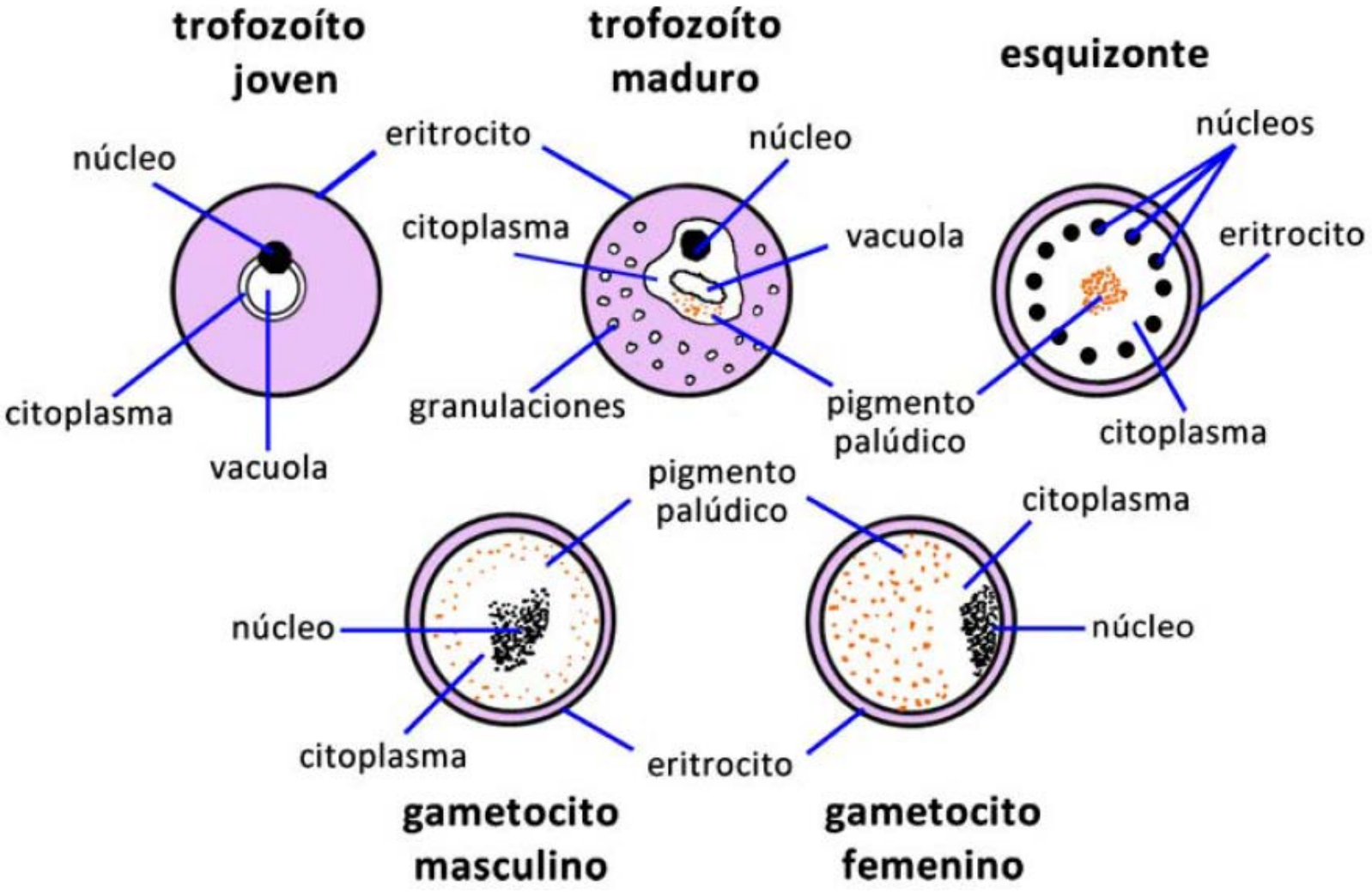
Ciclo de Vida / Relación Parásito - Hospedador



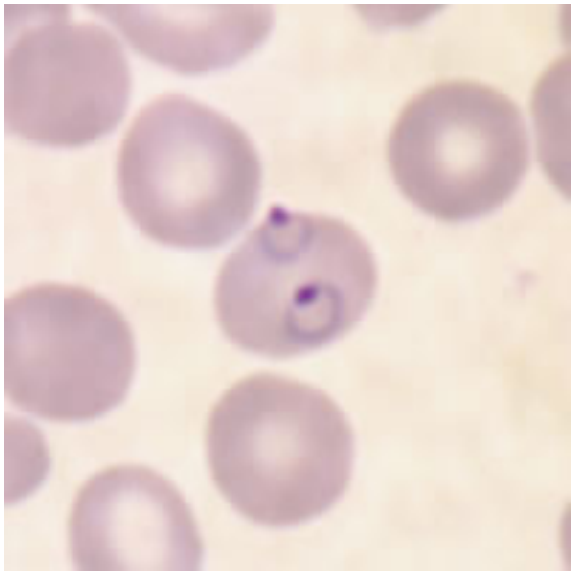

Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
Ruptured schizont
4
3
Schizont
Mosquito Stages
12 Ruptured oocyst
Release of sporozoites
1
Mosquito takes a blood meal (injects sporozoites)
11 Oocyst
C
Sporogonic Cycle
10 Ookinete
8
Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete 9
Exflagellated microgametocyte
Human Blood Stages
5
Immature trophozoite (ring stage)
B
Erythrocytic Cycle
Mature trophozoite
6
Ruptured schizont
Schizont
7
Gametocytes
7
Gametocytes
P. falciparum
P. vivax
P. ovale
P. malariae

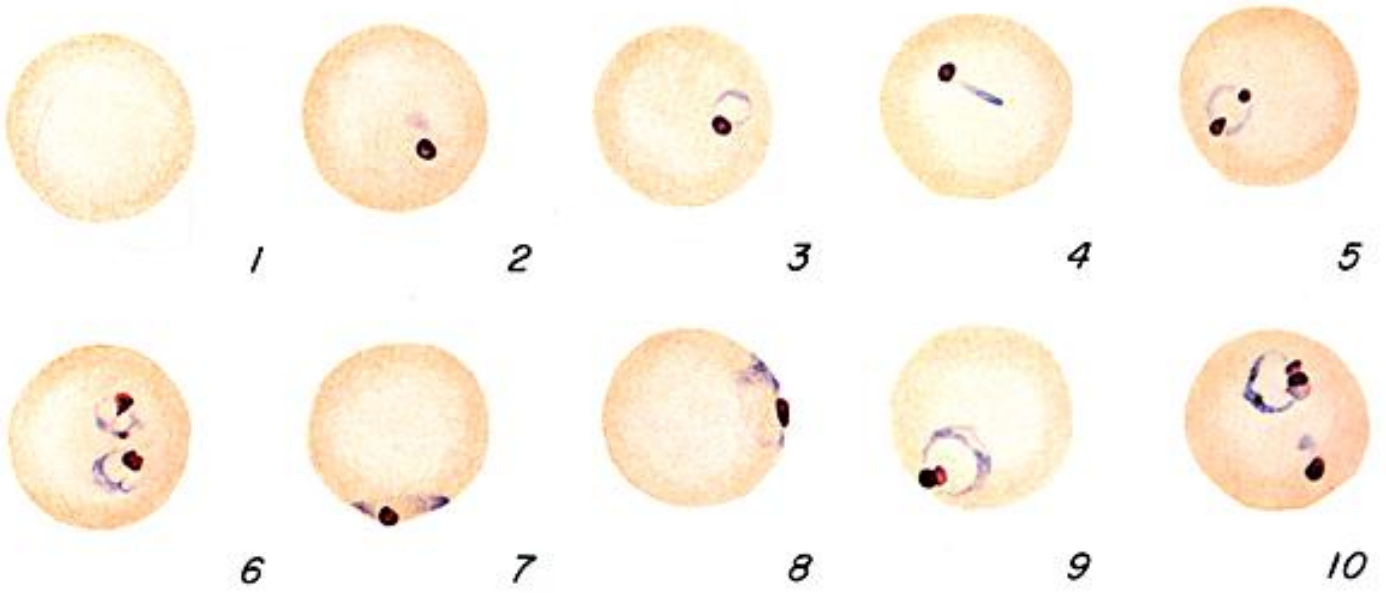
Distribución geográfica de la Malaria

Formas del parasito en eritrocitos

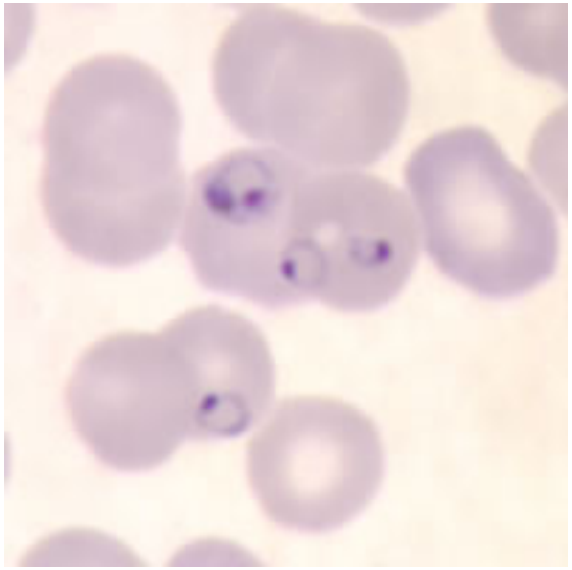


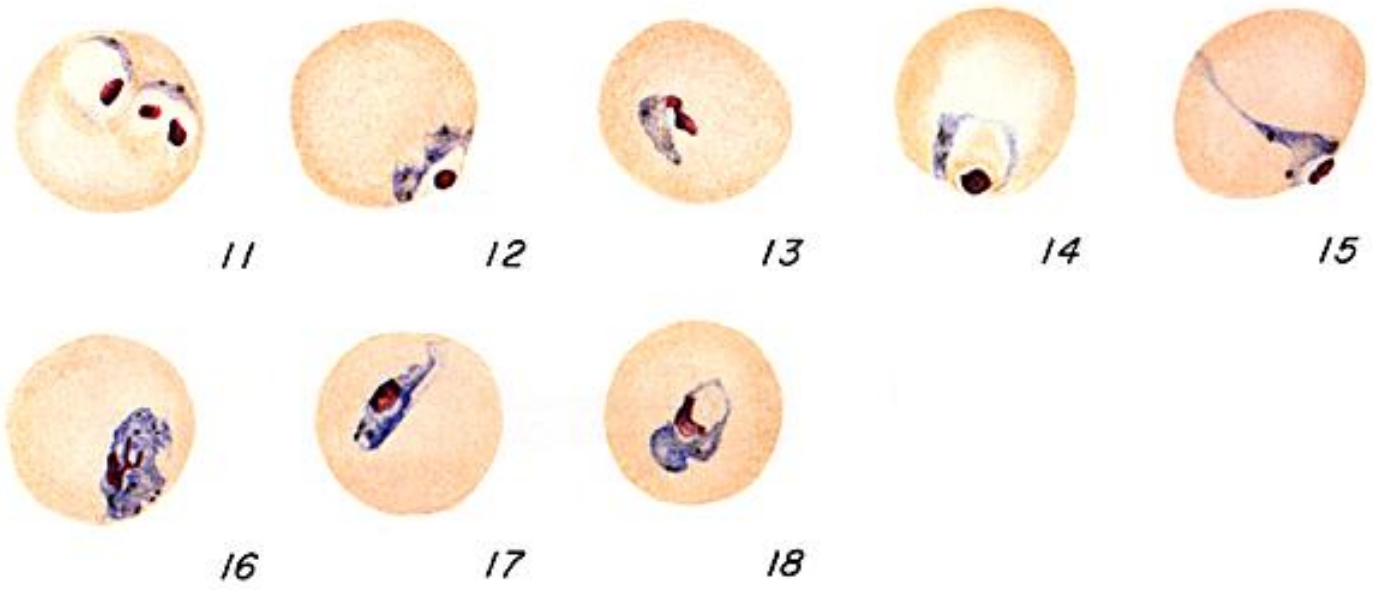
P. falciparum
Maduración de anillos

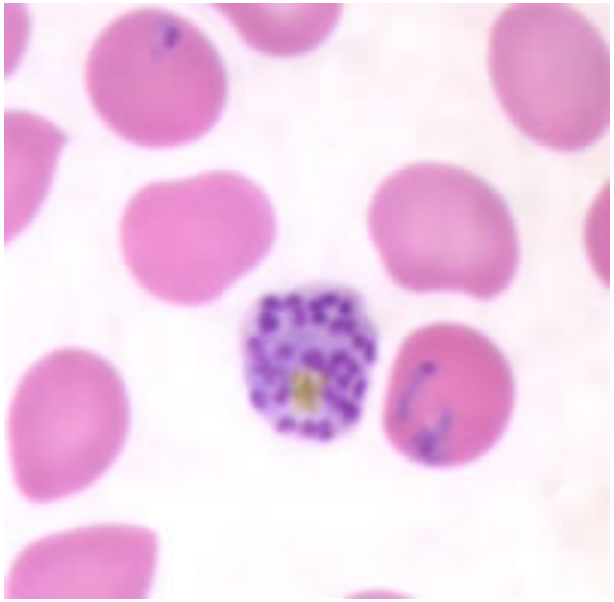




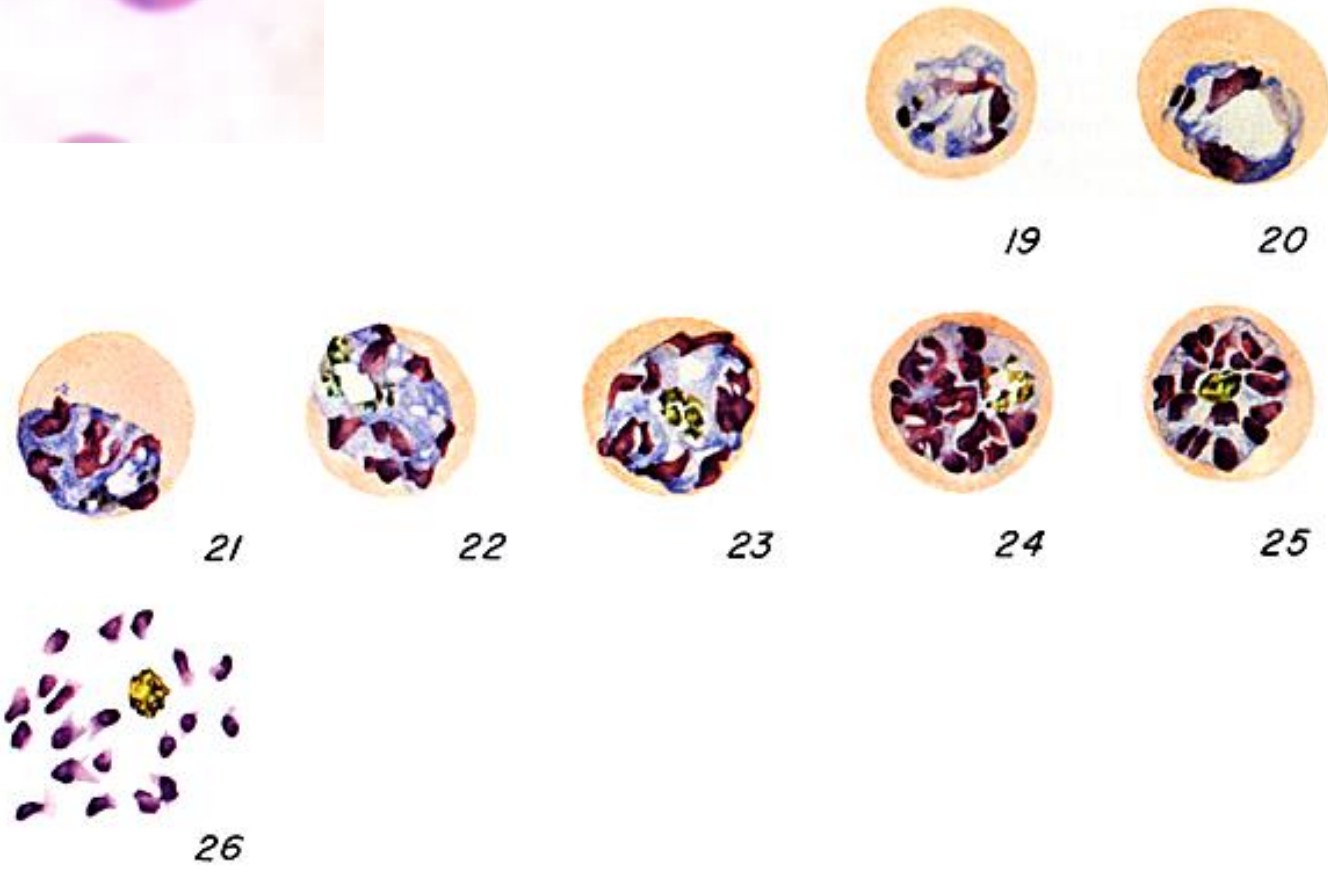
P. falciparum
Maduración de trofozoitos



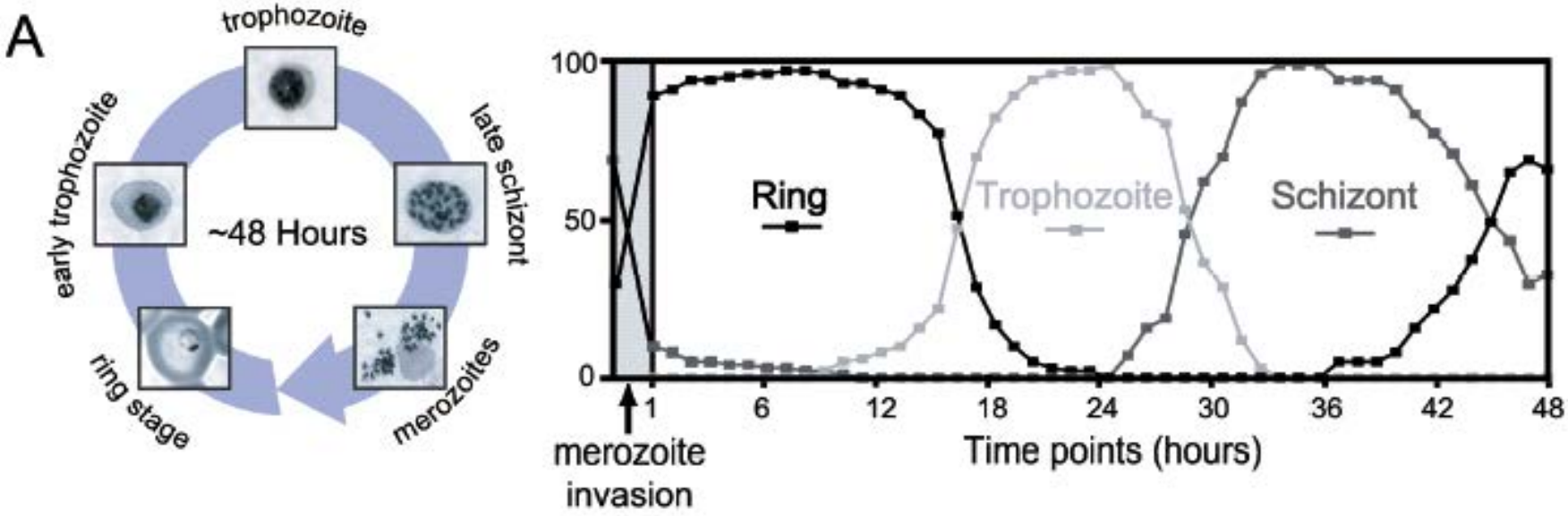




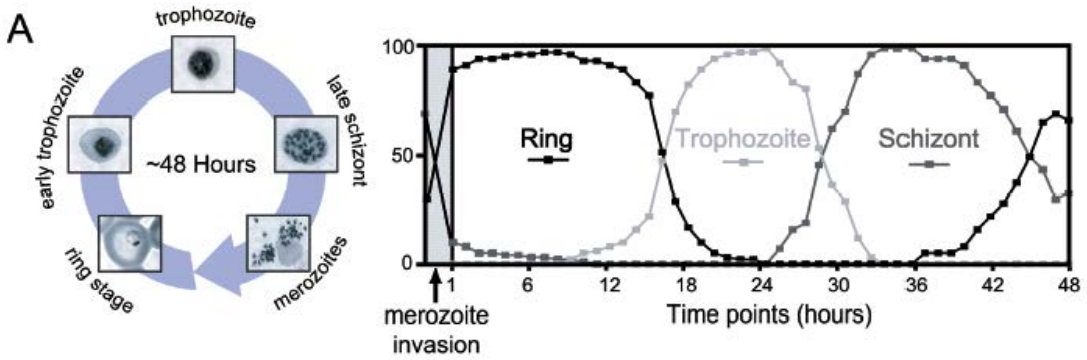
P. falciparum.
Maduración de esquizontes

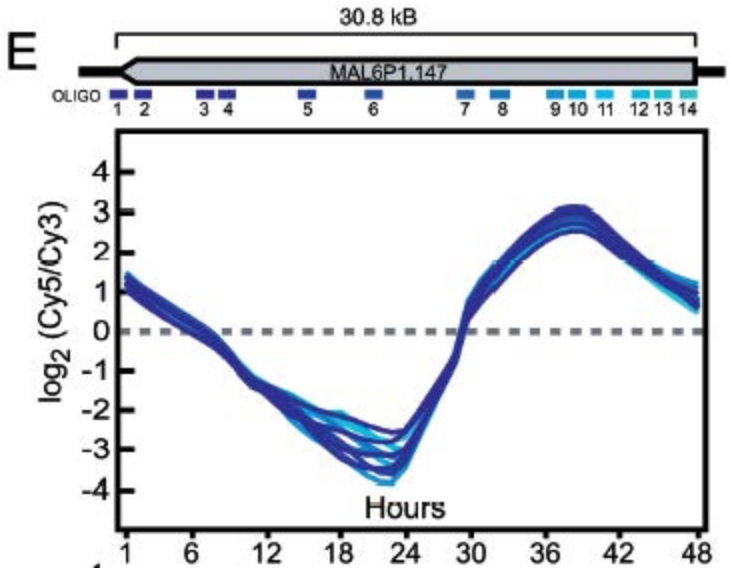


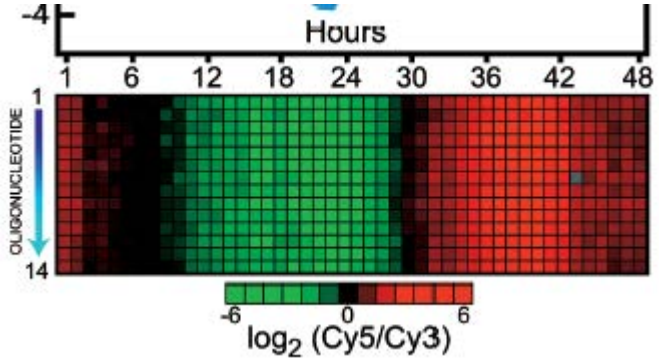
P. falciparum - Ciclo heritrocitario



Consistencia de los resultados: Hypotetical protein MALPP1.147 (14 oligonucleotides)

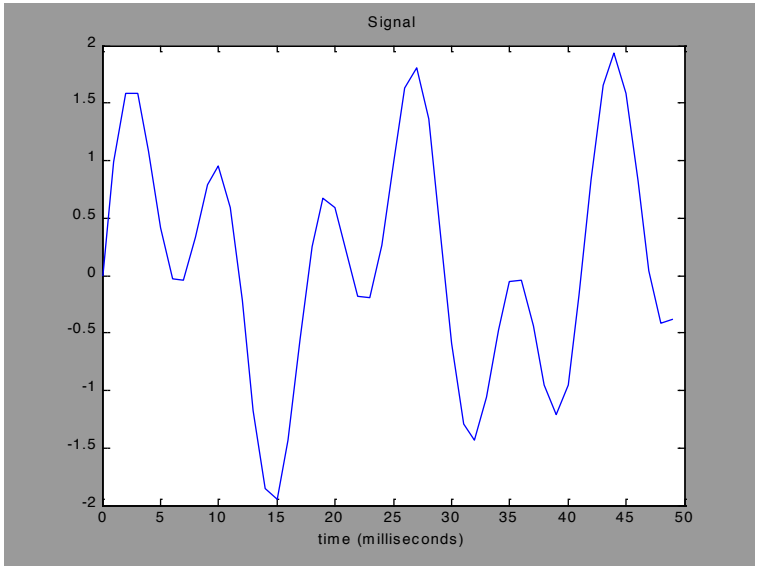




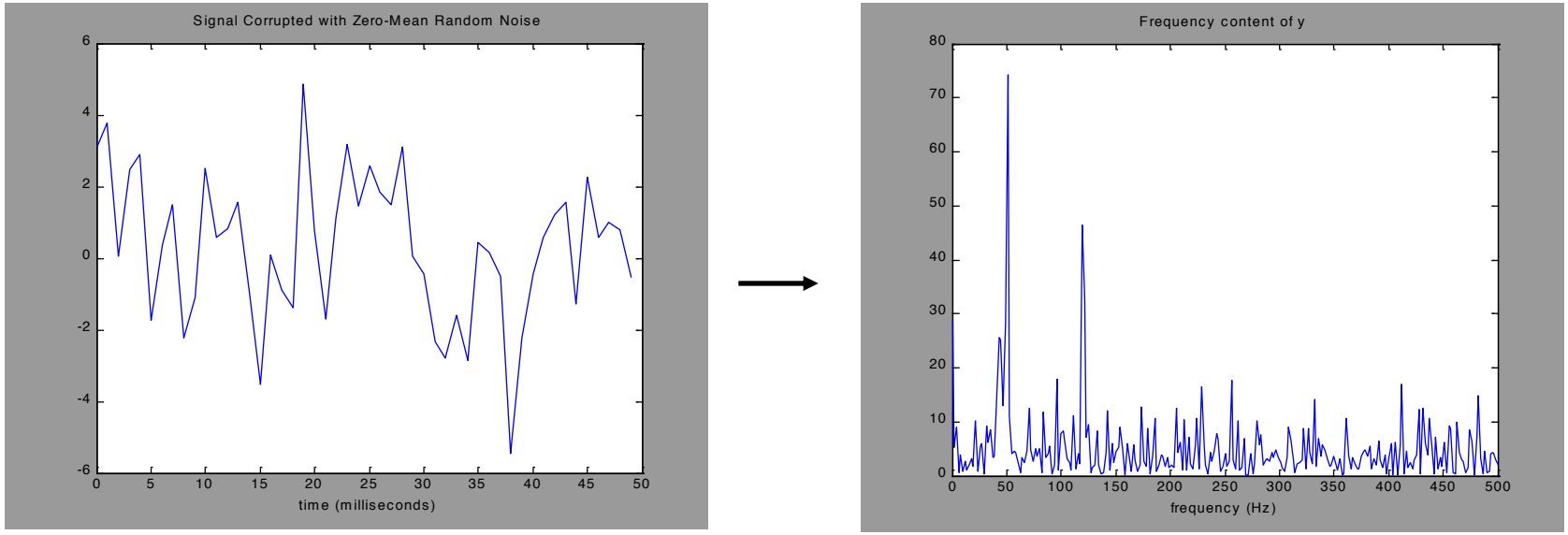


r (average pairwise correlation) = **0.89 ± 0.02**

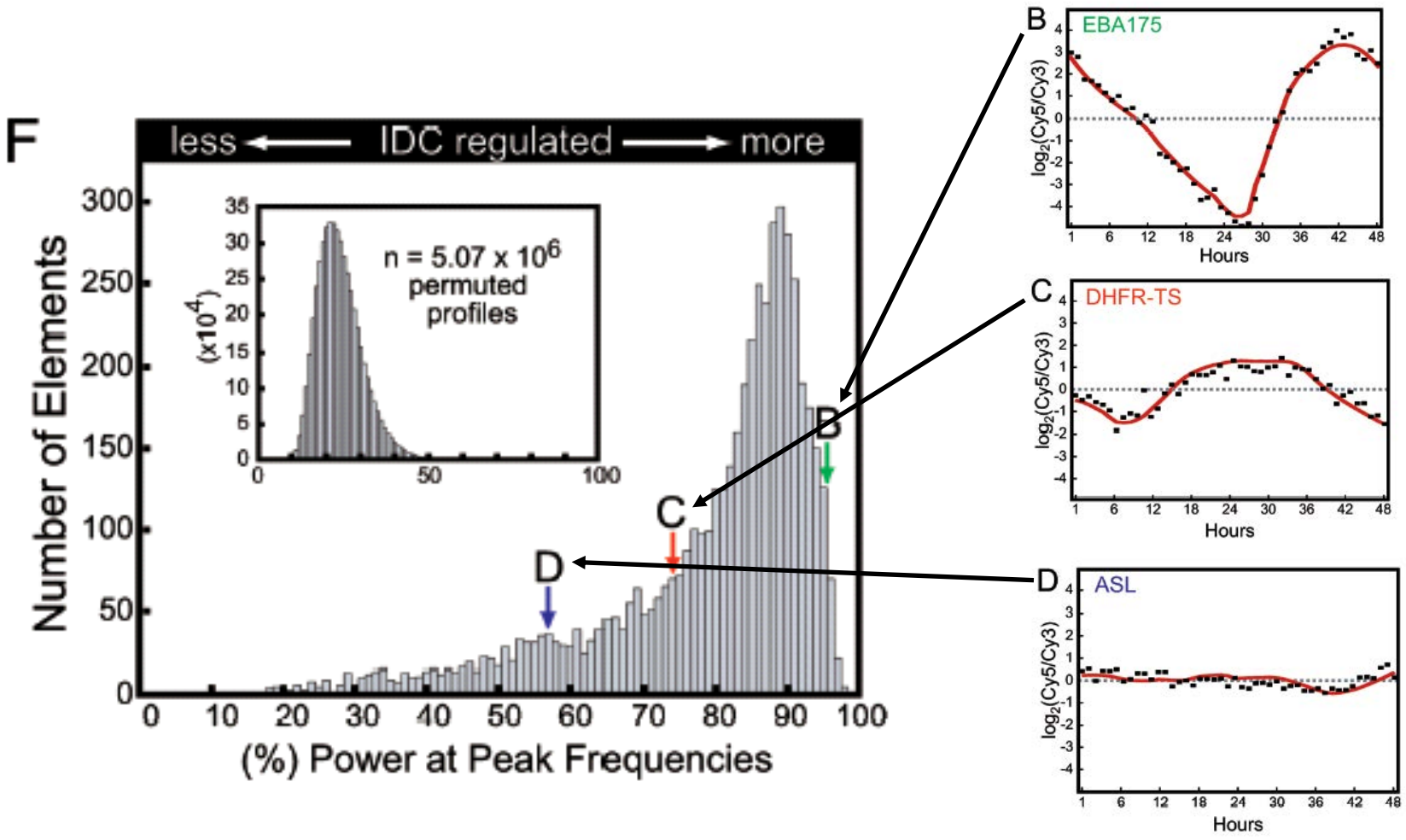
Transformada de Fourier



Transformada de Fourier

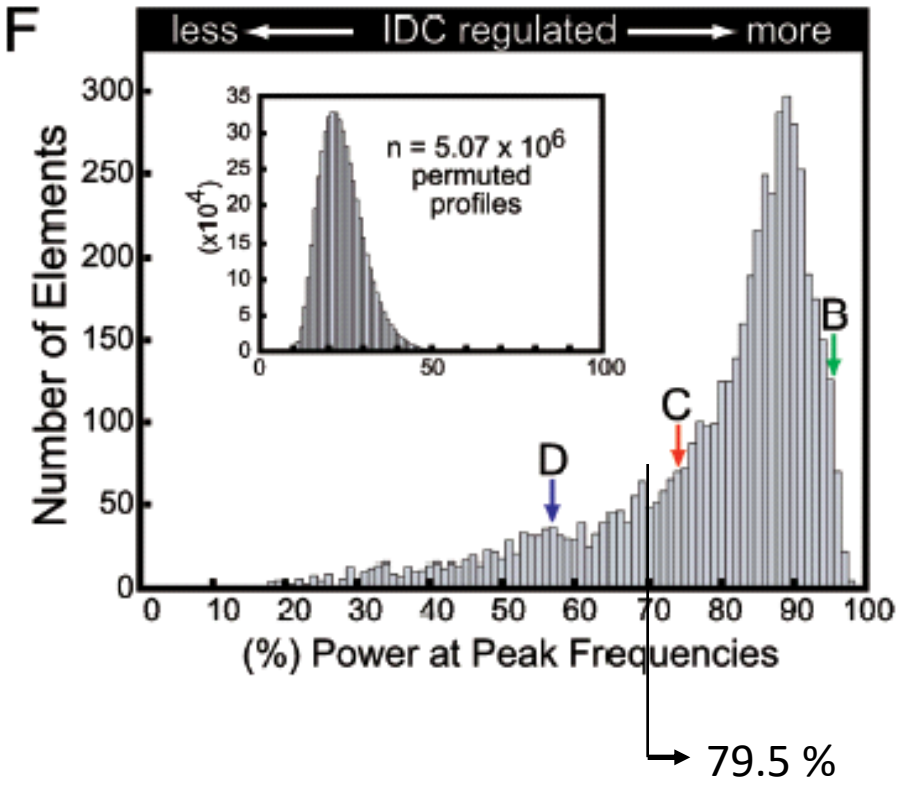


Ranking de los espectros cíclicos según el % PPF

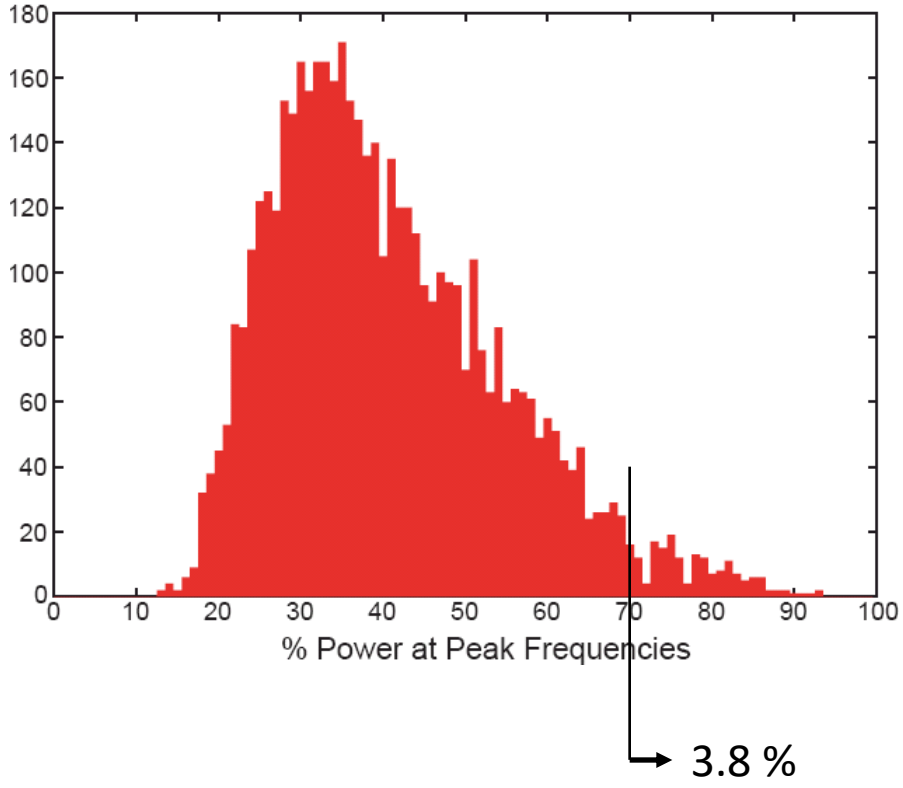


Ranking de los espectros cíclicos según el % PPF

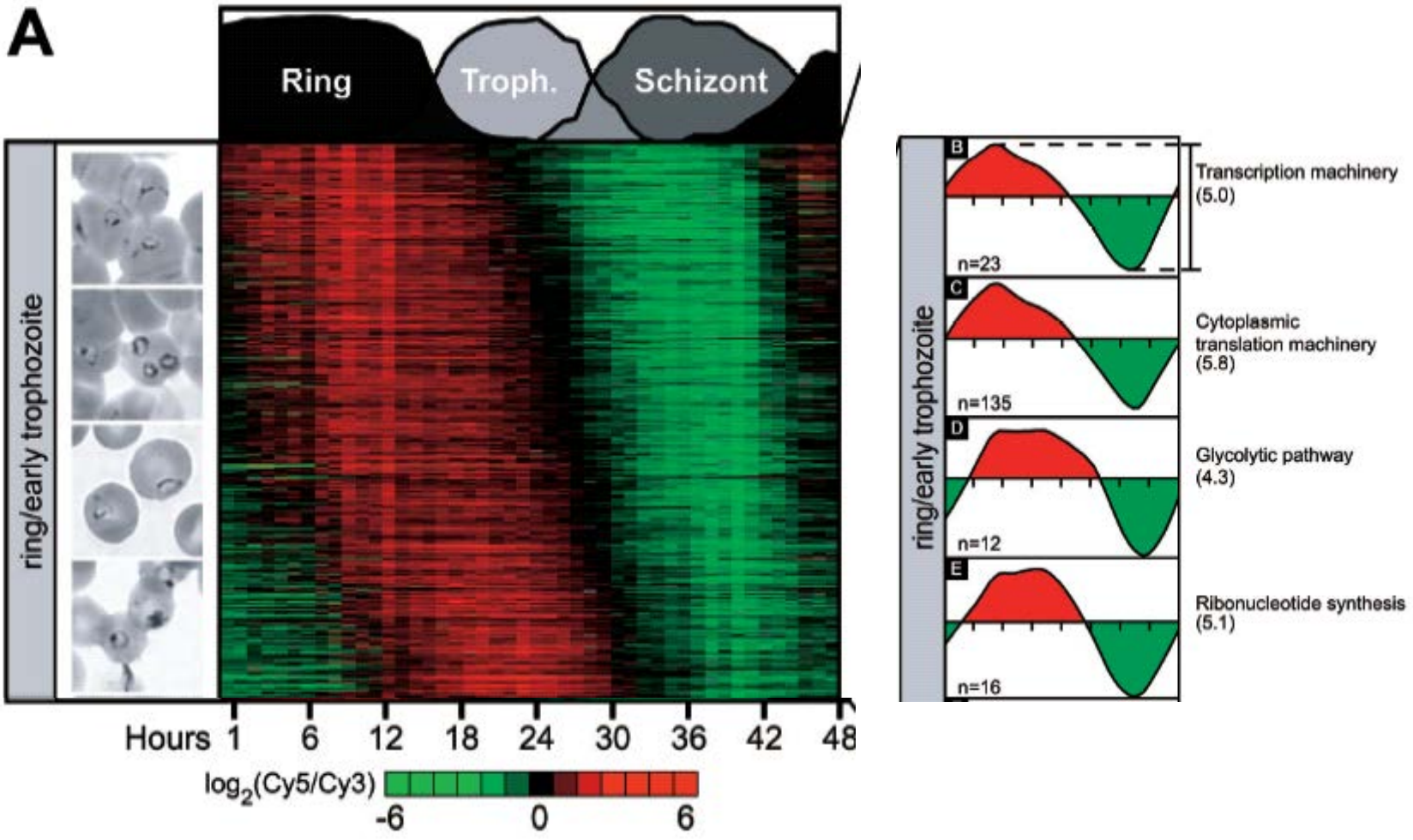
P. falciparum



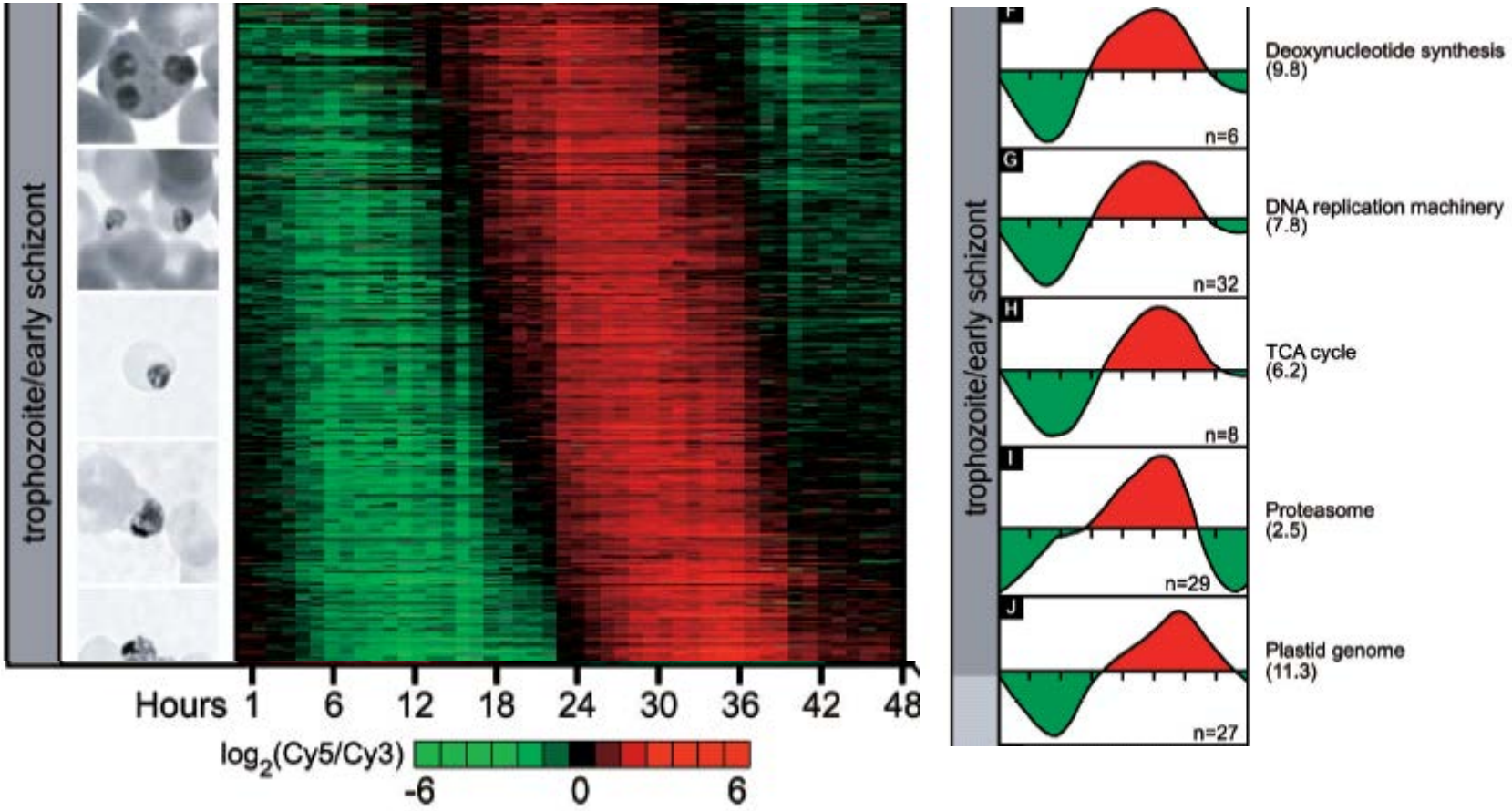
Yeast



Genes específicos del ring tardío

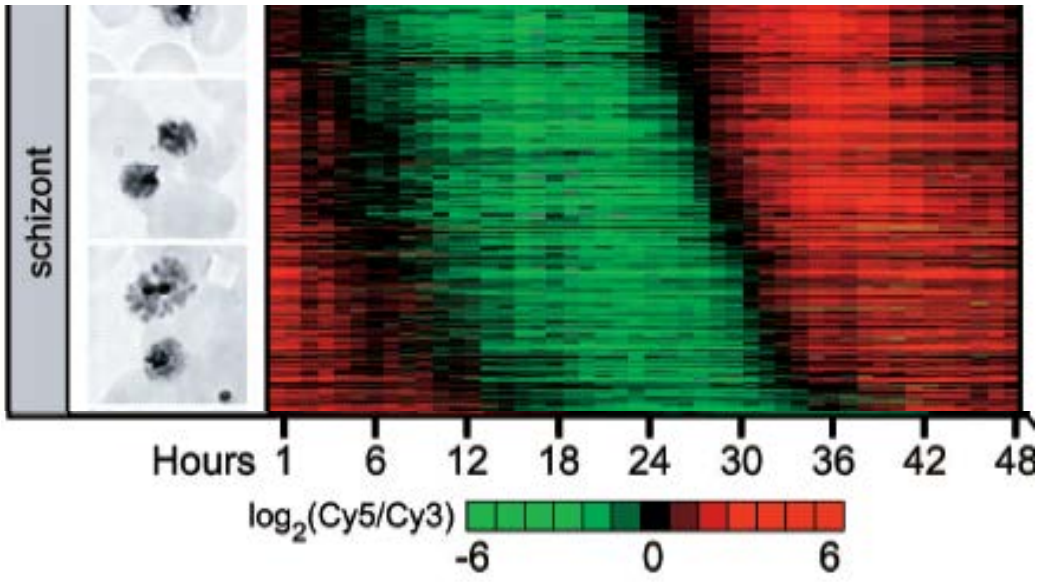


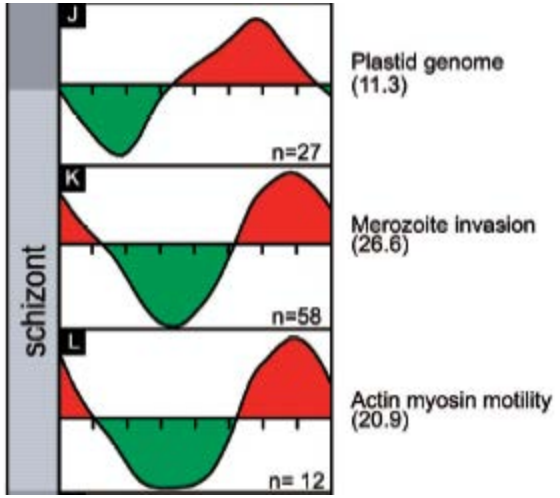
Genes específicos de trofozoito y esquizonte temprano



Genes específicos del esquizonte

aprox 500 ORFs



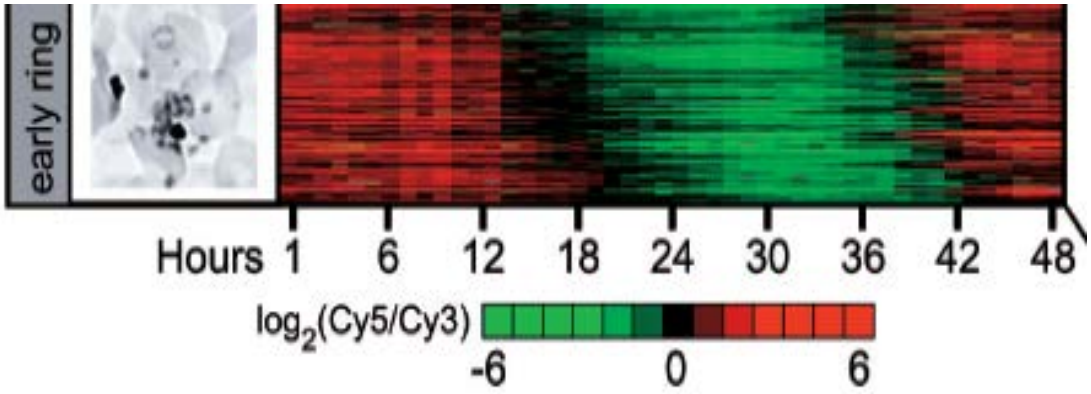


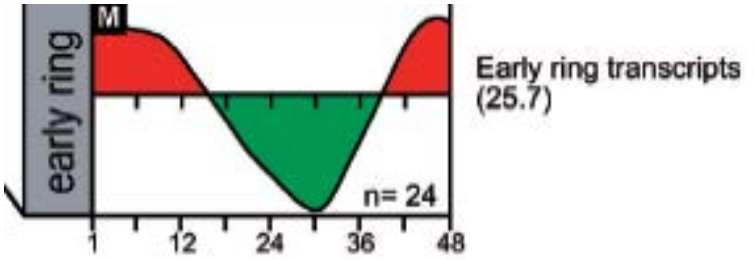
29 ORFs Proteosome

58 ORFs Invasión

Kinases / SERA

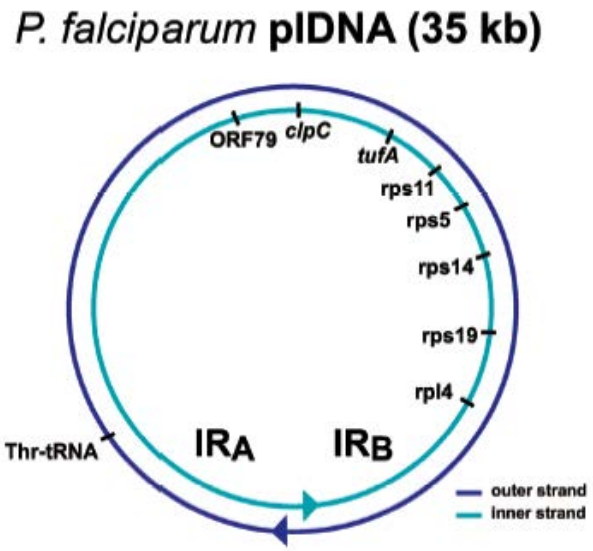
Genes específicos del ring temprano

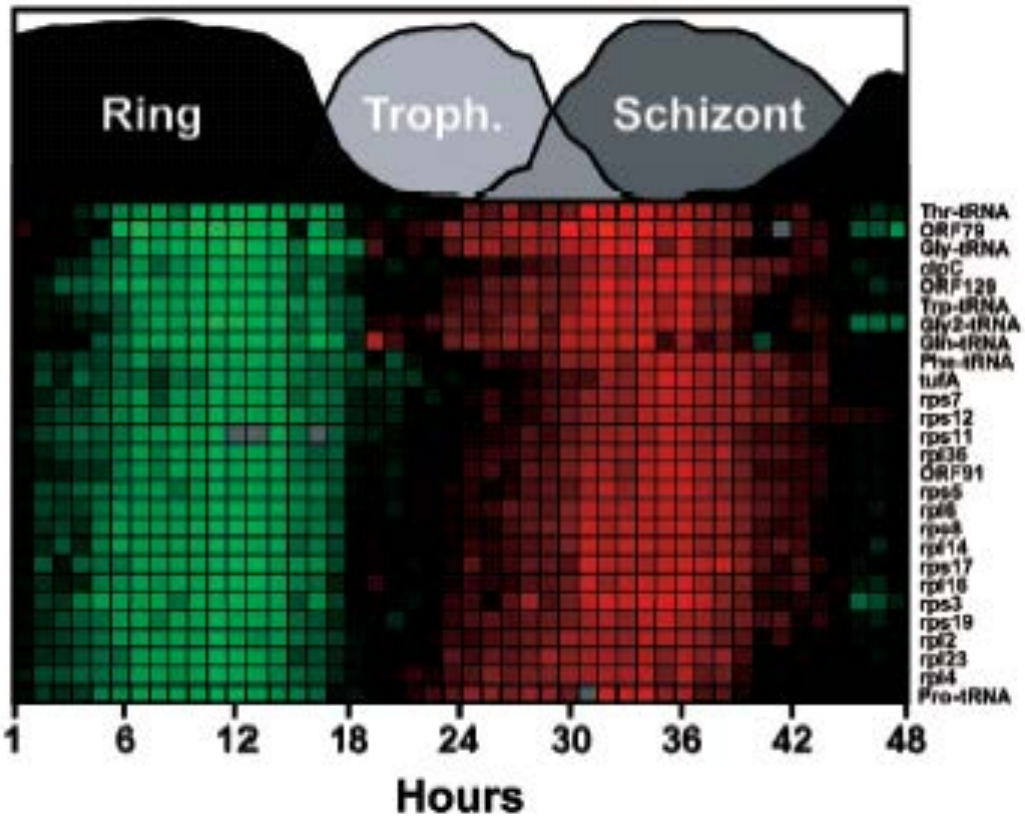




300 ORFs del esquizonte tardio permancen activos

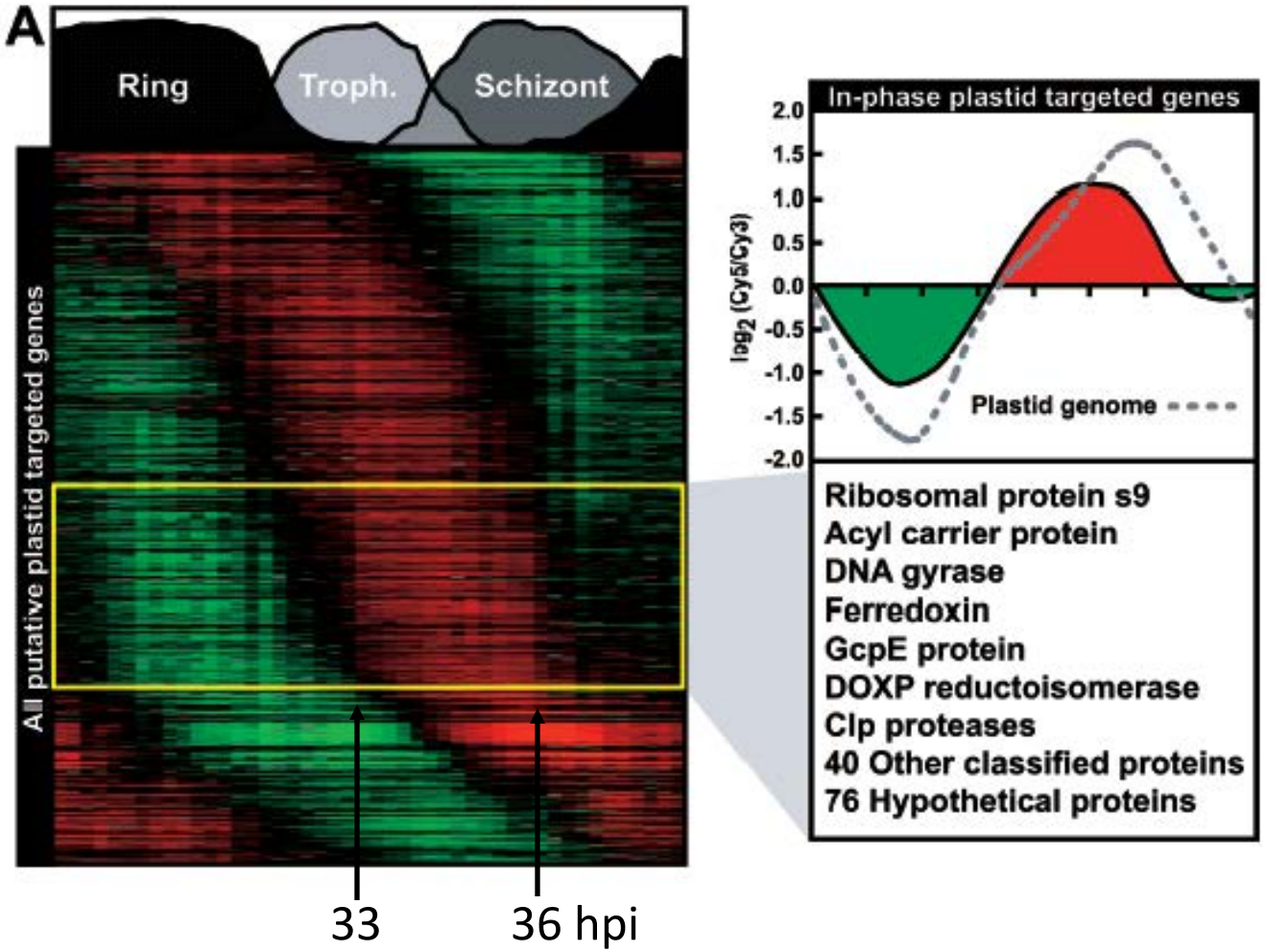
Coordinación de la regulación transcripcional del genoma plastídico



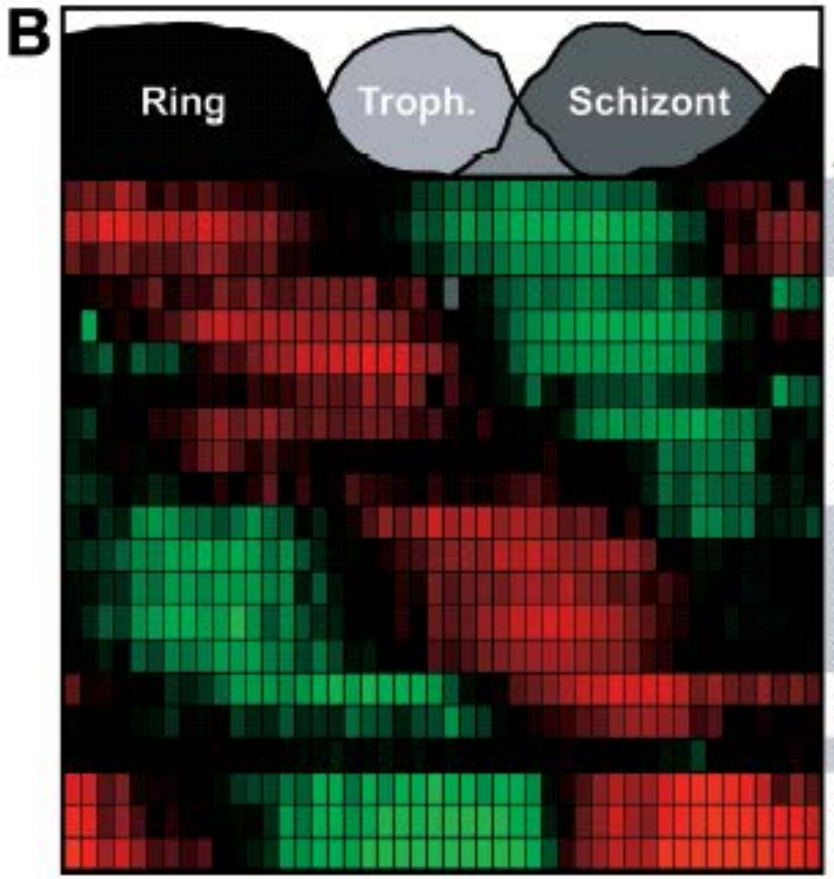


Faseograma de putativos genes de localización plastídica

550 ORFs predichos por presencia de péptido tránsito

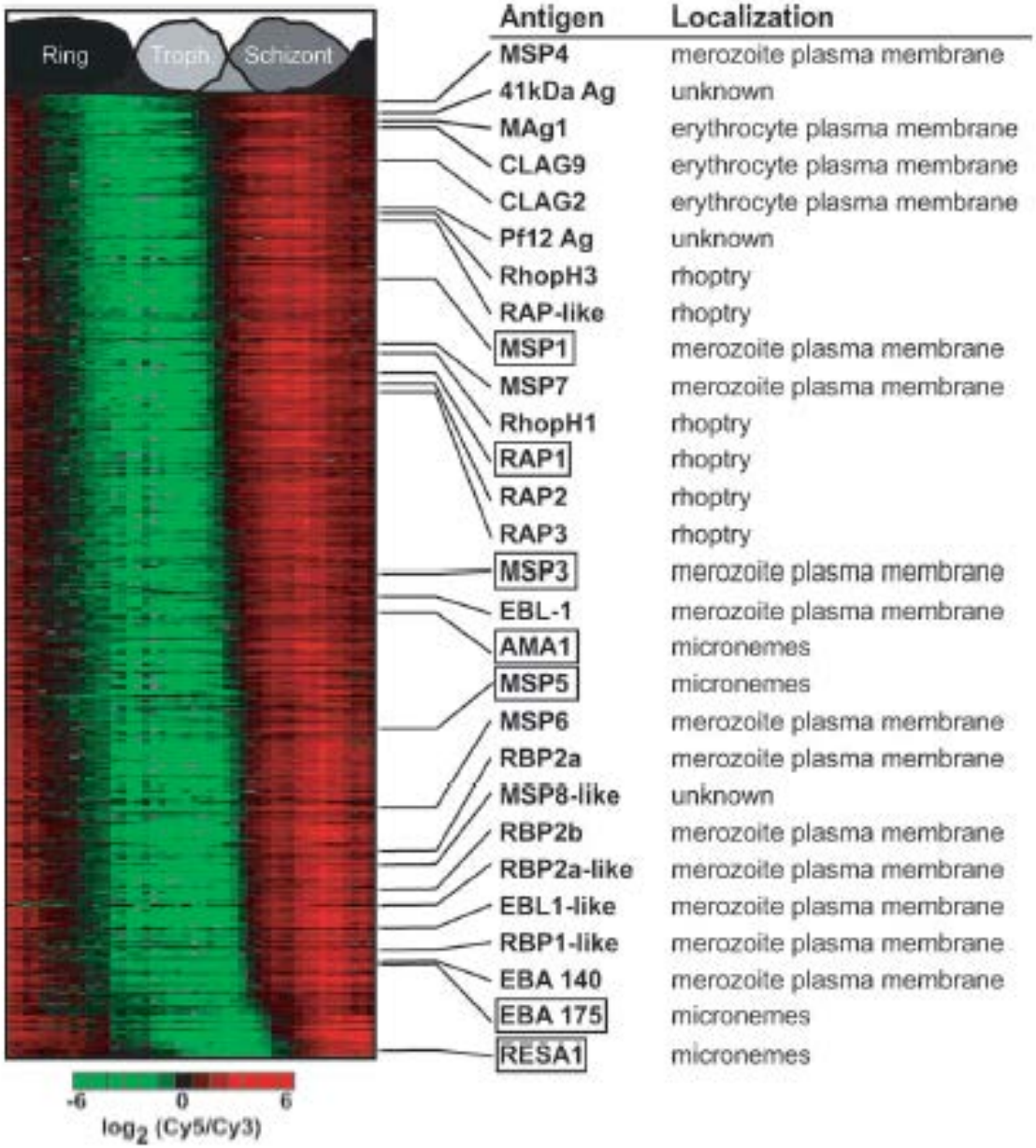


Faseograma de proteasas



Protease	Putative Function
falcipain-1	Merozoite invasion
plasmepsin I	Hemoglobinase
plasmepsin IV	Hemoglobinase
m1-family peptidase	Hemoglobinase
falcipain-2 homologue	Unknown
falcipain-2	Hemoglobinase
plasmepsin II	Hemoglobinase
HAP protein	Hemoglobinase
plasmepsin V	Unknown
falcilysin	Hemoglobinase
Clp endopeptidase	Protein maturation
Clp endopeptidase	Protein maturation
Clp endopeptidase	Protein maturation
signal peptidase	Protein maturation
signal peptidase	Protein maturation
Pfsub1 homologue	Unknown
plasmepsin IX	Unknown
falcipain-3	Hemoglobinase
Pfsub1	Merozoite invasion
Pfsub2	Merozoite invasion
plasmepsin X	Unknown

Faseograma de putativos antígenos protectores



Antigen	Localization
MSP4	merozoite plasma membrane
41kDa Ag	unknown
MAg1	erythrocyte plasma membrane
CLAG9	erythrocyte plasma membrane
CLAG2	erythrocyte plasma membrane
Pf12 Ag	unknown
RhopH3	rhoptry
RAP-like	rhoptry
MSP1	merozoite plasma membrane
MSP7	merozoite plasma membrane
RhopH1	rhoptry
RAP1	rhoptry
RAP2	rhoptry
RAP3	rhoptry
MSP3	merozoite plasma membrane
EBL-1	merozoite plasma membrane
AMA1	micronemes
MSP5	micronemes
MSP6	merozoite plasma membrane
RBP2a	merozoite plasma membrane
MSP8-like	unknown
RBP2b	merozoite plasma membrane
RBP2a-like	merozoite plasma membrane
EBL1-like	merozoite plasma membrane
RBP1-like	merozoite plasma membrane
EBA 140	merozoite plasma membrane
EBA 175	micronemes
RESA1	micronemes

High correlation with
7 well known antigens

262 ORFs

189 function unknown

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Molecular & Biochemical Parasitology 152 (2007) 35–46
MOLECULAR & BIOCHEMICAL PARASITOLOGY

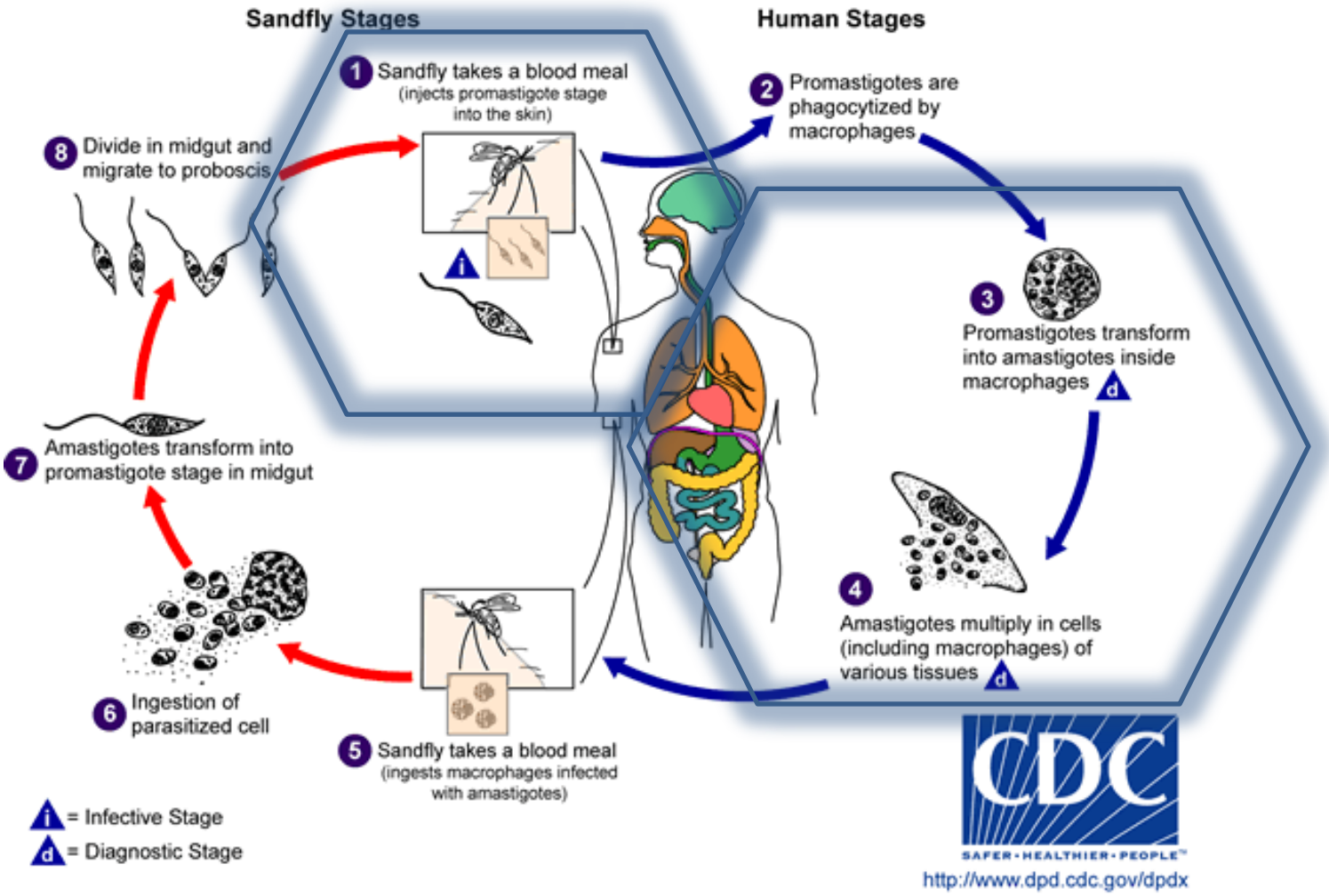
Genomic and proteomic expression analysis of *Leishmania* promastigote and amastigote life stages: The *Leishmania* genome is constitutively expressed

Kirk Leifso[a,b], Gabriela Cohen-Freue[c], Nisha Dogra[a,b], Angus Murray[a,b], W. Robert McMaster[a,b,*]

[a] *Immunity and Infection Research Centre, Vancouver Coastal Health Research Institute, Canada*
[b] *Department of Medical Genetics, 451-2660 Oak Street, Vancouver, BC, Canada V6H 3Z6*
[c] *Department of Statistics, University of British Columbia, Vancouver, BC, Canada*

Received 28 July 2006; received in revised form 10 November 2006; accepted 13 November 2006
Available online 8 December 2006

Leishmania ssp



Leishmania major Promastigotes Vs axenic Amastigotes

Promastigotes: 26°C, pH 7 (insect-like insect-like environment)

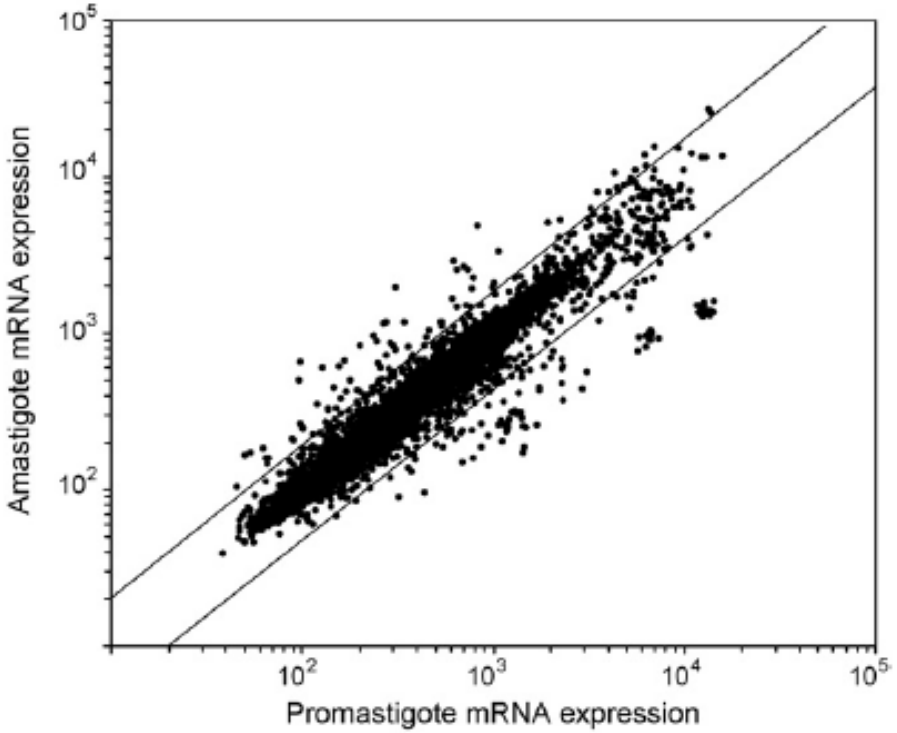
Axenic Amastigotes 37°C, pH 5.5, 5% CO (intralysosomal-like environment)

hora 1: Expresion de la familia de proteínas específicas de amastigote A2

hora 5-24: Transformacion morfológica

hora 24-72: Liberacion de LPG

Leishmania major Promastigotes Vs axenic Amastigotes



1.4% of total genes are differentially expressed in amastigotes

1.5% of total genes are differentially expressed in promastigotes

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Molecular & Biochemical Parasitology 146 (2006) 198–218
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Expression profiling by whole-genome interspecies microarray hybridization reveals differential gene expression in procyclic promastigotes, lesion-derived amastigotes, and axenic amastigotes in *Leishmania mexicana*

Timothy R. Holzer[a], W.R. McMaster[b], James D. Forney[a,*]

[a] *Purdue University, Department of Biochemistry, 175 S. University St., West Lafayette, IN 47907-2063, United States*
[b] *University of British Columbia, Department of Medical Genetics Immunity and Infection Research Centre, 2660 Oak Street, Vancouver, BC, Canada V6Z 3Z6*

Received 3 June 2005; received in revised form 27 November 2005; accepted 16 December 2005
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PLoS one

The Cell Cycle Regulated Transcriptome of *Trypanosoma brucei*

Stuart K. Archer[1]*, Diana Inchaustegui[1], Rafael Queiroz[2], Christine Clayton[1]

1 Zentrum für Molekulare Biologie Heidelberg, DKFZ-ZMBH Allianz, Heidelberg, Germany, **2** Deutsches Krebsforschungszentrum, DKFZ-ZMBH Allianz, Heidelberg, Germany

Cultivos cincronizados por hambreado

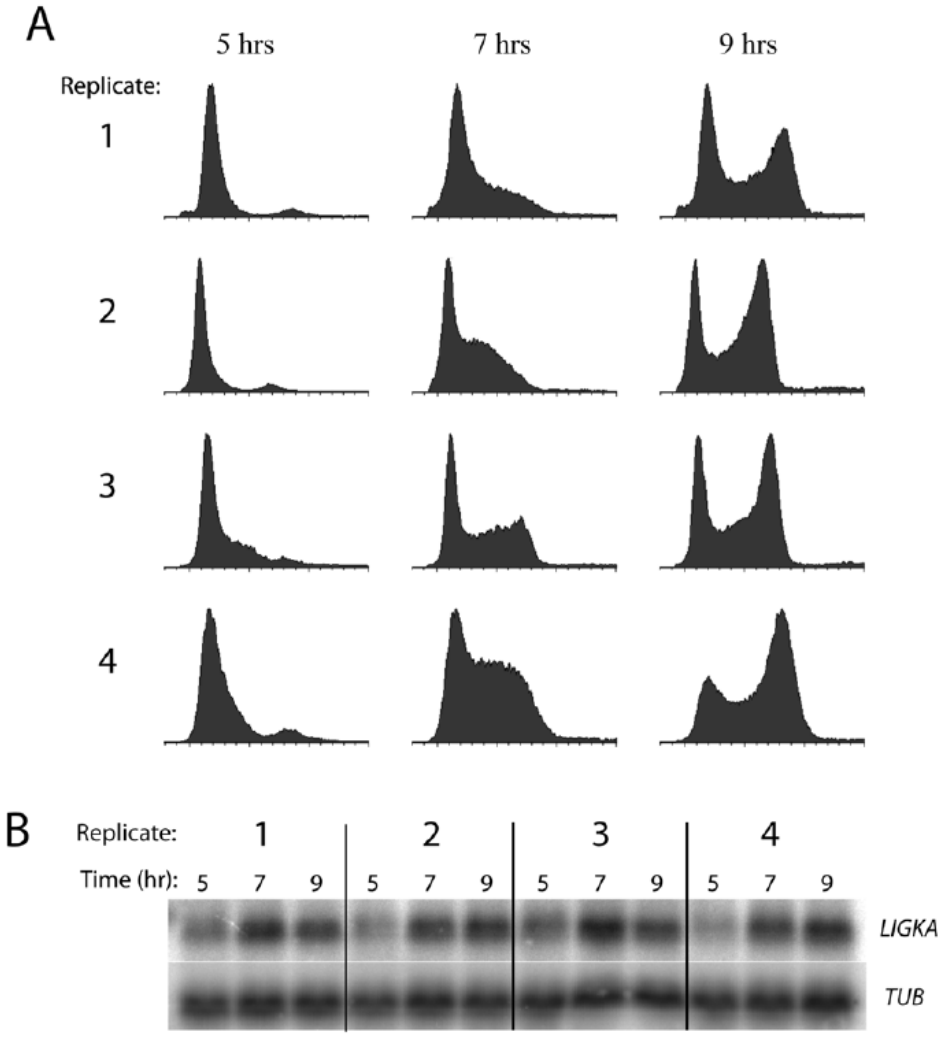


Figure 1. Synchronization of procyclic cells by starvation. Four biological replicates were performed in which cells were released from starvation at time t = 0 and samples collected for flow cytometry and RNA isolation at t = 5, 7 and 9 hours post-release. **A:** Flow cytometry profiles of synchronized procyclic cells. Propidium iodide fluorescence (indicating DNA content) is measured on the *x*-axes and cell count is plotted on the *y*-axes. **B:** Northern hybridization of a known cell-cycle regulated transcript (*LIGKA*) against RNA from synchronized cells.
doi:10.1371/journal.pone.0018425.g001

Cultivos cincronizados por Counterflow Centrifugal Elutriation

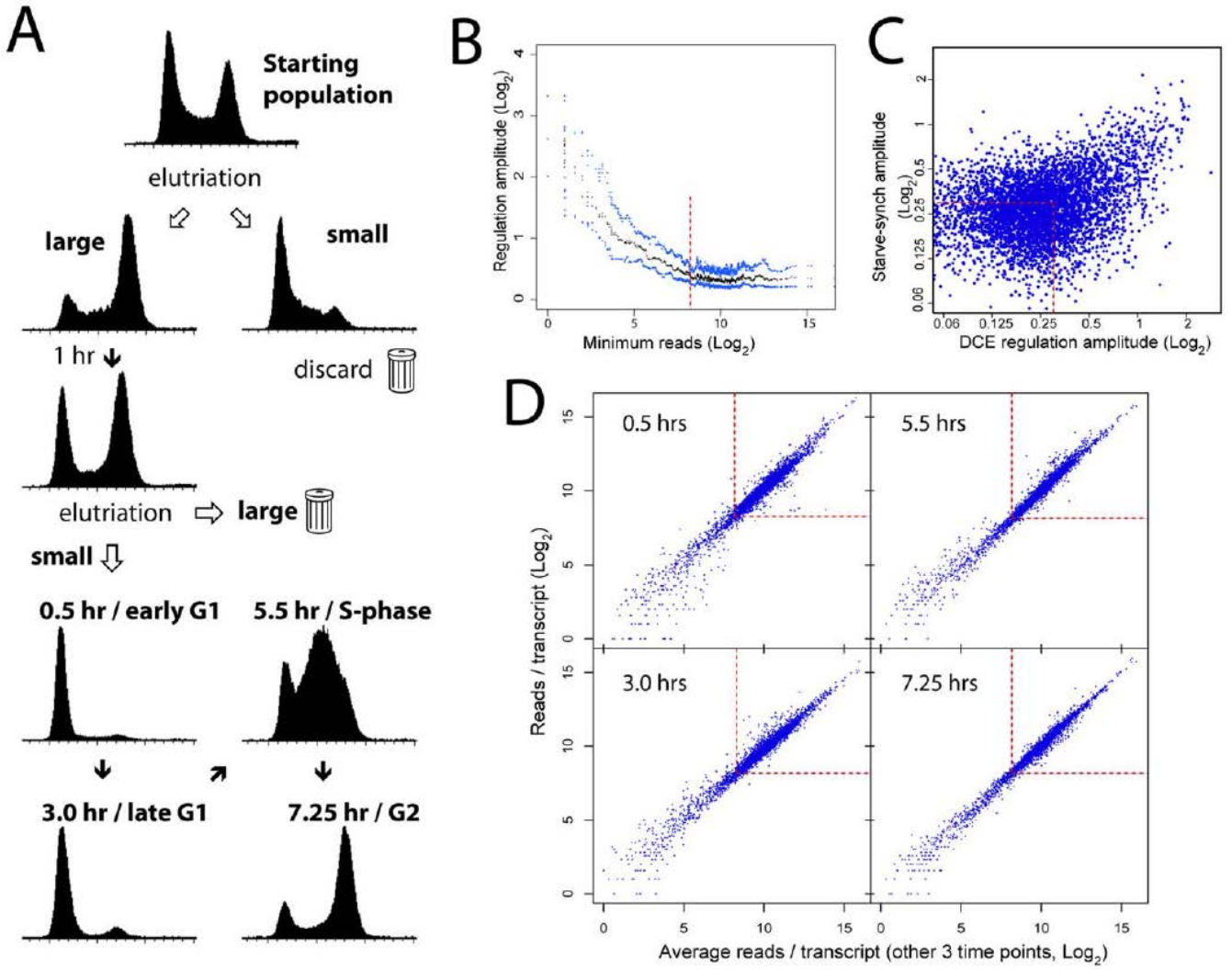


Figure 2. Expression profiling by DCE and RNA-seq. A: Flow cytometry profiles of procyclic cells throughout the DCE selection procedure. Propidium iodide fluorescence (indicating DNA content) is measured on the *x*-axes and cell count on the *y*-axes. **B:** Regulation amplitude (the difference between the maximum and minimum expression value throughout the cell cycle) was calculated for each gene. After ranking genes according to minimum read count, the median (black) and upper and lower quartile (blue) amplitude values across a moving window of 100 genes was calculated. Genes with fewer than ~300 reads in any time point (red line) gave amplitudes that were most likely to be a function of sequencing effort and were therefore excluded from further analysis. **C:** Comparison of gene regulatory amplitude between the starve-synch/microarray-analyzed cells (time points 5, 7 and 9 hrs) and DCE-synch/RNA seq-analyzed cells (time points 3, 5.5 and 7.25 hrs). Genes passing quality control in the RNA-seq experiment and with less than 1.23-fold regulation (0.3 $\log_2$ units - red lines) in both experiments (red box) were selected as a non-regulated control group for subsequent UTR sequence analyses (motif searching). **D:** Comparison of read counts per transcript for each time-point with the average read counts from the other 3 time points. Red boxes contain transcripts with >300 reads.

doi:10.1371/journal.pone.0018425.g002

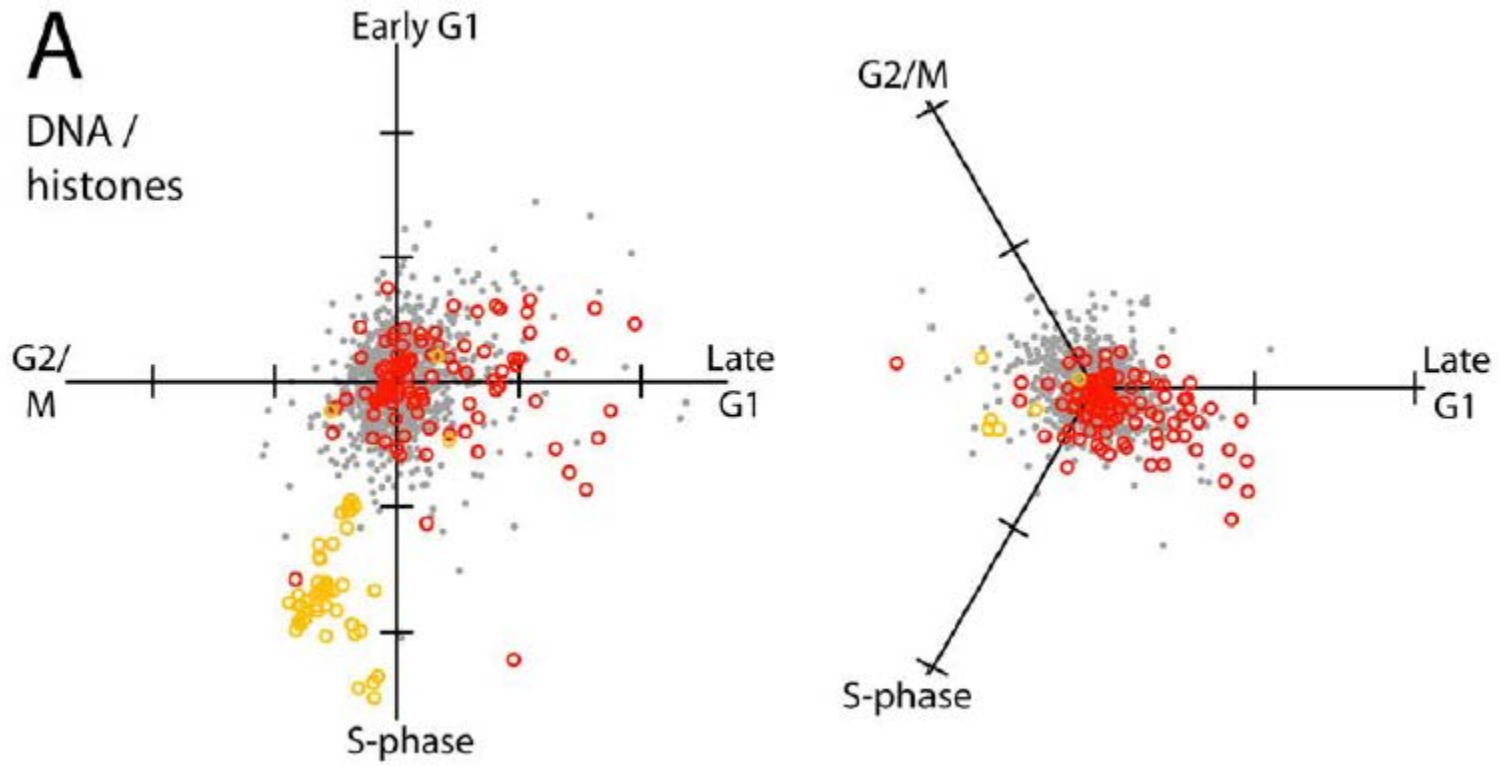
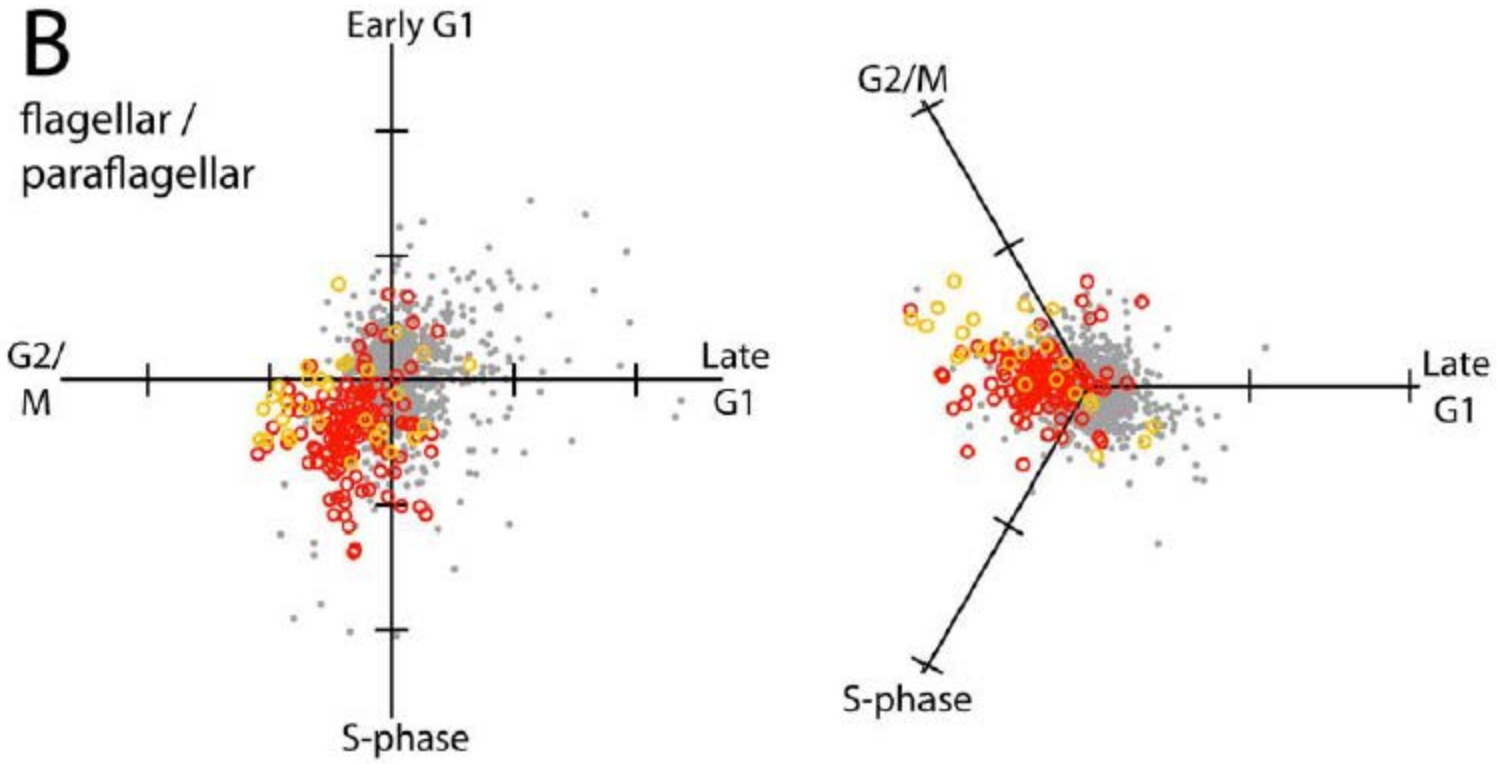


Figure 5. Vectorial representation of regulation of selected functional groups of transcripts in the cell cycle, from DCE/RNA-seq data (left panels) or starve-synch/microarray data (right panels). Each time-point in the cell cycle that was analyzed was arranged as a vector pointing outwards from the origin. Gene expression values were plotted by vector addition; tick marks on axes are one $\log_2$ unit. Reference profiles from 1000 randomly selected genes are plotted in grey; genes in specific functional groups are plotted as coloured circles. **A:** Red: Transcripts annotated with "DNA" in "product description" or in gene ontology fields of the TriTryp database (relevance score >40). Orange: Histone-encoding transcripts. **B:** Red: Transcripts encoding flagellar proteins [32]. Orange: Transcripts encoding Snl2-dependent paraflagellar rod proteins [33]. **C:** Putative mediators of mitosis and cytokinesis, and RBPs. Red: Transcripts encoding Aurora kinases and chromosomal passenger complex proteins. Orange: Polo-like kinase. Blue: Selected RBPs. (×) PUF9, (+) RBP45 homologue, (o) DRDB17.



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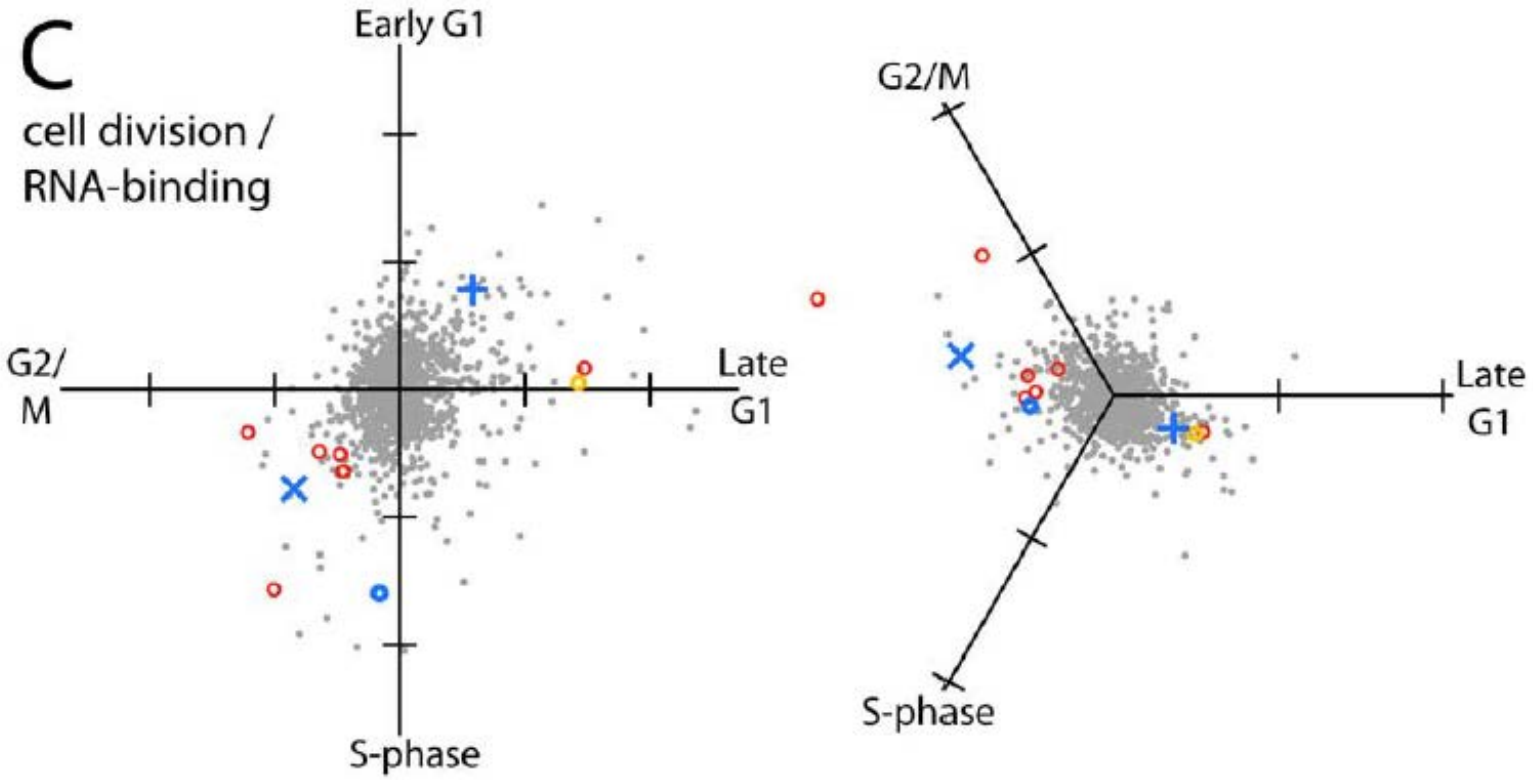

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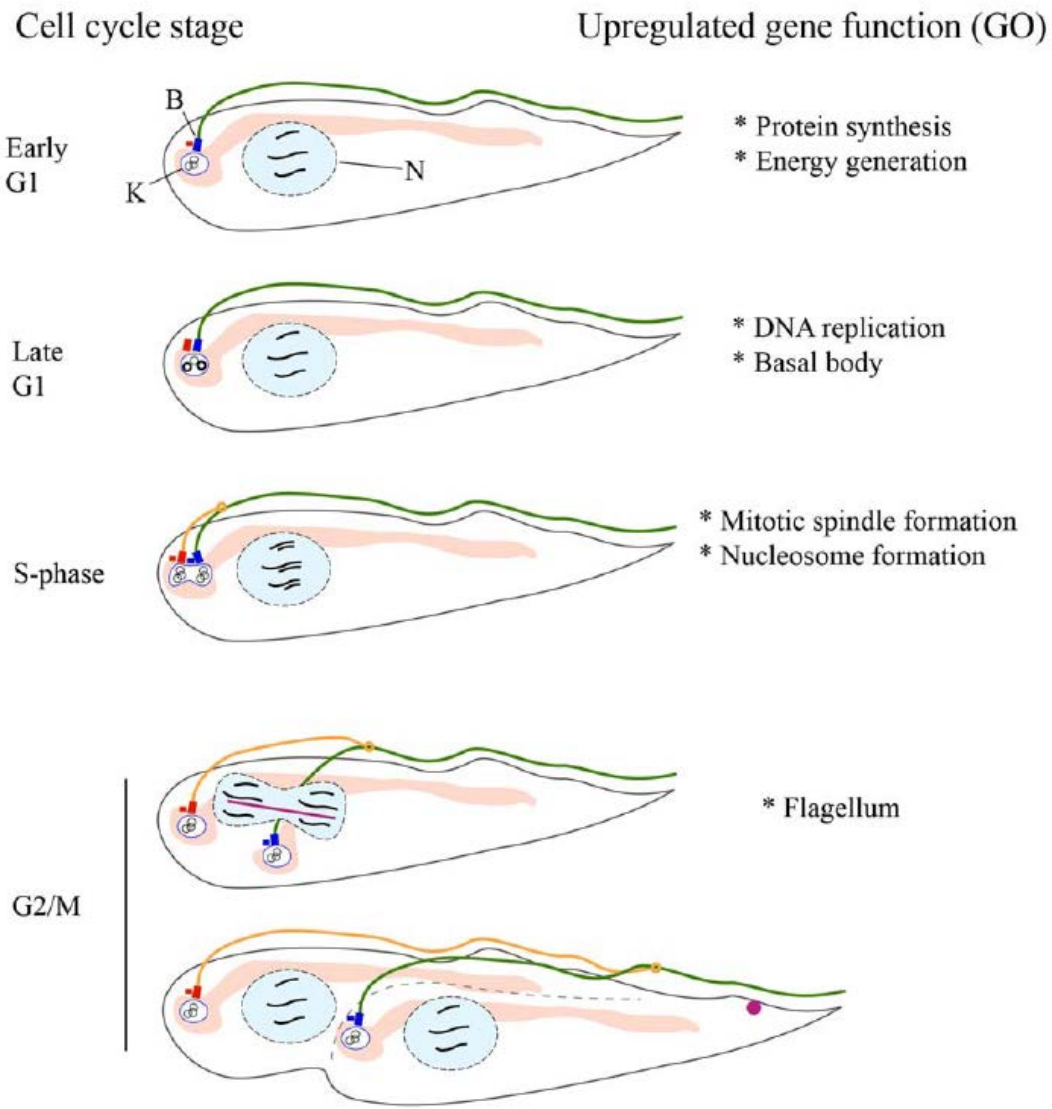
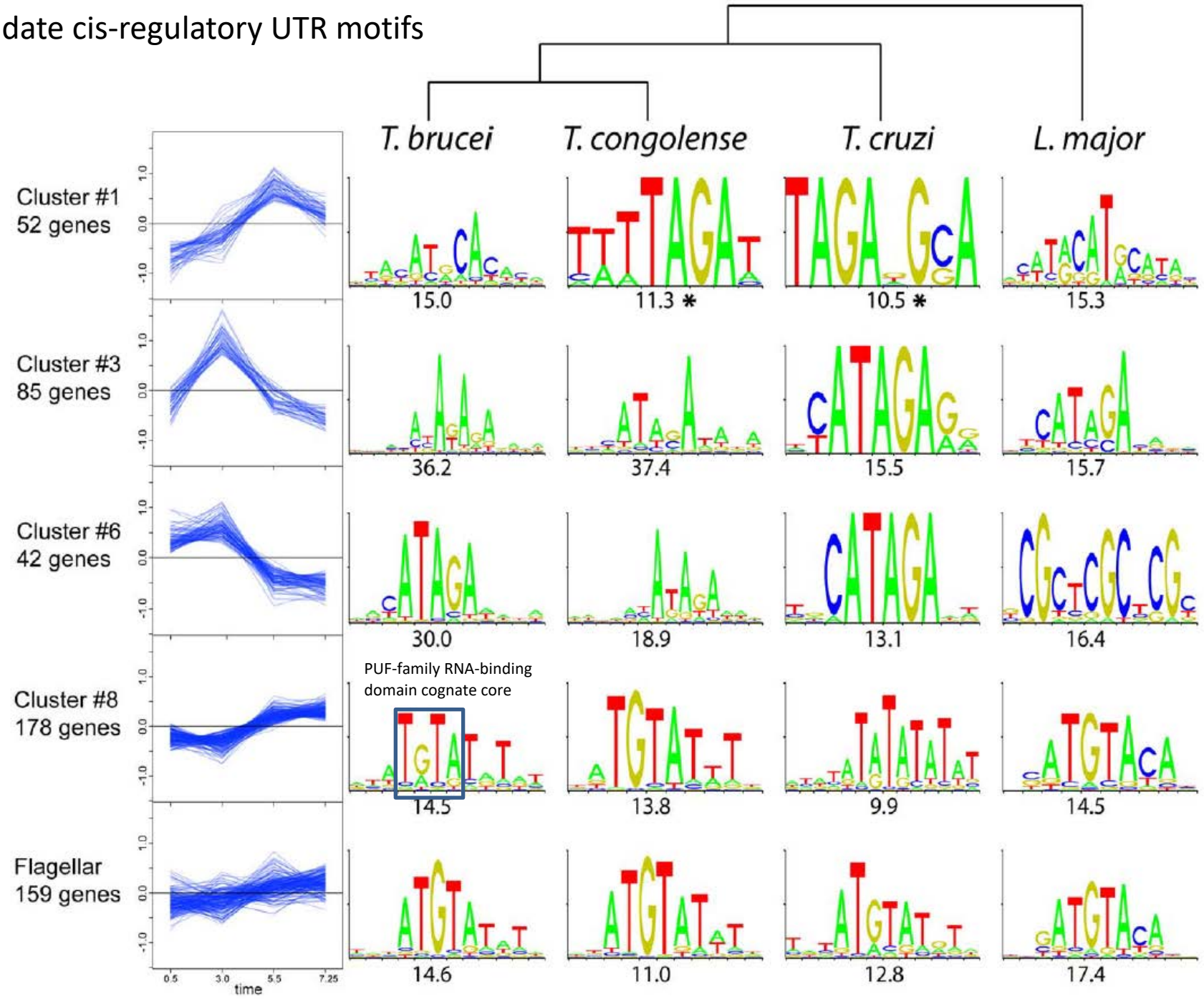
Genes regulados en las distintas etapas del ciclo celular



Candidate cis-regulatory UTR motifs



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The trypanosome transcriptome is remodelled during differentiation but displays limited responsiveness within life stages

V Lila Koumandou, Senthil Kumar A Natesan, Tatiana Sergeenko and Mark C Field*

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* Corresponding author

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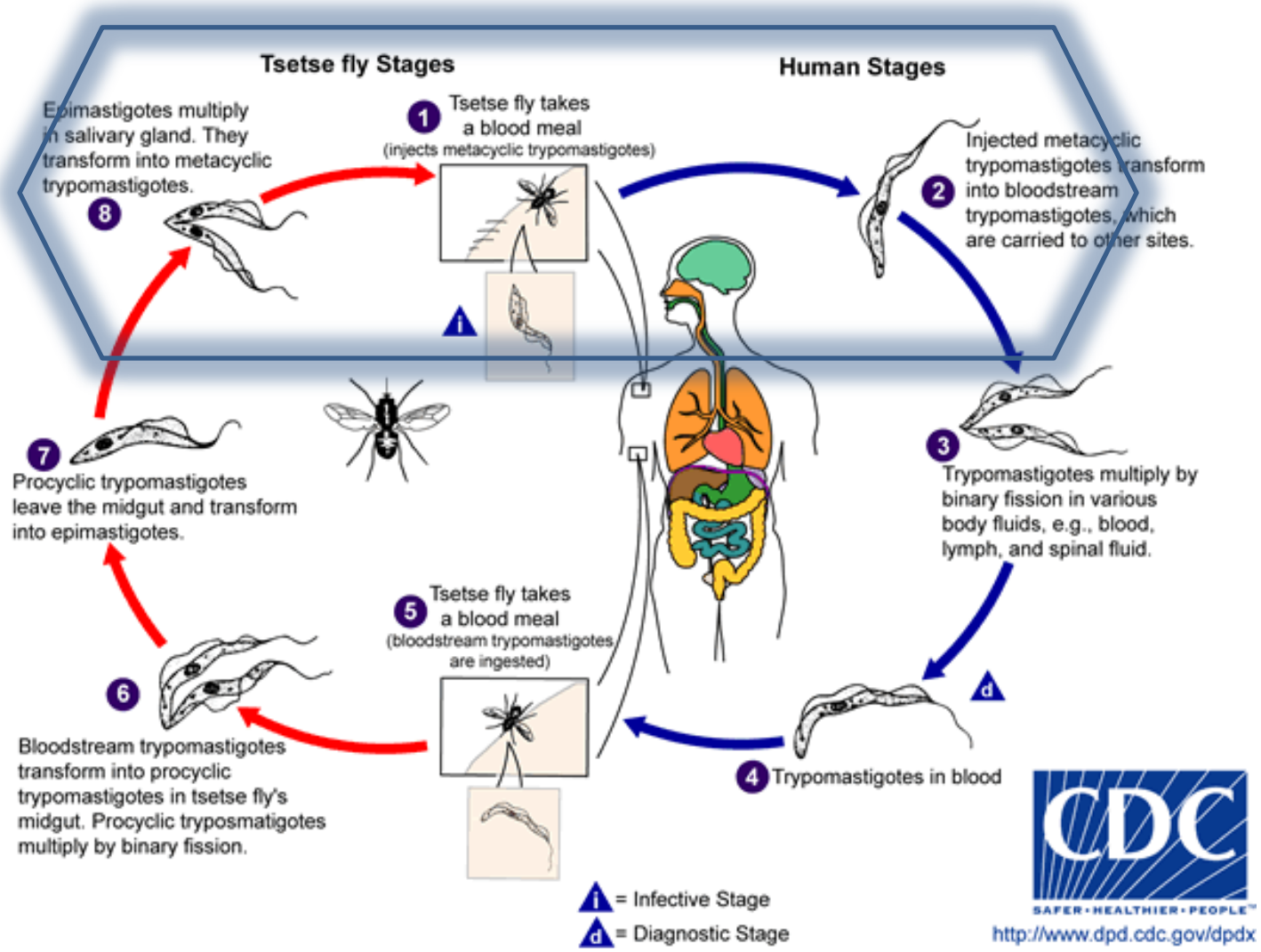
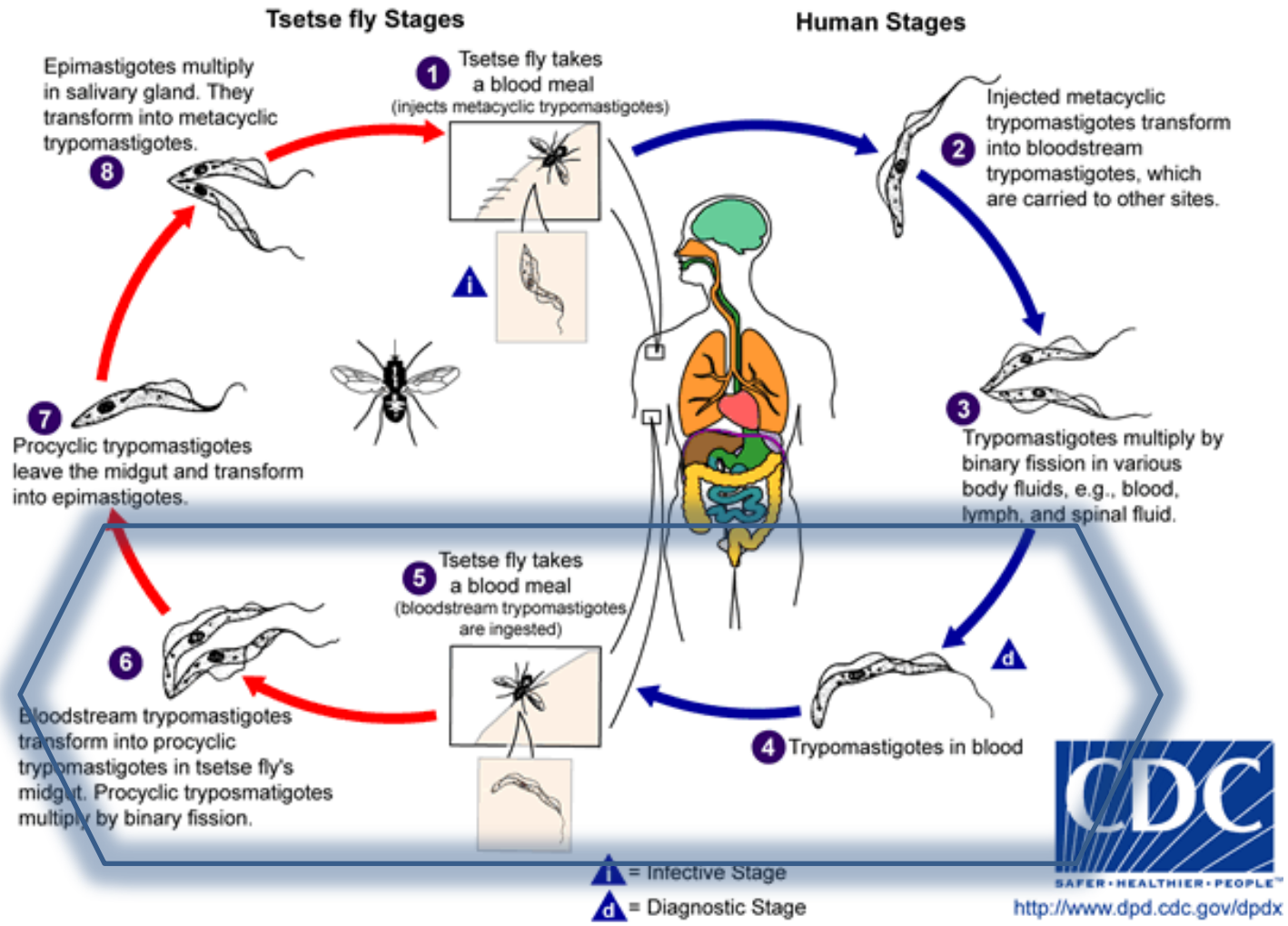
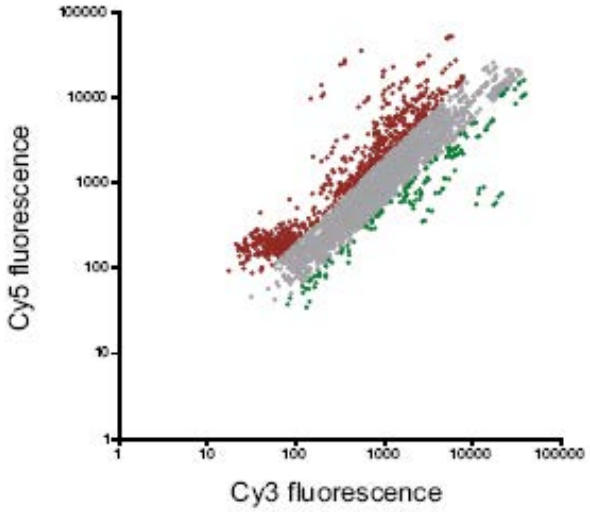
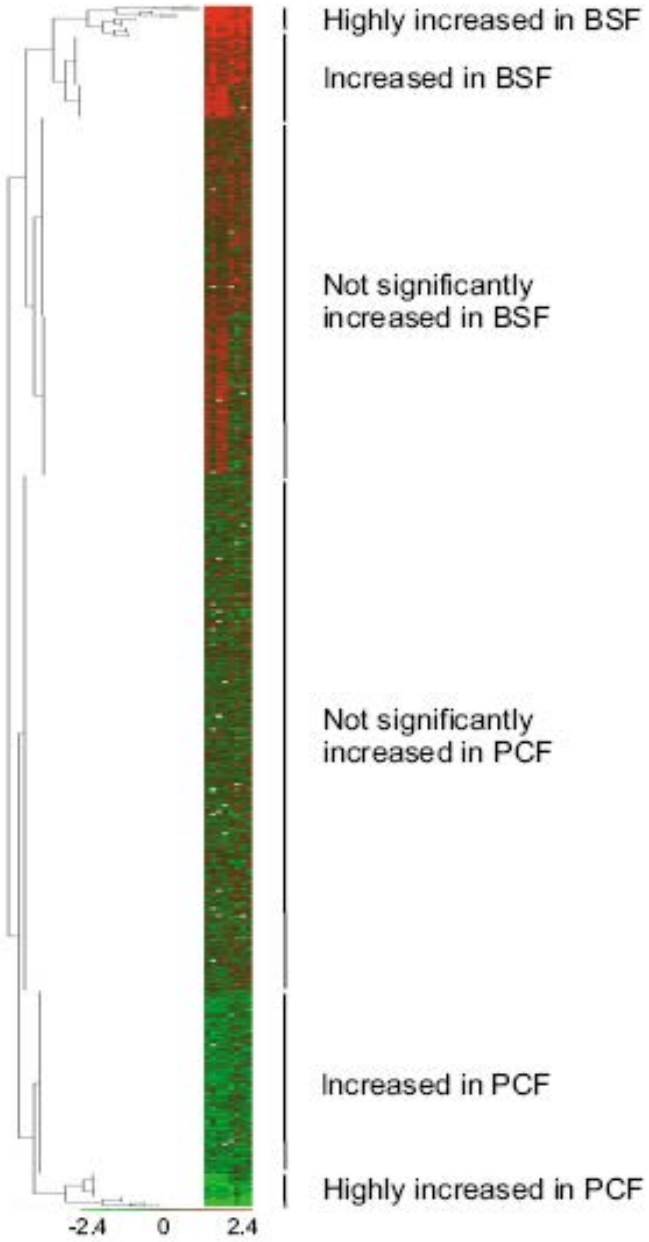
Trypanosoma brucei

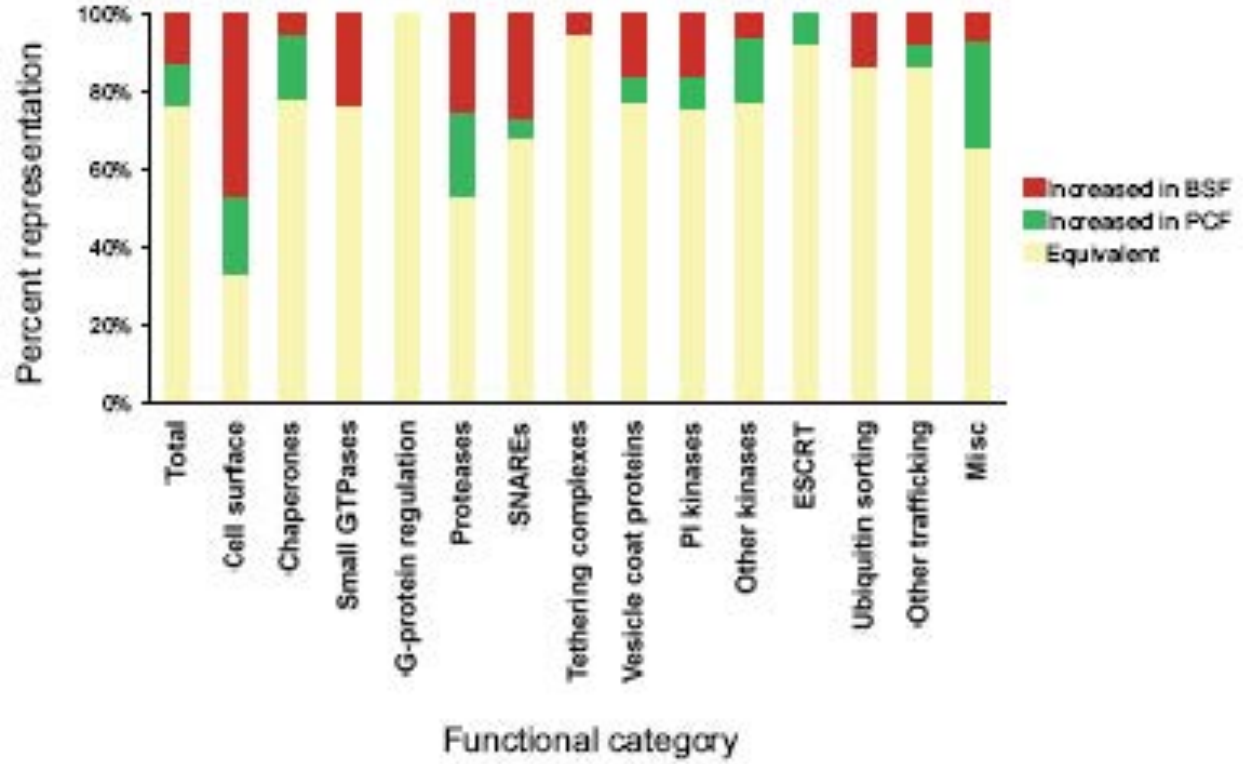


Trypanosoma brucei




Cy5 fluorescence
Cy3 fluorescence
100000
10000
1000
100
10
1
1
10
100
1000
10000
100000


Highly increased in BSF
Increased in BSF
Not significantly increased in BSF
Not significantly increased in PCF
Increased in PCF
Highly increased in PCF
-2.4
0
2.4


Percent representation
100%
80%
60%
40%
20%
0%
Total
Cell surface
Chaperones
Small GTPases
G-protein regulation
Proteases
SNAREs
Tethering complexes
Vesicle coat proteins
PI kinases
Other kinases
ESCRT
Ubiquitin sorting
Other trafficking
Misc
Increased in BSF
Increased in PCF
Equivalent
Functional category

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doi:10.1093/nar/gkq237

Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites

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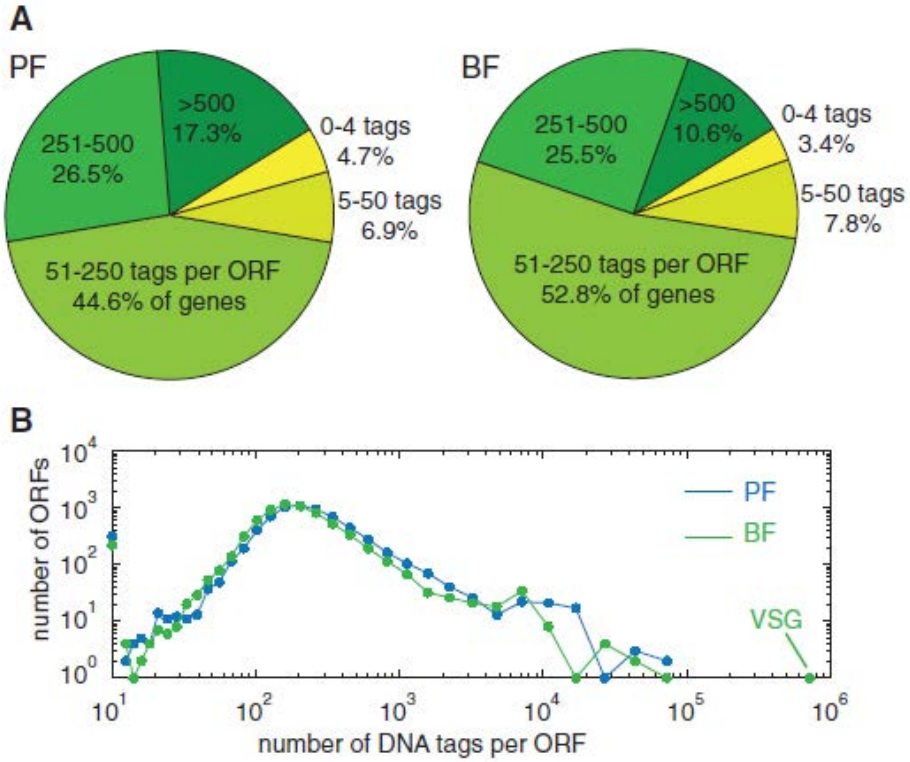
Received September 1, 2009; Revised February 23, 2010; Accepted March 19, 2010

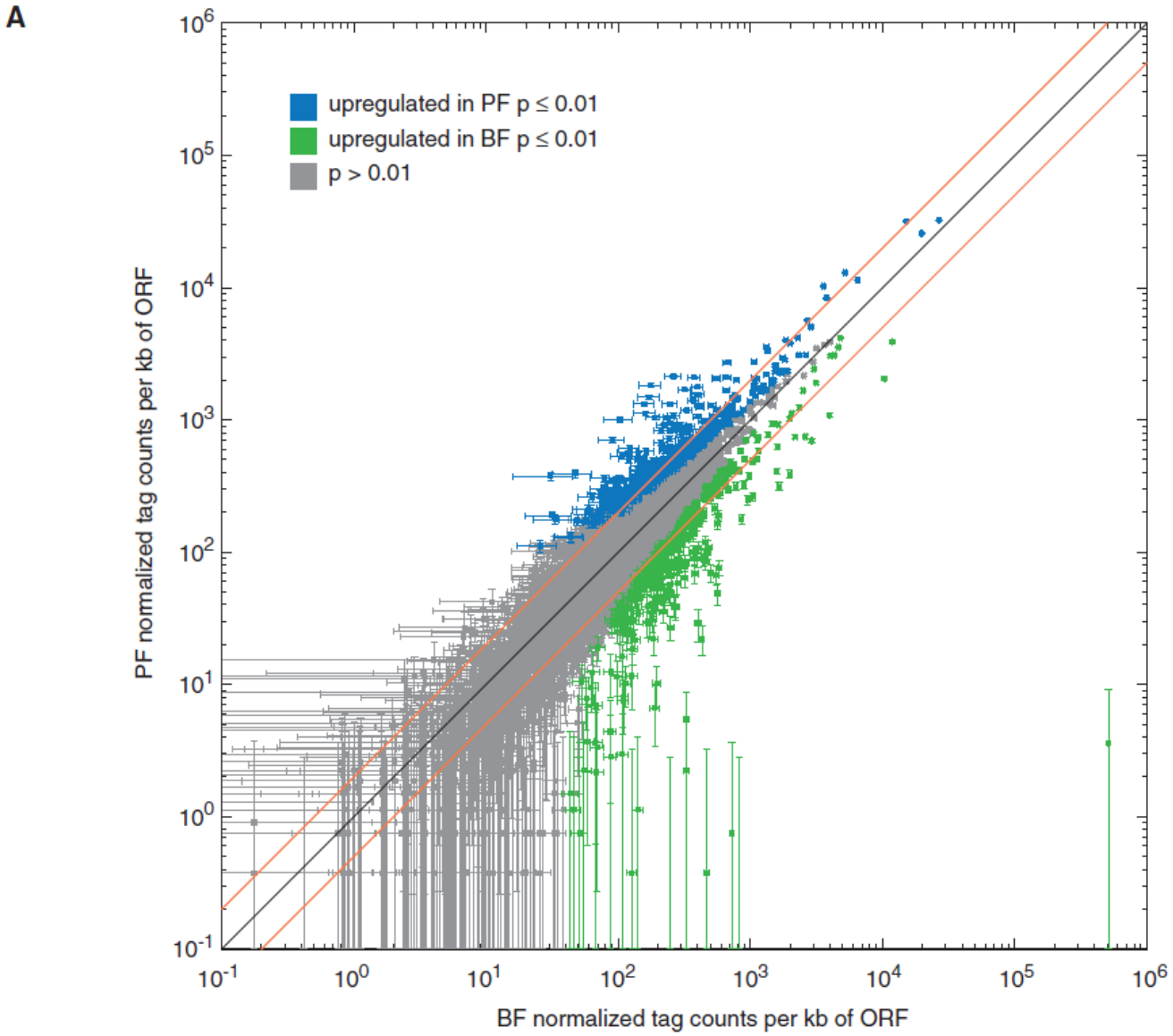
Table 1. Enumeration of sequenced tags

	Bloodstream form	Procyclic form
Biological replicates	3	4
Technical replicates	5	11
Total bases sequenced	727 822 044	5 816 182 540
Tag length	36 bp	32, 36 or 76 bp
Unique tags	11 108 029	12 661 997*
Tags used for expression analyses	5 592 775	7 334 554*

*Excluding the 65 691 627 76-bp tags. Although the majority of these were unique, because of their length, they were poly(A)-primed and not used for expression analysis.



De 7571 genes, 425 (5,6%) tienen expresión diferencial (> 2 fold, p >0,01)



221 upr in BF

204 upr in PF

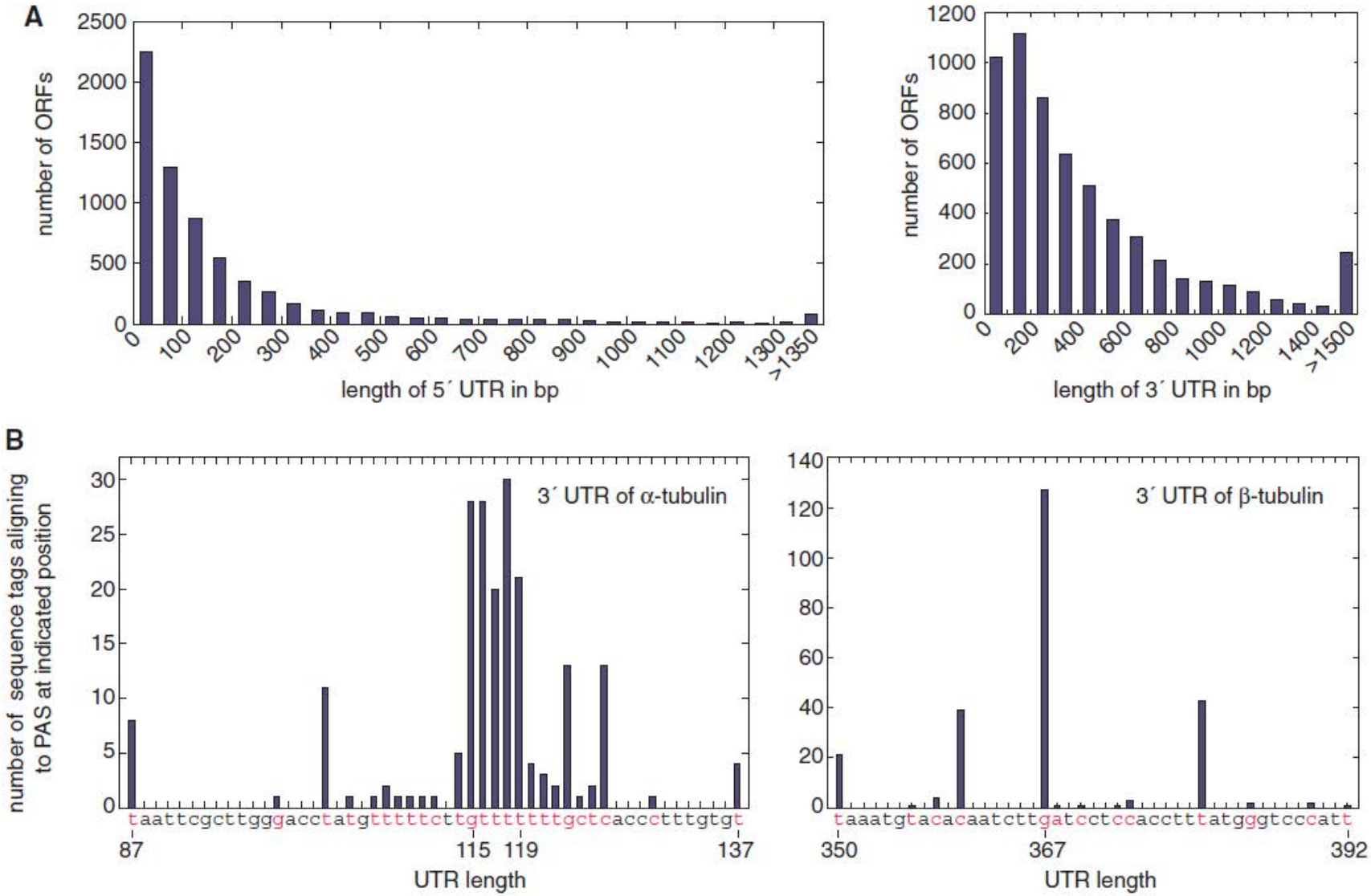


Figure 4. Length of 5′ and 3′ UTRs. **(A)** Histogram showing the length distribution of 5′ UTR (left panel; $n = 6\,644$; window 50 nt) and 3′ UTR (right panel; $n = 5911$; window 100 nt) for the predominant SAS and PAS. If multiple SAS or PAS occurred at the same frequency the length of the shortest UTR was used for this histogram. **(B)** Quantification of multiple PAS used by β-tubulin (left panel) and α-tubulin (right panel). Nucleotides labeled in red indicate PAS. For β-tubulin, additional sequence tags indicated PAS at 318 bp (1 tag), 323 bp (3), 334 bp (1) and 476 bp (1) downstream of the ORF.

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The steady-state transcriptome of the four major life-cycle stages of *Trypanosoma cruzi*

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Email: Todd A Minning - tminning@uga.edu; D Brent Weatherly - dbrentw@uga.edu; James Atwood - atwood@ccrc.uga.edu; Ron Orlando - orlando@ccrc.uga.edu; Rick L Tarleton* - tarleton@cb.uga.edu

* Corresponding author

Published: 7 August 2009

Received: 9 April 2009

Trypanosoma cruzi

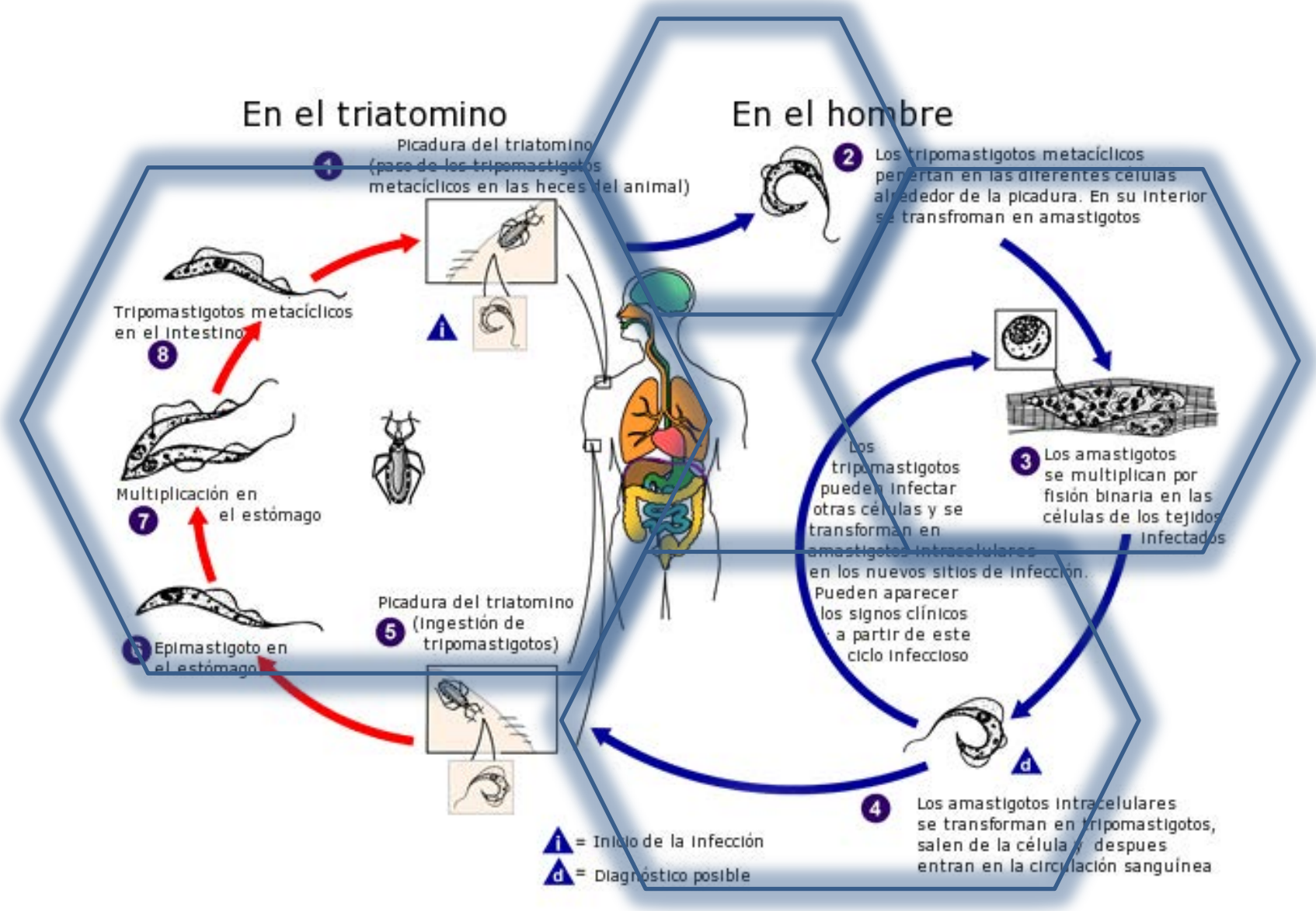


Table 1: With or without the contribution of large gene family members, greater than 50% of the *Trypanosoma cruzi* genes detected on whole-genome, oligonucleotide microarrays were significantly stage-regulated at the RNA level.

		# spots (percent of total)	# genes (percent of total)
all	SIG[1]	4273 (51.6)	6708 (52.2)
	NON-SIG[2]	4000 (48.4)	6141 (47.8)
	Total	**8273 (100)**	**12849 (100)**
large gene families[3]	SIG	739 (50.1)	1010 (51.5)
	NON-SIG	735 (49.9)	950 (48.5)
	Total	**1474 (100)**	**1960 (100)**
minus large gene families	SIG	3534 (52.0)	5698 (52.3)
	NON-SIG	3265 (48.0)	5191 (47.7)
	Total	**6799 (100)**	**10889 (100)**

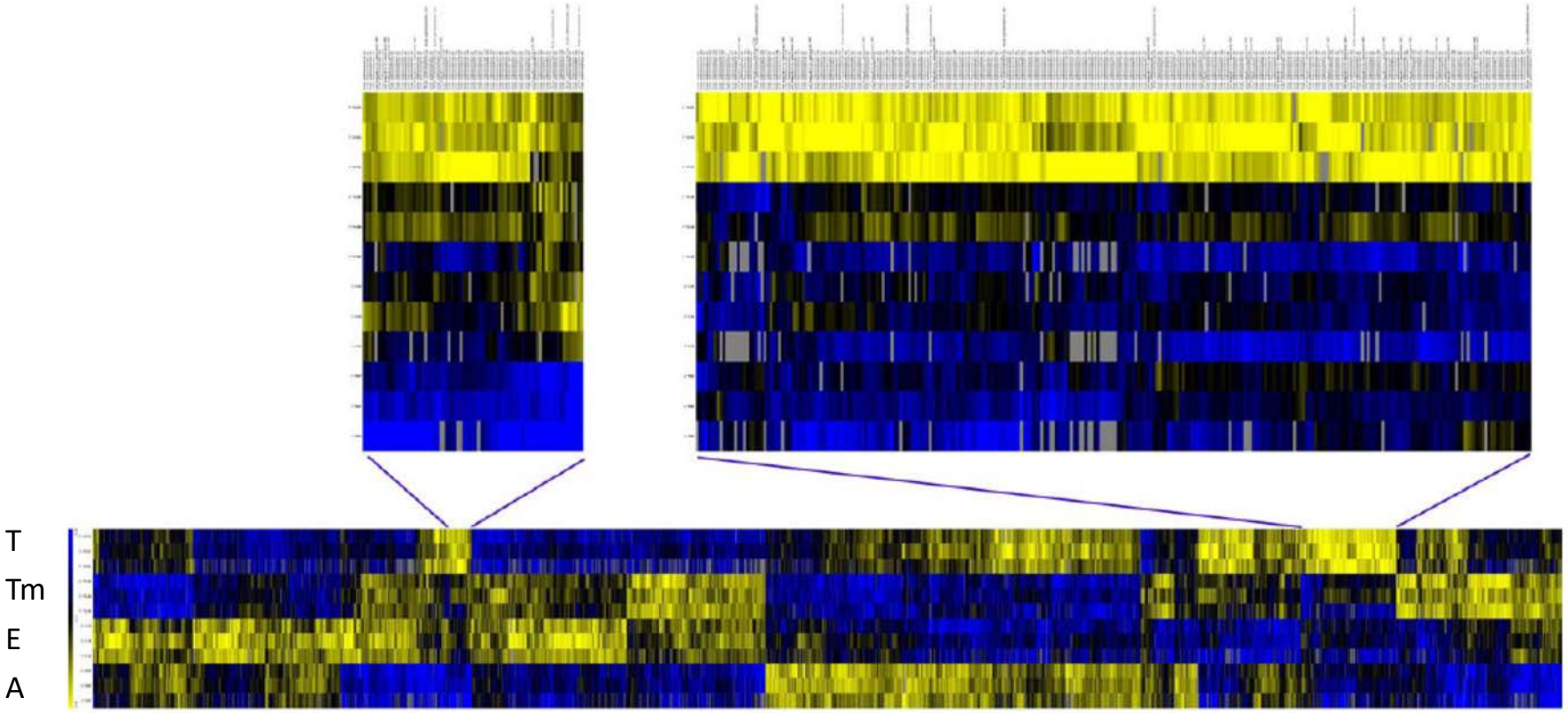
The oligonucleotide sequences were aligned against the *T. cruzi* genome by BLAST analysis [58]. Oligonucleotides showing 80% or greater identity with more than three *T. cruzi* gene ID's were not counted.

[1]Significantly stage-regulated as determined by SAM analysis (FDR = 0.4%).

[2]Non-significantly stage-regulated as determined by SAM analysis (FDR = 0.4%).

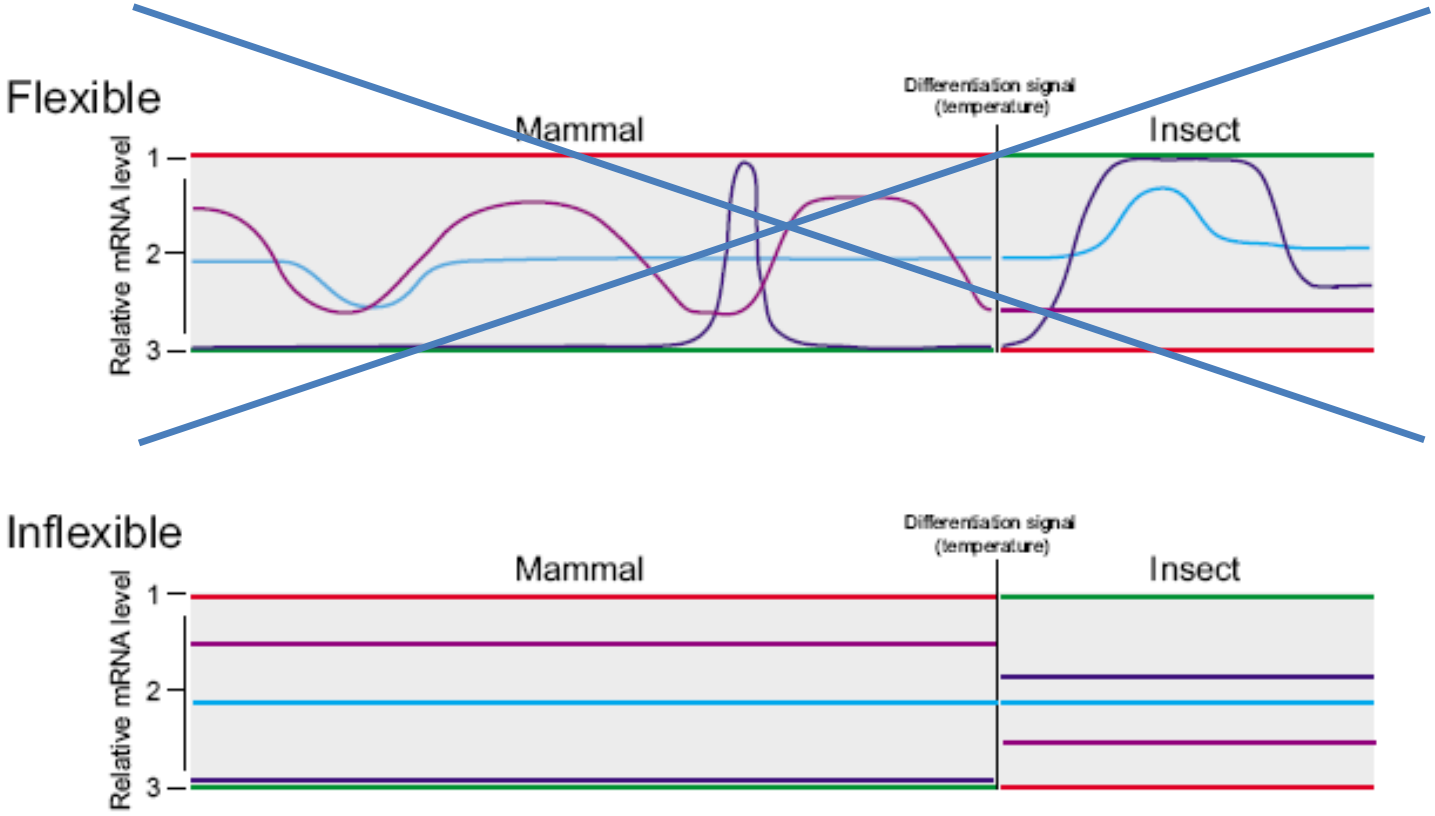
[3]Oligonucleotides mapping to members of large gene families (number of members > 30).

De los genes aumentados en dos estadios el 76% (1083/1423) corresponden a estadíos del mismo hospedador y 20% (282/1423) a función biológica similar



Heat map of genes significantly regulated during the life-cycle of *Trypanosoma cruzi*. Ratios are log2 (stage/reference), thus yellow bars represent upregulation and blue bars represent downregulation. Two trypomastigote upregulated clusters are expanded. The spots annotated as 'not mappable to annotated ORF' were designed from earlier versions of the *T. cruzi* genome sequence and subsequently designated as 'obsolete' by TIGR. These spots no longer mapped within an annotated ORF in the final version of the genome sequence, e.g. they were in intergenic regions (such as untranslated regions) and/or were antisense (would hybridize with first strand cDNA from transcripts from the non-coding strand).

Kinetoplastid´s gene expression model



Análisis de transcriptomas en Hongos

Estudio de los transcriptomas de *Histoplasma capsulatum*

Comparative Transcriptomics of Infectious Spores from the Fungal Pathogen *Histoplasma capsulatum* Reveals a Core Set of Transcripts That Specify Infectious and Pathogenic States

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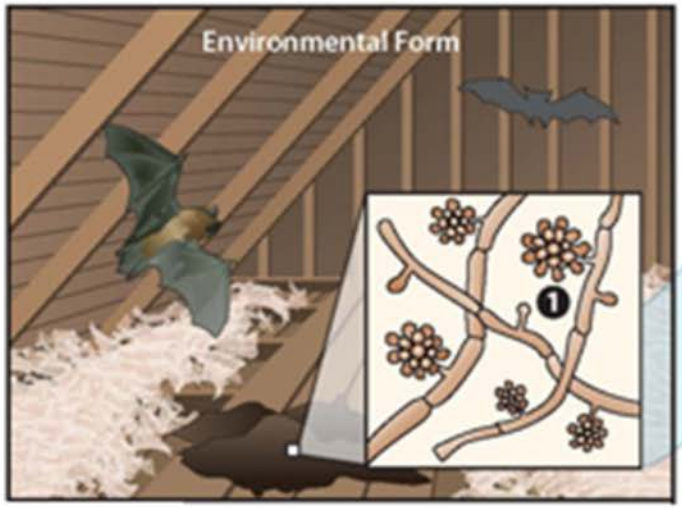
[*]Department of Microbiology and Immunology, University of California San Francisco, San Francisco, California, USA; [&]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

Estudio de los transcriptomas de *Histoplasma capsulatum*



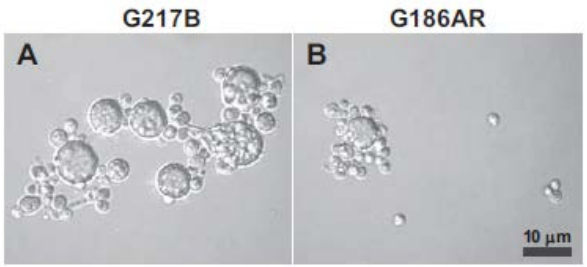
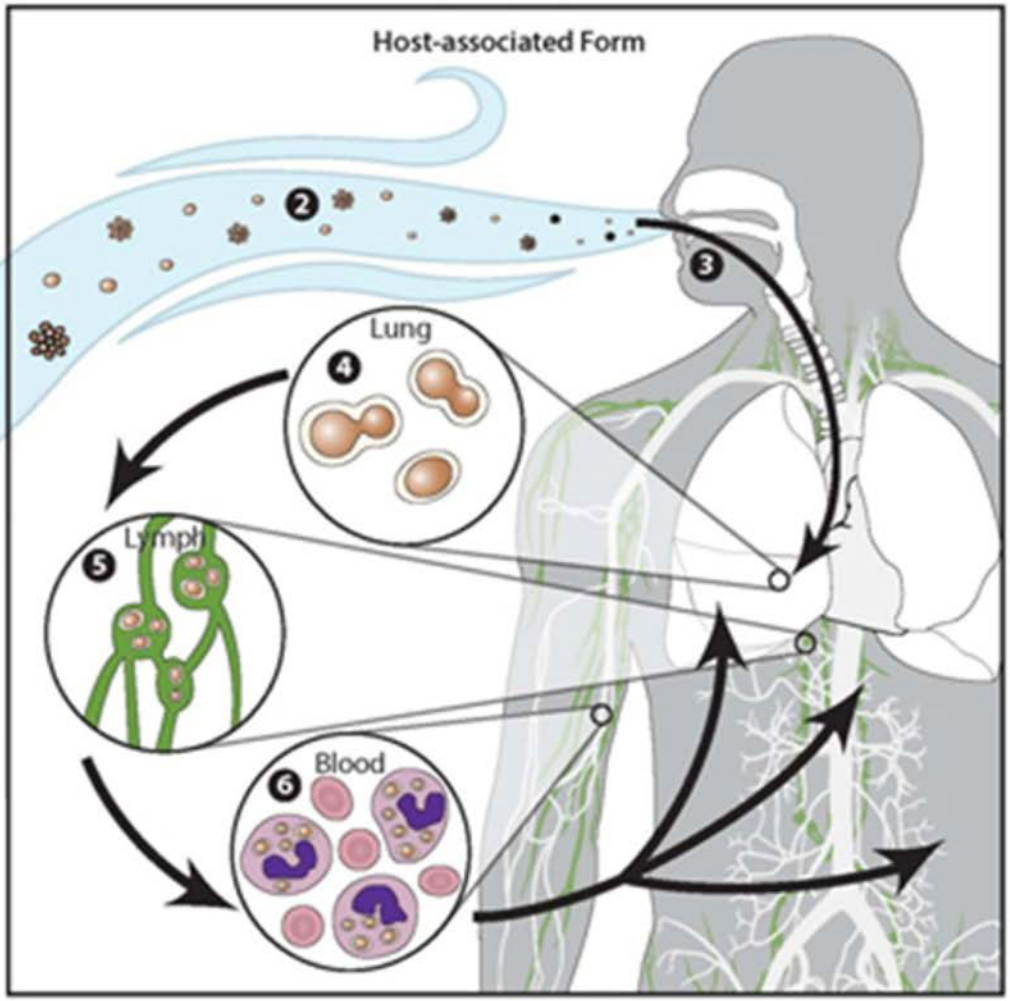


FIG 1 Purified microconidia and macroconidia were generated from *H. capsulatum*. Purified macroconidia and microconidia of the G217B (A) and G186AR (B) strains are shown.



Estudio de los transcriptomas de *Histoplasma capsulatum*

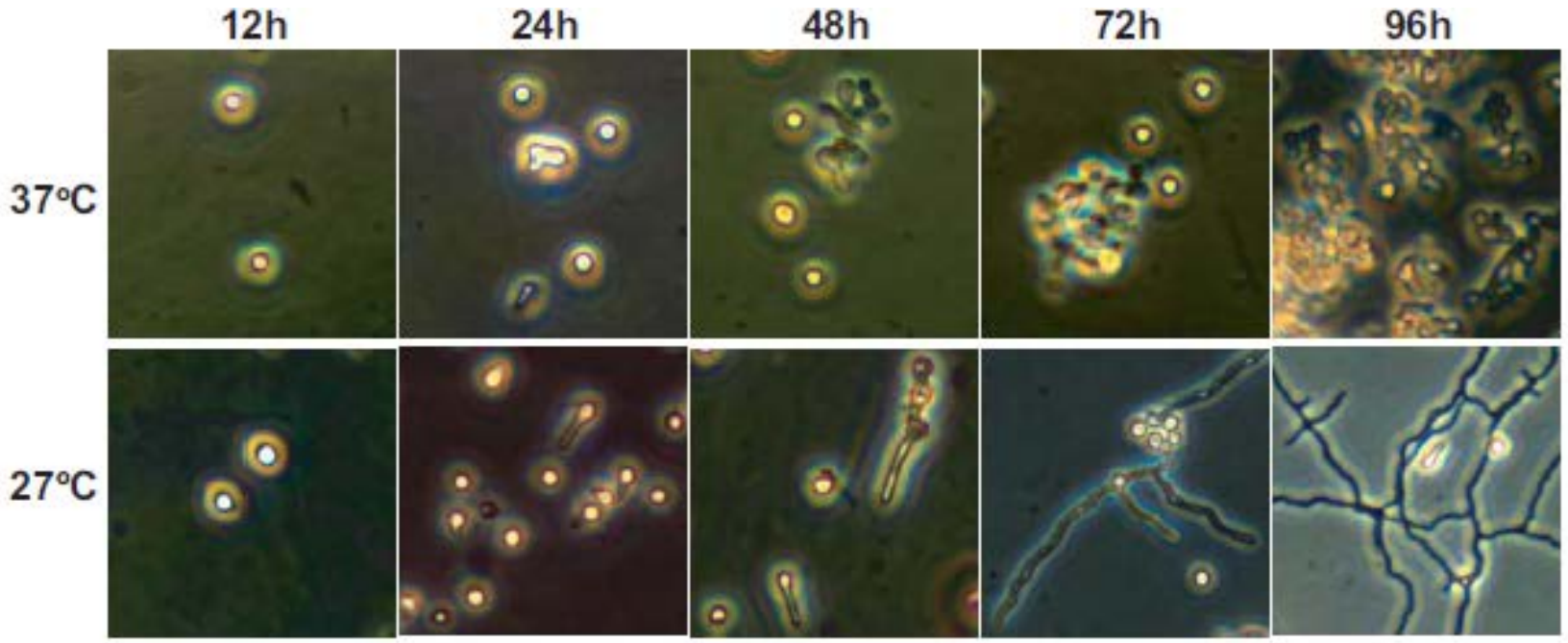
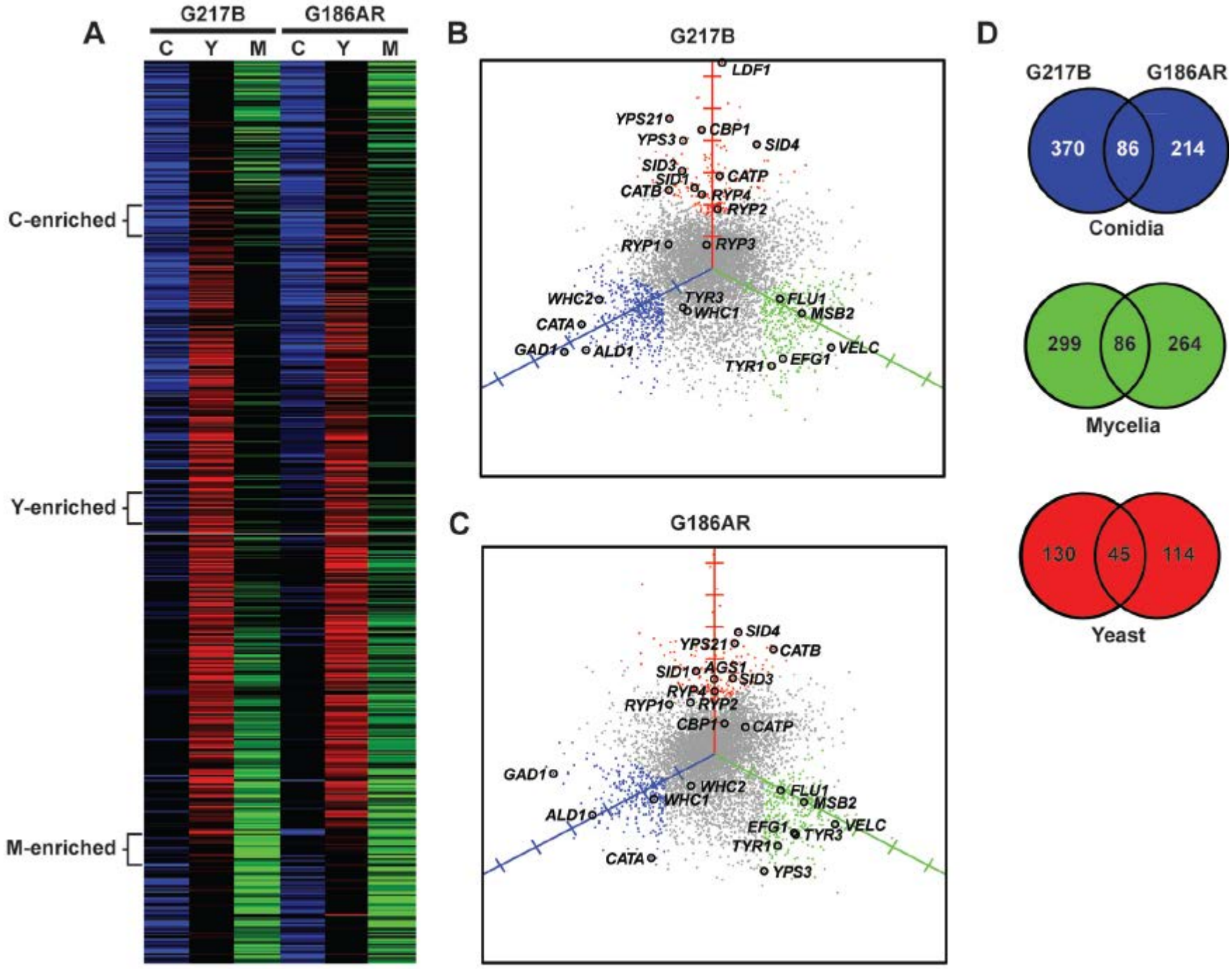
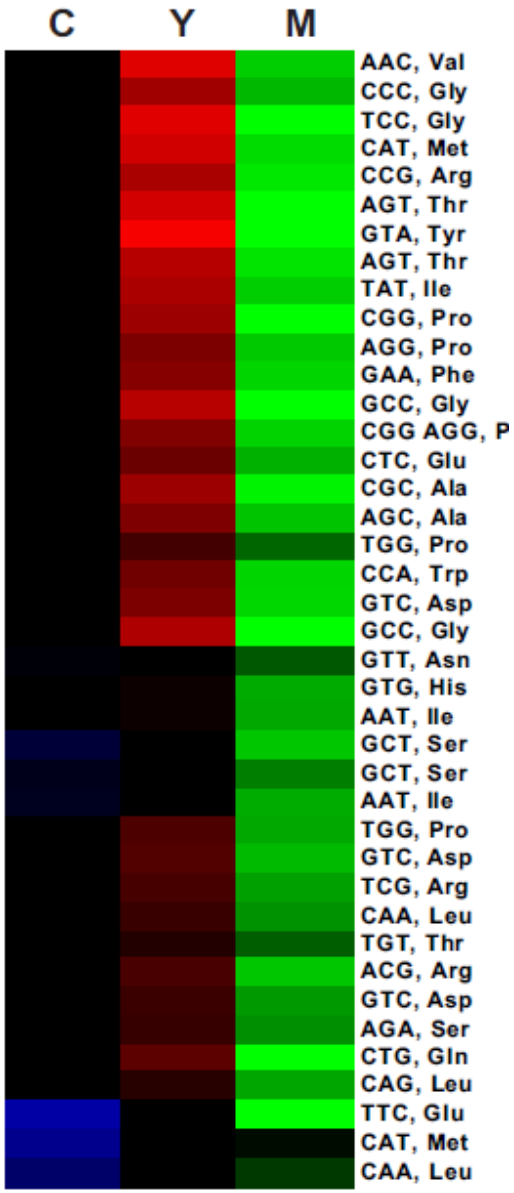


FIG 2 Conidia germinate *in vitro* into filaments at 27°C and into yeast-phase cells at 37°C. A time course of germination of G217B conidia was performed at the indicated temperatures.


A
G217B
G186AR
C
Y
M
C
Y
M
C-enriched
Y-enriched
M-enriched
B
G217B
LDF1
YPS21
CBP1
YPS3
SID4
SID3
SID1
CATP
CATB
RYP4
RYP2
RYP1
RYP3
WHC2
TYR3
FLU1
WHC1
MSB2
CATA
VELC
GAD1
ALD1
TYR1
EFG1
C
G186AR
SID4
YPS21
CATB
SID1
AGS1
SID3
RYP4
RYP1
RYP2
CBP1
CATP
GAD1
WHC2
FLU1
WHC1
MSB2
ALD1
EFG1
VELC
TYR3
TYR1
CATA
YPS3
D
G217B
G186AR
370
86
214
Conidia
299
86
264
Mycelia
130
45
114
Yeast

Expresión diferencial de los tRNAs en los distintos estadíos



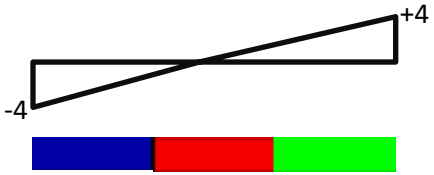


FIG 7 tRNAs are differentially expressed in conidia, yeast, and mycelia. The heat map shows the enriched expression in mycelia and depleted expression in conidia of 40 tRNA transcripts of G217B. Relative transcript levels in conidia (C), mycelia (M), and yeast (Y) are displayed. Intensities are $\log_2$ BAGEL-estimated relative expression levels from 0 (black) to 4 (saturated). tRNAs are labeled with cognate amino acids and anticodons as predicted by tRNAscan-SE.

Expresión diferencial de los miembros de la familia de la tirocinasa

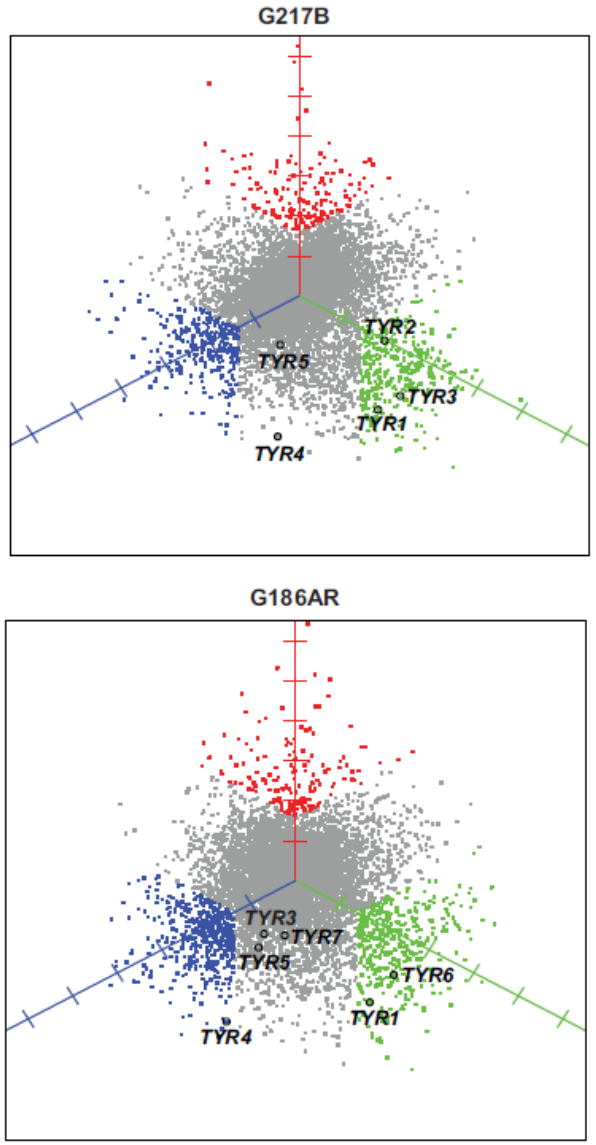


FIG 6 The tyrosinase gene family shows phase-specific expression. Relative enrichment plots are shown for G217B (A) and G186AR (B). Yeast, mycelial, and conidial axes are drawn in red, green, and blue, respectively; axis ticks indicate $\log_2$ units of enrichment. Genes were plotted by projecting the BAGEL-estimated relative expression values on the corresponding axes (the condition of lowest expression always has a $\log_2$ enrichment value of zero). Yeast, mycelial, and conidial enriched genes, based on the 3-fold enrichment criterion, are colored red, green, and blue, respectively. Tyrosinases are highlighted with black circles and labeled.

The Transcriptional Stress Response of *Candida albicans* to Weak Organic Acids

Fabien Cottier,* Alrina Shin Min Tan,* Jinmiao Chen,* Josephine Lum,* Francesca Zolezzi,* Michael Poidinger,*,† and Norman Pavelka*,‡,1

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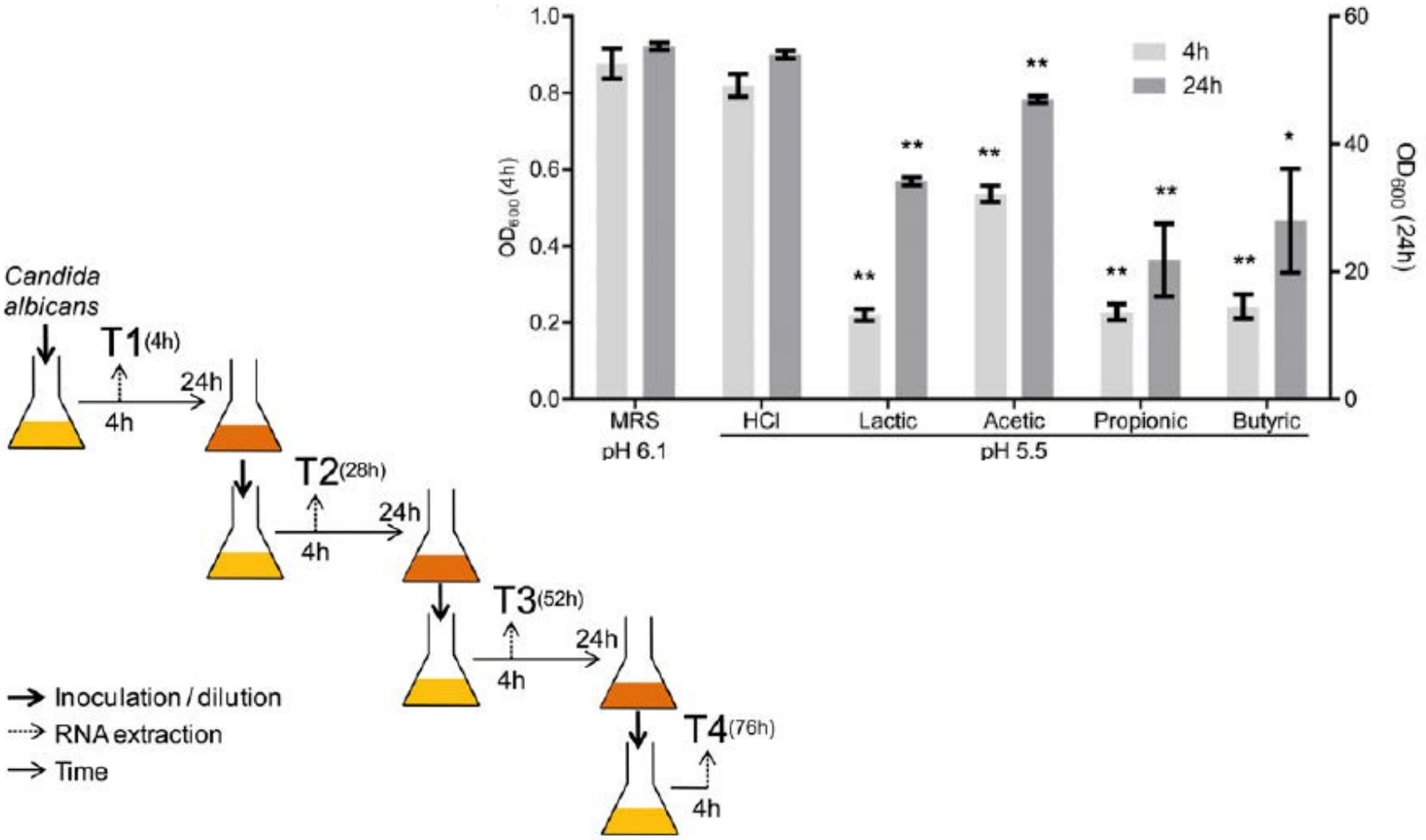
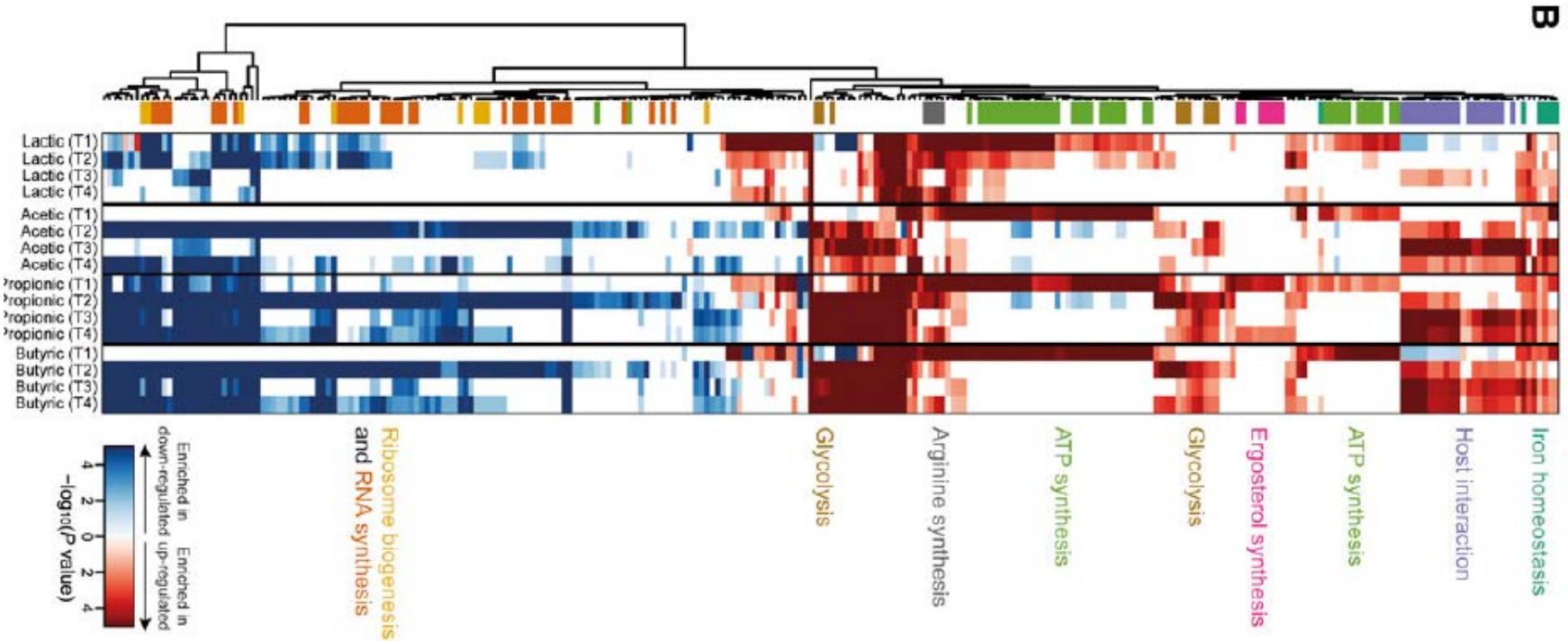
ORCID ID: 0000-0003-4542-9970 (N.P.)

Crecimiento de *Candida albicans* en presencia de distintos Ácidos Orgánicos Suaves



Expresión diferencial de genes en presencia de distintos Ácidos Orgánicos Suaves



Expresión diferencial de genes en presencia de distintos Ácidos Orgánicos Suaves