

**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**

Robert Duncan, PhD
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

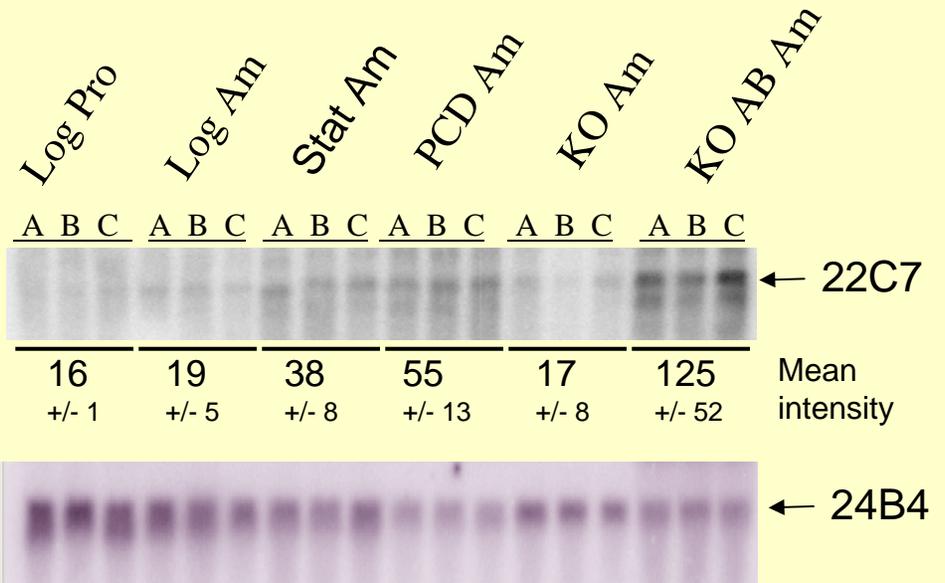
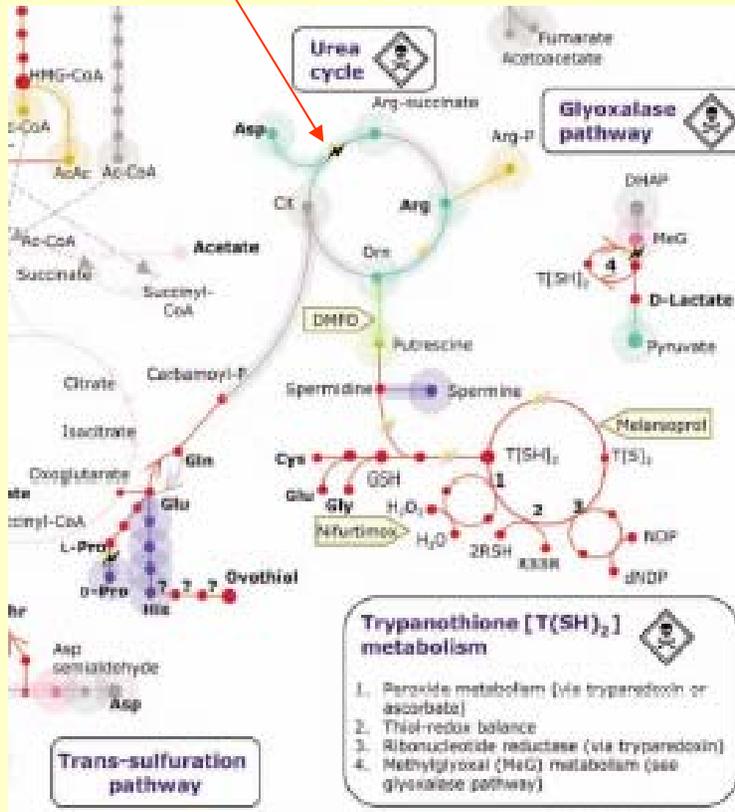
Rationale for microarray characterization of the centrin-deleted cell line

- 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)

Argininosuccinate synthase

Science 7/15/05



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

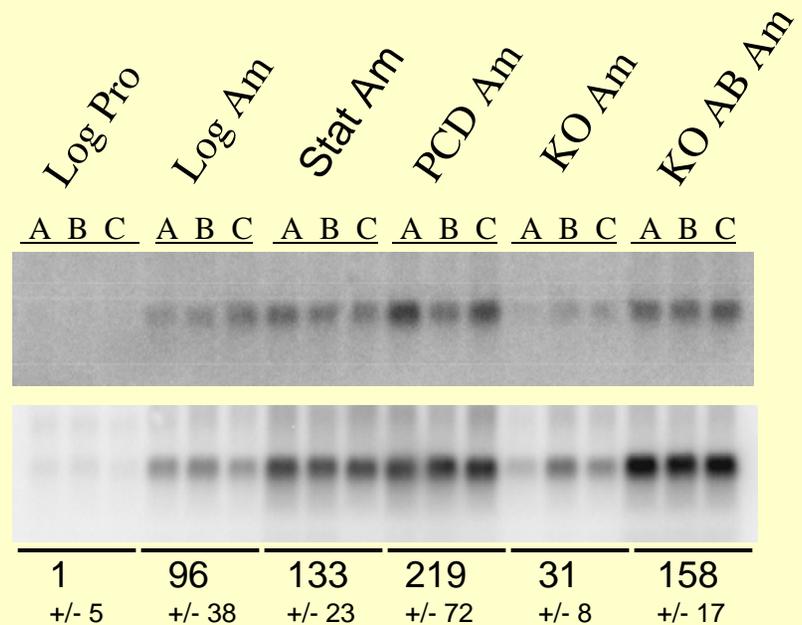
Protein similarity (%)

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

Probe
Ld 46G8
library clone

Ld p27 ORF

Mean
intensity



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 pestis* and *pseudotuberculosis* (**plague**)

Bioterror Viruses

POX: Pox viruses

VAC: Vaccinia

VAR: Variola (**Smallpox**)

MPV: Monkeypox Viruses

CPV: Cowpox Viruses

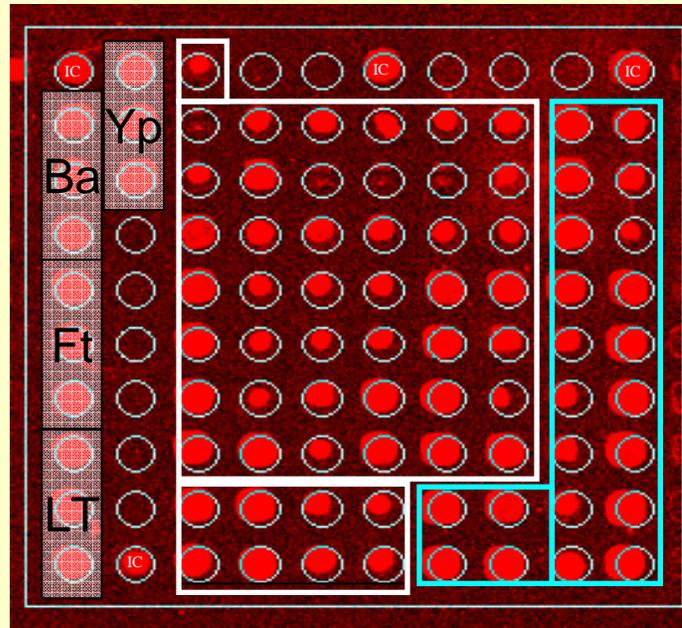
NOVAC: All Pox viruses but Vaccinia

EBO: Ebola Viruses

VE: Venezuelan Equine Enceph. Virus

VETD: VE Trinidad Donkey

MBG: Marburg Viruses



IC 4 internal control probes (Human rRNA gene)

Blood Borne Viruses

WNV: West Nile Viruses

HCV: Hepatitis C Viruses

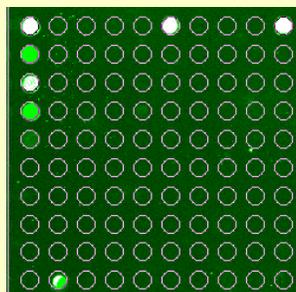
HBV: Hepatitis B Viruses

HIV: Human Immunodeficiency Viruses

HTLV: Human T-cell Leukemia Viruses

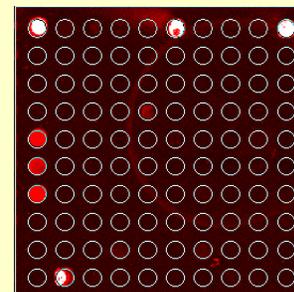
Results of detection in pathogen-spiked blood – 50 cells/ml

Bacillus anthracis



livestock vaccine strain

Francisella tularensis



Live Vaccine Strain

Yersinia pseudotub.

