

Resistomes revealed by DNA Microarrays

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trends in
MICROBIOLOGY

VIRULENCE, INFECTION AND PATHOGENESIS



DRUG RESISTANCE: THE NEW APOCALYPSE

TOP GUNS OF ANTIMICROBIAL RESISTANCE (WHO, 2000)

- **Pneumonia (PRP)**
- **Diarrhoeal diseases**
- **AIDS**
- **Tuberculosis**
- **Malaria**
- **Viral Hepatitis**
- **Nosocomial infections (VRE, MRSA)**
- **Leishmaniasis**
- **Gonorrhoea**
- **Common worms**

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RESISTOMES OF PATHOGENS

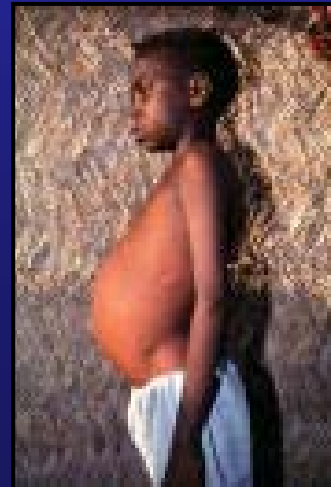
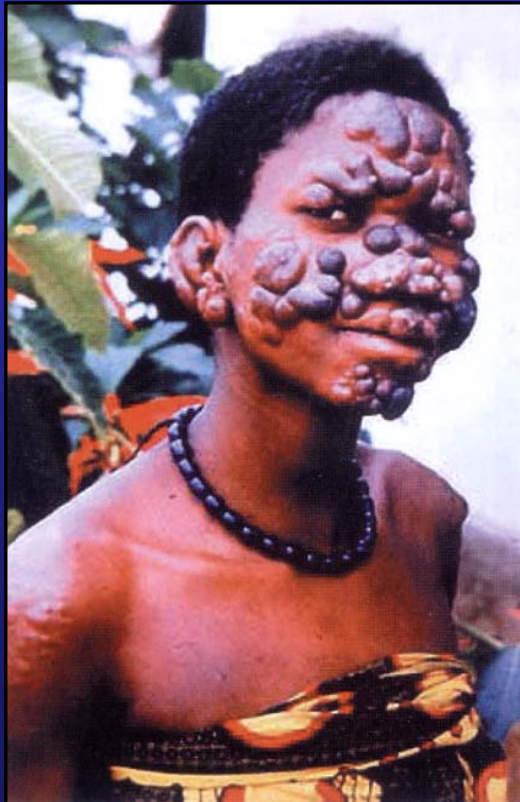
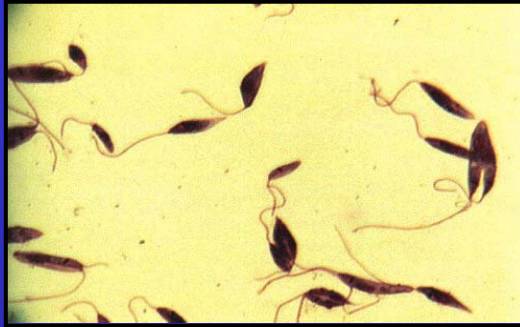
GLOBAL APPROACHES

- **MICROARRAYS**
 - TRANSCRIPTIONAL PROFILING
- **PROTEOMICS**
 - 2D GELS + MS

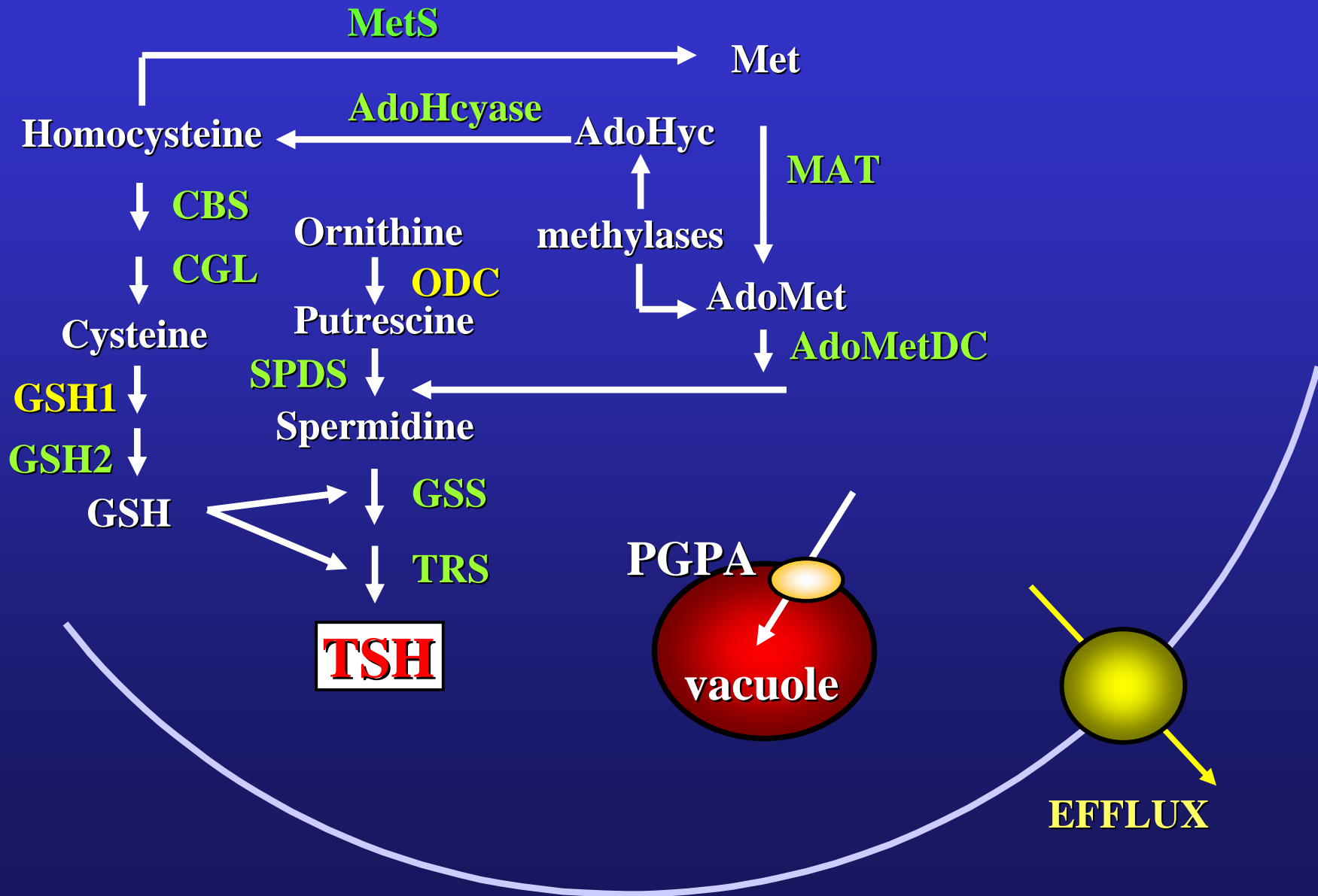
RESISTOMES

- PRIMARY MECHANISMS
- COMPENSATORY MUTATIONS

Leishmania



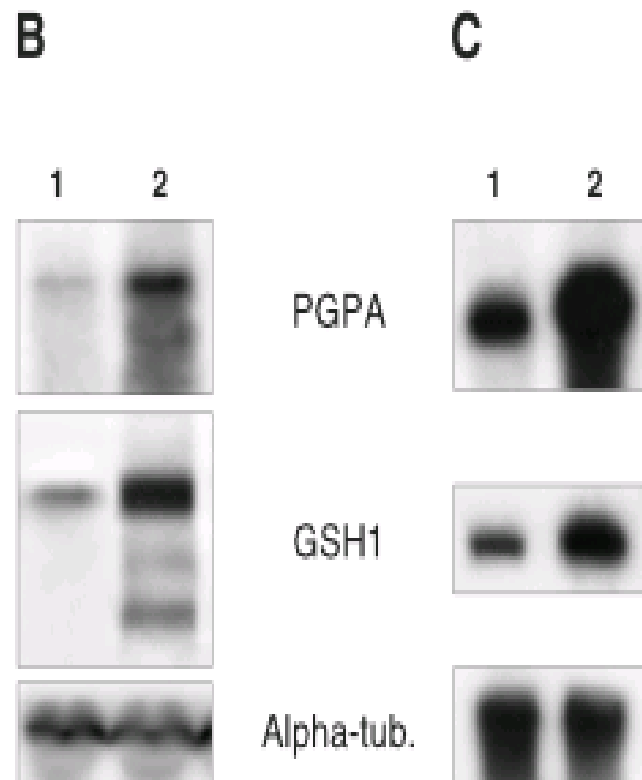
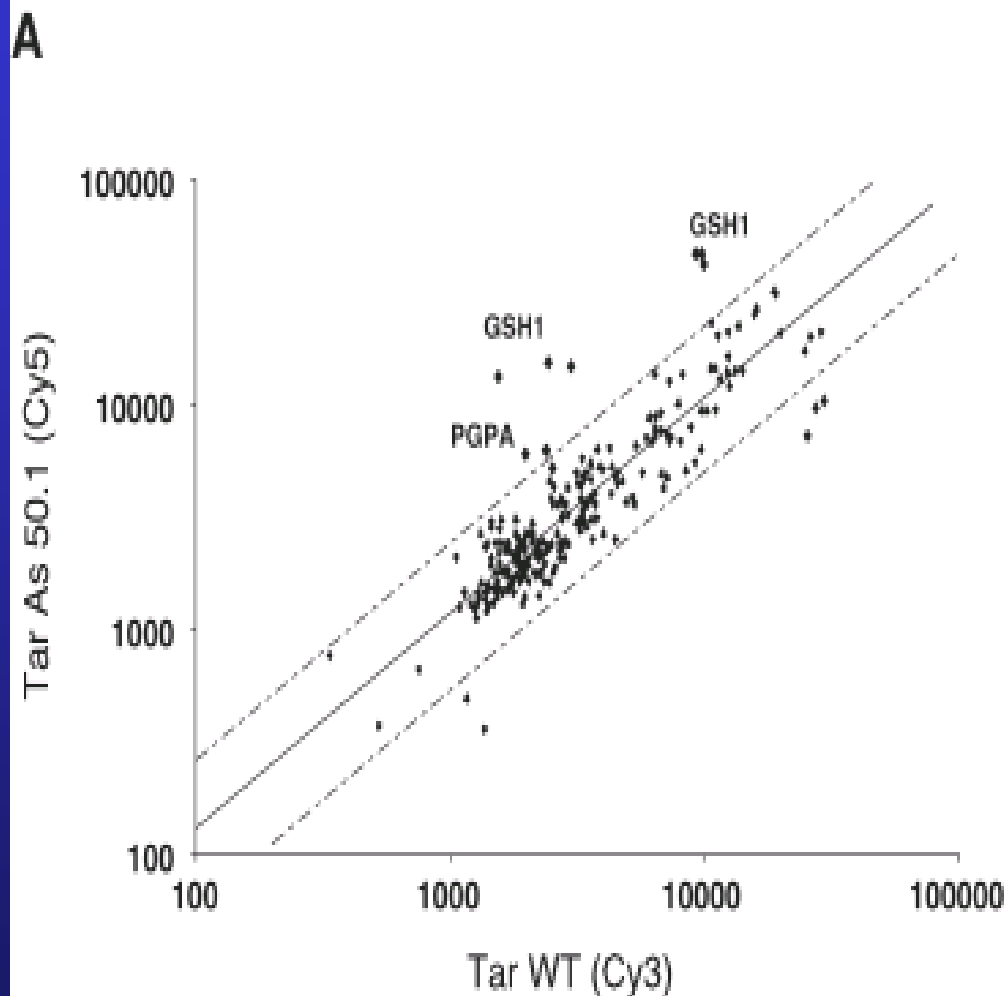
Trypanothione metabolism in *Leishmania*



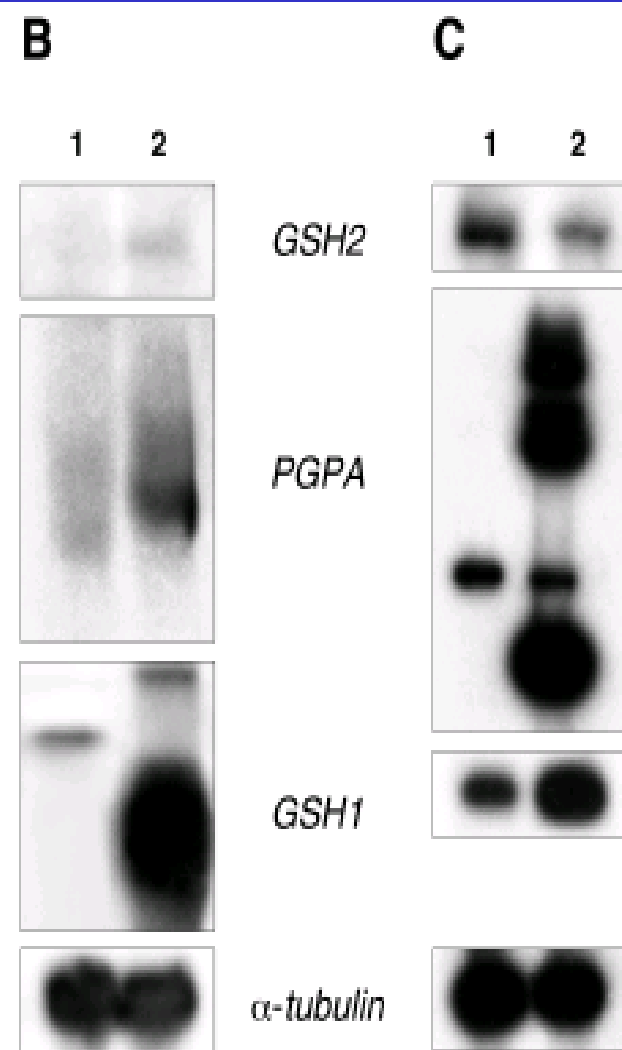
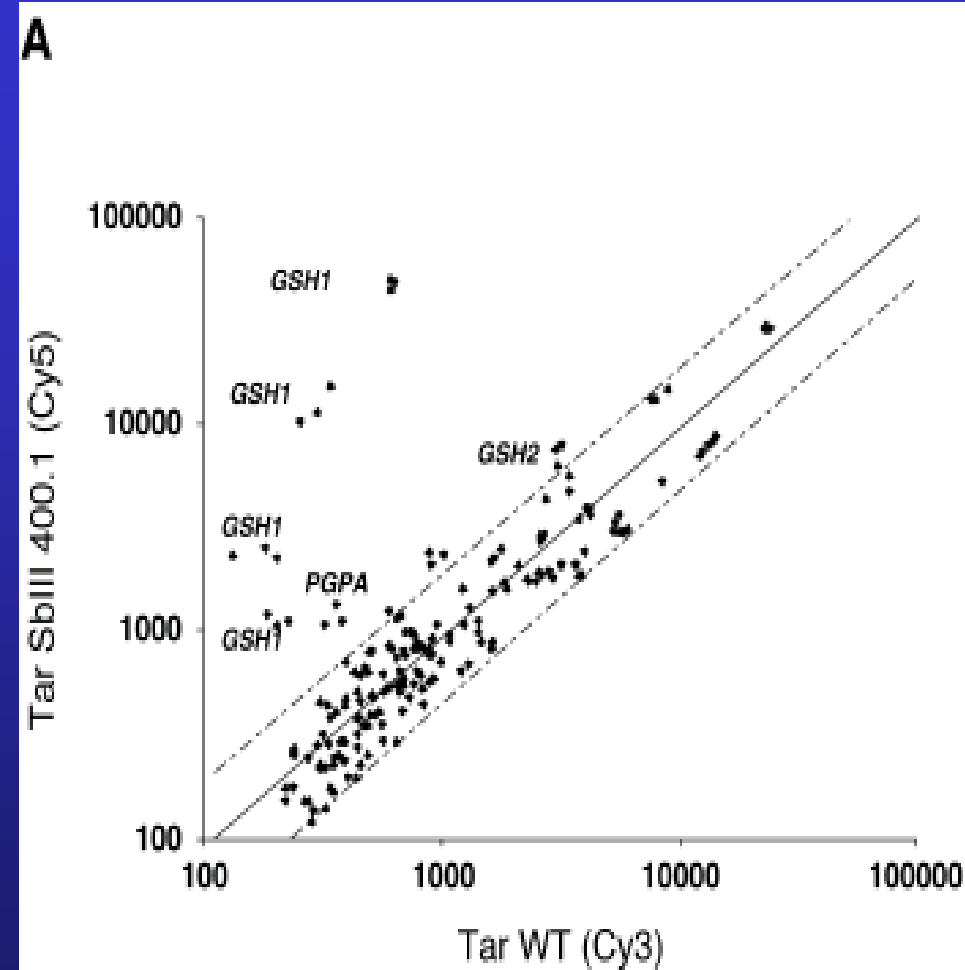
***Leishmania* custom microarrays**

- ~ 50 metabolic genes
 - TSH biosynthesis
 - ABC proteins
 - Folate metabolism
 - Tubulin, actin
 - *A. thaliana* spikes
(*CAB1*, *NAC1*; Stratagene)
 - Printing
 - At least in triplicate, supergrid
 - 50% DMSO
 - CMT-GAPs II (Corning)
- 2-3 fragments / gene
 - 500 bp fragments

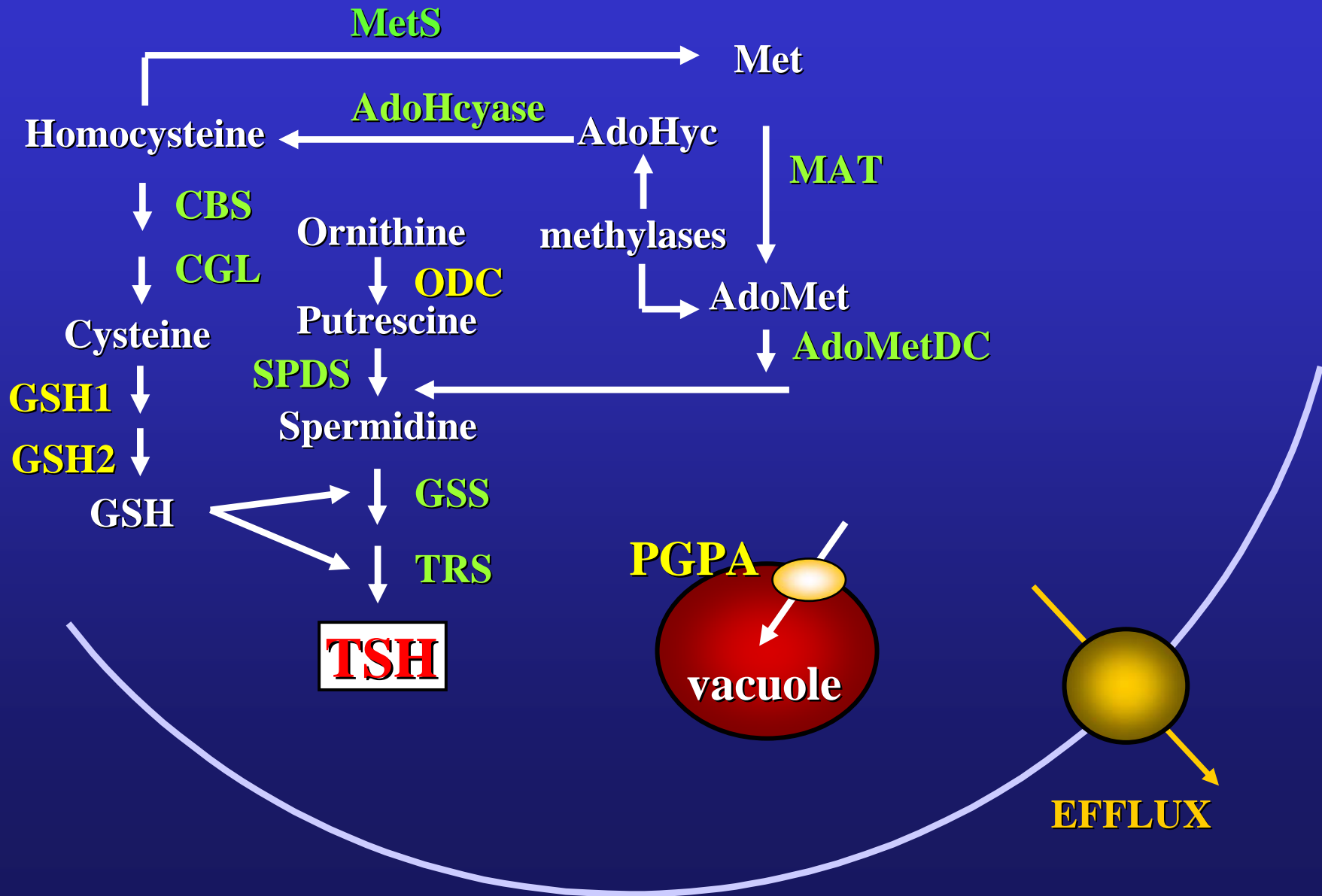
Differential gene expression in *L. tarentolae* As50.1



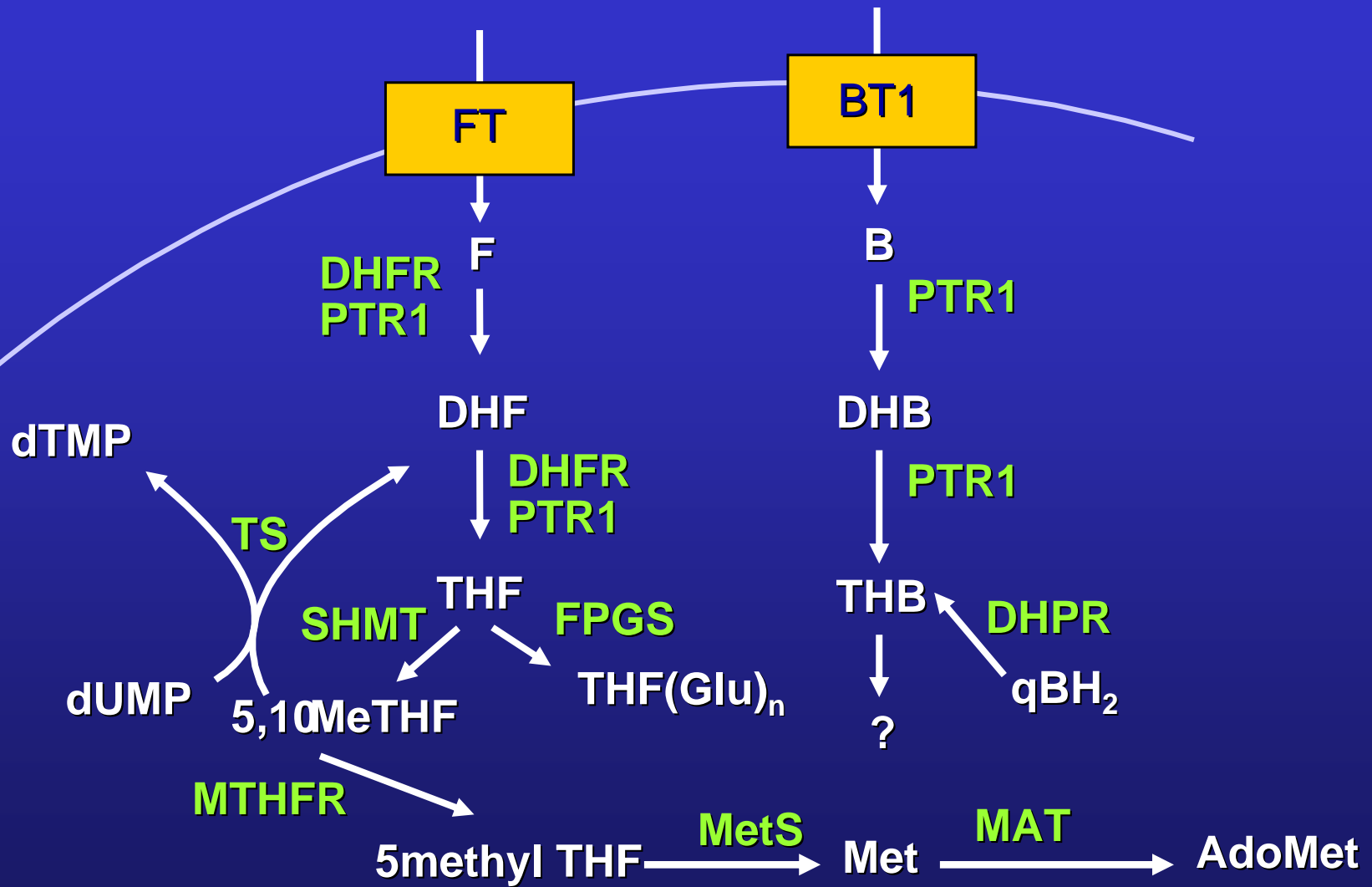
Differential gene expression in *L. tarentolae* SBIII400.1



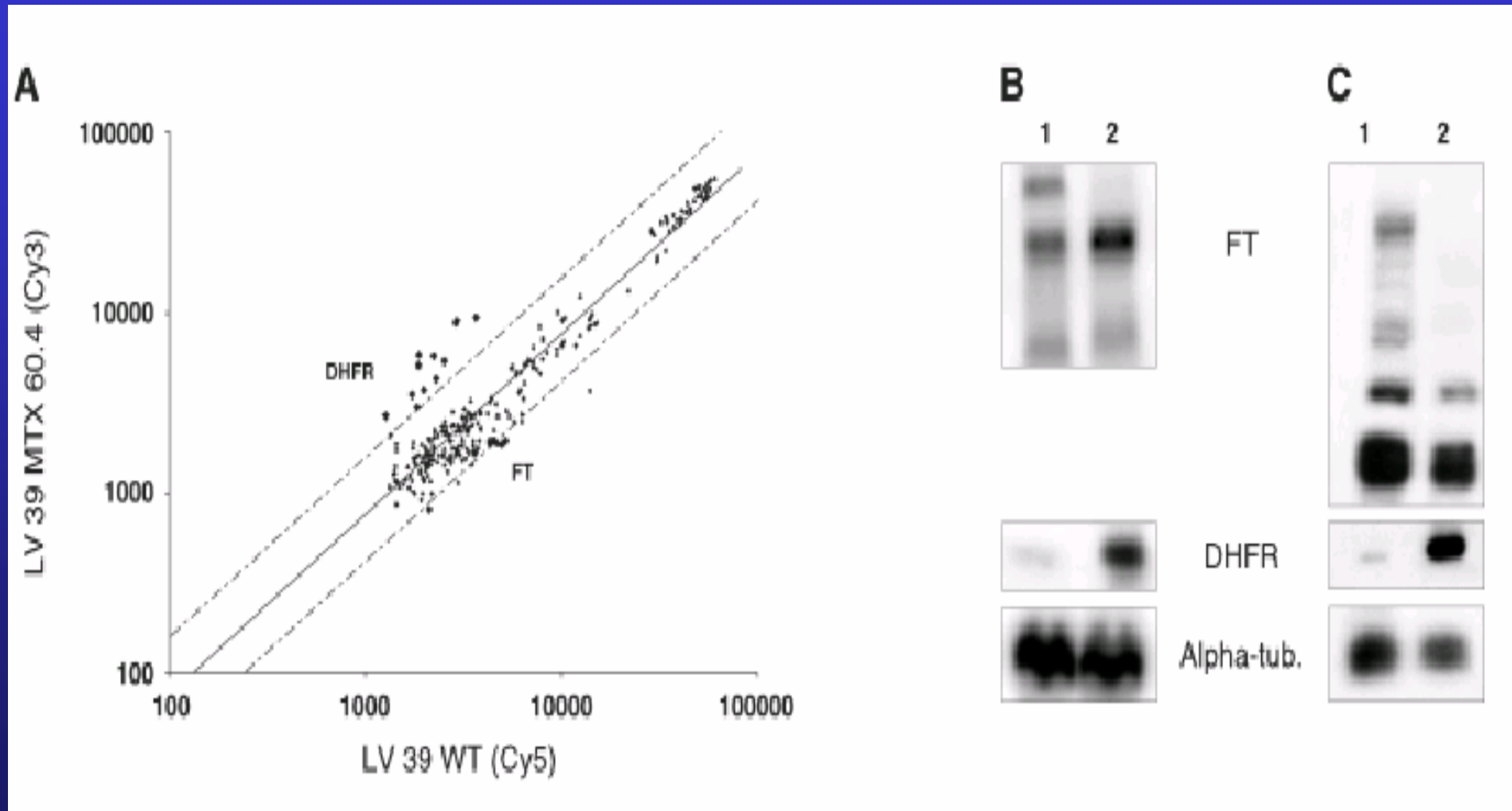
Trypanothione metabolism in *Leishmania*



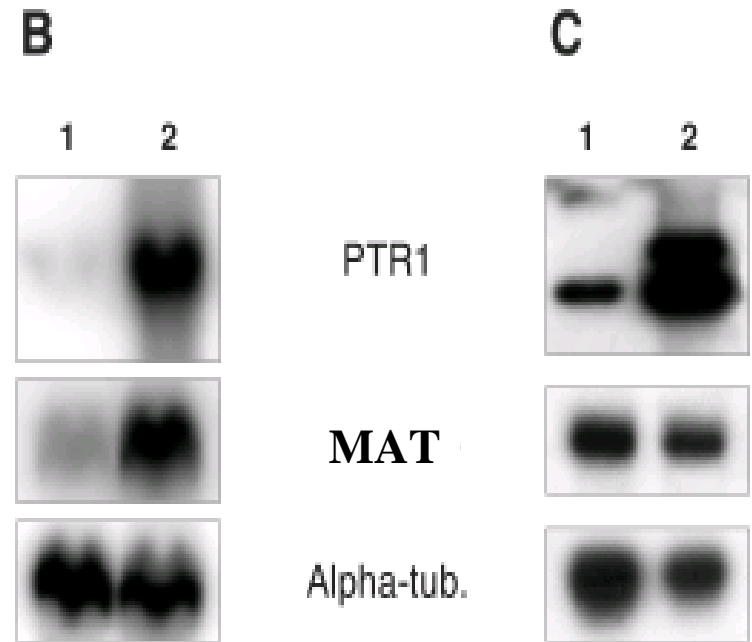
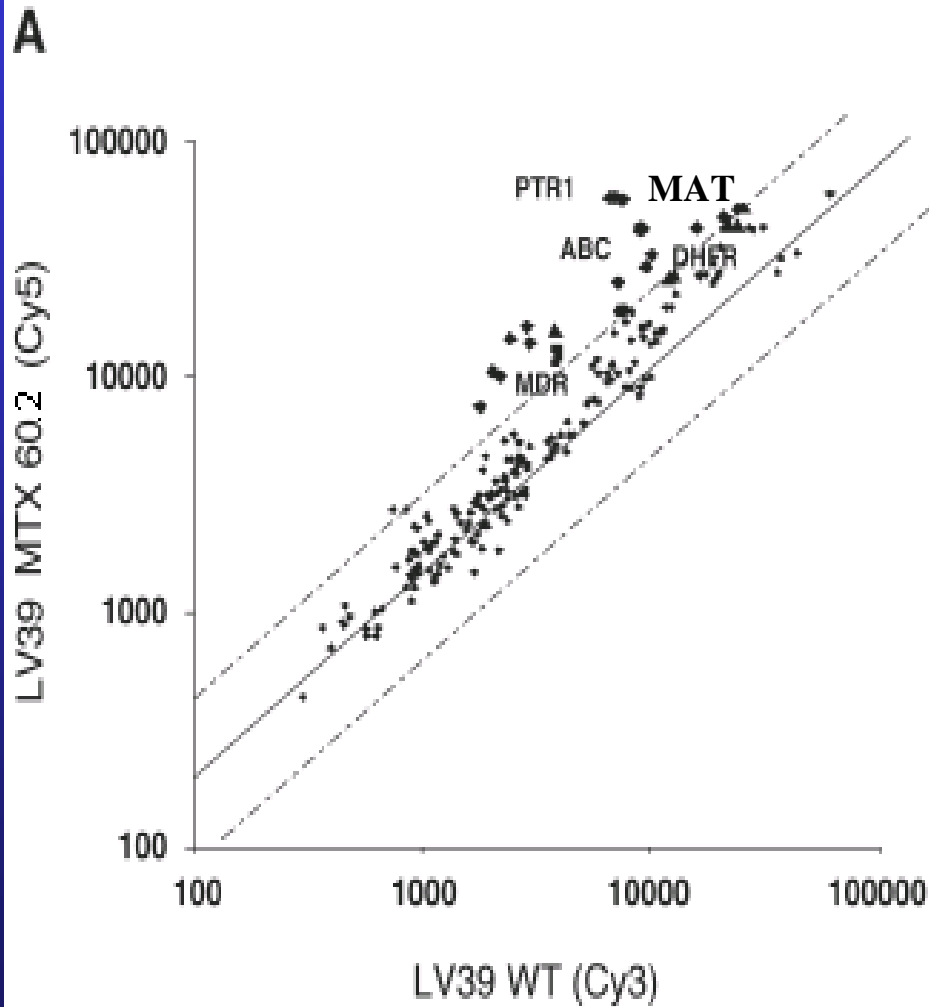
Folate metabolism in *Leishmania*



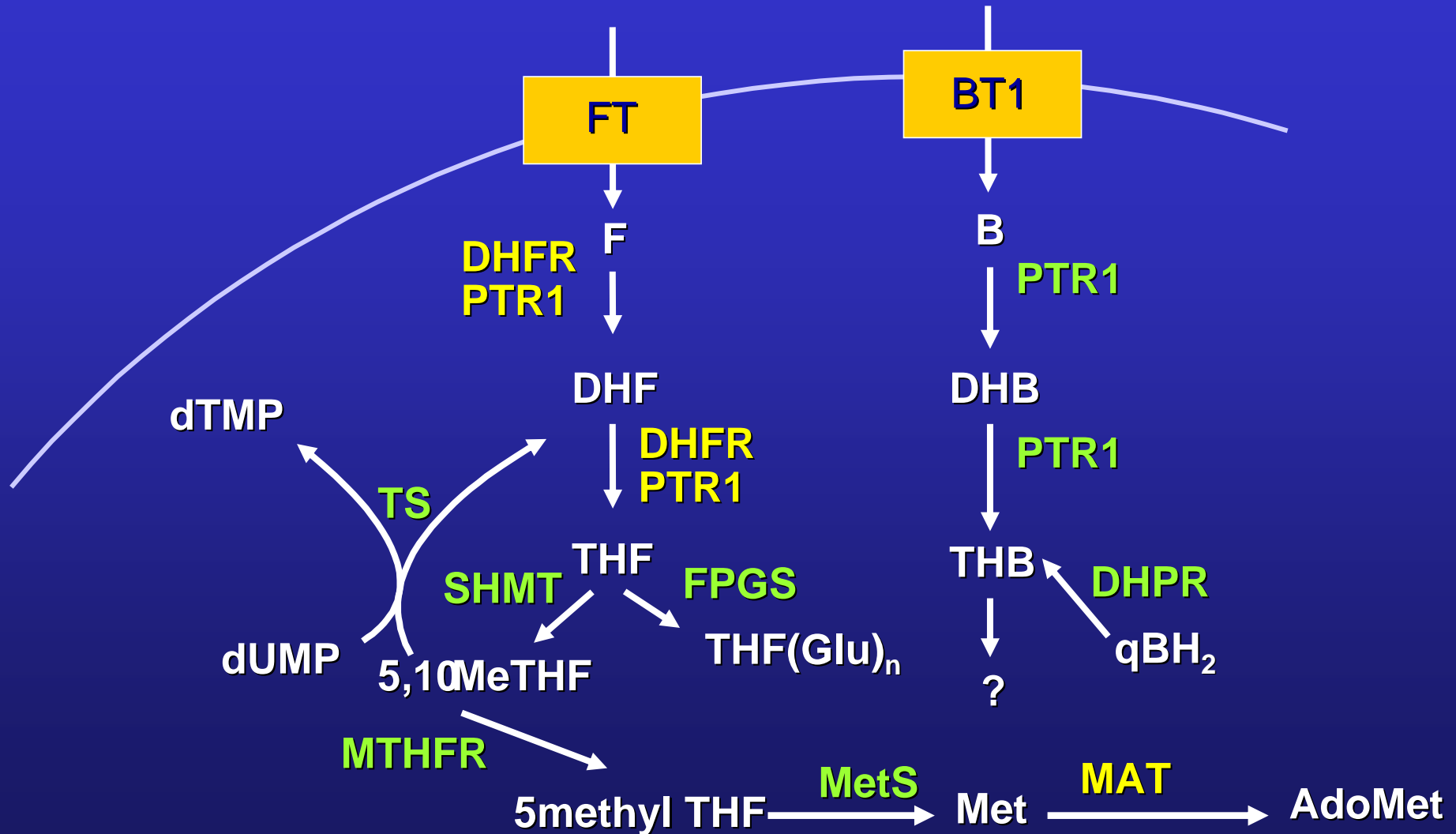
Differential gene expression in *L. infantum* LV39 MTX60.4



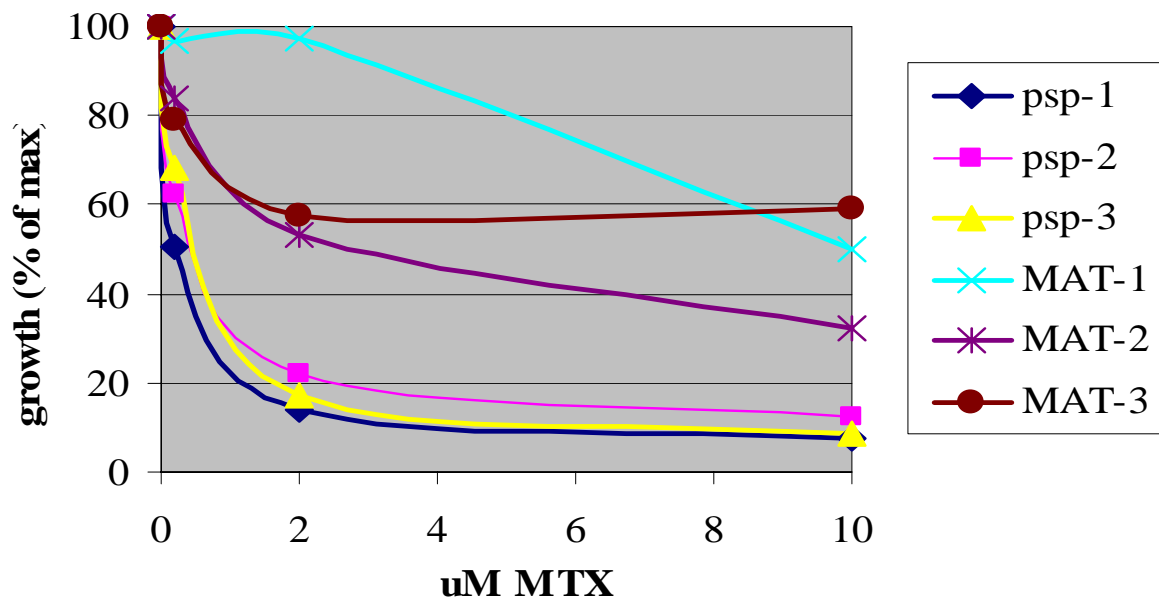
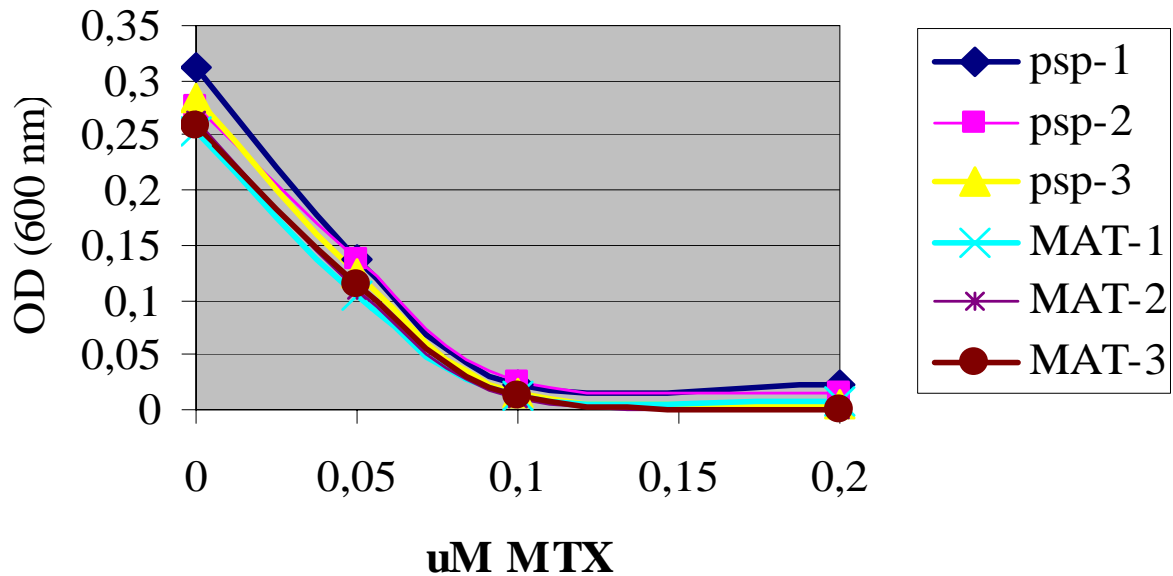
Differential gene expression in *L. infantum* LV39 MTX60.2



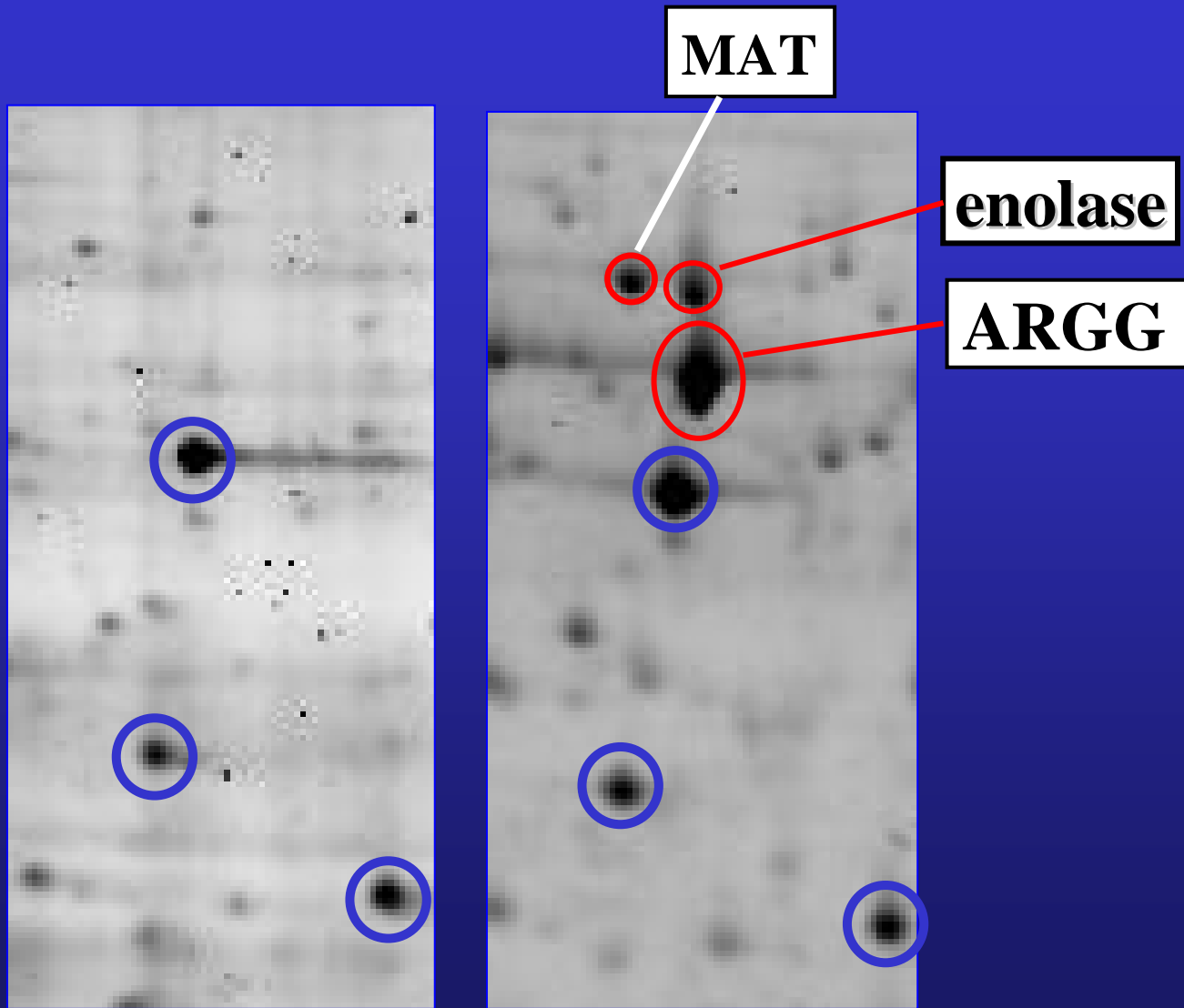
Folate metabolism in *Leishmania*



MTX sensitivity



Towards the resistome



WT

MTX 60.2

Conclusions / Perspectives

- Custom microarrays are useful to study resistance in pathogens

- **Primary resistance mechanisms**

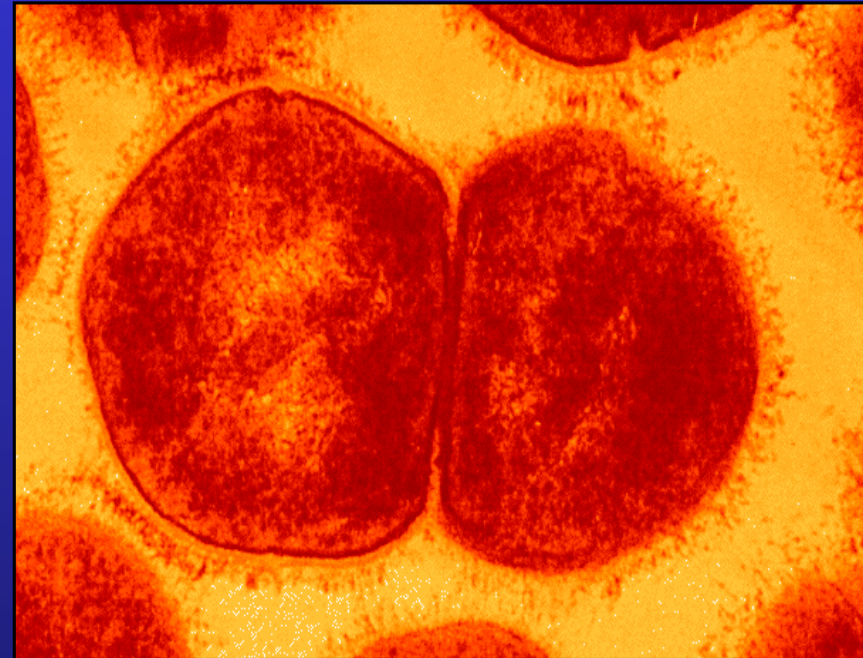
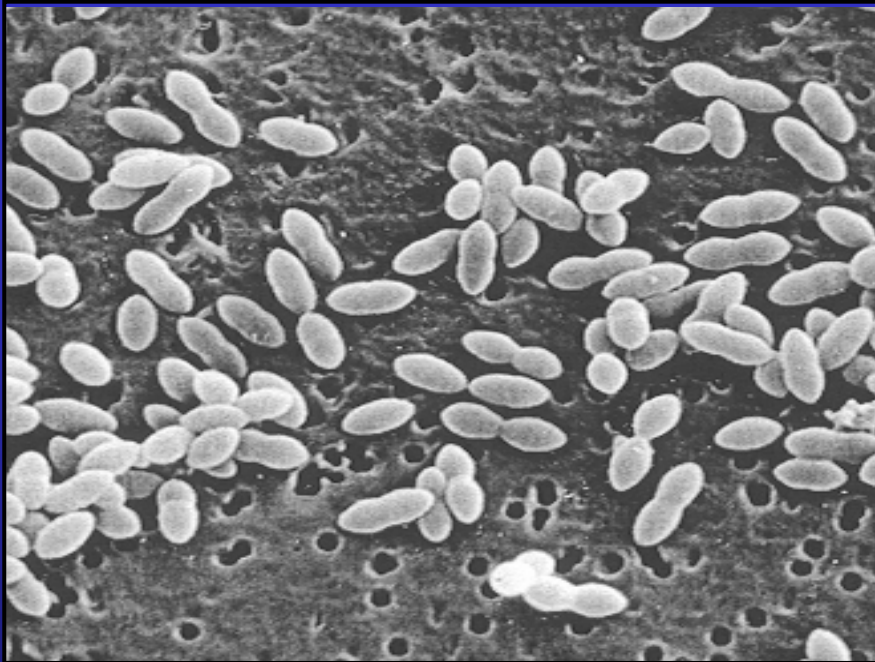
- PGPA
- PTR1
- GSH1
- FT
- GSH2
- DHFR

- **Compensatory mutations**

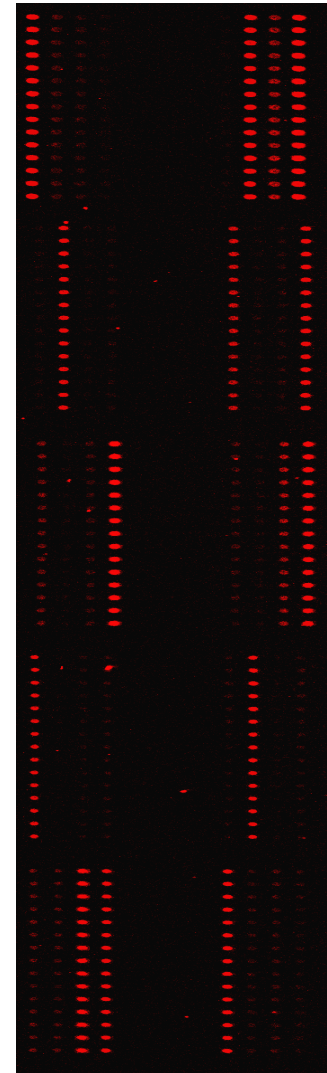
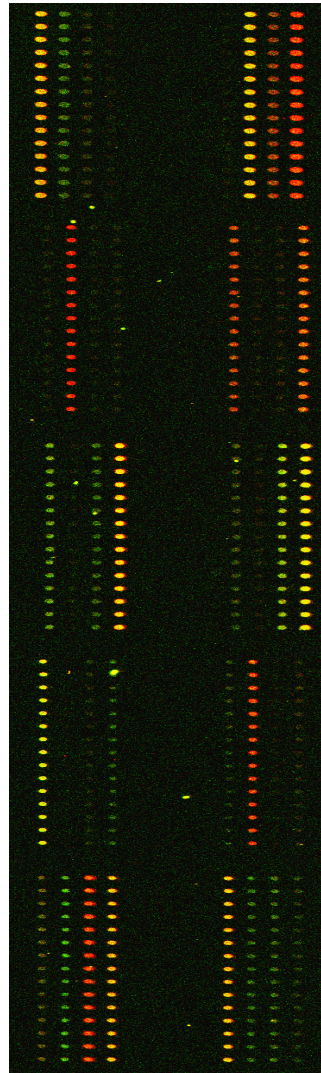
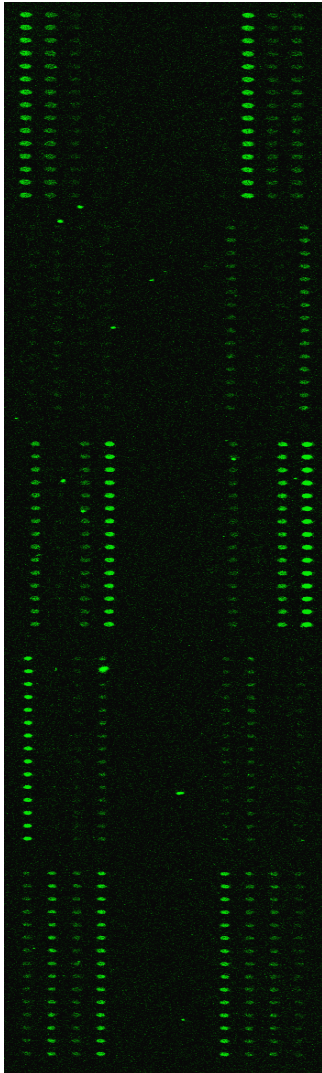
- MAT

—————> **CUSTOM OLIGO (70 MERS) ARRAYS**

Streptococcus pneumoniae



Streptococcus pneumoniae (PFGRC-TIGR)



Poster session

**PNA Biochips for the detection of unlabelled DNA
using a novel polymeric transducer**

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François J. Picard

Mario Leclerc

Michel G. Bergeron

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