Molecular Genetic Epidemiology of Tuberculosis in Elephants

William S. Modi, PhD

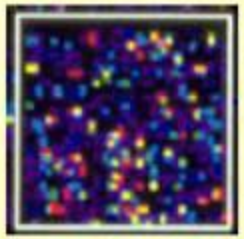
Using Genomic Technology to Understand and Manage Tuberculosis in Elephants

- Overview of next generation sequencing technology
- Host genetics and infectious disease
- TB in elephants

Leveraging Genomic Information

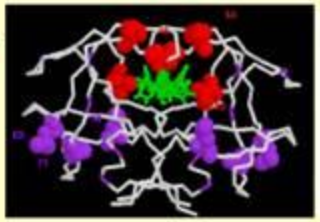
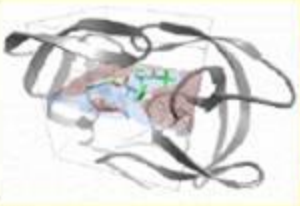
Novel Diagnostics

- Microchips & Microarrays - DNA
- Gene Expression - RNA
- Proteomics - Protein


Novel Therapeutics

- Drug Target Discovery
- Rational Drug Design
- Molecular Docking
- Gene Therapy
- Stem Cell Therapy

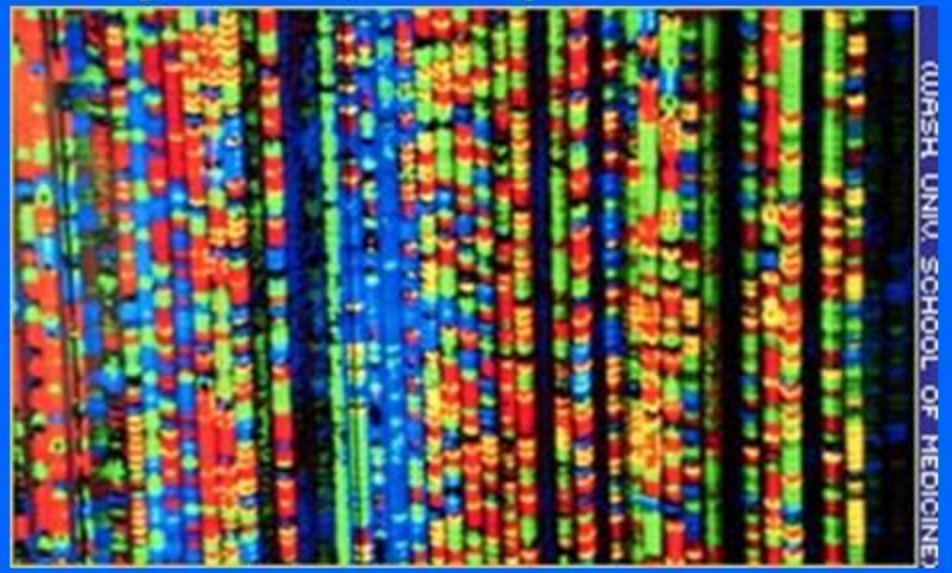

Understanding Metabolism

Understanding Disease

- Inherited Diseases - OMIM
- Infectious Diseases
  - Pathogenic Bacteria
  - Viruses


Next Generation DNA Sequencing

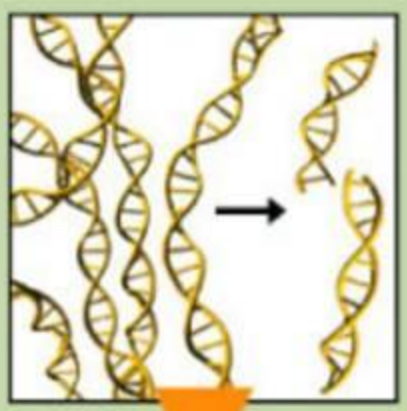
- **Paradigm Shift**
  - **High throughput**
  - **High accuracy**
  - **Lower cost (*2001 human genome sequence cost $200 million, today $50,000*)**

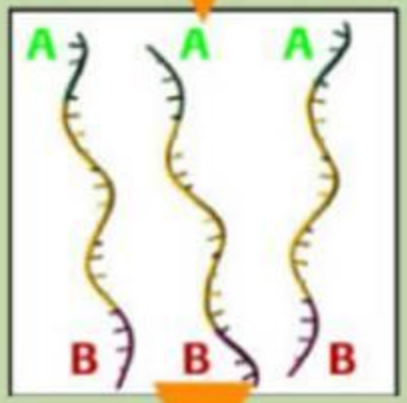


Nanotechnology

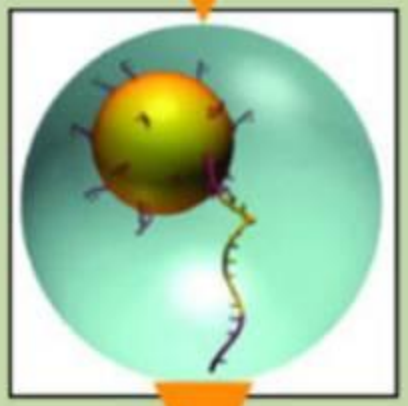
- –shear DNA into small pieces
- –bind individual DNA molecules to solid surface
- –amplify each molecule into a cluster
- –copy each base and detect signals for A, C, T, & G
- –precise high-resolution imaging

Sample Input
and Fragmentation

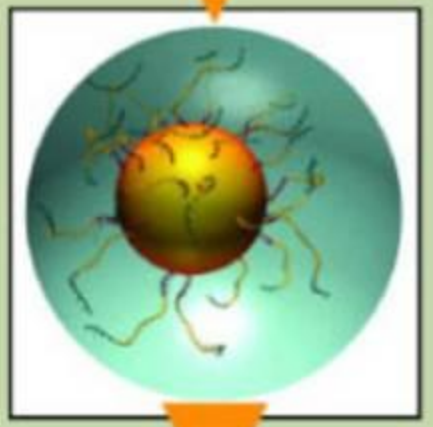




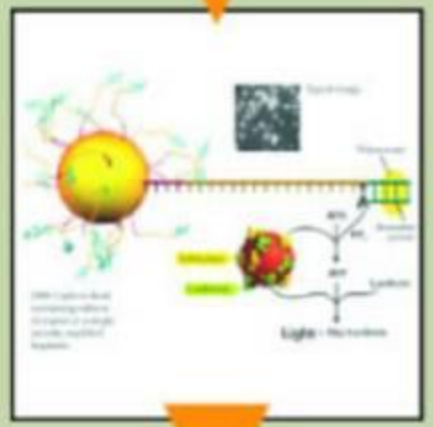
Library Preparation



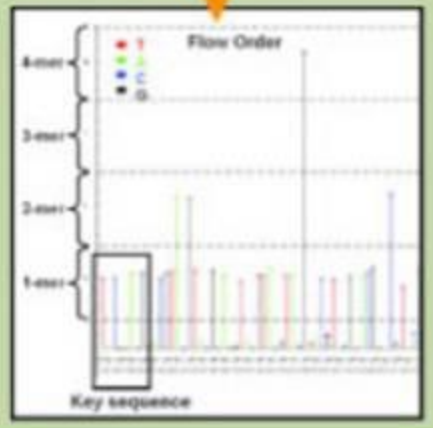
One Fragment =
One Bead



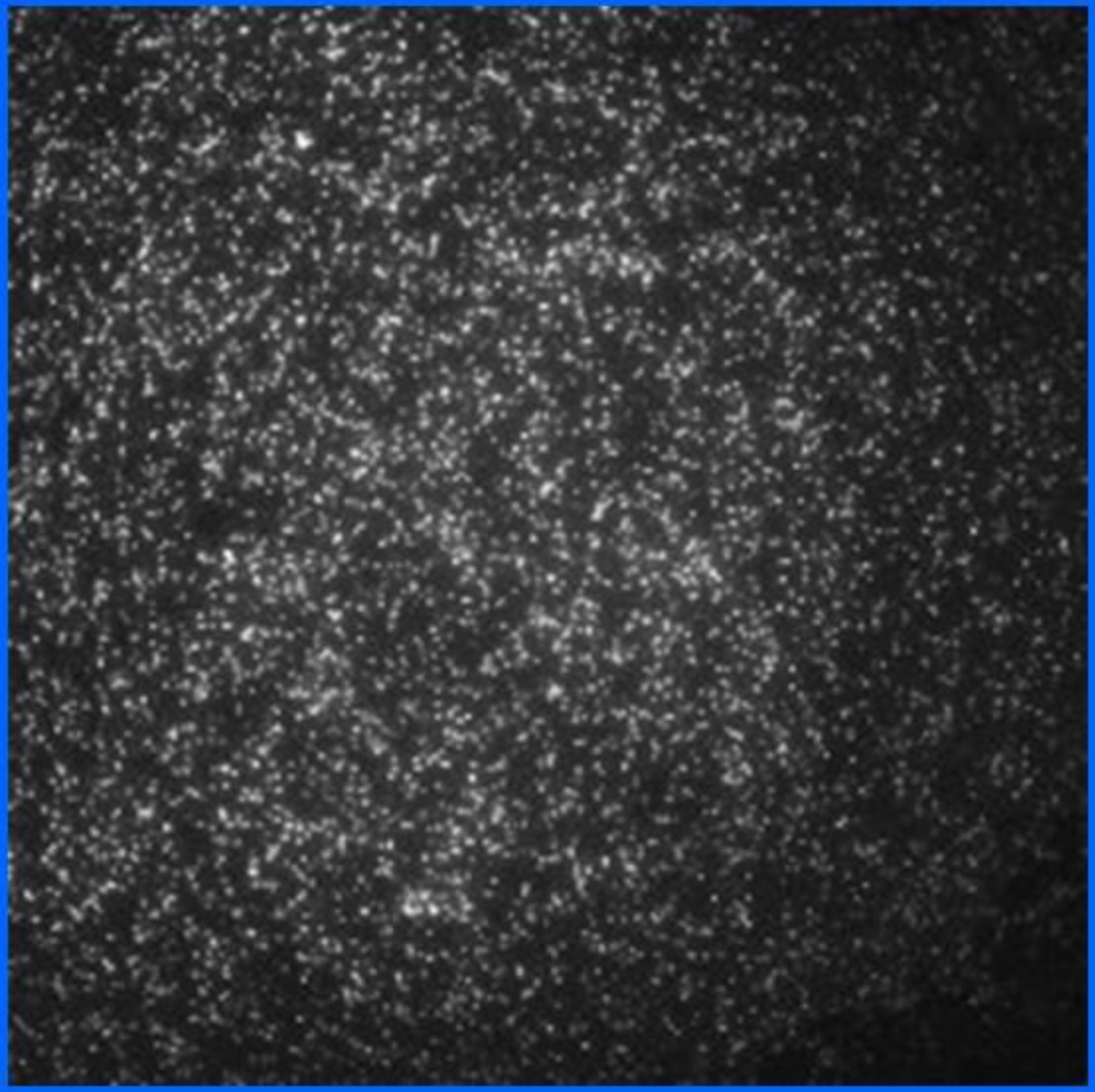
emulsion PCR
(amplification)

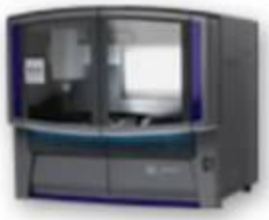


One Bead = One Read



Tiles on Illumina Sequencer



Specifications	Systems	
Specifications[1]	 **5500xl SOLiD™ System**	 **5500 SOLiD™ System**
Specification Sheet	Information Sheet (PDF)	Information Sheet (PDF)
Description	Production system for translational research	Flexible system for every lab
System Accuracy[2]	Up to 99.99%	Up to 99.99%
Throughput/Day[3]	Up to 20-30 Gb	Up to 10-15 Gb
Samples/Run[4]	• 2 Genomes • 24 Exomes • 12 Transcriptomes	• 1 Genome • 12 Exomes • 6 Transcriptomes

Applied Biosystems

Informatics is the Bottleneck

- Sequence data generated faster than they can be analyzed
- Customized bioinformatic analysis needed for each project

Complete genomics

Revolutionizing human genome discovery

ADVANCE … ATA MANAGE…

Our advanced … customers ca… …ols ensure of data genera… …al amounts

Human Genome Sequencing & Analysis Service

Dedicated to **complete human genome sequencing and analysis** provided as an innovative, **end-to-end, outsourced service model**. Complete

Receive Research-Ready Genomic Data

We offer our customers an end-to-end, outsourced solution that delivers **research-ready genomic data**.

News and Events

» Complete Genomics

BCM
Baylor College of Medicine

2009

Human Genome
Sequencing Center

Bovine Genome Project

HGSC Home

Genome Data

Human

Nonhuman Primates

Other Mammals

Armadillo

Bovine

Dolphin

Hyrax

Kangaroo Rat

Megabat

About the Project

The Human Genome Sequencing Center is working to sequence and annotate the bovine genome, *Bos taurus*. The bovine genome assembly and analysis and the study of cattle genetic history were published in April 24, 2009 issue of *Science*.

Sequencing of the bovine (Hereford) genome consumed a large part of 2004-06 HGSC resources. The project was staged to produce an initial 3x WGS assembly followed by a second 6x WGS assembly to allow gene predictions for preliminary annotation, and a final assembly including BAC sequences for improved local assembly refinement. The 3x assembly was used by Ensembl to test their pipeline on low coverage genome assemblies.

Photo by Michael MacNeil, USDA

Featured Publications

Liu, Y, Qin, X, Song, XZ, Jiang, H, Shen, Y, Durbin, KJ, Lien, S,

华大基因 BGI | Premier Scientific Partner

Home | Plant & Animal | Microbe | Disease | Event | Publication | Apply

- Research
  - Plant
  - Animal
- BGI-G10K
  - Fishes
  - Teleost
  - Amphibian
  - Mammal
  - Reptile
  - Bird
- Core Technology
- BGI Demo

Plant & Animal >> 1,000 Plant and Animal Project

World's Largest Genomics Lab in Hong Kong

Selected by	Scientific name	Common name	Family	Genome size(Gb)	Sequencing status
BGI	*Acinonyx jubatus*	Cheetah	Felidae	2.5	In progress
BGI	*Acipenser sinensis*	Chinese sturgeon	Acipenseridae	1.6-7.0*	Proposed *,
BGI	*Ailuropoda melanoleuca*	Giant panda	Ursidae	2.9	Complete and Published
G10K	*Ailurus fulgens*	Red panda	Ailuridae	No data	Proposed *
BGI	*Alligator sinensis*	Chinese alligator	Alligatoridae	~2.5*	Proposed *
G10K	*Amia calva*	Bowfin	Amiidae	1.4-1.28 pg	Proposed *
BGI	*Anas platyrhynchos domestica*	Peking duck	Anatidae	~1.4	Sequencing finished
G10K	*Andrias davidianus*	Giant salamander	Cryptobranchidae	49	Funded
G10K	*Anguilla anguilla*	European freshwater eel	Anguillidae	1.1-1.6	Proposed *
BGI	*Anser anser domesticus*	Domestic goose	Anatidae	1.4*	Funded
BGI	*Aptenodytes forsteri*	Emperor penguin	Spheniscidae	No data	In progress
BGI	*Aristichthys nobilis*	Bighead carp	Cyprinidae	1	In progress
G10K	*Ascaphus truei*	Tailed frog	Ascaphidae	~4.0	Proposed *
G10K	*Aspidoscelis tigris*	Western whiptail	Teiidae	No data	Proposed *
G10K	*Astyanax mexicanus*	Blind cave fish	Characidae	1.05-1.77 pg	Proposed *
G10K	*Atelopus zeteki*	Golden frog	Bufonidae	~5.0*	Proposed *
G10K	*Bombina orientalis*	Oriental fire-bellied toad	Bombinatoridae	~8.2	Proposed *
BGI	*Bos grunniens*	Domestic yak	Bovidae	~3.5*	In progress
BGI	*Bufo gargarizans*	Mongolian toad	Bufonidae	4.9	Funded
G10K	*Calypte anna*	Anna's hummingbird	Trochilidae	~1.0**	Proposed *

~ = averaged estimate; * = estimate based on congeneric species

Host Genomics and Infectious Disease

- Susceptibility to infection and disease progression are *multi-factorial* phenotypes
- Multiple loci with varying effect sizes and environmental factors

letters to nature

Nature **382**, 722 - 725 (22 August 1996); doi:10.1038/382722a0

Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene

MICHEL SAMSON[*], FRÉDÉRICK LIBERT[*], BENJAMIN J. DORANZ[†], JOSEPH RUCKER[†], CORINNE LIESNARD[‡], CLAIRE-MICHÈLE FARBER[§], SENTOB SARAGOSTI[||], CLAUDINE LAPOUMÉROULIE[¶], JACQUELINE COGNAUX[£], CHRISTINE FORCEILLE[£], GAETAN MUYLDERMANS[£], CHRIS VERHOFSTEDE[£], GUY BURTONBOY[£], MICHEL GEORGES[*], TSUNEO IMAI[**], SHALINI RANA[††], YANJI YI[††], ROBERT J. SMYTH[††], RONALD G. COLLMAN[††], ROBERT W. DOMS[†], GILBERT VASSART[*‡‡] & MARC PARMENTIER[*]

[*] IRIBHN and Services de [‡‡] Génétique Médicale, [‡]Virologie and [§] Immunodéficiences, Université Libre de Bruxelles, Campus Erasme, 808 route de Lennik, B-1070 Bruxelles, Belgium [†] Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA [||] Institut Cochin de Génétique Moléculaire, Hôpital Cochin, 75014 Paris, France [¶] INSERM U120, Hôpital Robert Debré, 48 Bd Sérurier, 75935 Paris, France [£] Belgian AIDS Reference Laboratories. [*] Department of Genetics, Faculty of Veterinary Medicine, University of Liège, Belgium [**] Department of Surgery II, Nagoya University School of Medicine, Japan [††] Pulmonary and Critical Care Division, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Science. 1996 Sep 27;273(5283):1856-62.

Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene.

Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gom
Donfield S, Vlahov D, Kaslow R, Saah A, Rinaldo C, Detels R, O'Brien SJ.

Laboratory of Genomic Diversity, National Cancer Institute (NCI), Frederick, MD 21702-1201, USA.

CCR5 *Δd32* mutation and resistance to HIV-1 infection, 1996

Forward Genetic Dissection of Immunity to Infection in the Mouse

S.M. Vidal,[1,*] D. Malo,[2,*] J.-F. Marquis,[3] and P. Gros[3,*]

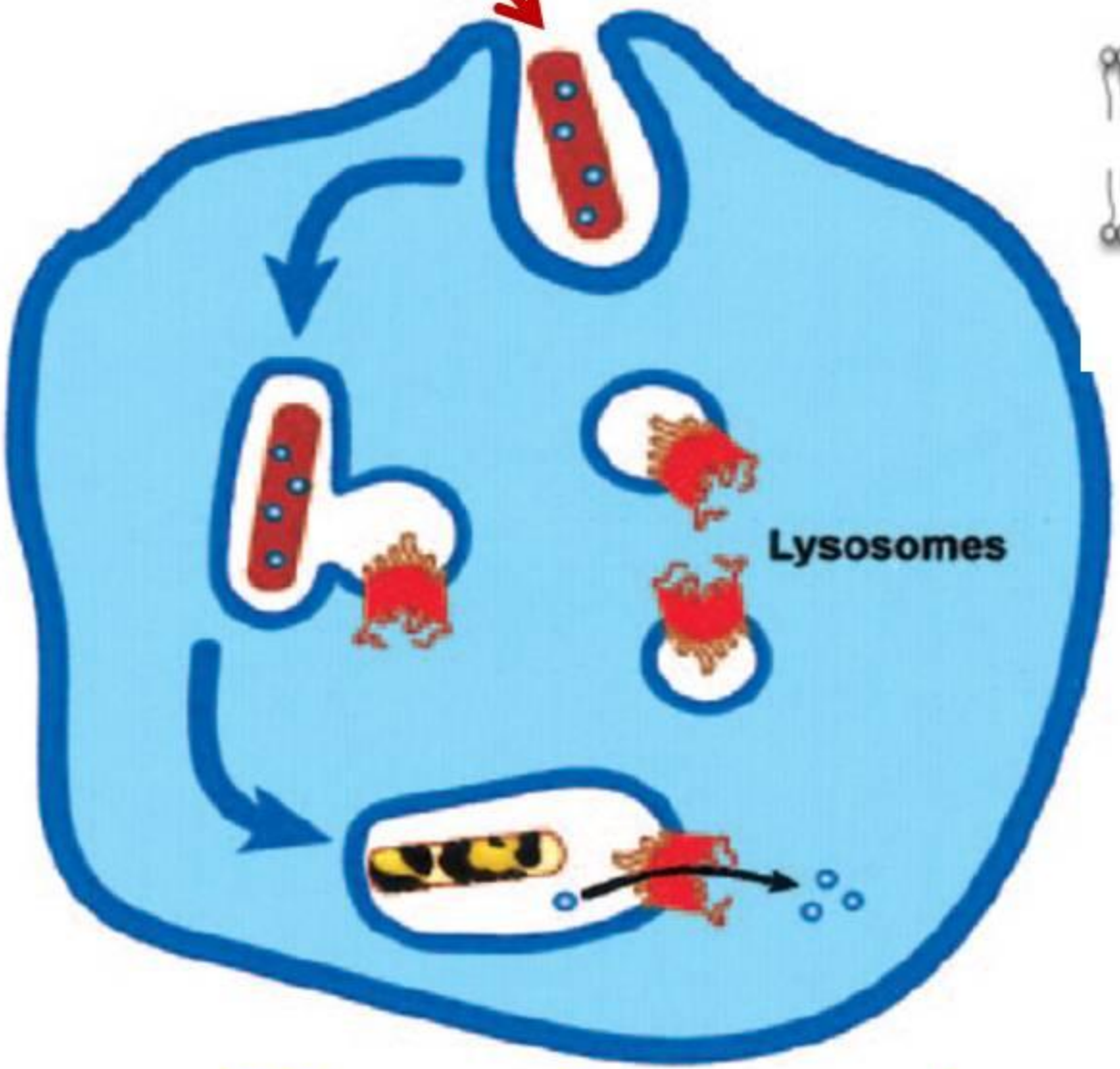
[1]Department of Microbiology and Immunology, [2]Research Institute of the McGill University Health Center and Department of Human Genetics, and [3]Department of Biochemistry, McGill University, Montreal, Quebec, Canada H3G 1Y6;
email: philippe.gros@mcgill.ca

Annual Review Immunology, 2008

Table 1 Host resistance loci revealed by forward genetics approaches[a]

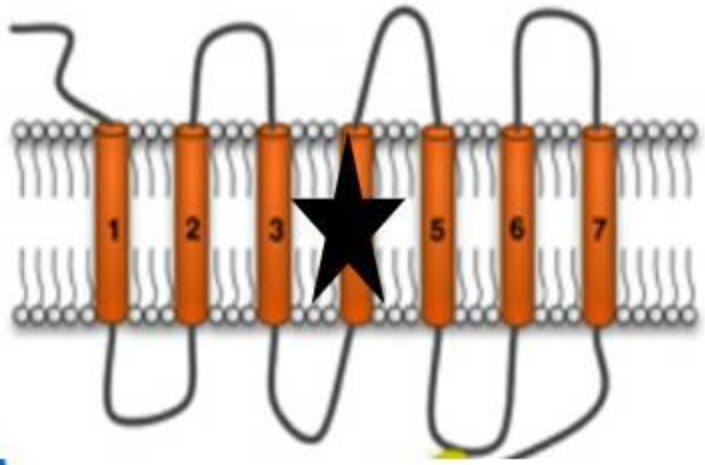
Pathogen(s)	Locus (Gene)	Main cell type(s)	Protein function or biological process/mechanism of action	Reference(s)
Salmonella Typhimurium *Leishmania donovani* *Mycobacterium bovis* (BCG)	*Ity-Lsh-Bcg* (*Nramp1*)	M	Iron transporter/regulation of intraphagosomal iron	16, 17, 20
Salmonella Typhimurium	*Lps* (*Tlr4*)	M	Surface receptor for bacterial LPS/cellular recognition of LPS	63, 64, 70, 75
Salmonella Typhimurium	*xid* (*btk*)	B	Tyrosine kinase/regulation of B cell development	60, 65, 66
Legionella pneumophila	*Lgn1* (*Ilrrc1c*)	M	NLR protein/intracellular antigen recognition	113, 114
Bacillus anthracis	*Lin1* (*Nalp1b*)	M	NLR protein/sensing of anthrax toxin	125, 126, 128
Mycobacterium bovis (BCG) *Salmonella* Typhimurium *Rattus chabaudi AS* *Mycobacterium tuberculosis*	*Myls* (*Icsbp/IRF8*)	M	Transcriptional regulator/regulation of the IL-12 and IFN-γ pathway	130, 134, 135
Mycobacterium tuberculosis *Listeria monocytogenes*	*ms1* (*Ifnr1*)	M	Transcriptional regulator/transcriptional activation in response to intracellular pathogens	139, 144–146
Candida albicans *Listeria monocytogenes*	*Cs* (*C5a*)	unknown	Components of complement cascade/proinflammatory activity	156–158, 161, 162
Rattus chabaudi AS	*Char4* (*Pklr*)	E	Pyruvate kinase/glycolysis in erythrocytes (role for ATP production)	167–170
Rattus chabaudi AS	*Char9* (*Vnn1/Vnn3*)	E	Pantetheinases/production of the antioxidant cysteamine	167, 171
Toxoplasma gondii	*Tyk2* (*Tyk2*)	M	Jak kinase/cellular signaling by cytokine receptors	180, 181
Orientia tsutsugamushi	*Rit* (*Sgp1*)	M, T, NK	Phosphoprotein/recruitment of leukocytes and T cell polarization	187, 189, 190, 192, 193
Chlamydia trachomatis	*Cerq3* (*Irgb10*)	M and others	p47GTPase/mediator of the inhibitory effects of IFN-γ	197, 199
Orthomyxovirus (influenza)	*Mx* (*Mx1*)	M	GTPase/inhibition of viral genome transcription	206–209
Coronavirus (MHV)	*Hc2* (*Ceacam1*)	EP	Transmembrane glycoprotein (with Ig domains)/adhesion molecule, signal regulatory protein	236–239
West Nile Virus (WNV)	*Bst* (*Oas1b*)	M	Oligoadenylate synthetase/part of the OAS/RNase L system of RNA decay pathway stimulated by type I IFN	257–259
Cytomegalovirus (MCMV)	*Cmv1* (*Ly49h*)	NK	MHC class I receptor/recognition of infected cells by NK cell receptors	272, 274–276, 277–280
Staphylococcus aureus	*Olf* (*Cd36*)	M	Scavenger receptor type B/regulation of the Tlr2/6-dependent signaling pathway	299
Vesicular stomatitis virus (VSV) Vaccinia virus	*Lps2* (*Trif*)	M	Toll-receptor–associated activator of IFN/regulation of the Tlr3- and Tlr4-dependent signaling pathway	300, 305–308
Cytomegalovirus (MCMV)	*Cpg1* (*Tlr9*)	M	Transmembrane receptor/recognition of pathogen-derived molecules	301, 309

M. bovis



Mouse Macrophage

G169D



mutation in *Nramp-1* gene increases susceptibility to infection

Contents lists available at ScienceDirect

Preventive Veterinary Medicine
2010
journal homepage: www.elsevier.com/locate/prevetmed

Candidate gene polymorphisms (*BoIFNG*, *TLR4*, *SLC11A1*) as risk factors for paratuberculosis infection in cattle

Pablo J. Pinedo[a], Claus D. Buergelt[b], G. Art Donovan[a], Pedro Melendez[a], Laurence Morel[c], Rongling Wu[d], Taimour Y. Langaee[e], D. Owen Rae[a,*]

[a] *Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, United States*
[b] *Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, United States*
[c] *Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, United States*
[d] *Statistics IFAS, University of Florida, United States*
[e] *Department of Pharmacy Practice, Center for Pharmacogenomics, College of Pharmacy, University of Florida, Gainesville, Florida, United States*

Elephant Genomic Study

- Next generation genome sequencing
- Compare exposed-uninfected with exposed-infected

Hypothesis:

- Because elephants are secondary host for *Mtb* and prevalence in Asia seems constant, is genetic basis for susceptibility *MONOGENIC*??

Prevalence of TB in elephants

- Captive USA (38/225) = 17%
- Chitwan, Nepal ~ 15-20%
- India ~ 15-20%

Exposure History

- Three categories:
  - –unexposed, uninfected
  - –exposed, uninfected
  - –exposed, infected
- Variable in USA institutions
- Uniform among Asian captives

Chitwan, Nepal

U.S. Zookeepers Wary of Herpes Virus Attacking Asian Elephants (NY Times, February 26, 2009)

St. Louis Zoo

Jade, a 2-year-old Asian elephant at the St. Louis Zoo, was fighting a deadly herpes infection but appears to be recovering.

DNA sequence data suggests 6 species of EEHV diverged 20 million years ago (Hayward et al)

Gene(s) influencing susceptibility to infection

- Breeding and movement of animals
- Diagnostic tests
- Vaccine development
- Drug efficacy and side effects
- Basic research in genetic immunology

What's Needed?

- Accurate clinical data
- Genomic DNA

Eur. J. Immunol. 2009. 39: 1991–2058

Infectious disease: Tuberculosis

Animal models of tuberculosis

Douglas Young

Division of Mycobacterial Research,

MRC National Institute for Medical Research, London, UK

Modeling human TB in experimental animals (Young 2009)

	Drugs	Vaccines	Latent infection
Mice	Routinely used model, works for current drugs	Routinely used model	
Rats	Established PK/PD model; potential for increased use?		
Guinea pigs		Routinely used model, large "window" for BCG	
Rabbits	Useful for assessing lesion-specific activities		
Cattle		Experimental challenge and natural transmission models	
Non-human primates	Expensive; use for proof-of-concept	Mimics human response (highly diverse); use for proof-of-concept	The only model that reproduces human phenomena

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Biotechnology
2010

Tuberculosis: global approaches to a global disease

Denise E Kirschner[1], Douglas Young[2] and JoAnne L Flynn[3]

Mycobacterium tuberculosis is a remarkably successful human pathogen. The interaction with the human host is complex and much remains unknown. Recent advances in systems biology have allowed the integration of data from humans and animal models into computational approaches. For example, mathematical models provide a platform for *in silico* manipulation of host–pathogen interactions to gain insight into this infection across temporal and biologic scales. Here, we review recent studies on global approaches toward identifying comprehensive responses of both host and bacillus during infection, and the potential for incorporation of these data into many types of useful computational systems. Systems biology approaches provide a unique opportunity to study interventions that may improve therapy and vaccines against this major killer.

Tuberculosis (TB)

What are TB, MDR TB, and XDR TB?

Definitions of Tuberculosis, Multidrug-Resistant TB (MDR TB) and Extensively Drug-Resistant TB (XDR TB)

View Drug-Resistant TB — A Visual Tour

Understanding TB

- History of TB
- Causes
- Transmission
- Symptoms
- Diagnosis
- Treatment
- Prevention

The New Challenge for TB Research

An ancient disease, TB remains one of the major causes of disability and death worldwide. One third of the world's population are infected with TB. Each year, over 9 million people around the world become sick with TB and there are almost 2 million TB-related deaths. NIAID is examining TB at its fundamental levels through research aimed at developing faster diagnostic tools, better vaccines, and shorter treatment times. More about NIAID TB Research and Scientific Findings.

Multidrug-resistant and Extensively Drug-resistant Tuberculosis Research Agenda (PDF)

NIAID's Role in Addressing TB, Drug-Resistant TB, and TB in People with HIV/AIDS

TB Research at NIAID

- Research Goals
- Basic Research
- Diagnostic Research
- Advances in Treatment
- Prevention: Vaccine Development
- Global Research
- Scientific Illustrations of Drug-Resistant TB
- NIAID Labs
- Networks, Consortia and Partners
- Resources
- Training
- Meetings
- Scientific Literature

Acknowledgments

- Susan Mikota (Elephant Care International)
- David Abraham (University of Calgary)
- San Diego Zoo Global