# **A**. Discovery of New Genes related to *Leishmania* Pathogenesis and as Biomarkers of Attenuation Using a Genomic Microarray

# **B**. Multiplex PCR Microarray Assay to Detect Pathogens in Blood

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Site Visit Presentation

# Meeting the Challenge of Leishmaniasis: harvesting the benefits of the genomic era

**Our knowledge of the *Leishmania* genome:**

| | |
|---|---|
| 8333 | Open Reading Frames |
| 307 (4%) | Experimentally characterized |
| 2618 (31%) | Inferred from homology |
| 4673 (56%) | Conserved hypothetical |

**A method to rapidly identify virulence related genes: The Microarray**

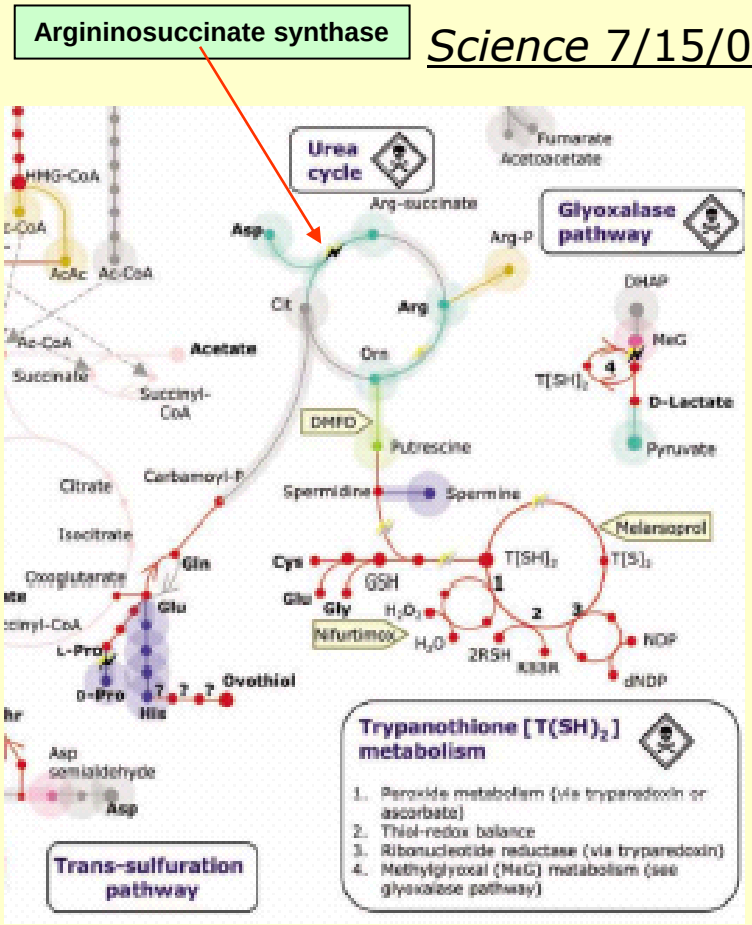
**Goals:**

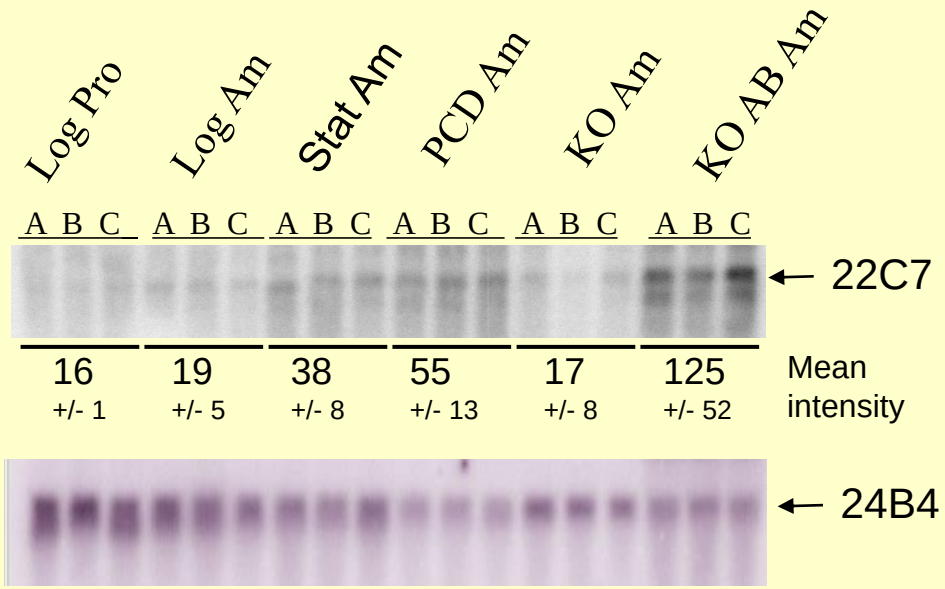
- **Genetic mechanisms of *Leishmania* pathogenesis**
- **Genetic characterization of live attenuated vaccine candidates**
- **Better diagnostics based on genetic technology**
- **Biomarkers of vaccine safety**

# Rationale for microarray characterization of the centrin-deleted cell line

- Urgent need for a vaccine against leishmaniasis—live, attenuated approach
- Vaccine candidate must be safe—genetically stable to avoid reversion
- Global gene expression and identified biomarkers to measure genetic stability
- Focus CBER research to make a unique scientific contribution

# *L. d.* Argininosuccinate synthase (22C7)



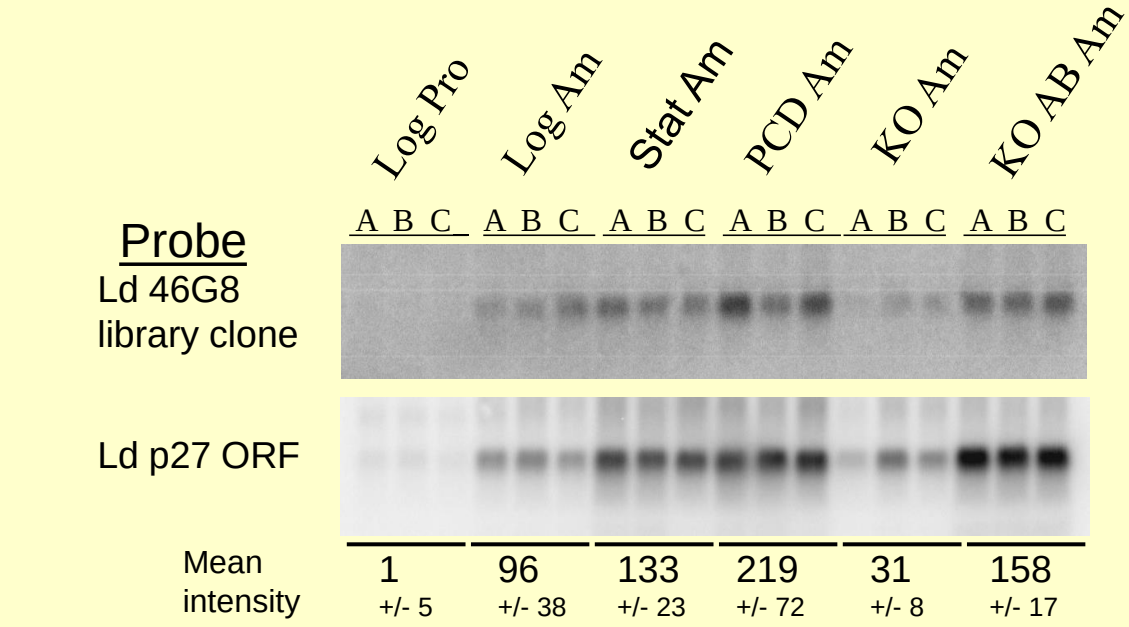

Placement on a critical metabolic pathway and reproducible pattern of expression make *L.d.* argininosuccinate synthase a potential biomarker of attenuation

# The hypothetical conserved, 27.6kD protein

## (46G8) L. d. homologue of LmjF28.0980

Protein similarity (%)

| Lin | Lmj | Tb | Tc | |
|---|---|---|---|---|
| 100 | 96 | 79 | 78 | Ld |
| | 96 | 79 | 78 | Lin |
| | | 79 | 76 | Lmj |
| | | | 90 | Tb |




Trypanosomatid restriction and high level of conservation suggest a critical function unique to the flagellated parasite physiology and the reproducible pattern of expression make *L.d.* p27 a potential biomarker of attenuation

# Summary: characterization of gene expression in the centrin-deleted cell line

- Differentially expressed genes identified and validated
- Selected genes with potential as biomarkers of attenuation further characterized
- Characterized genes reveal physiological correlates of centrin deletion
- Characterization of newly described gene function may lead to better understanding of *Leishmania* pathogenesis

# Meeting the Challenge of Blood Safety: harvesting the benefits of new technologies

- Transfusion blood safety has improved with pathogen testing
- Increasing number of known potential infectious agents and emerging threats, including bioterrorism increases the burden of testing
- Urgent need for methods to streamline and consolidate testing: nucleic acid tests (NAT), real-time PCR, microarrays, nanotechnology
- Multiplex potential of a pathogen detection microarray assay

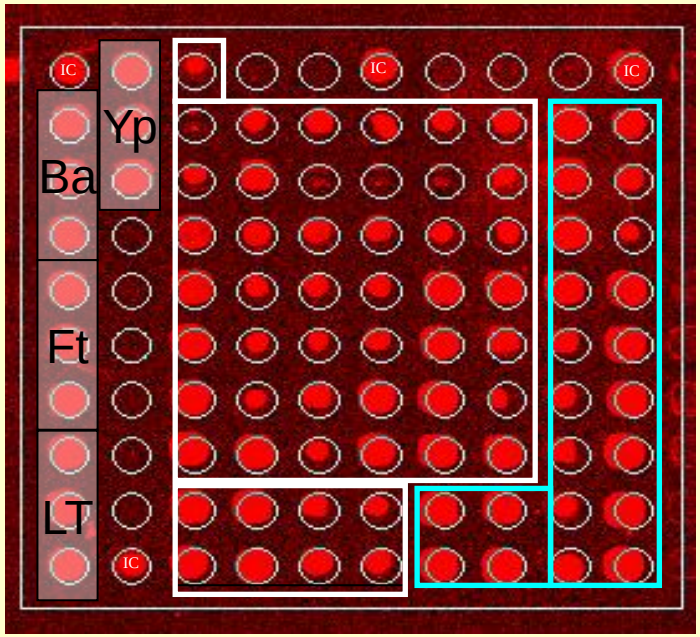
# Microarray for Detection of Blood-borne and BT Pathogens

**Bacteria, and Parasites**

Ba: *Bacillus anthracis* (**anthrax**)
Ft: *Francisella tularensis* (**tularemia**)
LT: *Leishmania* /*Trypanosoma*
Yp: *Yersinia pestes* and *pseudotuberculosis* (**plague**)

**Bioterror Viruses**

POX: Pox viruses
VAC: Vaccinia
VAR: Variola (**Smallpox**)
MPV: Monkeypox Viruses
CPV: Cowpox Viruses
NOVAC: All Pox viruses but Vaccinia
EBO: Ebola Viruses
VE: Venezuelan Equine Enceph. Virus
VETD: VE Trinidad Donkey
MBG: Marburg Viruses




**Blood Borne Viruses**

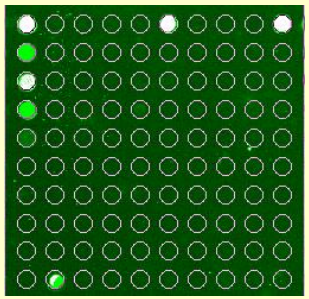
WNV: West Nile Viruses
HCV: Hepatitis C Viruses
HBV: Hepatitis B Viruses
HIV: Human Immunodeficiency Viruses
HTLV: Human T-cell Leukemia Viruses

IC **4 internal control probes (Human rRNA gene)**

## Results of detection in pathogen-spiked blood – 50 cells/ml

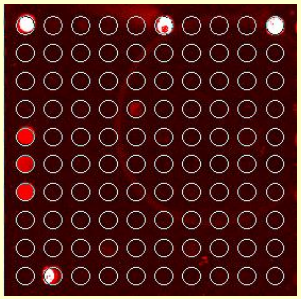
***Bacillus anthracis***

**livestock vaccine strain**



***Francisella tularensis***

**Live Vaccine Strain**



***Yersinia pseudotub.***