# The Lung Microbiome

*challenging old paradigms about microbes and the host respiratory tract*


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**Professor of Internal Medicine and Microbiology/Immunology**

**Division of Pulmonary and Critical Care Medicine**

**University of Michigan Medical School**

Univeristy of Michigan Microbiome Research Initiative

Infection and Immunity, Sept. 2004, p. 4996–5003 Vol. 72, No. 9
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.9.4996–5003.2004


# Role of Antibiotics and Fungal Microbiota in Driving Pulmonary Allergic Responses

Mairi C. Noverr,[1] Rachael M. Noggle,[1] Galen B. Toews,[1] and Gary B. Huffnagle[1,2]*

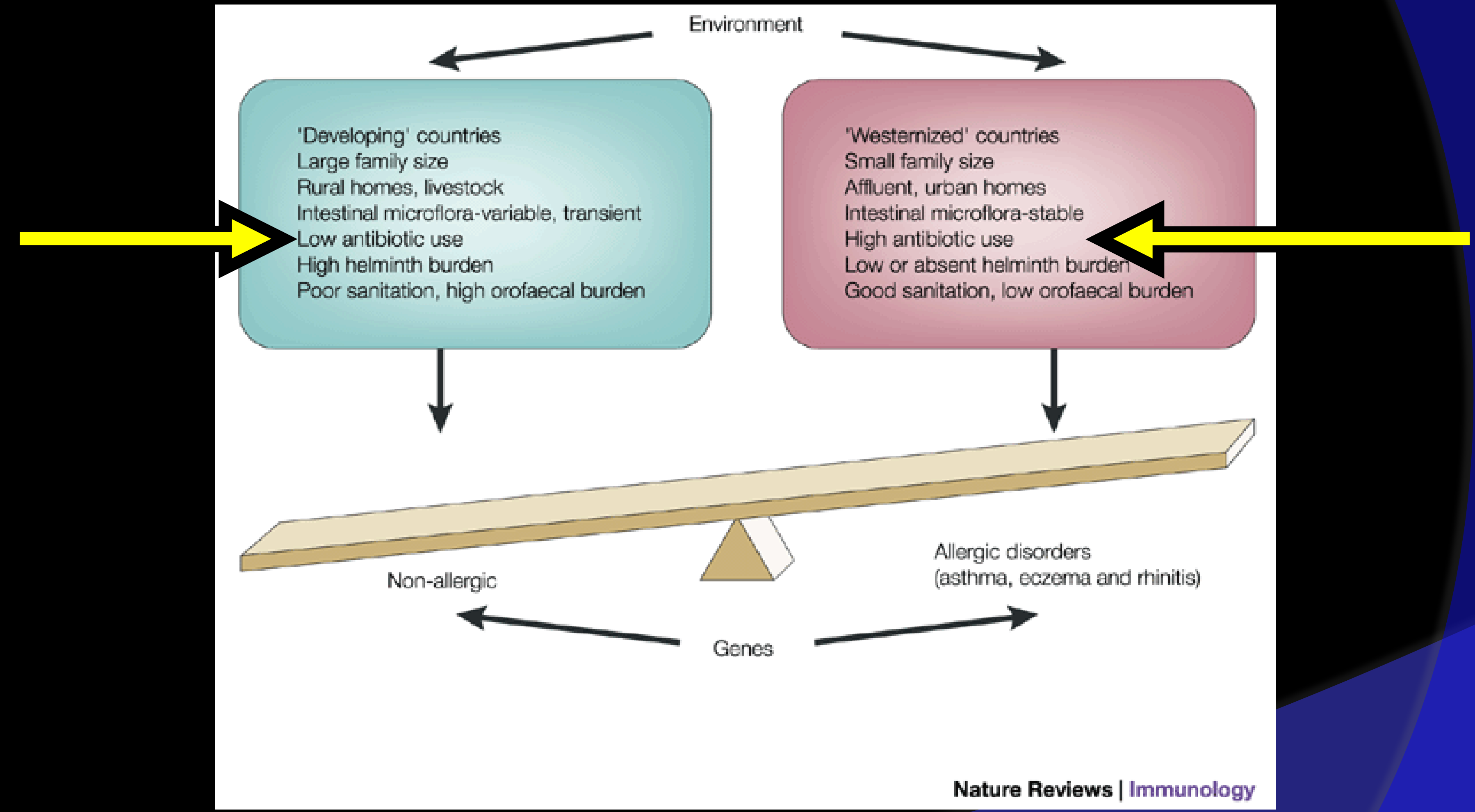
*Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine,[1] and Department of Microbiology and Immunology,[2] University of Michigan Medical School, Ann Arbor, Michigan*

Infection and Immunity, Jan. 2005, p. 30–38 Vol. 73, No. 1
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.1.30–38.2005


# Development of Allergic Airway Disease in Mice following Antibiotic Therapy and Fungal Microbiota Increase: Role of Host Genetics, Antigen, and Interleukin-13

Mairi C. Noverr,[1] Nicole R. Falkowski,[1] Rod A. McDonald,[1] Andrew N. McKenzie,[2] and Gary B. Huffnagle[1,3]*

# The Hygiene Hypothesis




Wills-Karp et al. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Imm 1, 69-75 (2001)

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Review
*TRENDS in Microbiology* Vol.12 No.12 December 2004
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# Does the microbiota regulate immune responses outside the gut?

**Mairi C. Noverr[1] and Gary B. Huffnagle[1,2]**

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[2]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0642, USA

# Subsequent Studies on the Effect of Antibiotics or Germ-Free State on Allergic Airway Response in Mice

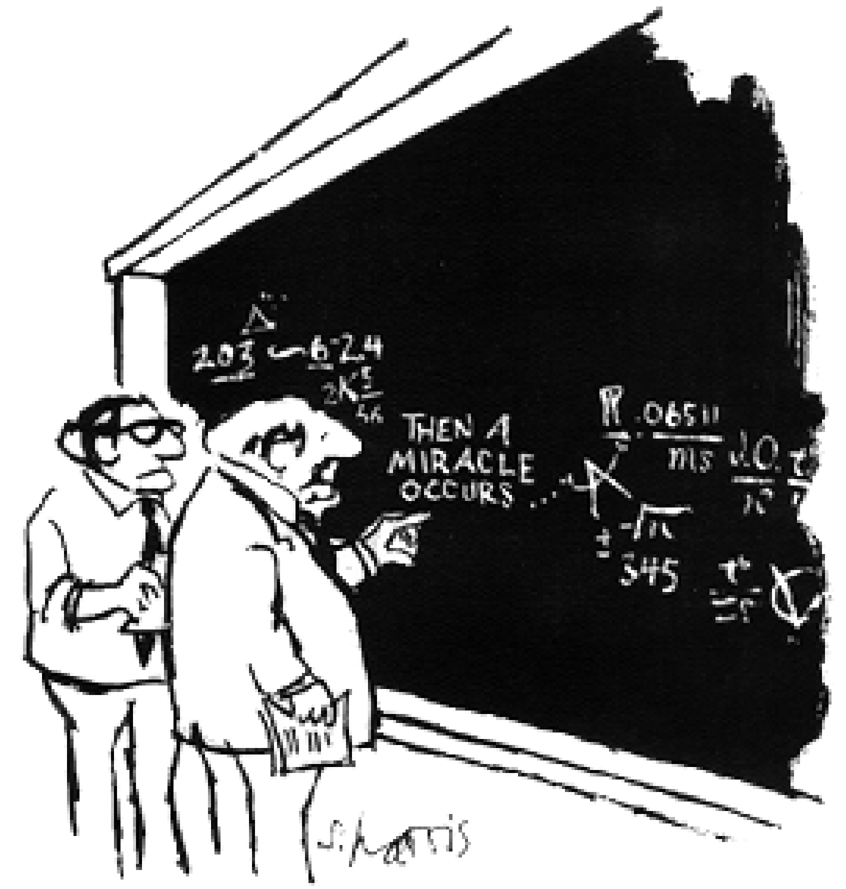
Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice.Karimi K, Inman MD, Bienenstock J, Forsythe P.. Am J Respir Crit Care Med. 2009 Feb 1;179(3):186-93.

Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models.Lyons A, O'Mahony D, O'Brien F, MacSharry J, Sheil B, Ceddia M, Russell WM, Forsythe P, Bienenstock J, Kiely B, Shanahan F, O'Mahony L.Clin Exp Allergy. 2010 May;40(5):811-9.

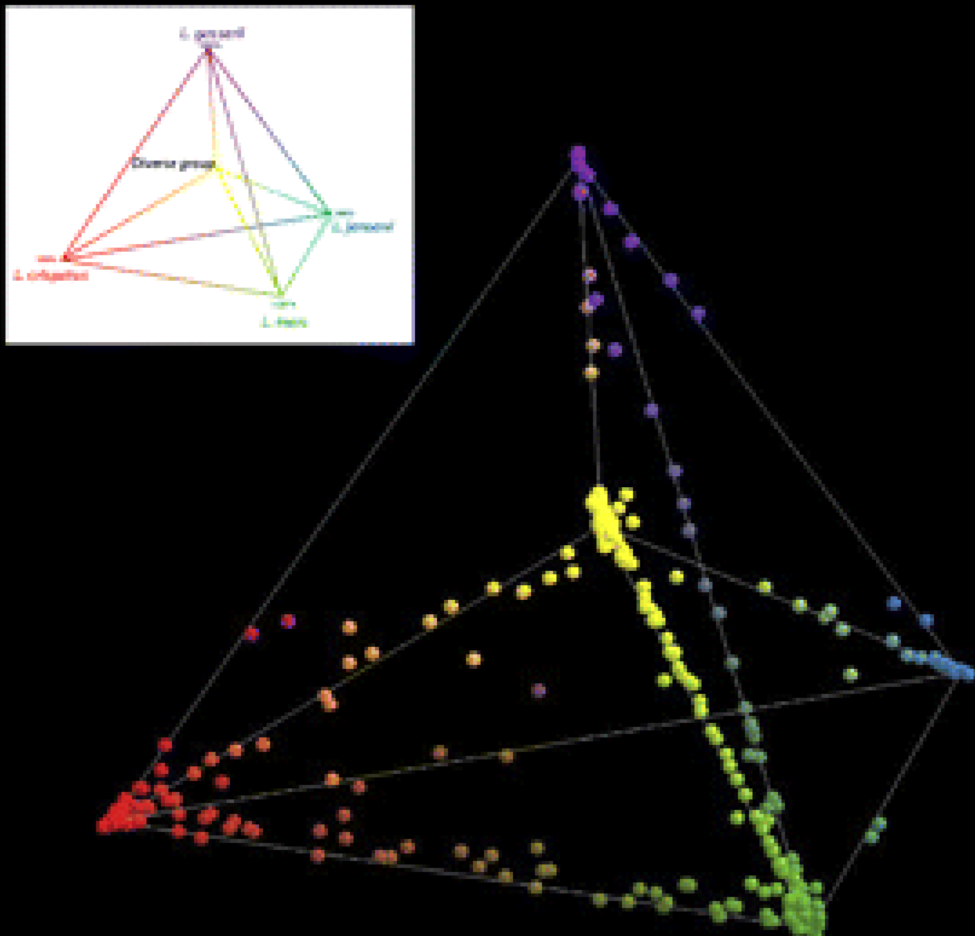
Dysregulation of allergic airway inflammation in the absence of microbial colonization.Herbst T, Sichelstiel A, Schär C, Yadava K, Bürki K, Cahenzli J, McCoy K, Marsland BJ, Harris. Am J Respir Crit Care Med. 2011 Jul 15;184(2):198-205.

Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma.Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, Gill N, Blanchet MR, Mohn WW, McNagny KM, Finlay BB. EMBO Rep. 2012 May 1;13(5):440-7.

# How does the GI microbiome modulate inflammation at distal sites?




"I think you should be more explicit here in step two."




Ravel J ... Forney LJ.
Proc Natl Acad Sci U S A. 2011 Mar 15;108

**GAP**

- Understanding HOW changes in the bacterial microbiota of the GI tract can affect distal mucosal sites

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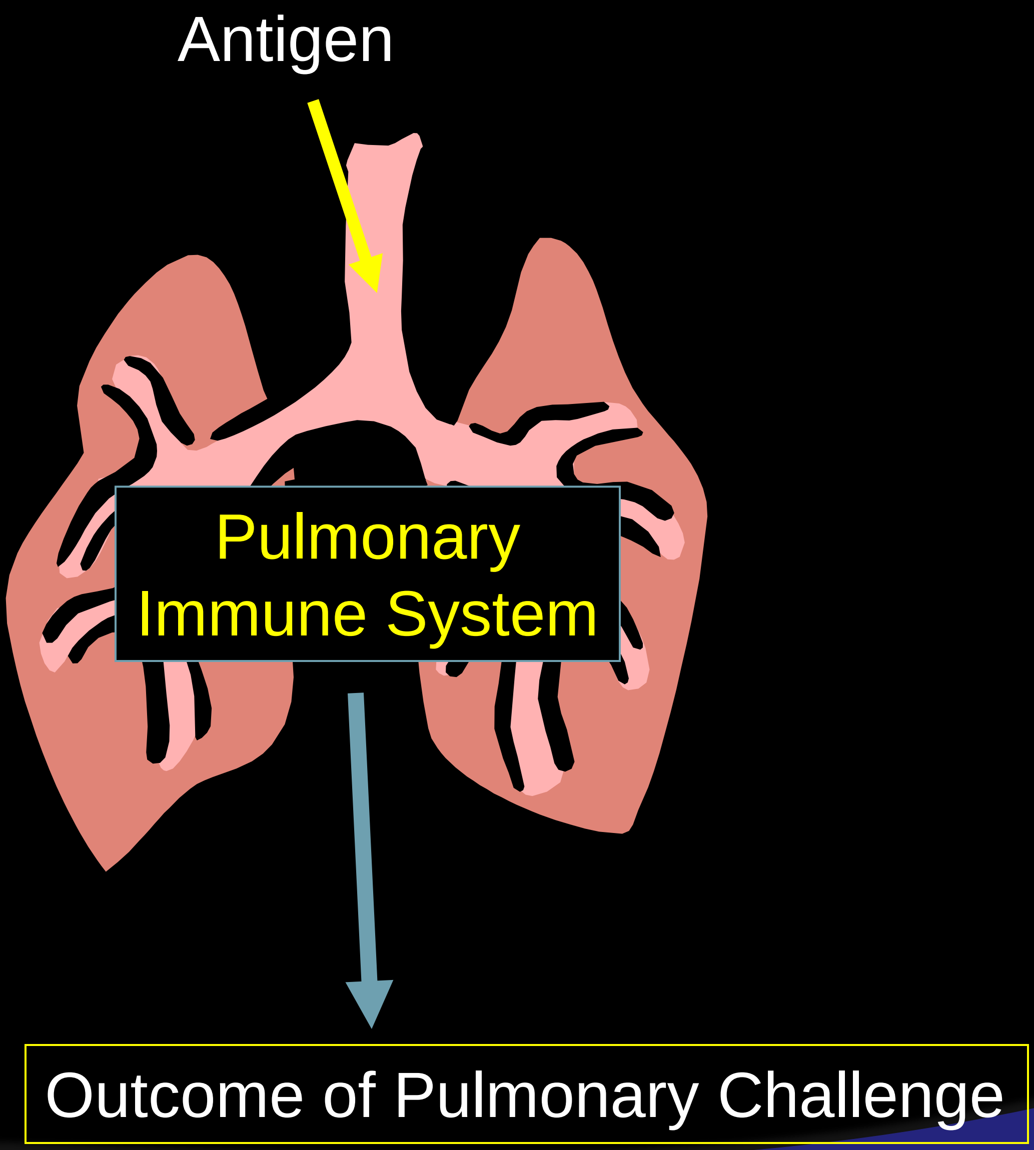
- Understanding HOW changes in the bacterial microbiota of the GI tract can affect distal mucosal sites

**CHALLENGE**

- Trying to get studies of complex in vivo systems reviewed positively by study sections dominated by reductionist biologists

# Gut-Lung Axis of Immunoregulation



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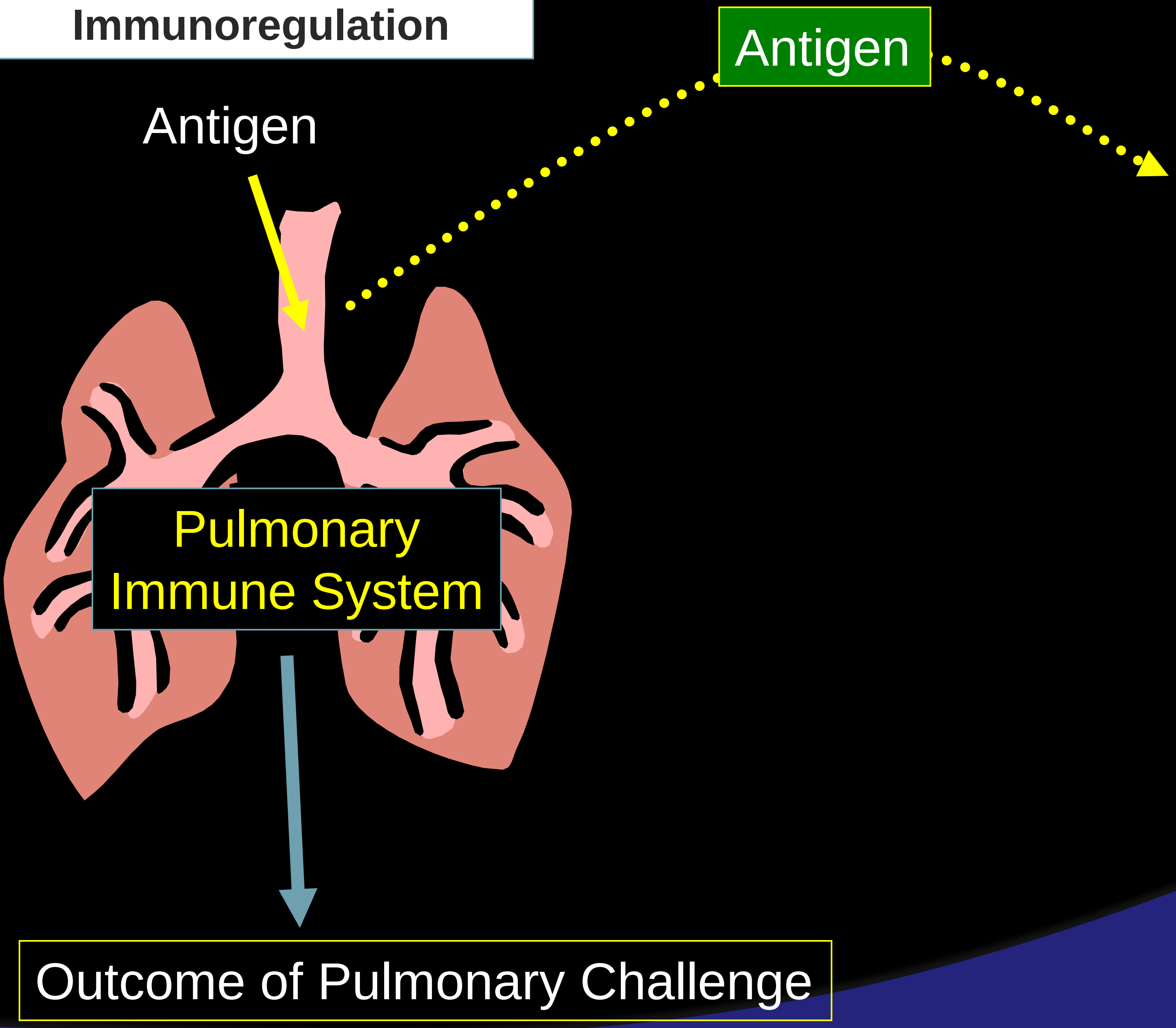

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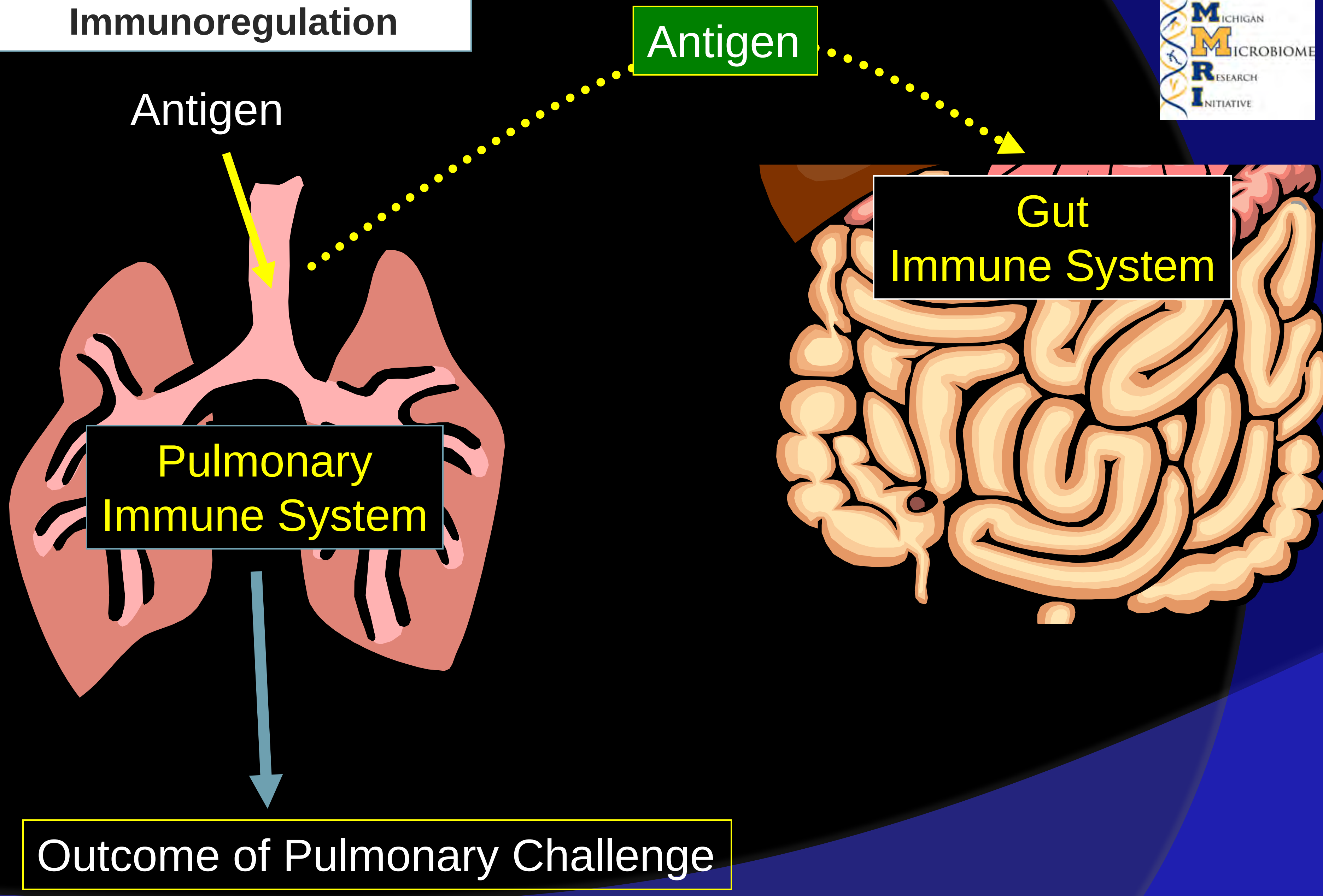

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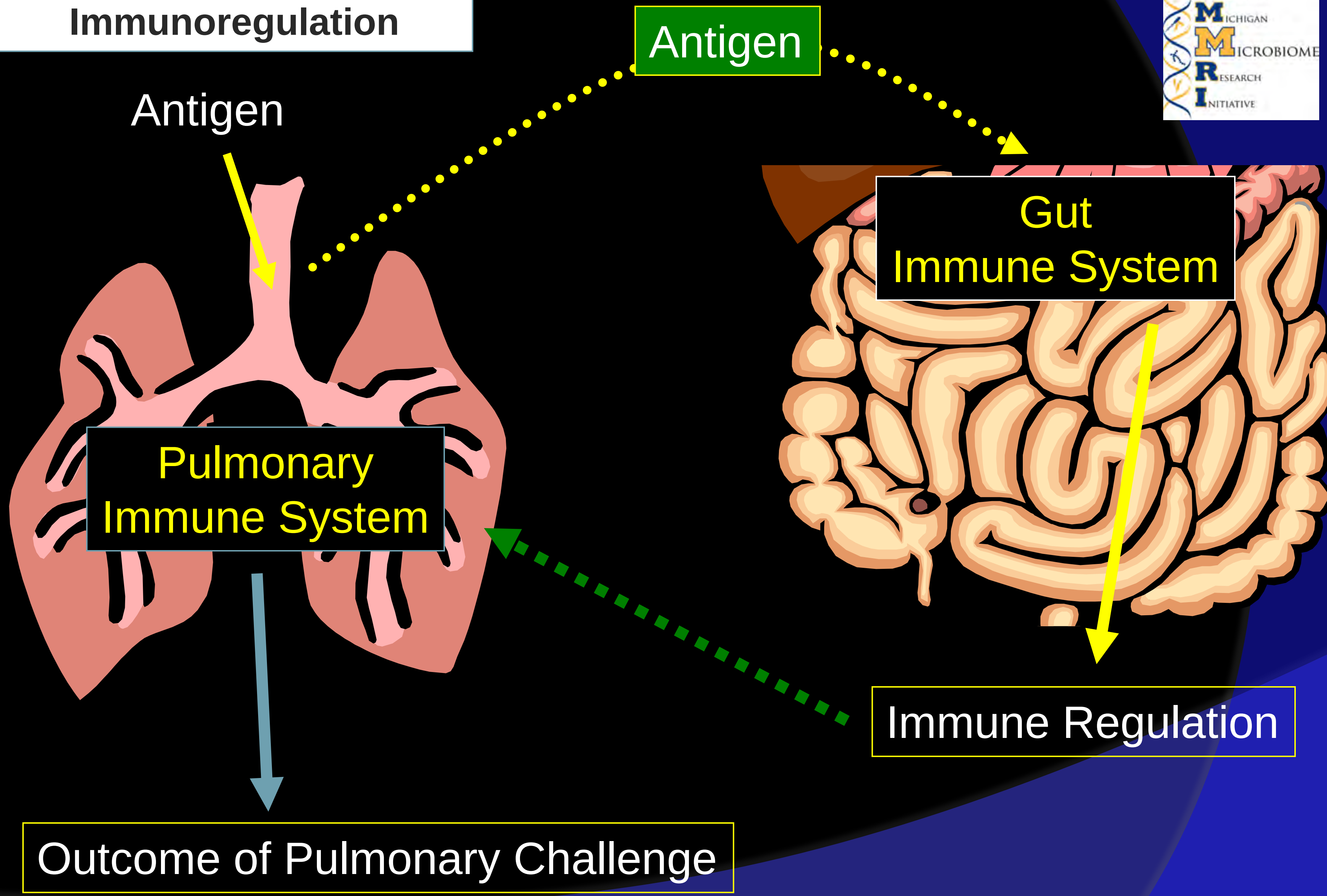


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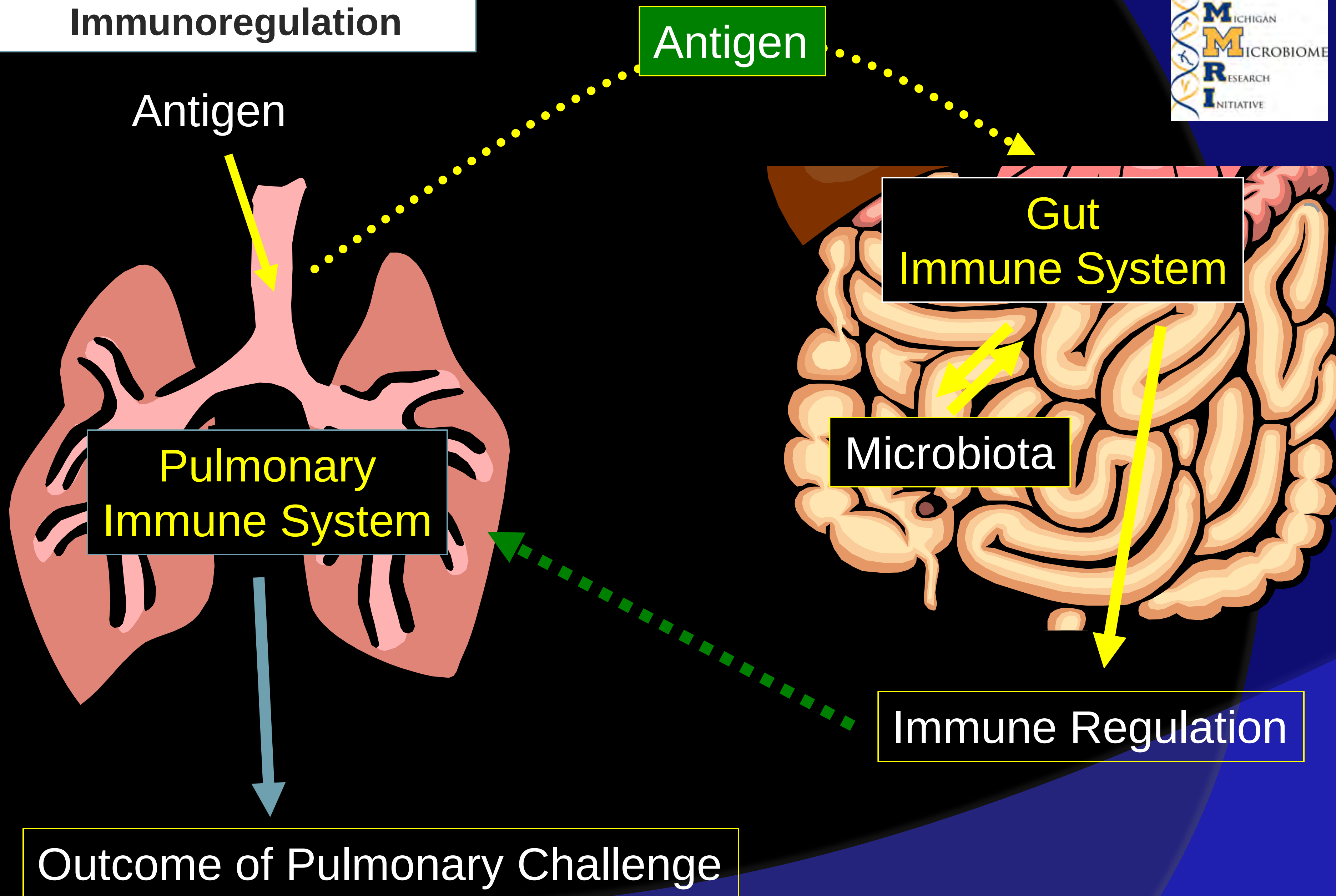

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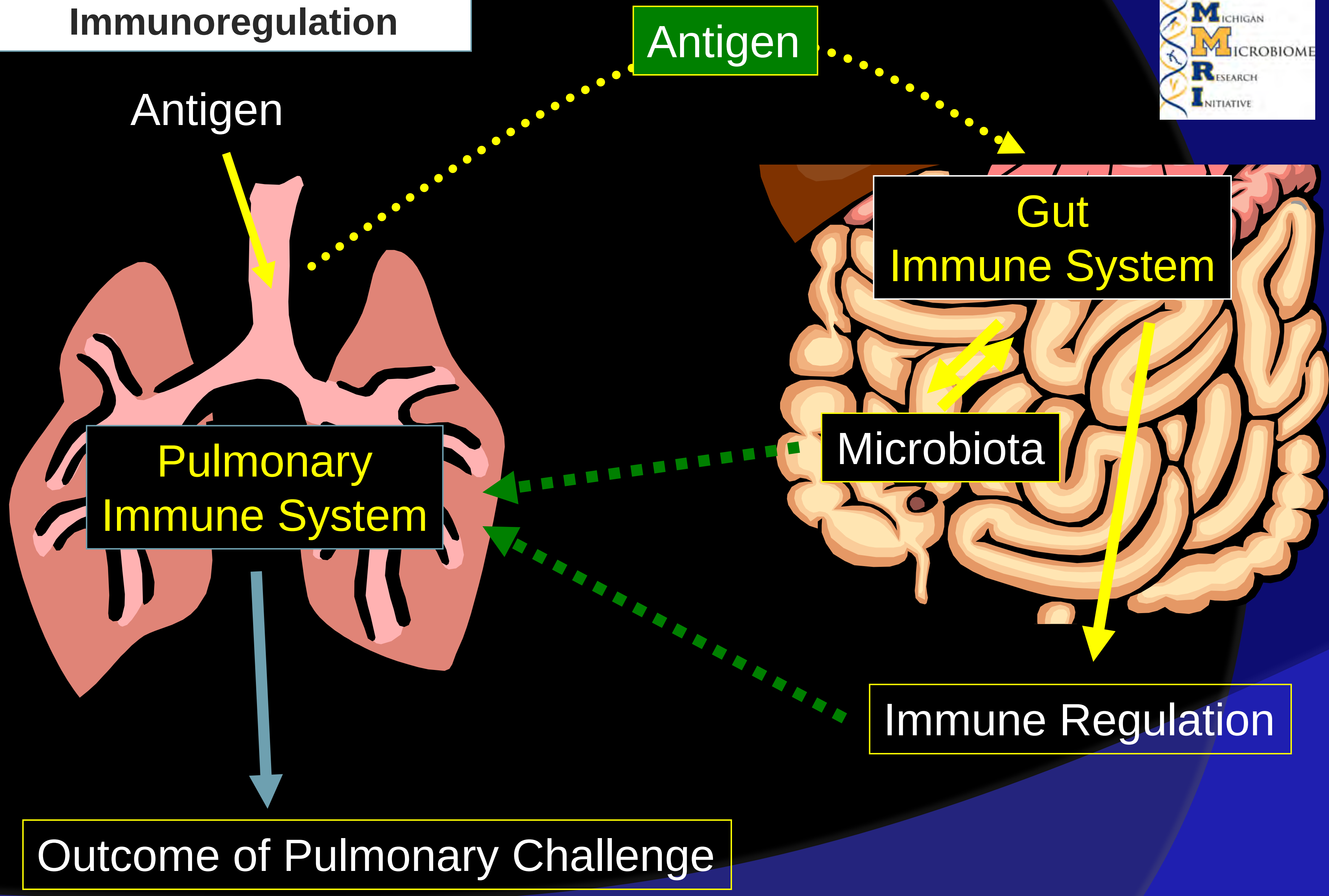


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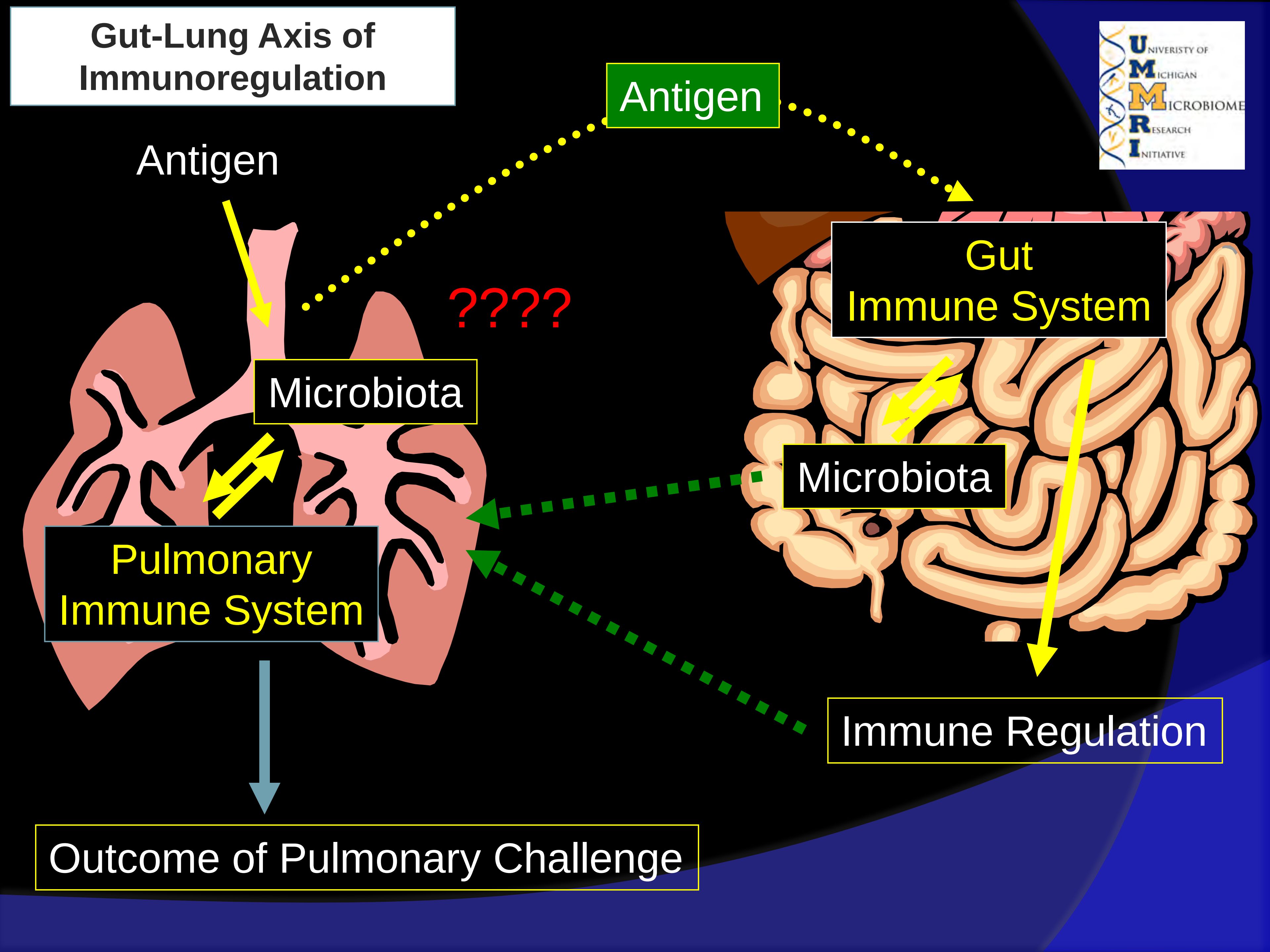

Gut-Lung Axis of Immunoregulation
Antigen
Antigen
????
Microbiota
Pulmonary Immune System
Gut Immune System
Microbiota
Immune Regulation
Outcome of Pulmonary Challenge
University of Michigan Microbiome Research Initiative

The normal lung is free from bacteria.

# The National Institutes of Health Human Microbiome Project

University of Michigan Microbiome Research Initiative

OPEN ACCESS Freely available online PLoS one

# Disordered Microbial Communities in Asthmatic Airways

**Markus Hilty[1], Conor Burke[2], Helder Pedro[3,4], Paul Cardenas[1], Andy Bush[1], Cara Bossley[1], Jane Davies[1], Aaron Ervine[2], Len Poulter[2], Lior Pachter[4], Miriam F. Moffatt[1], William O. C. Cookson[1]***

**1** National Heart and Lung Institute, Imperial College London, London, England, **2** Department of Respiratory Medicine, Connolly Hospital, Dublin, Ireland, **3** Instituto Gulbenkian de Ciência, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, **4** Department of Mathematics, University of California, Berkeley, California, United States of America

PLoS ONE | www.plosone.org January 2010 | Volume 5 | Issue 1 | e8578

# Respiratory Diseases in Which the Lung Microbiome Has Been Reported to be Altered

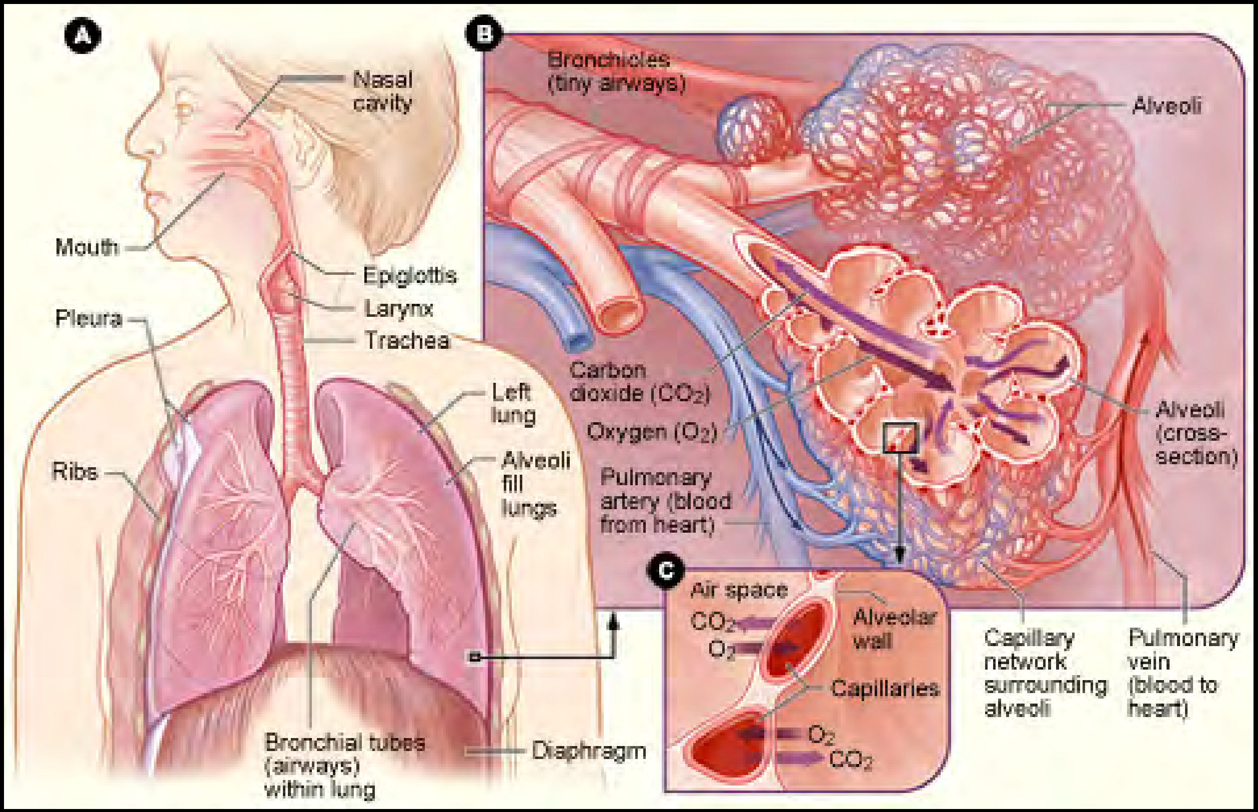
**Cystic Fibrosis**

**Asthma**

**Chronic Obstructive Pulmonary Disease**

**Bronchiolitis Obliterans Syndrome following Lung Transplantion**

# Airway Anatomy




-Anatomically contiguous surface

-Nasophrygeal cavity has a significant resident microbiota

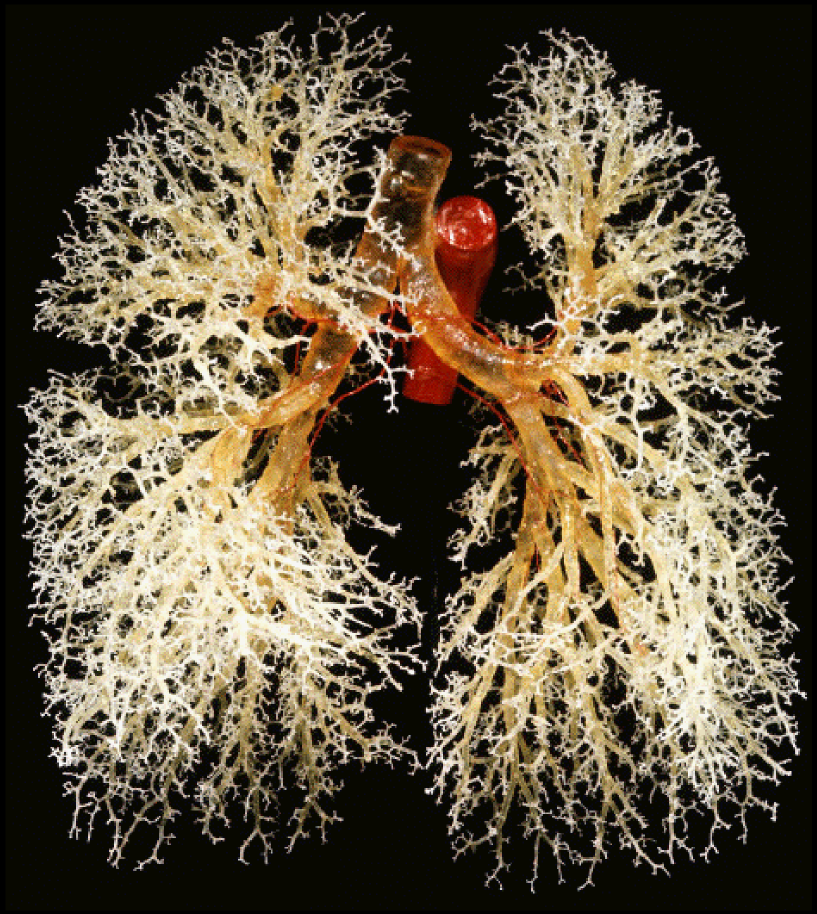
-A single "dam" between the upper and lower airways

-Cilia

- Turns and branches

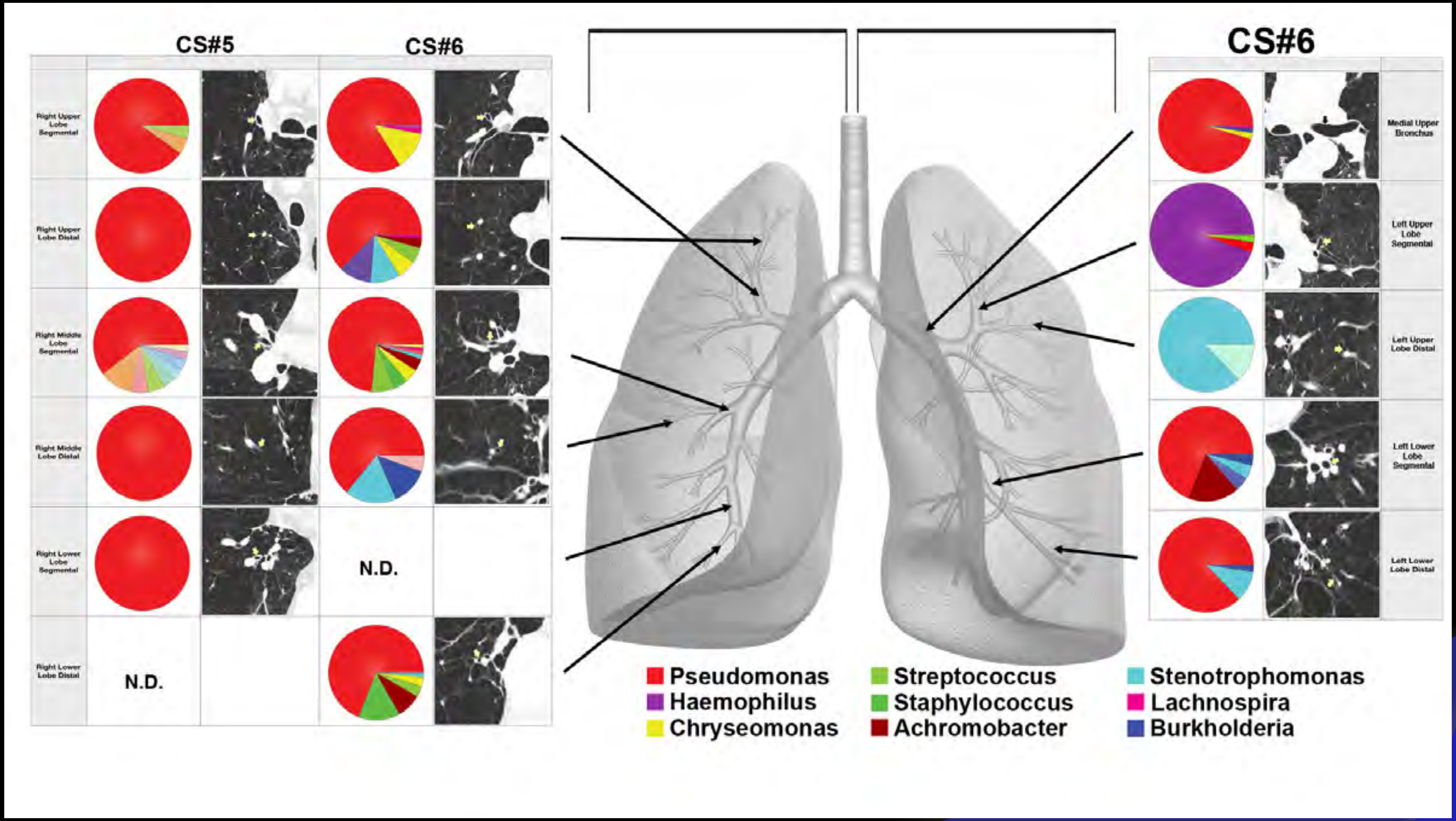
http://www.nhlbi.nih.gov/health/health-topics/topics/hlw/system.html

# Airway Anatomy



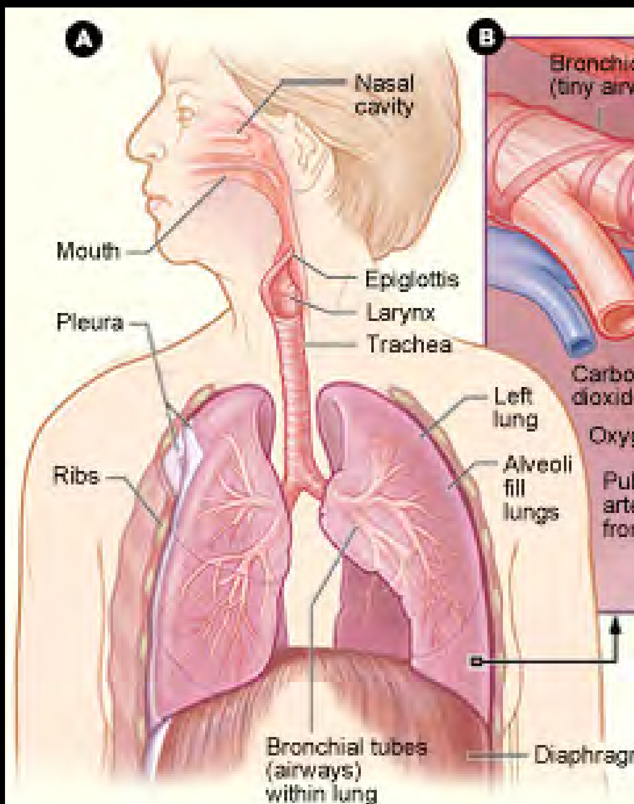
http://capturedlightning.com/frames/Lung-Branching.gif

# Anatomical Heterogeneity of the Lung Microbiome in Advanced COPD




Erb-Downward et al. PLoS One. 2011;6(2):e16384.

# Sources of Microbes in the Lungs




- Nasopharyngeal aspiration
- Inhalation
- Reflux & aspiration
- (Bloodstream)

# Can healthy lungs be considered sterile? If so, what do we mean by "sterile"?

Lack of microbial exposure?

Lack of microbial metabolism?

Lack of microbial replication?

Lack of microbial colonization?

What do we call the collection of persistent low grade microbial immigrants in the lungs?

# How to sample the airways

- Sputum (spontaneous and/or induced)
- Bronchoalveolar lavage
- Protected endobronchial brush
- Biopsy
- Sterile tissue sample

GAP

Determining the degree of bacterial transience vs. persistence vs. colonization in the lower airways

CHALLENGES

Type of sampling (invasive)

Infrequent (longitudinal = 2 to 3 samplings)

Potential for contamination of samples from nasal or oral microbiota

# Using Analysis of the Oral Microbiome to Remove the Noise from Lung Microbiome Samples

**Restricting Sampling Depth & Single-sided Outlier Test**

Charlson ES et al. Assessing bacterial populations in the lung by replicate analysis of samples from the upper and lower respiratory tracts. PLoS One. 2012;7(9):e42786.

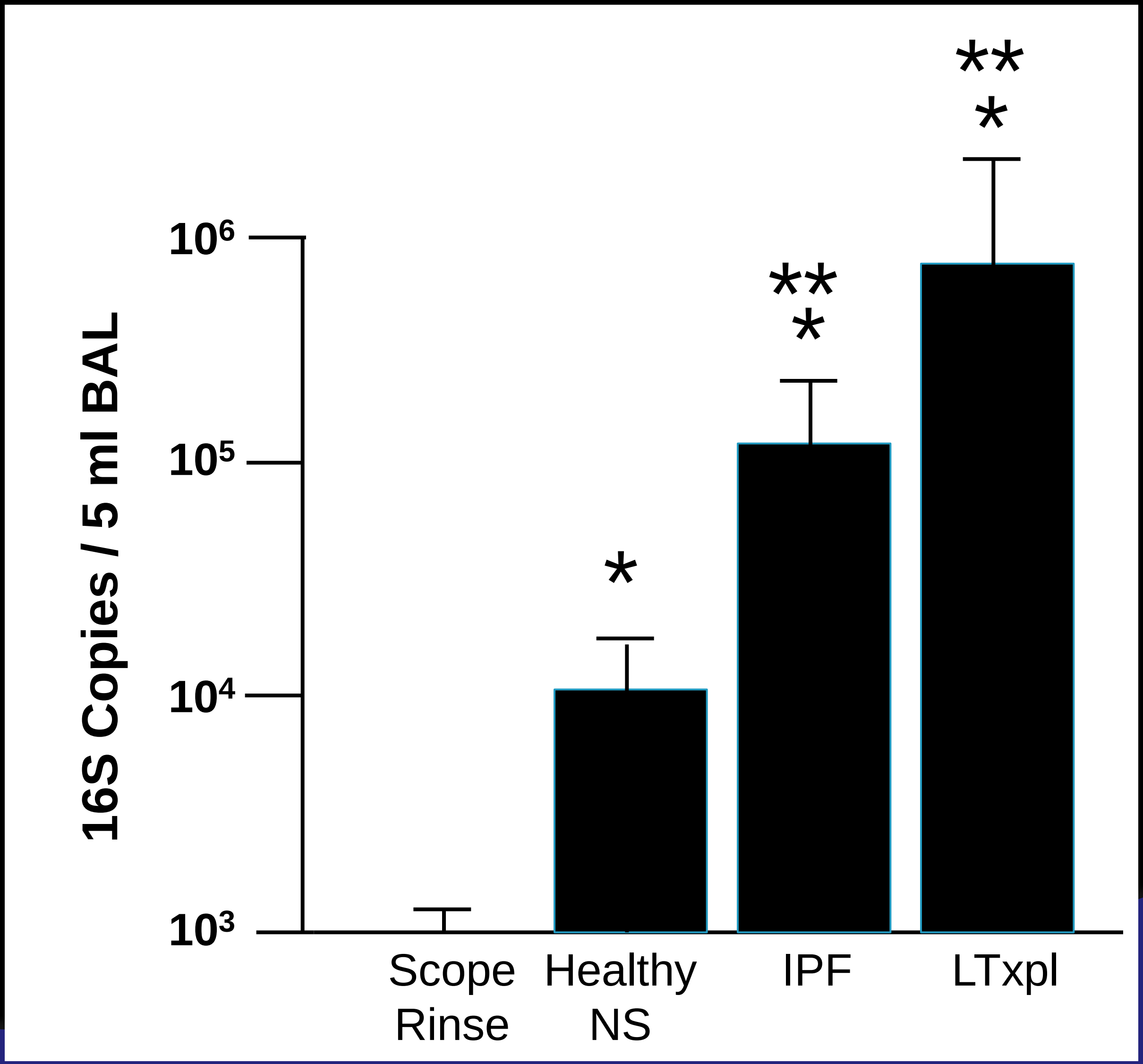
**Neutral Community Model**

Sloan WT et al. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol. 2006 Apr;8(4):732-40

Morris A et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Lung HIV Microbiome Project.Am J Respir Crit Care Med. 2013 May 15;187(10):1067-75.

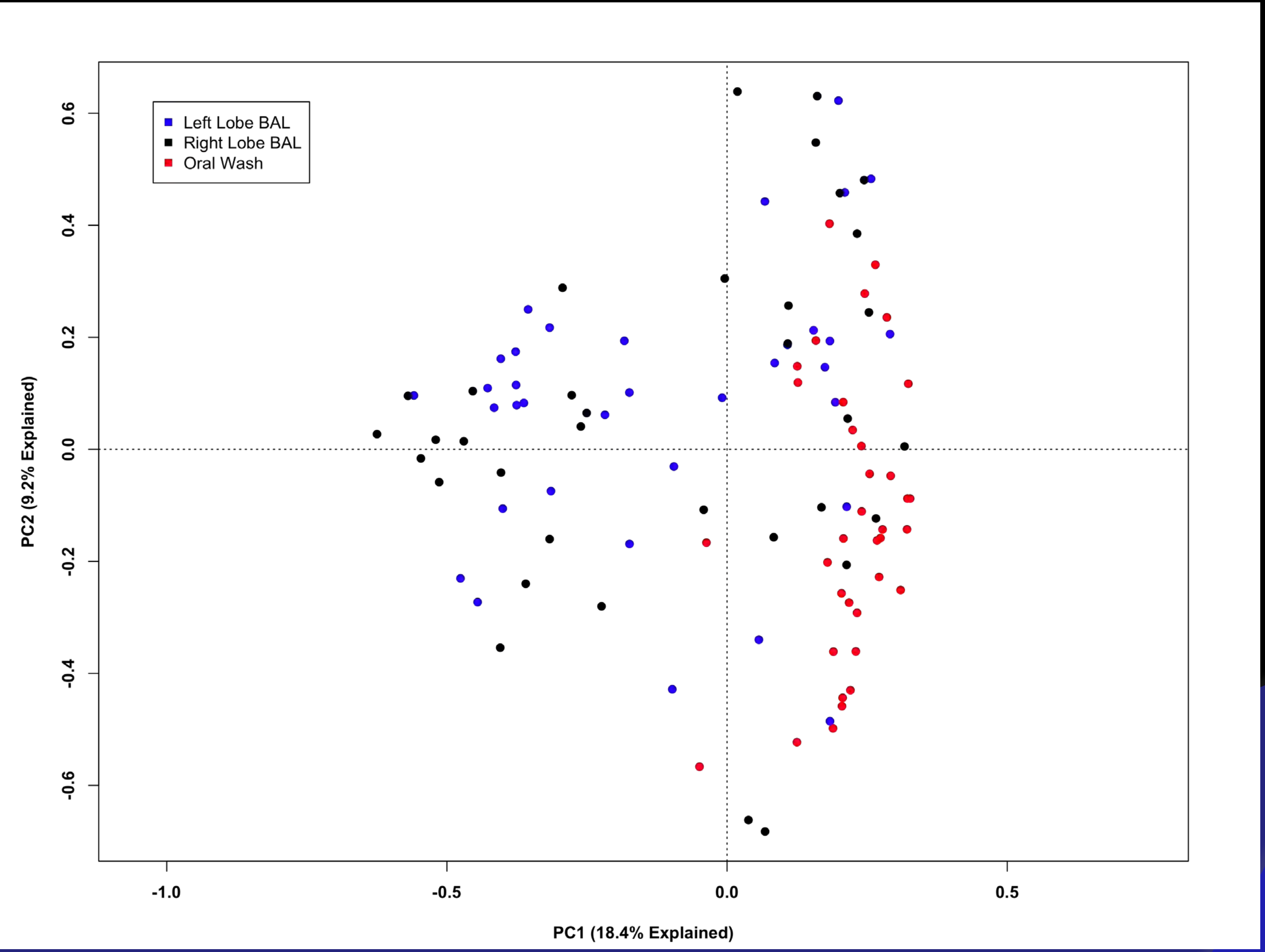
# Bacterial 16S Copy Numbers in the BAL fluid

UNIVERISTY OF MICHIGAN MICROBIOME RESEARCH INITIATIVE




Thanks!
(TaqMan protocol)
Ric Bushman, Penn

# Comparison of BAL vs. Oral Rinse Bacterial Communities



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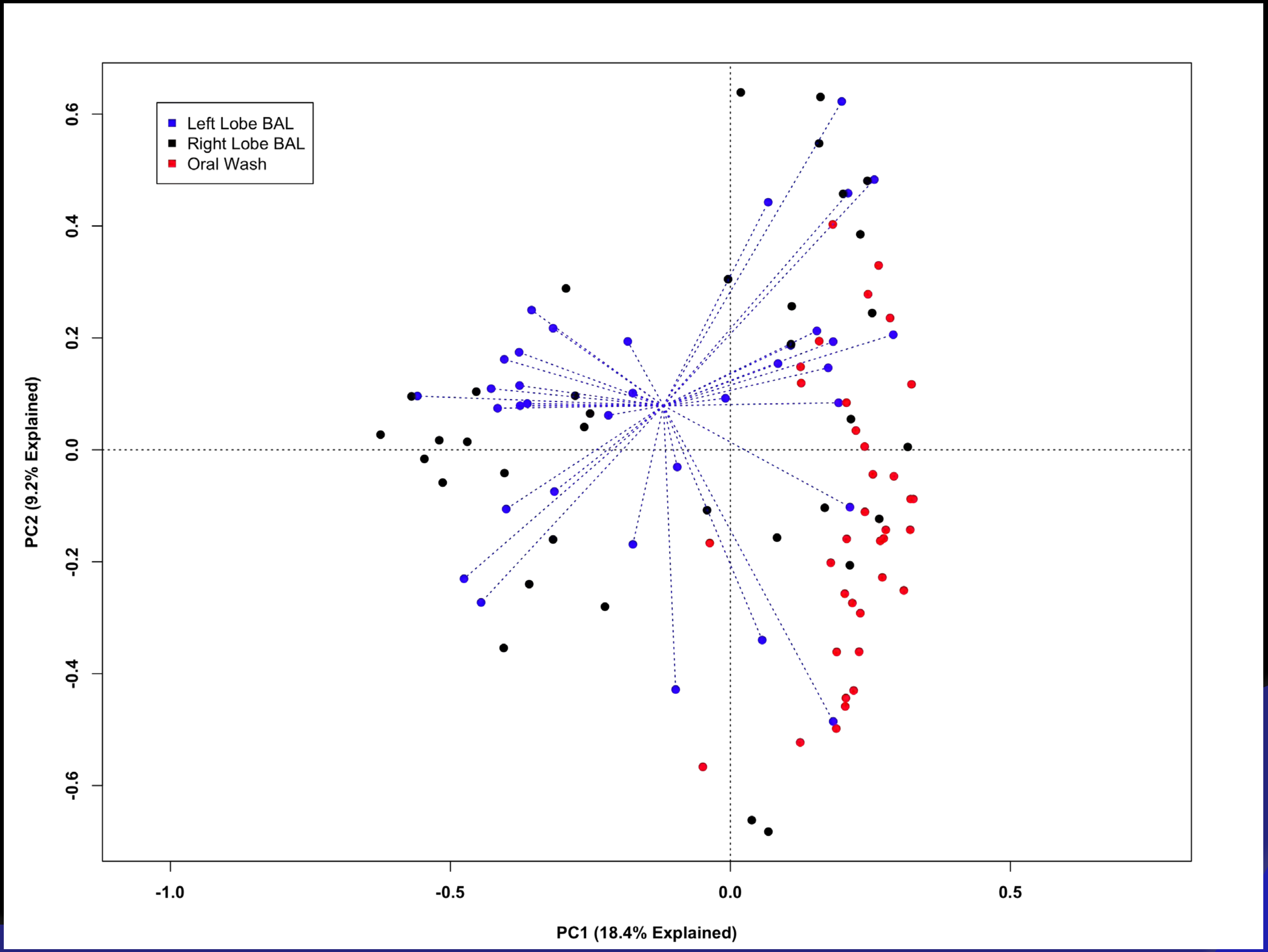


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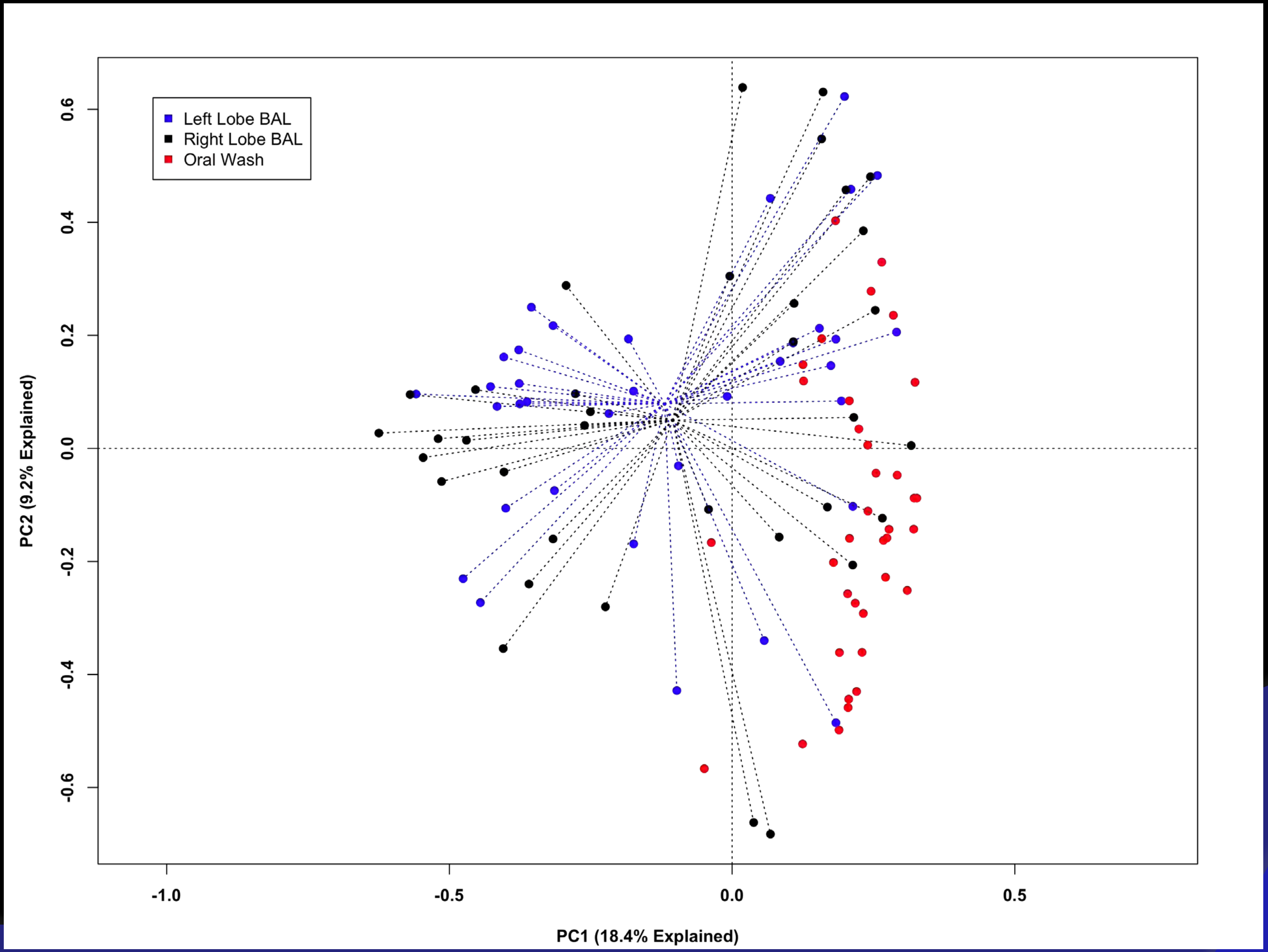

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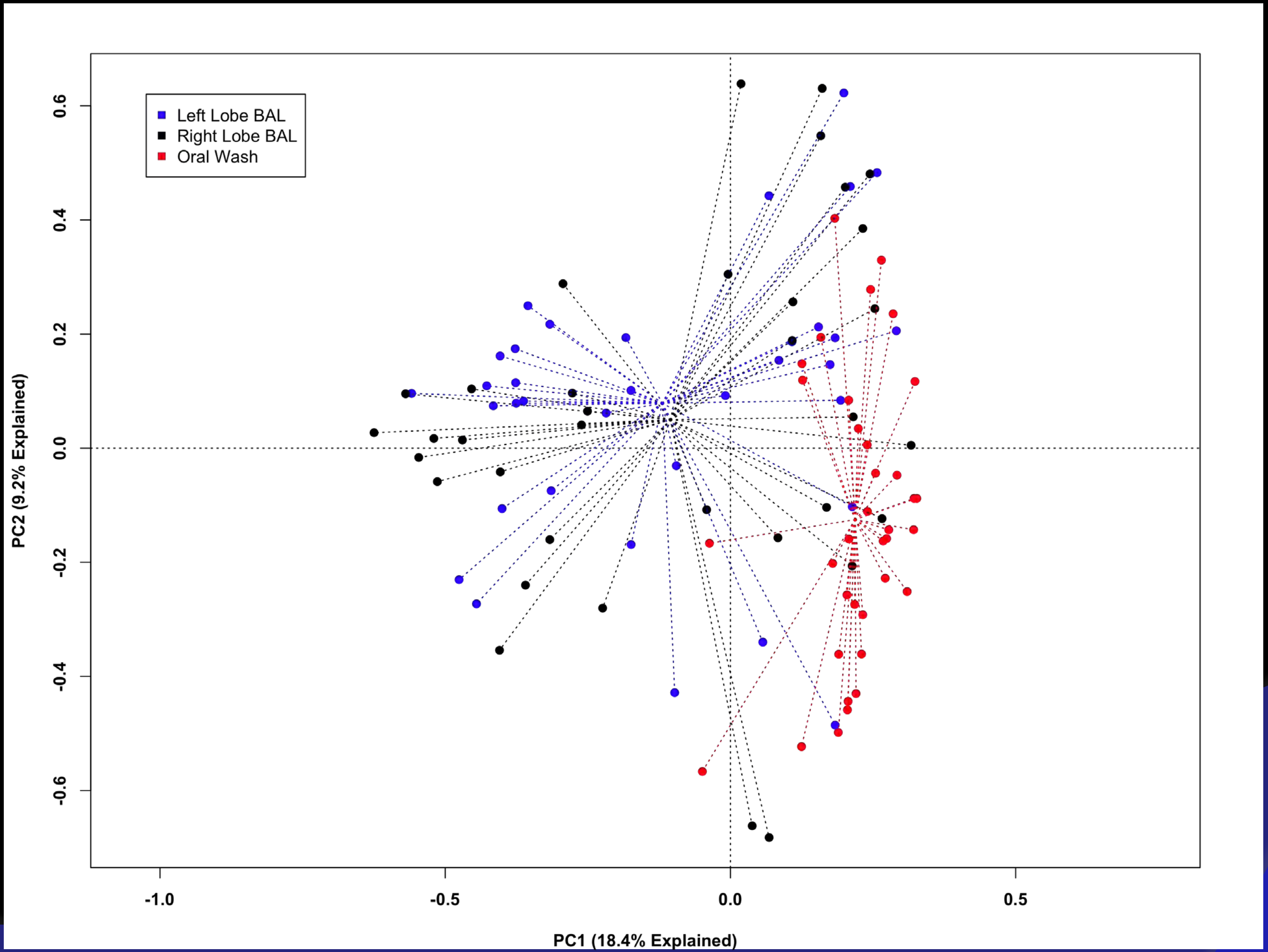

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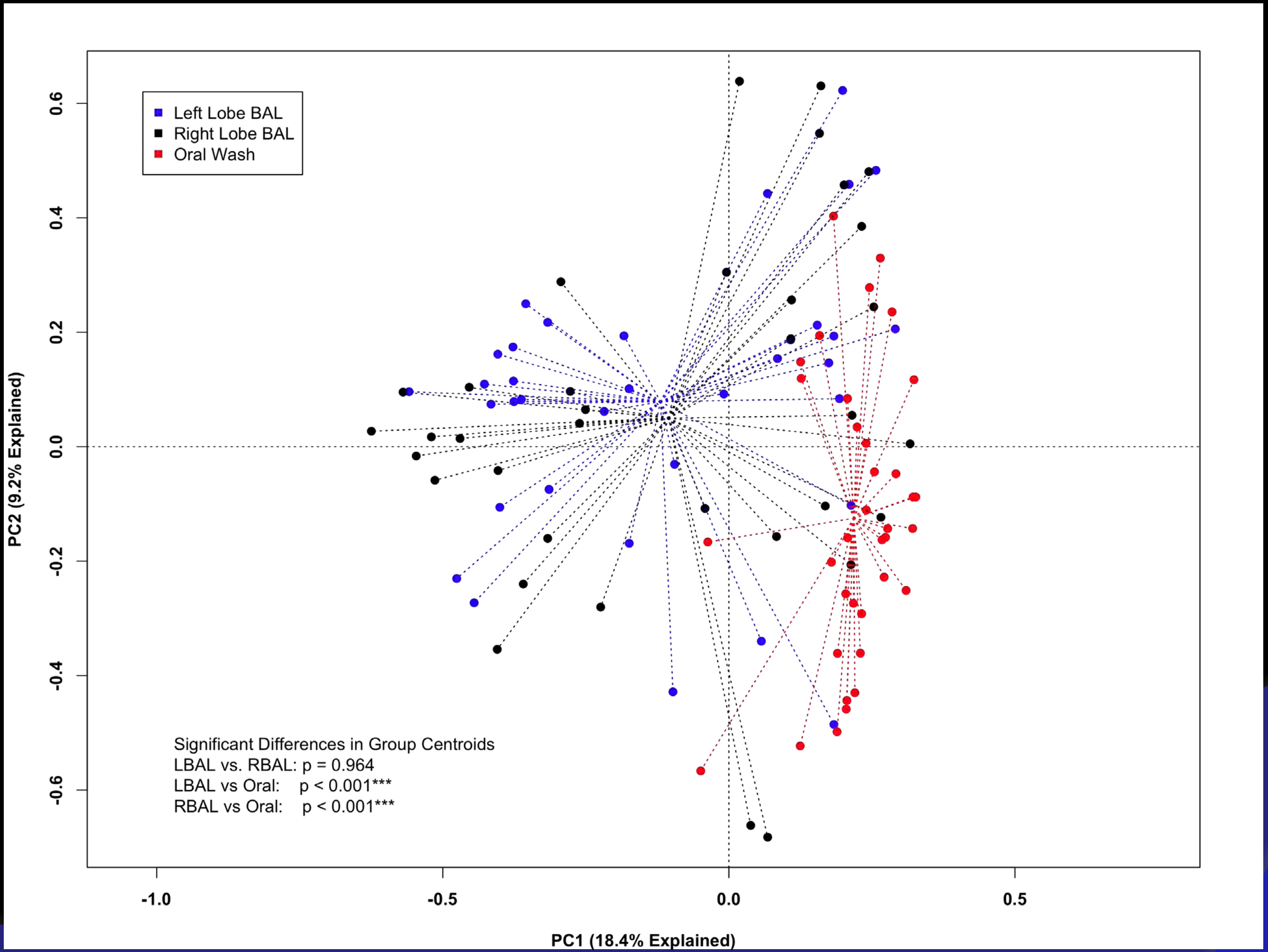

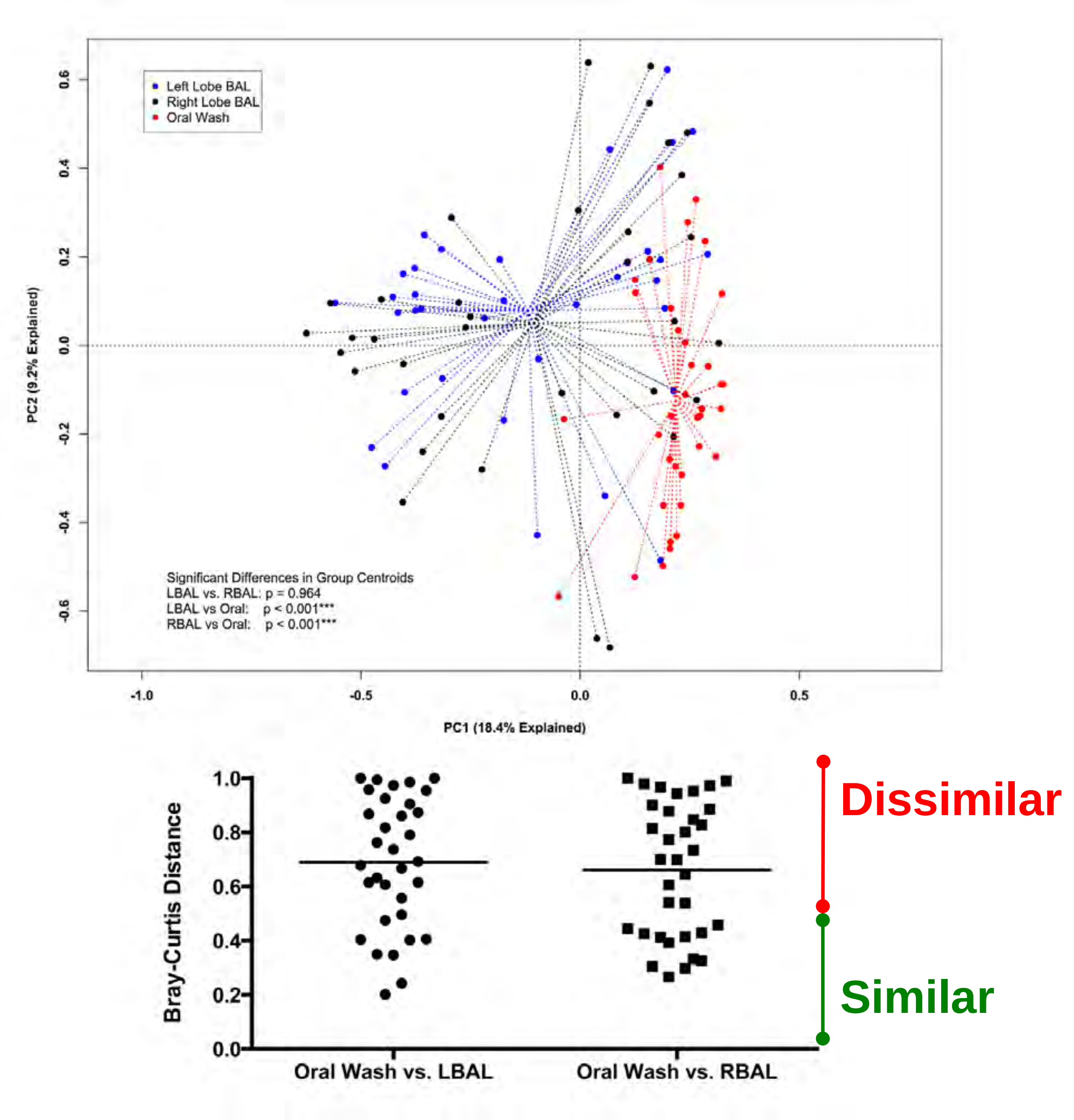


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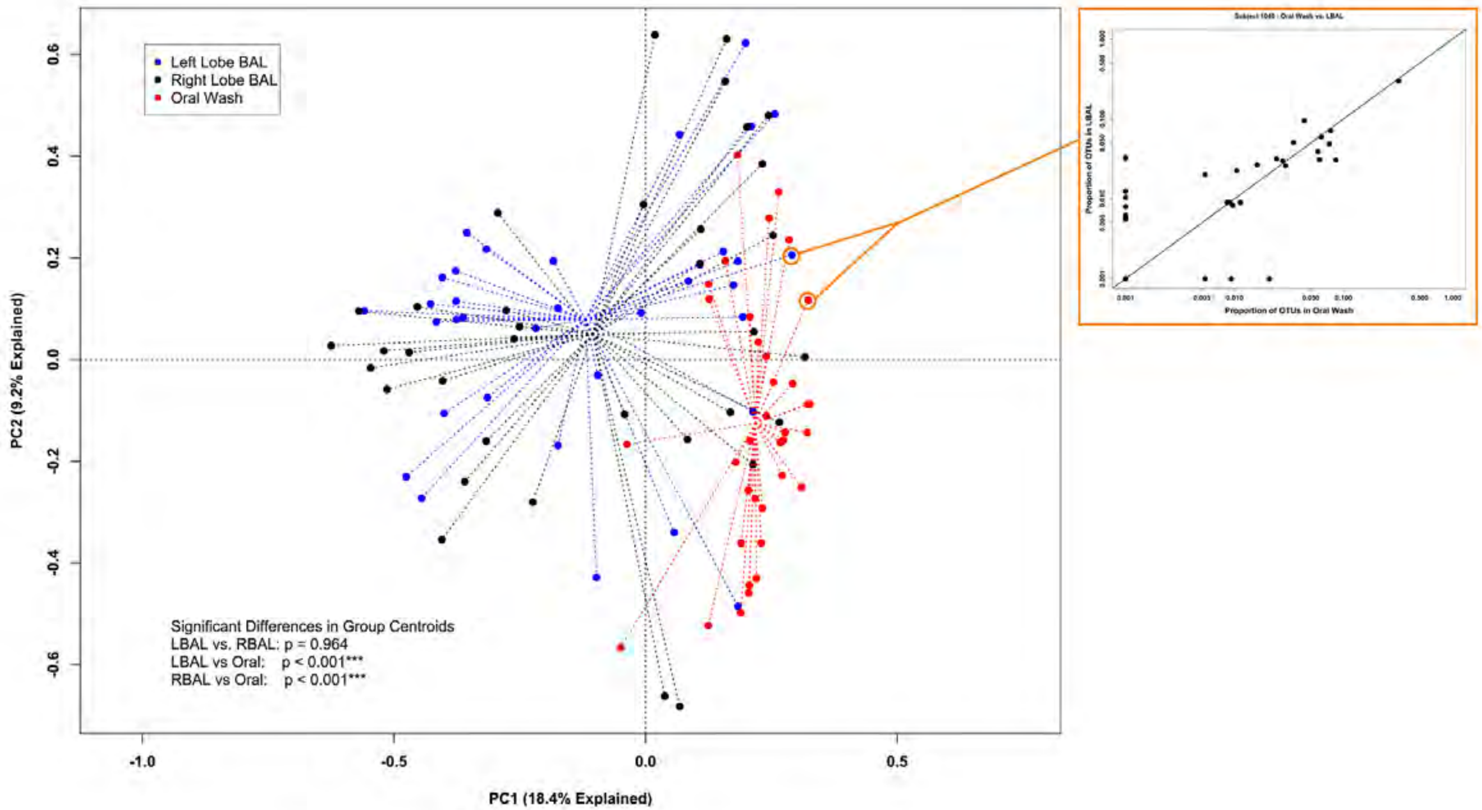
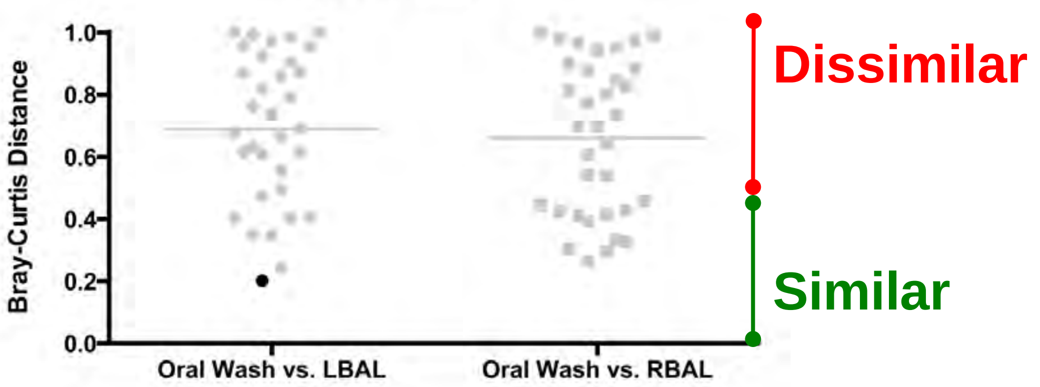
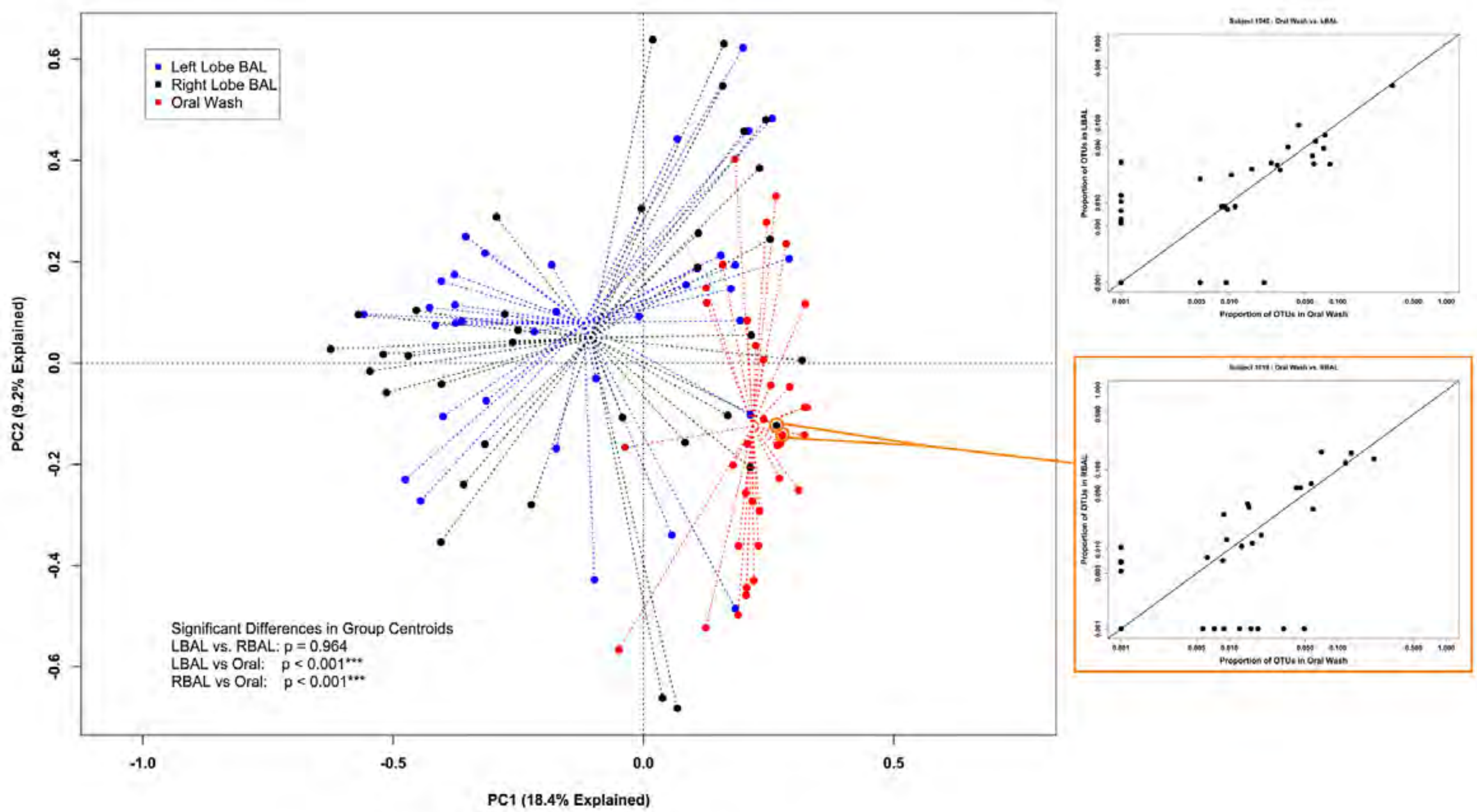

Left Lobe BAL
Right Lobe BAL
Oral Wash
Significant Differences in Group Centroids
LBAL vs. RBAL: p = 0.964
LBAL vs Oral: p < 0.001***
RBAL vs Oral: p < 0.001***
PC2 (9.2% Explained)
PC1 (18.4% Explained)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-1.0
-0.5
0.0
0.5


Bray-Curtis Distance
1.0
0.8
0.6
0.4
0.2
0.0
Oral Wash vs. LBAL
Oral Wash vs. RBAL
Dissimilar
Similar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Proportion of OTUs in Oral Wash
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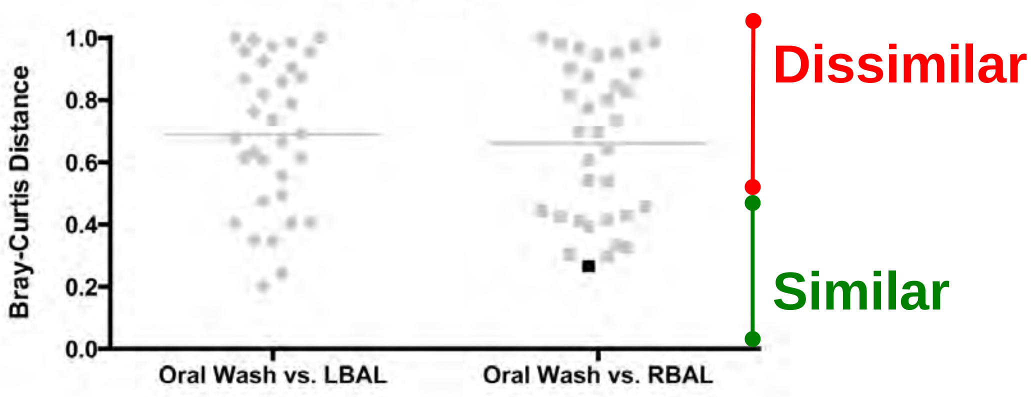
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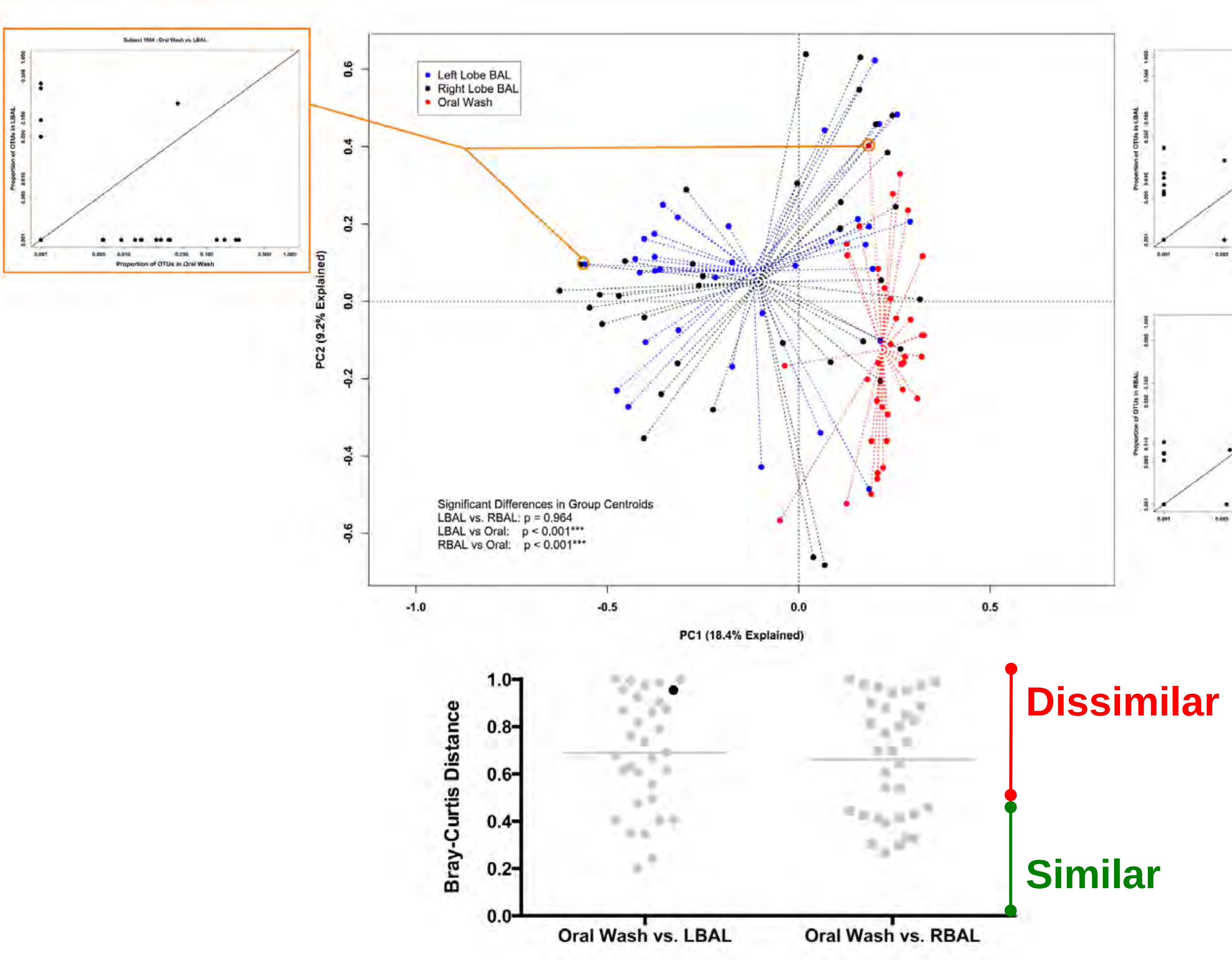
Similar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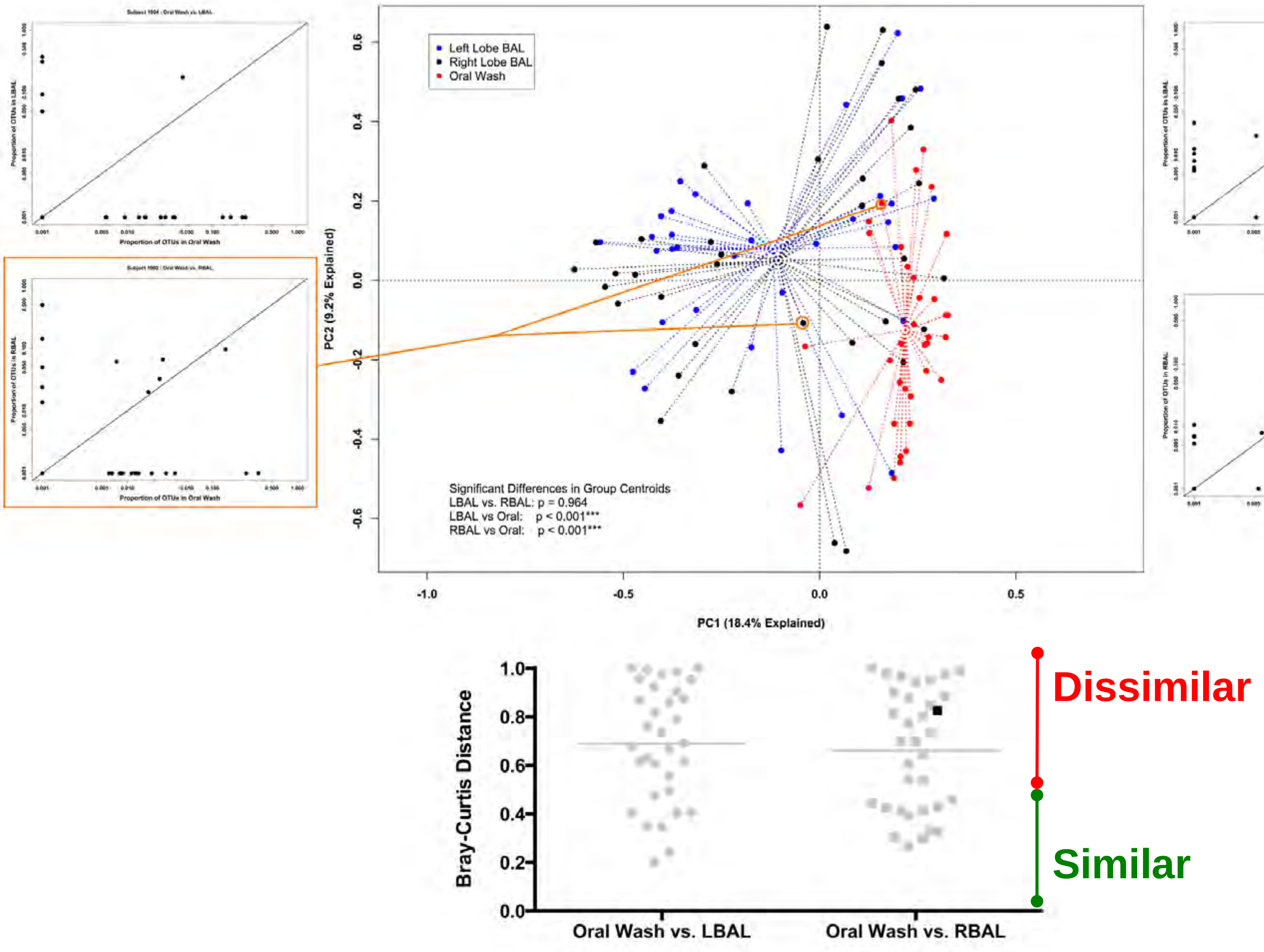
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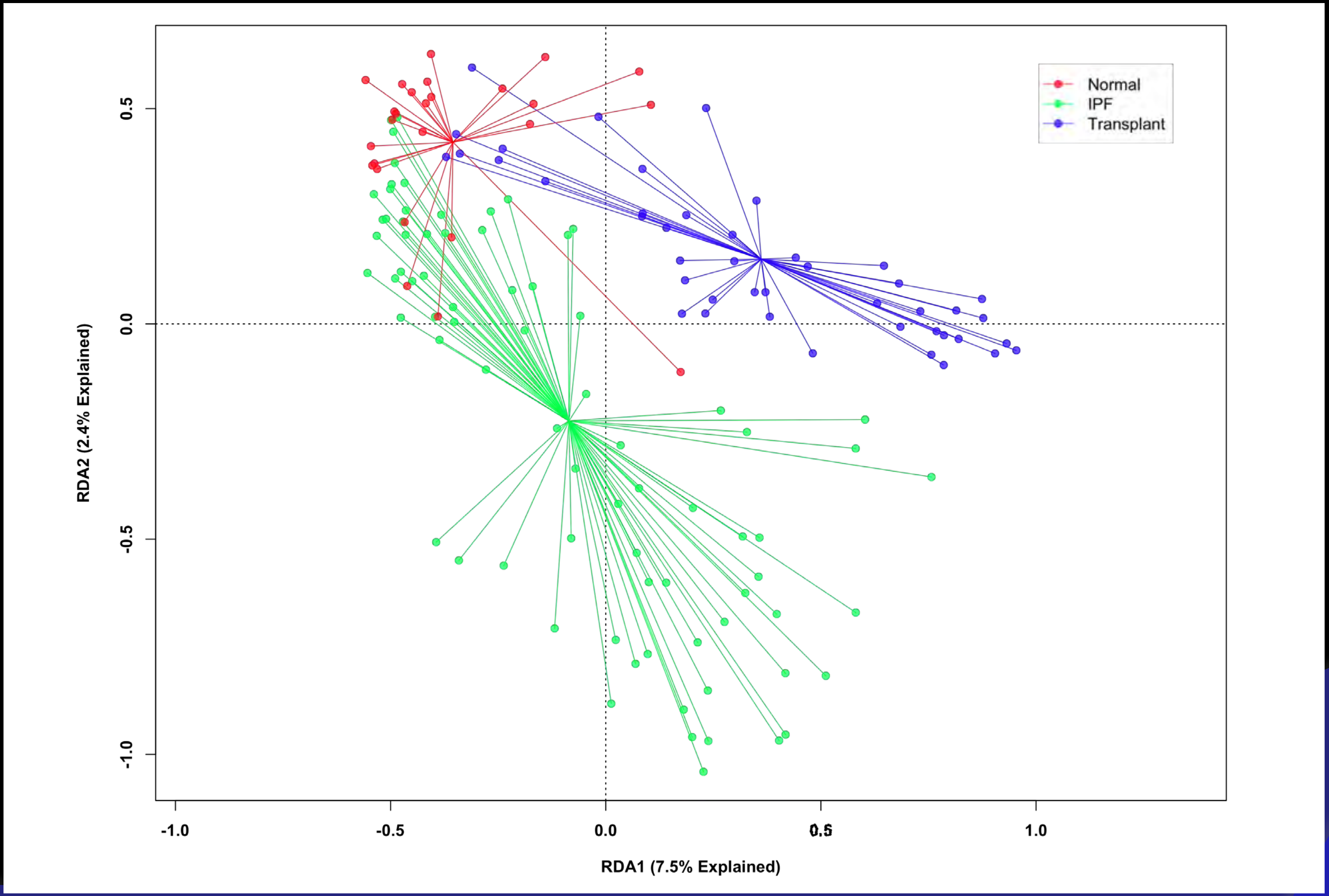
Oral Wash vs. LBAL
Oral Wash vs. RBAL
Dissimilar
Similar

When healthy, the microbial load in the lungs is low and BAL samples contain a predominance of bacterial taxa also found in the mouth.

Bacterial diversity in the lungs is very low.

In some individuals, there are some differences suggesting that selective pressures exist in the lungs for elimination, persistence, colonization and growth.

# Comparison of the BAL Microbiome in Healthy Non-Smoker, IPF & Lung Transplant Subjects



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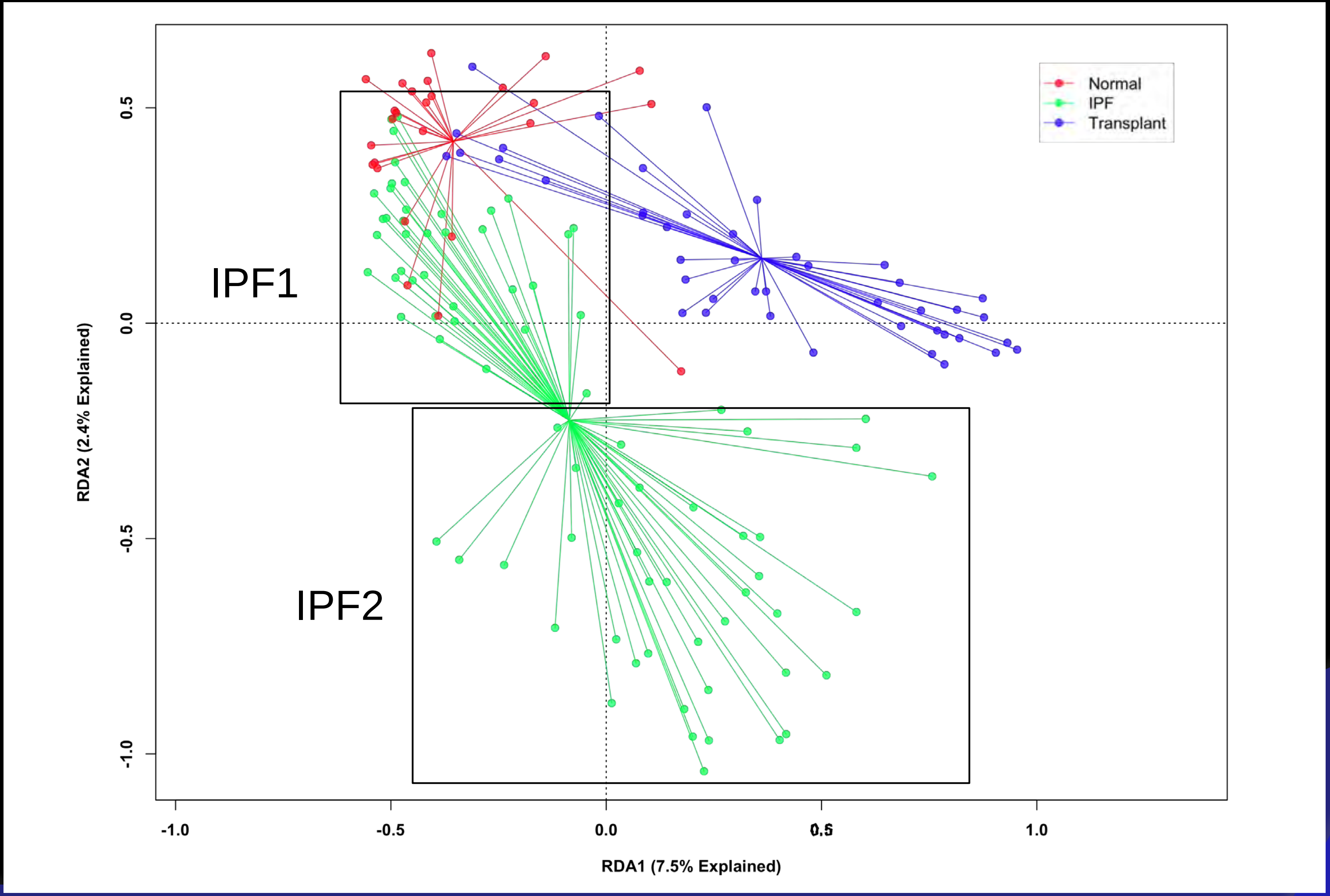
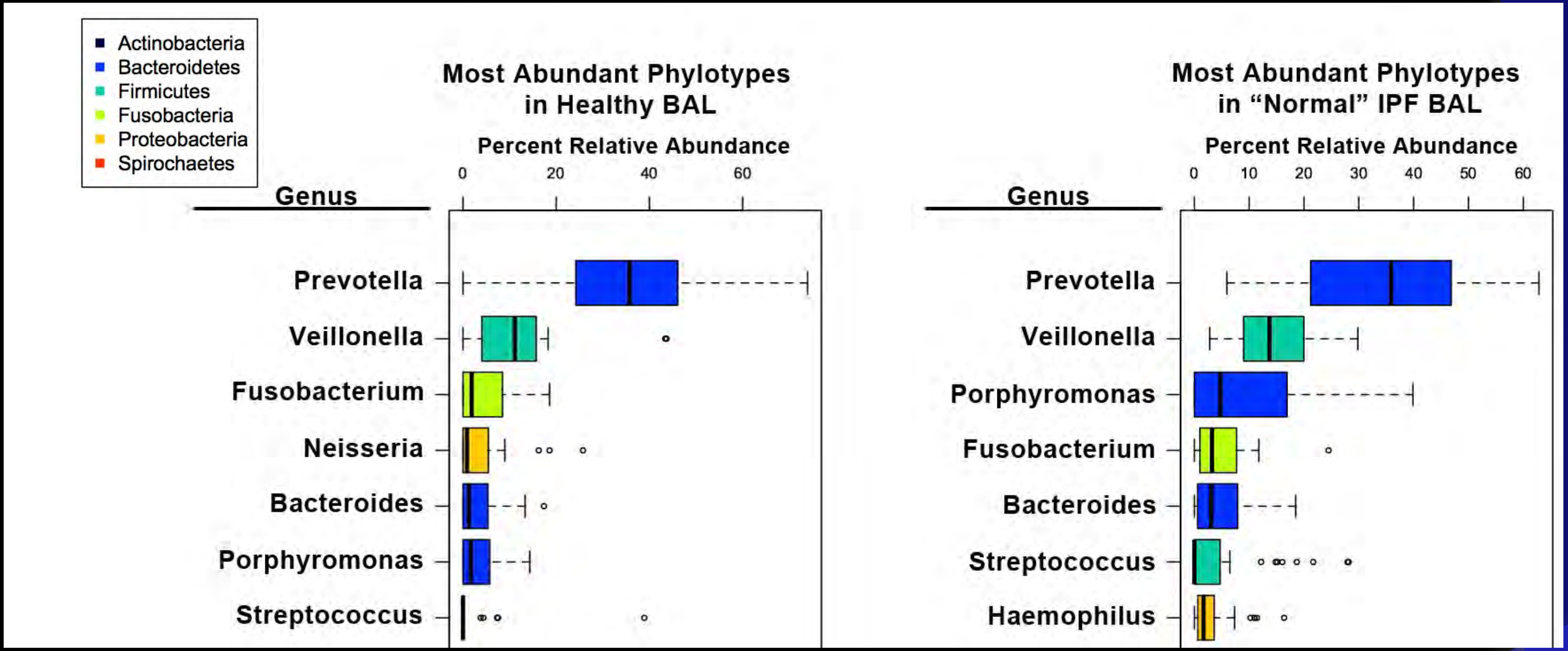


# Genus-level OTU Cluster Analysis of the BAL Microbiome in Healthy NS and IPF1 Subjects



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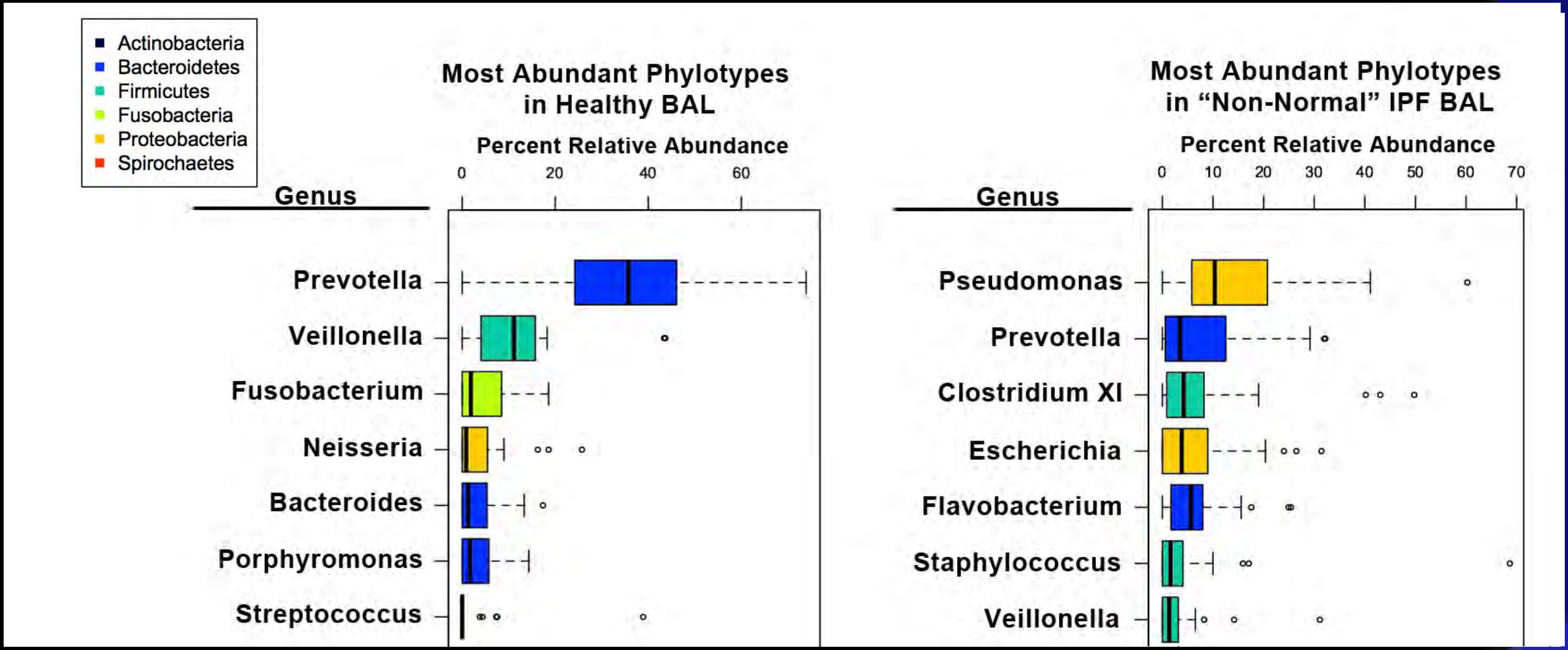
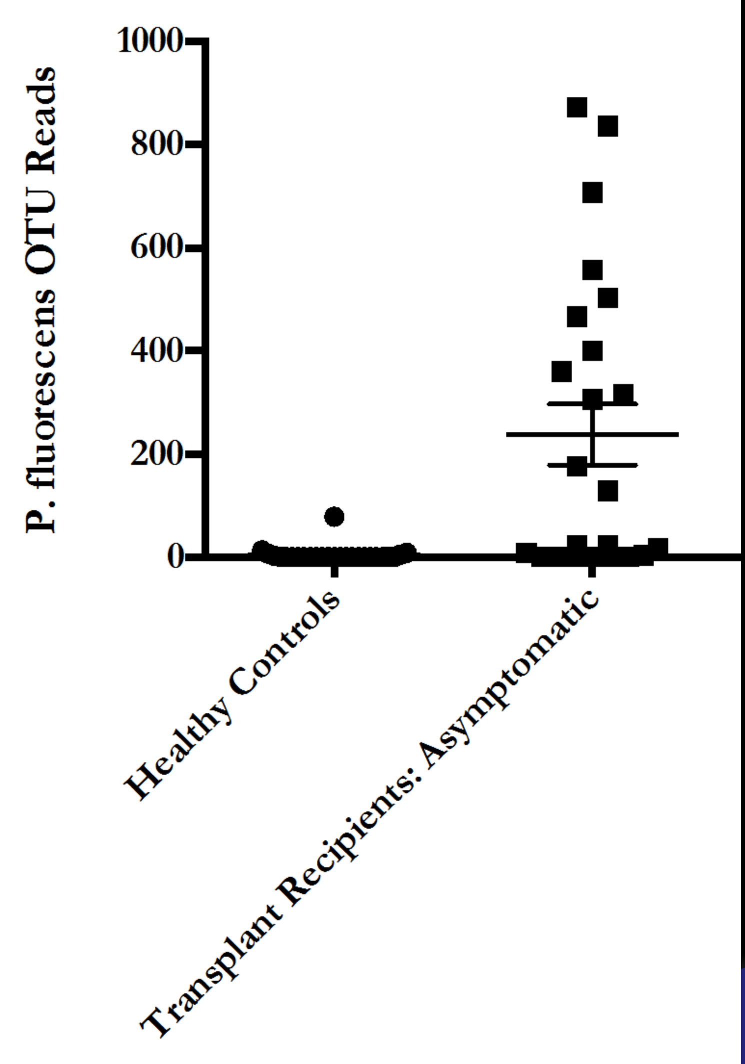
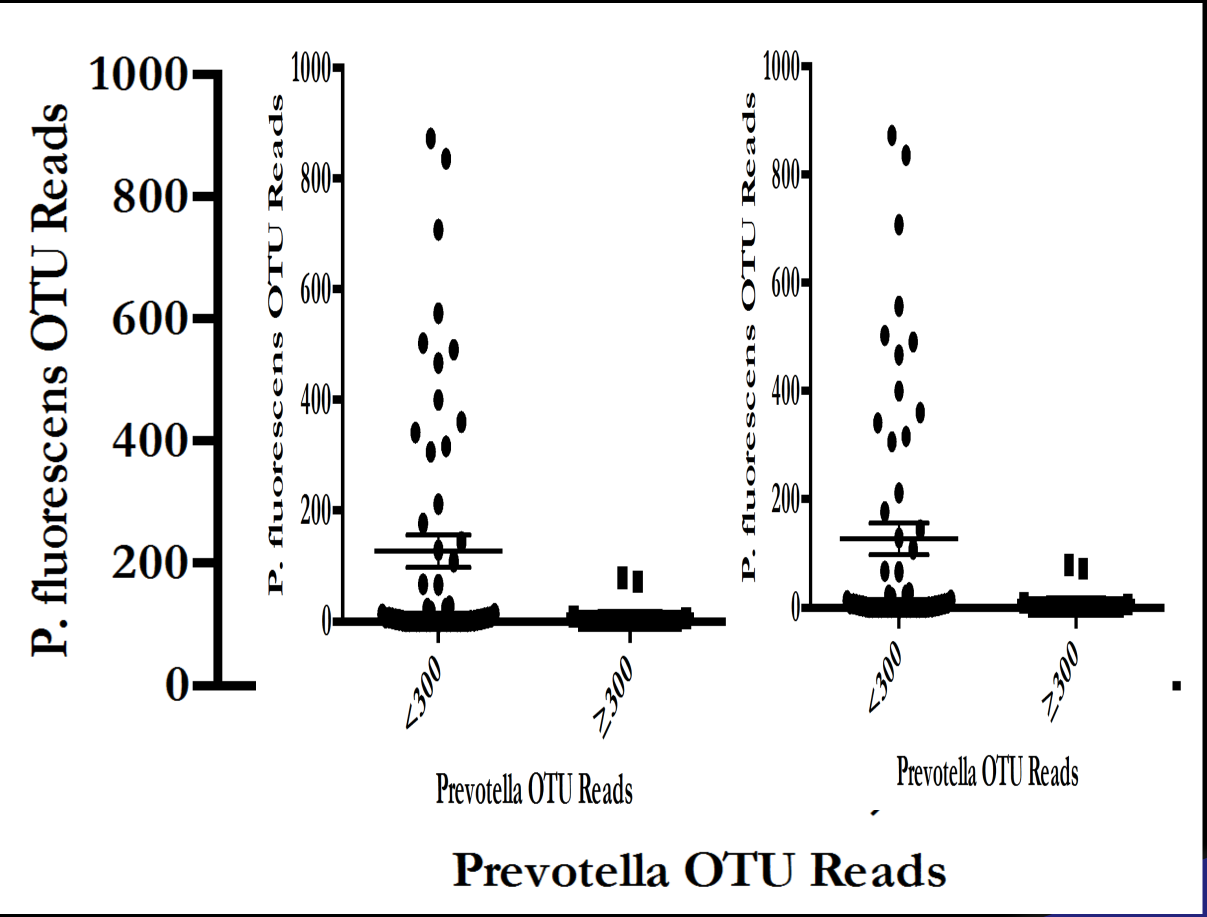


# The Lung Microbiome Following Lung Transplantation




1500 reads/sample

# The Lung Microbiome Following Lung Transplantation




1500 reads/sample

When diseased, the microbial load in the lungs increases and BAL samples now often contain numerous bacterial taxa not found in the mouth, indicating selective pressures in the lungs for persistence, colonization and growth.

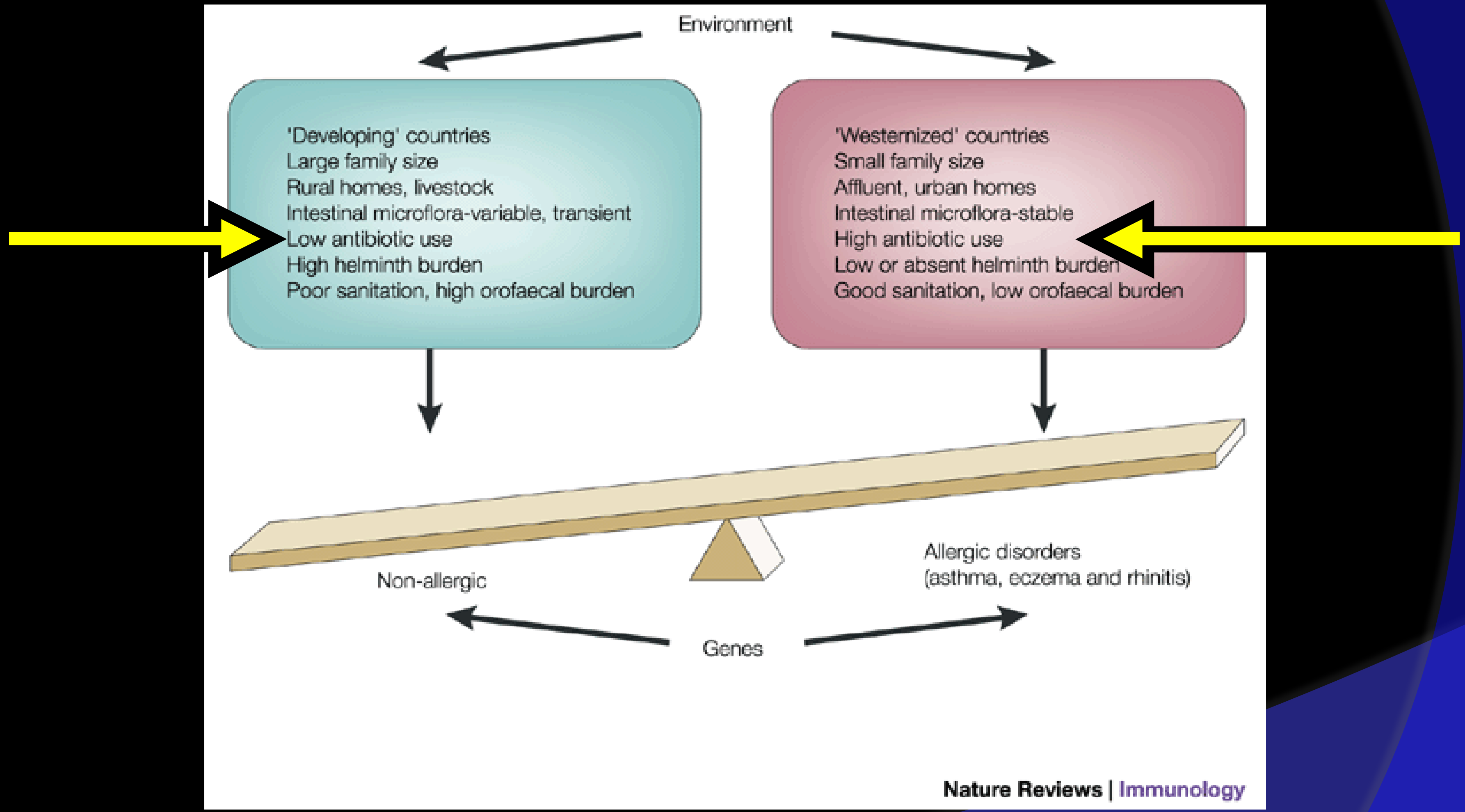
CHALLENGE

- Studies only involving human subjects will NEVER demonstrate causality, no matter how large the cohort.  In vivo (animal) studies, model organisms, and in vitro experiments are needed to delineate mechanisms.

NEED

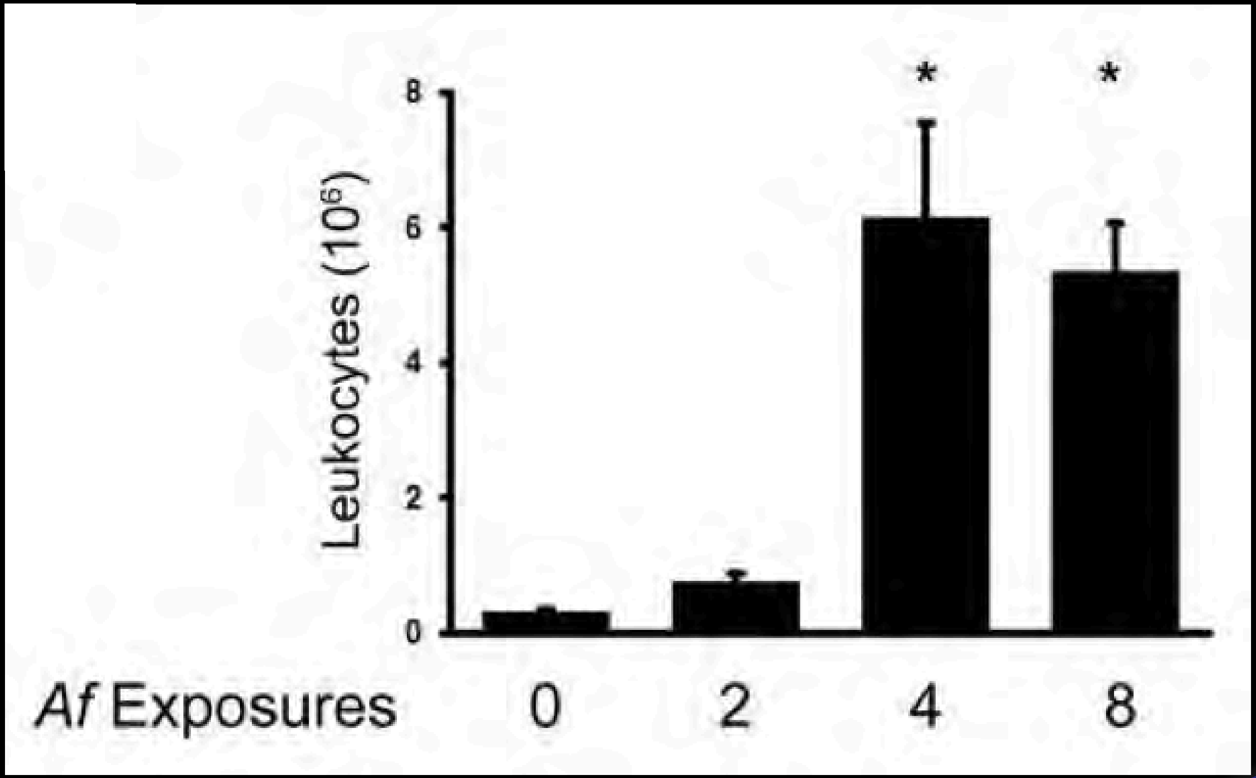
- Support for animal models in the study of the human microbiome

# The Hygiene Hypothesis




Wills-Karp et al. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Imm 1, 69-75 (2001)

# The Development of Pulmonary Inflammation Following Repeated *Aspergillus fumigatus* Conidia Exposure




Murdock et al. Infect Immun. 2011 Jan;79(1):125-35.
Shreiner et al. Infect Immun. 2012 Jan;80(1):388-97

# Effect of Chronic Pulmonary Inflammation on Changing the Lung Microbiota

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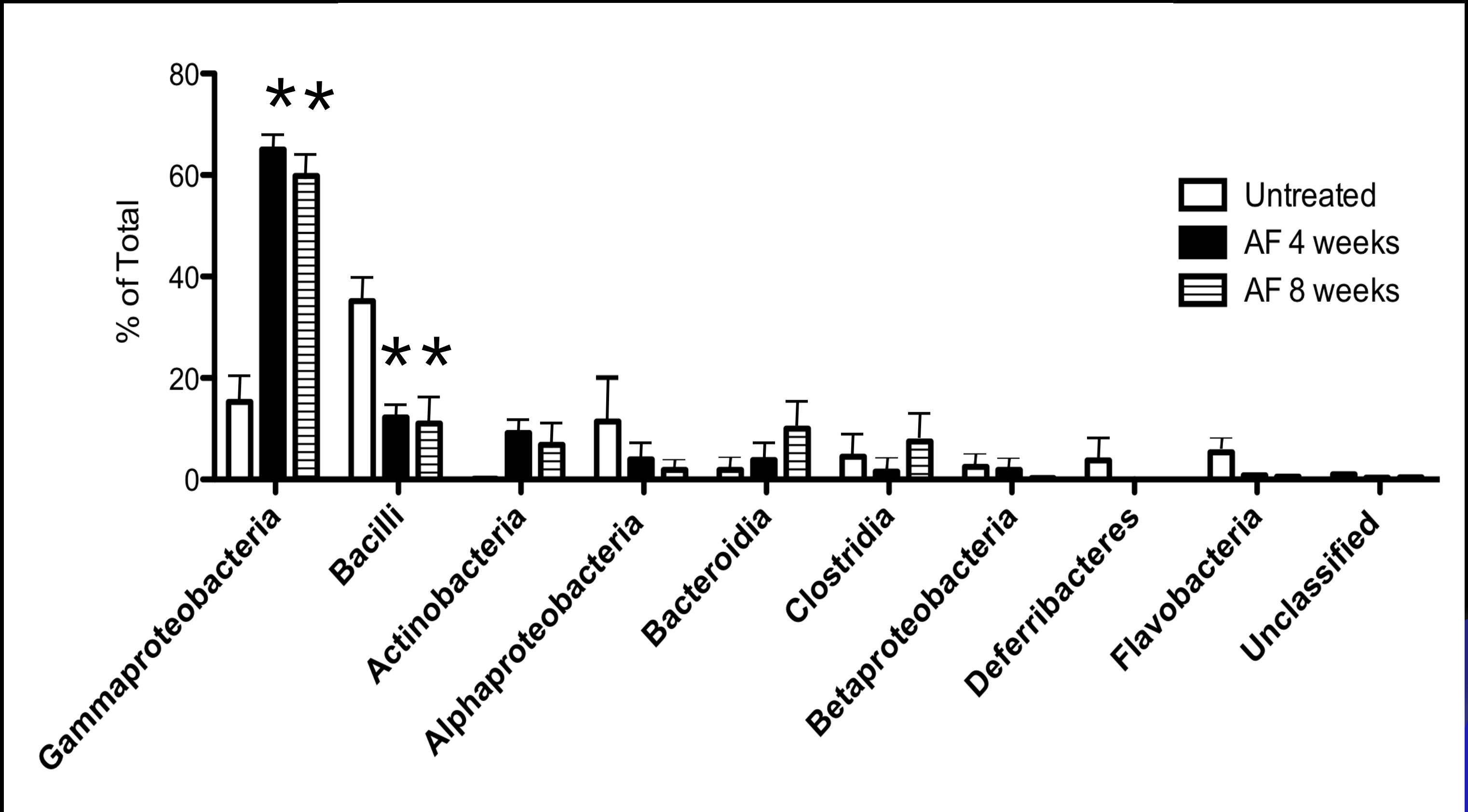

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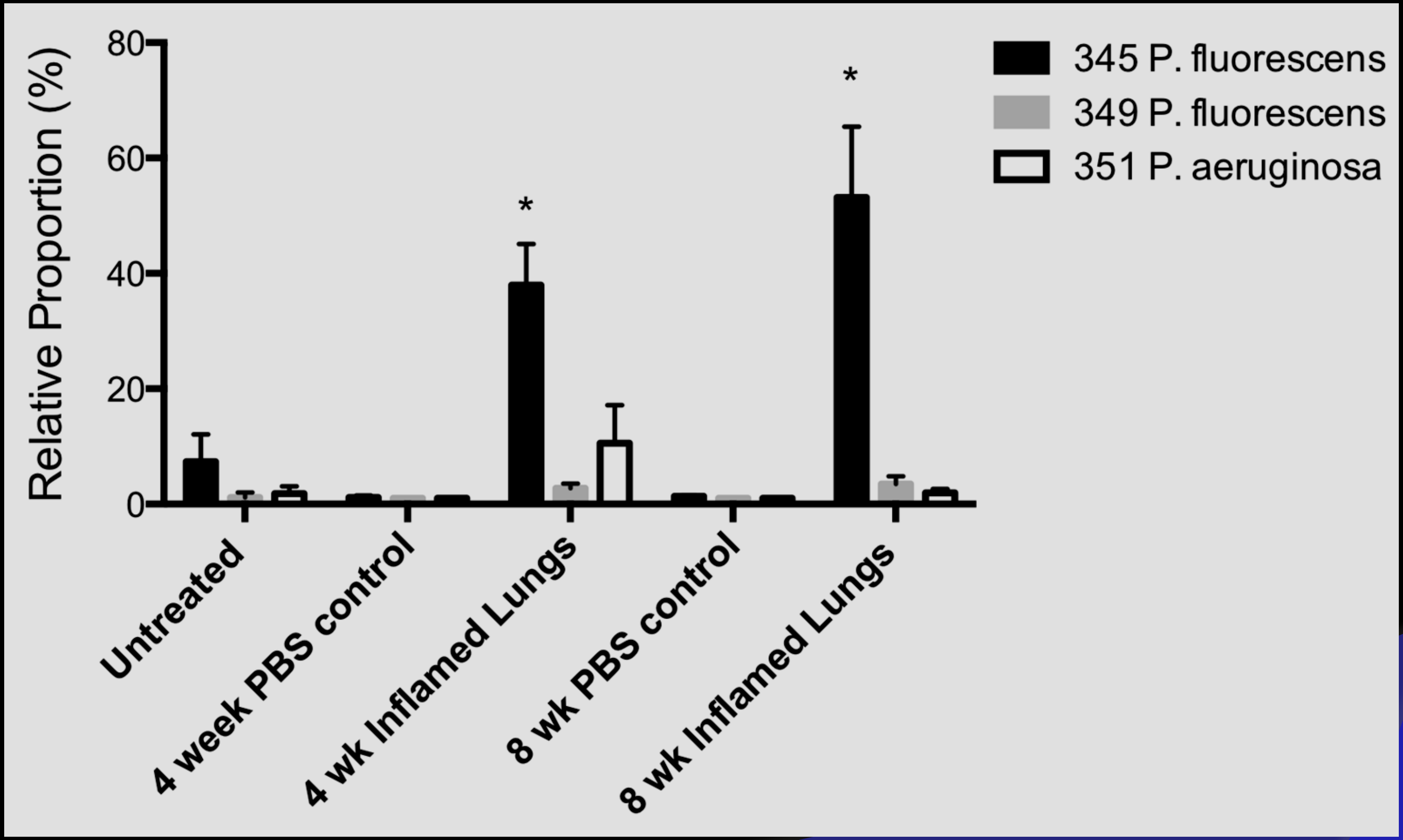
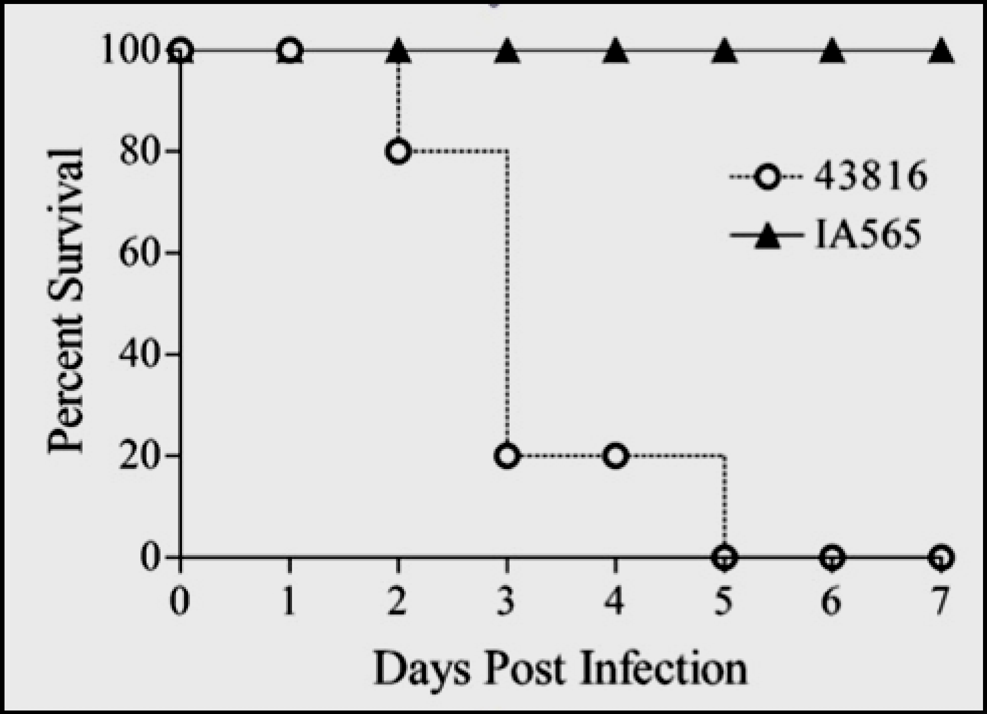


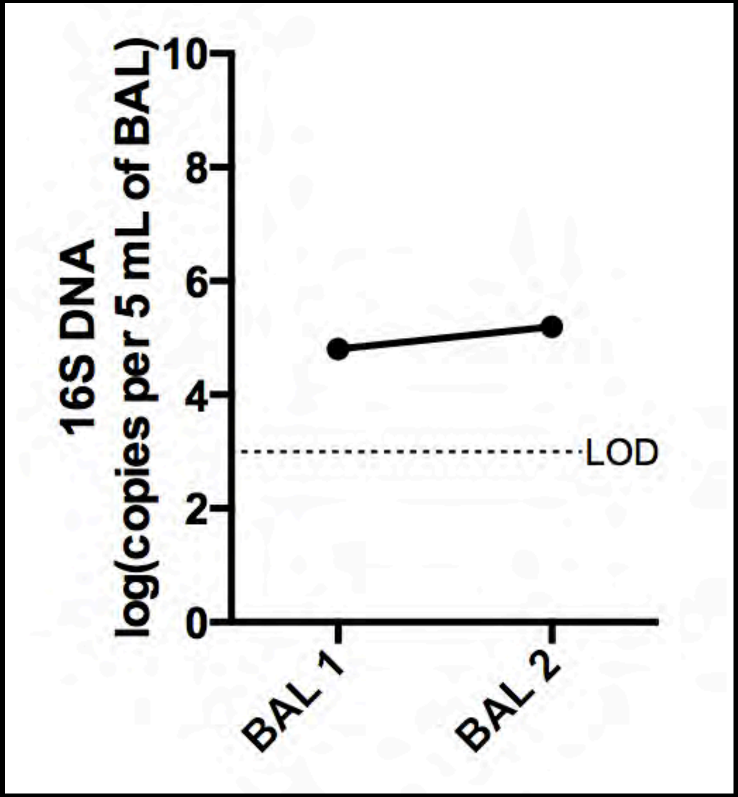
# *Klebsiella pneumoniae* as an etiologic agent of pneumonia

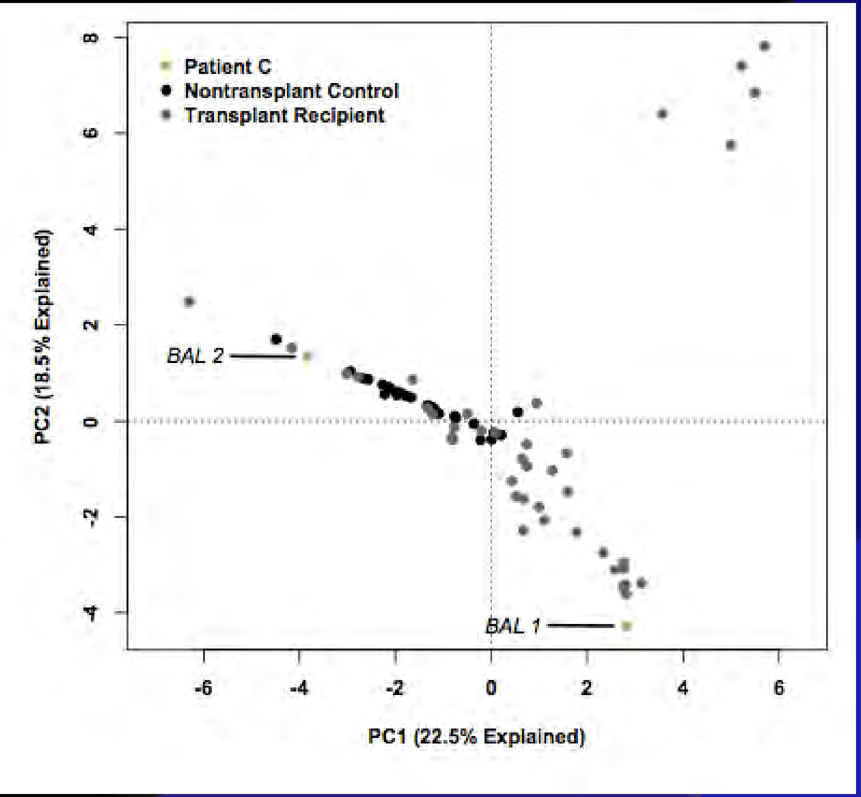
Two isolates of *K. pneumoniae* grown from two patients with presumed gram negative pneumonia (43816 and IA565)



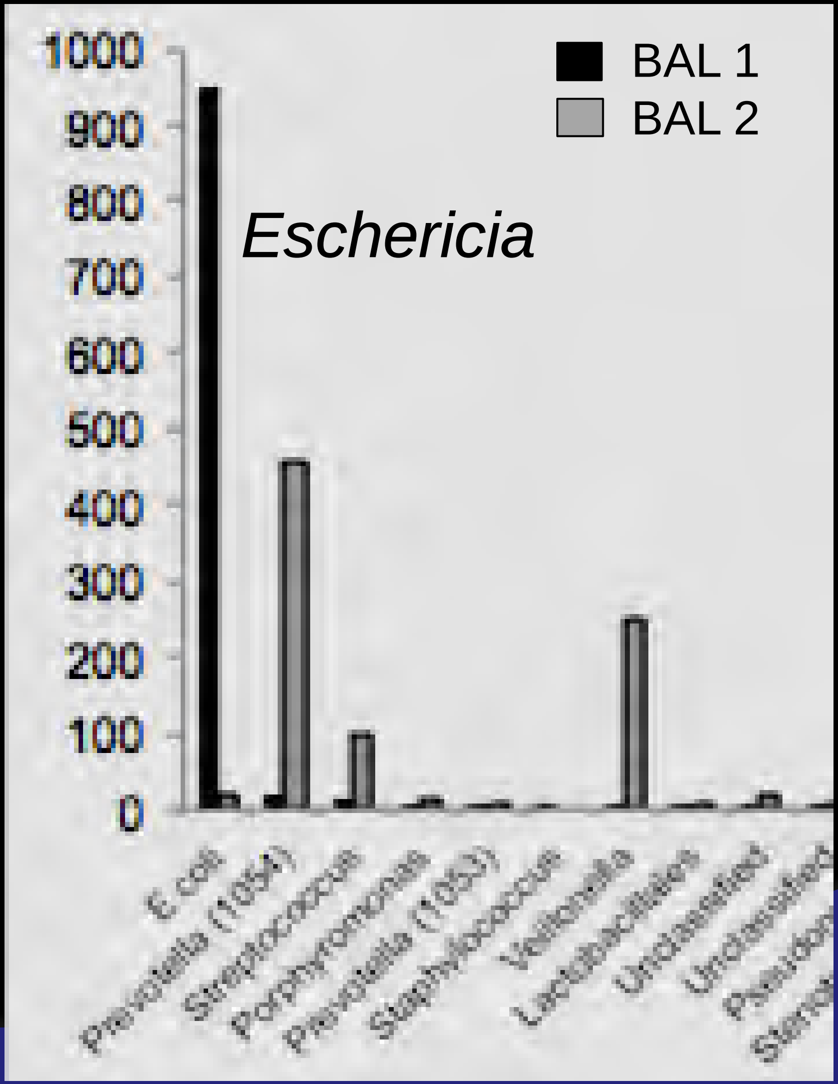

Lau HY et al. Microb Pathog. 2007;42(4):148-55.
Lau HY et al. Microbes Infect. 2008;10(12-13):1283-90.

# Pyrosequencing of a culture-negative pneumonia



# Pyrosequencing of a culture-negative pneumonia



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## GAPS

- understanding the implications of culturable and non-culturable states of bacteria in the lungs
- visualization of microbes in the lungs

## NEEDS

- new cultivation strategies

# OVERALL CHALLENGES

- more consistent and supportive peer review of microbiome/lung proposals
- accepting that sampling of the lower respiratory tract in humans will be imperfect and utilization of animal models to close the gap

# The Research Group

John Erb-Downward
Brittan Scales
Bob Dickson
Nicole Falkowski
Rod McDonald
Dayana Rojas
Amir Sadighi Akha
Andrew McDermott

Andrew Shreiner
Ryan Muraglia
Katy Higdon
Zach Britt

Fernando Martinez
Vincent Young
Jeff Curtis
Vibha Lama
MeiLan Han
Margaret Gyetko
Pat Schloss
Christine Freeman
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# The future of internal medicine…how will you think about disease?

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