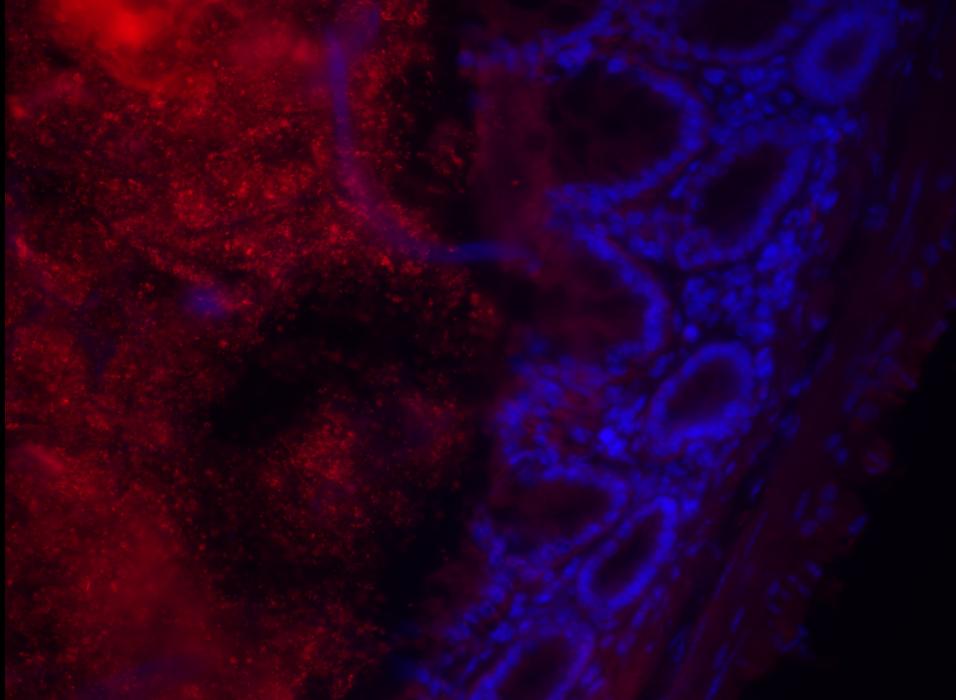


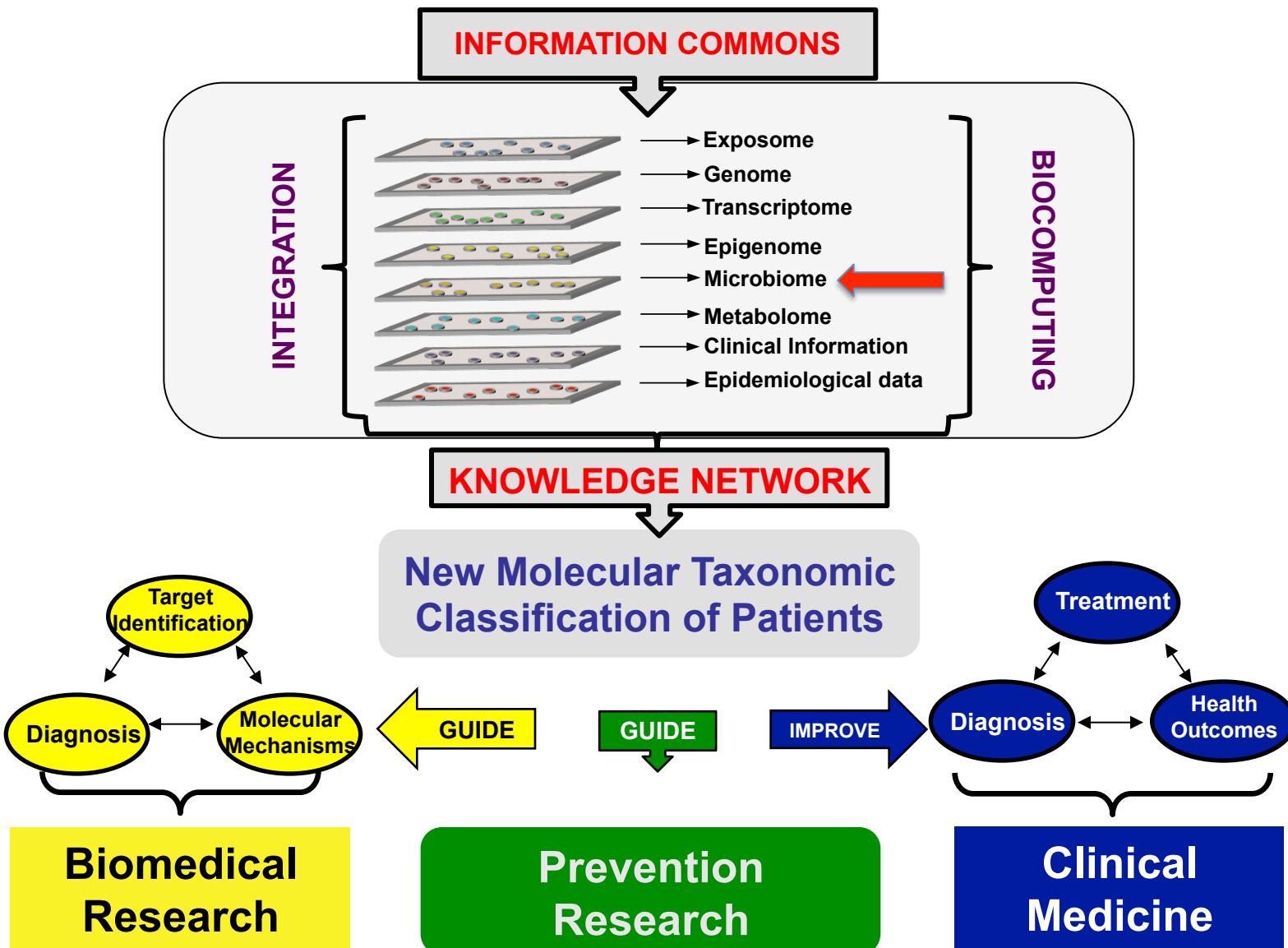
# Unraveling the microbiome to improve precision medicine for cancer diagnosis and prognosis



K. Leigh Greathouse, PhD, MPH, RD  
Laboratory of Human Carcinogenesis  
National Cancer Institute

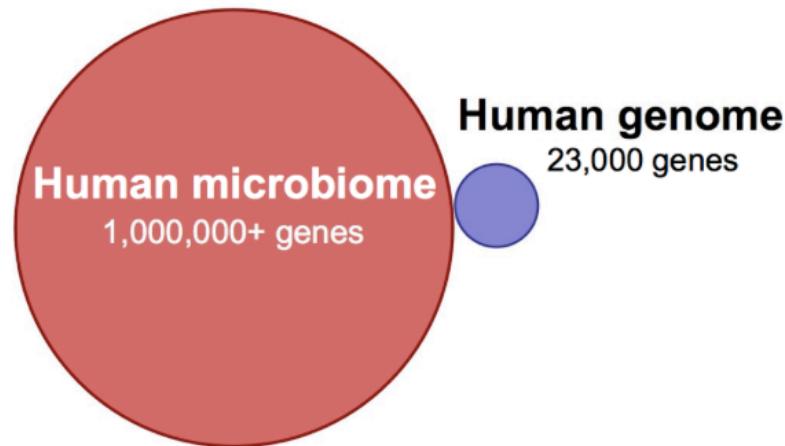


# A Precision Medicine Research Strategy



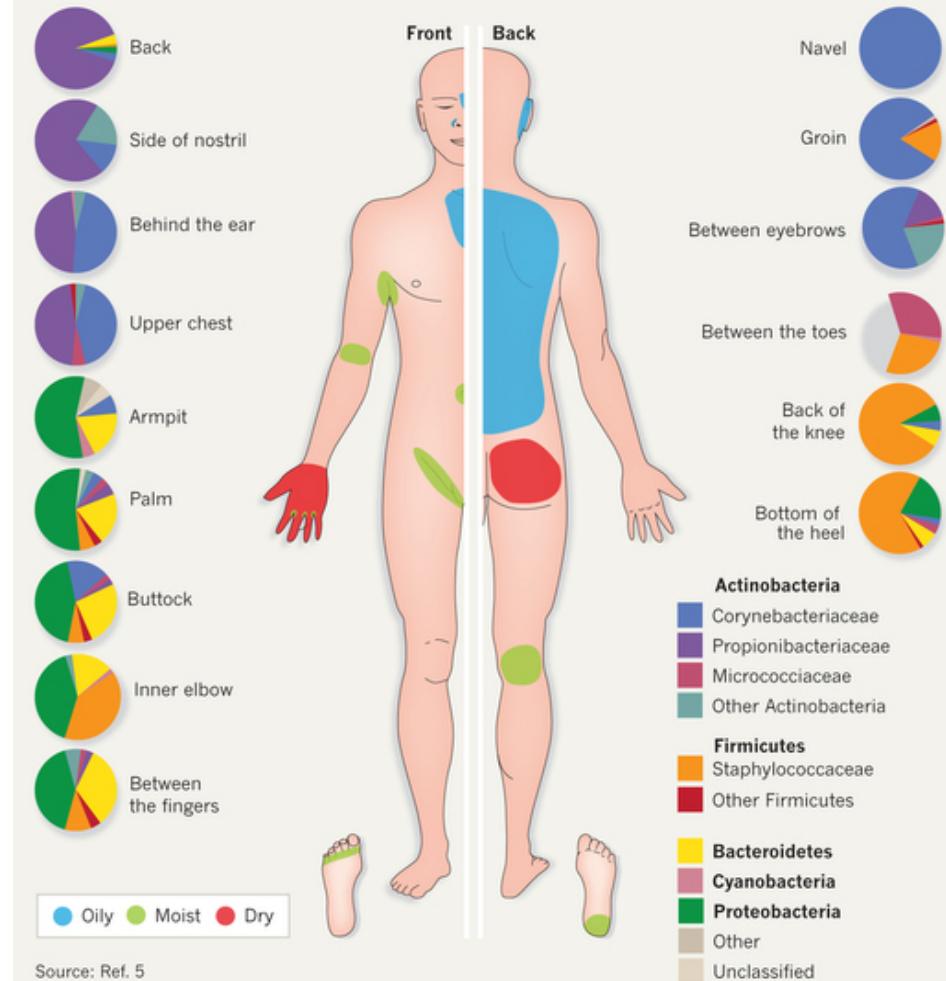
Modified: Toward Precision Medicine, National Research Council, National Academy of Science, 2011

# What *is* the human microbiome?



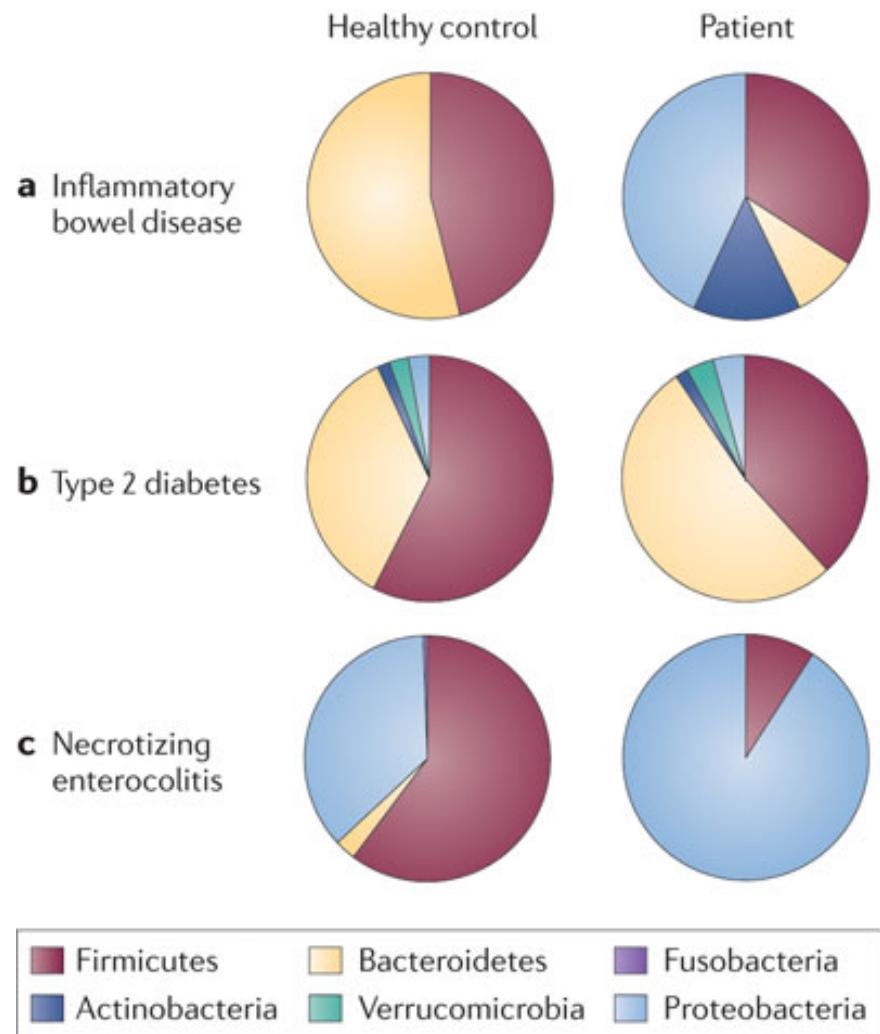
## MICROBIOME MAP

The human skin is rich with bacteria. The population and ratios vary by region, and depend on the whether the skin site is oily, moist or dry.



# What is the importance of the microbiome in disease?

- **Dysbiosis** – loss of commensal and gain of pathogenic microbes
- Generally **loss of diversity** is a characteristic of the disease state
- Characterizing microbial **biomarkers for disease prediction** (i.e. eczema)



# The microbiome: diagnosis and treatment of disease

## As a mechanism to ameliorate infection:

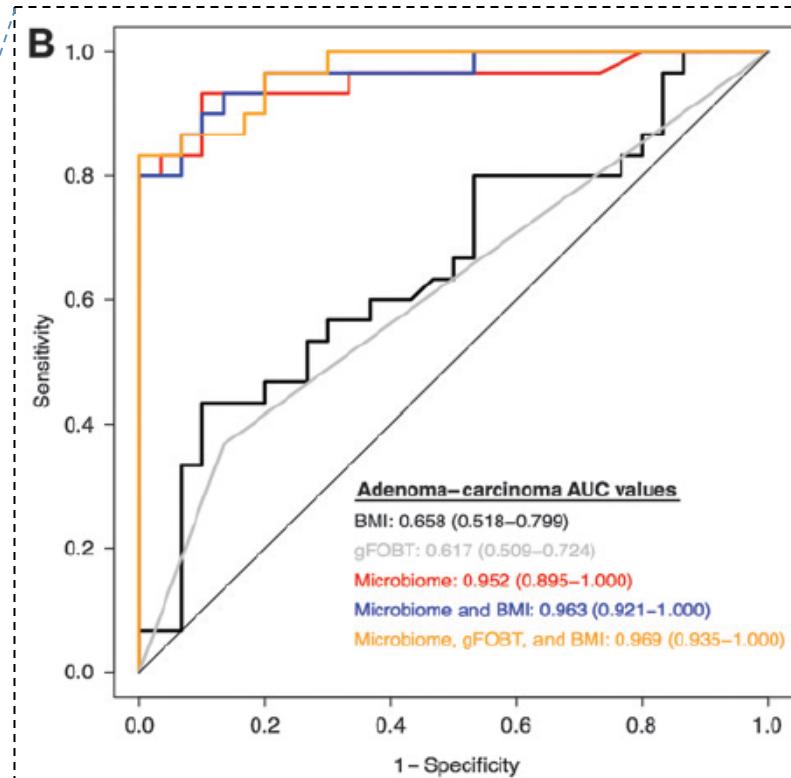
Stool substitute transplant therapy for the eradication of **Clostridium difficile** infection: 'RePOOPulating' the gut. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. *Microbiome*. 2013 Jan 9;1(1):3. doi: 10.1186/2049-2618-1-3.

## As a therapeutic for inflammatory bowel disease:

Intra-luminal Lactococcus lactis expressing interleukin (IL)-27 is a potential future therapeutic for inflammatory bowel disease. McLean MH, Hanson M, Gold B, Golubeva Y, Anver M, Wu X, Sun D, Steidler L, Durum S. 14<sup>th</sup> Annual NCI CCR Fellow and Young Investigators Colloquium. 2014.

## As a screening tool for colorectal cancer:

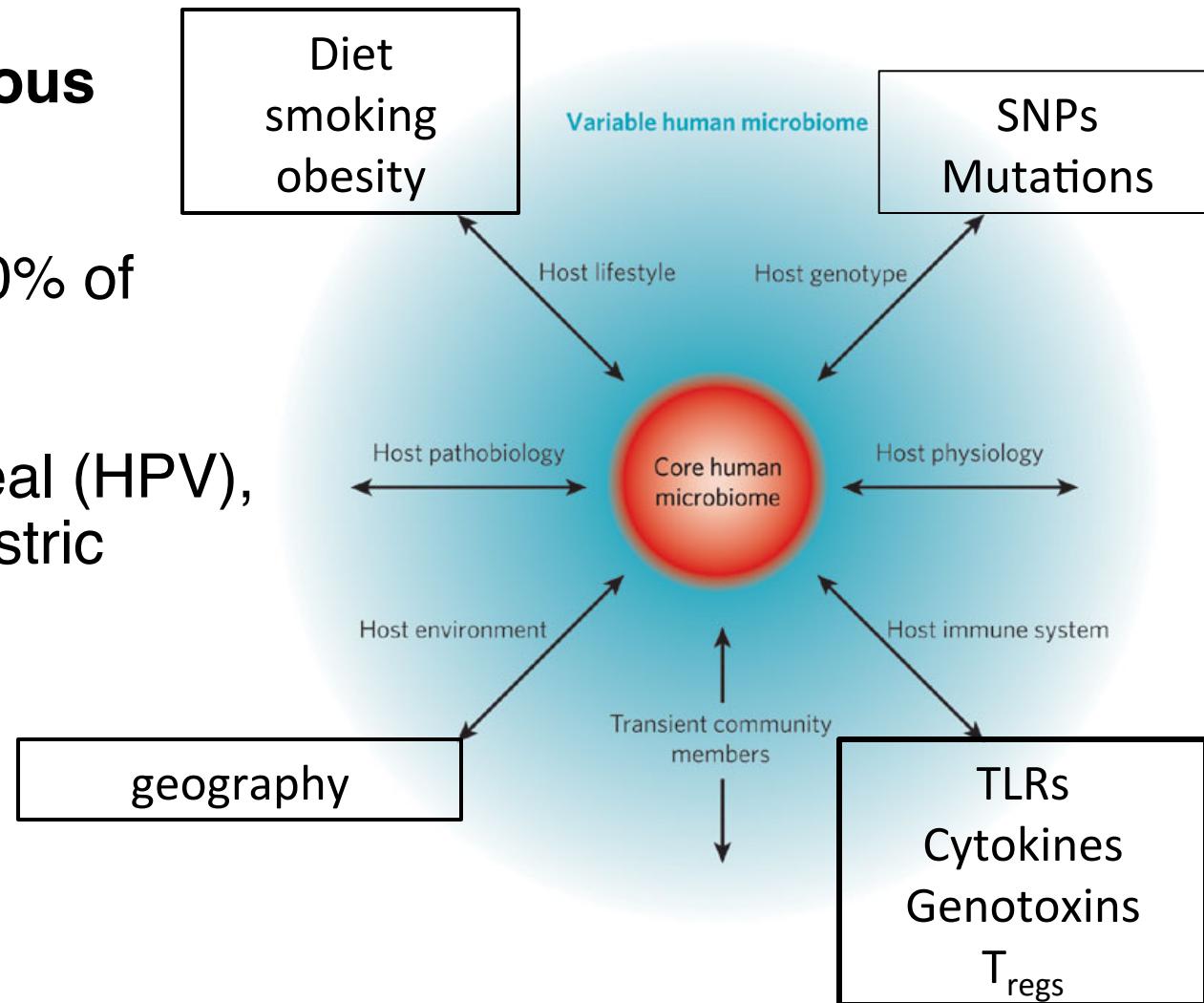
The Human Gut Microbiome as a Screening Tool for Colorectal Cancer. Joseph P. Zackular, Mary A.M. Rogers, Mack T. Ruffin IV, et al. *Cancer Prev Res* Published OnlineFirst August 7, 2014.



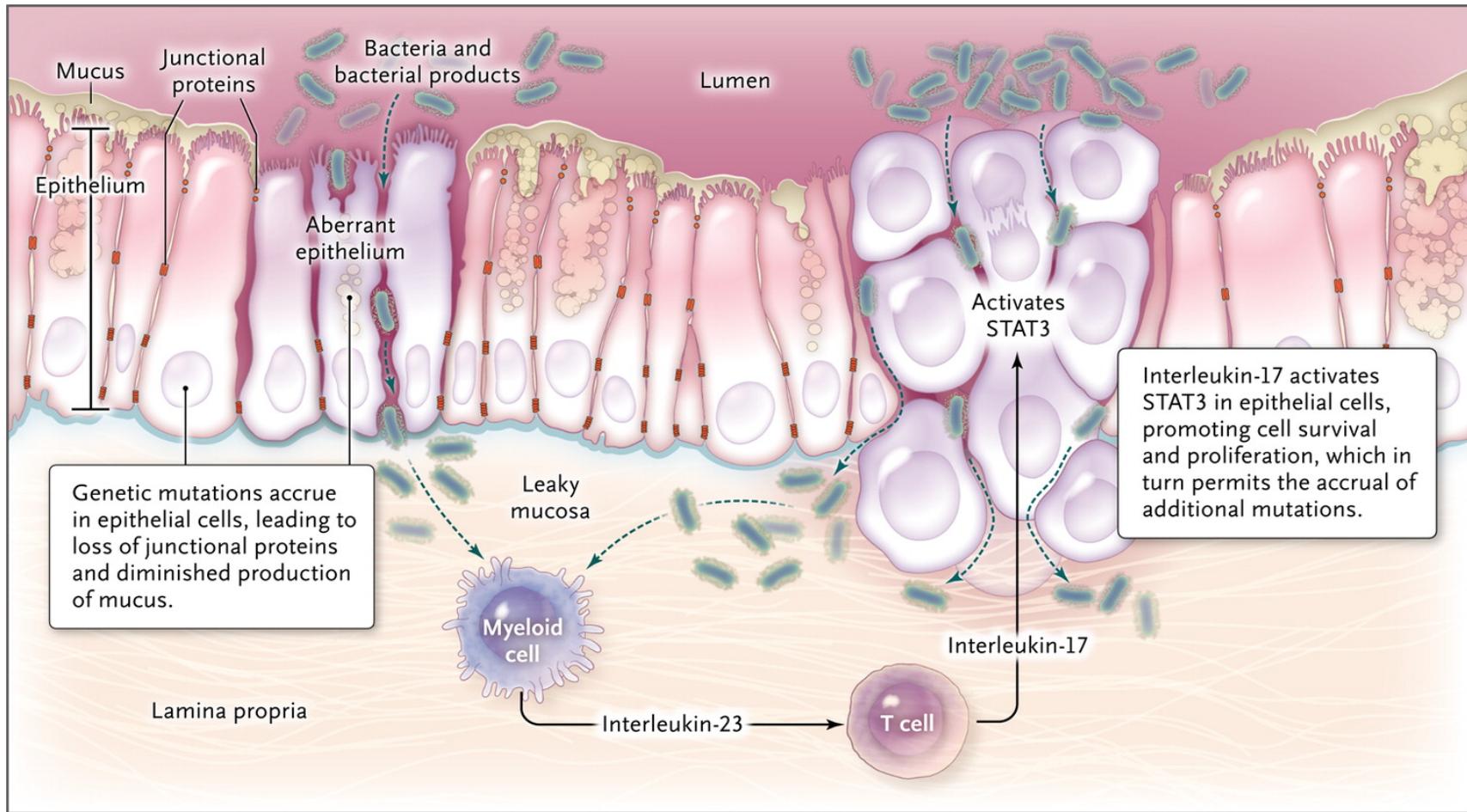
# Microbiome and Cancer: microbiome-environment interactions

## Microbes as infectious agents:

- Account for ~15-20% of cancers worldwide
- Cervical/Esophageal (HPV), liver (HBV) and gastric cancers (*H. pylori*)



# Inflammatory Model of the Initiation of Colorectal Cancer



Gallimore AM, Godkin A. N Engl J Med 2013;368:282-284.



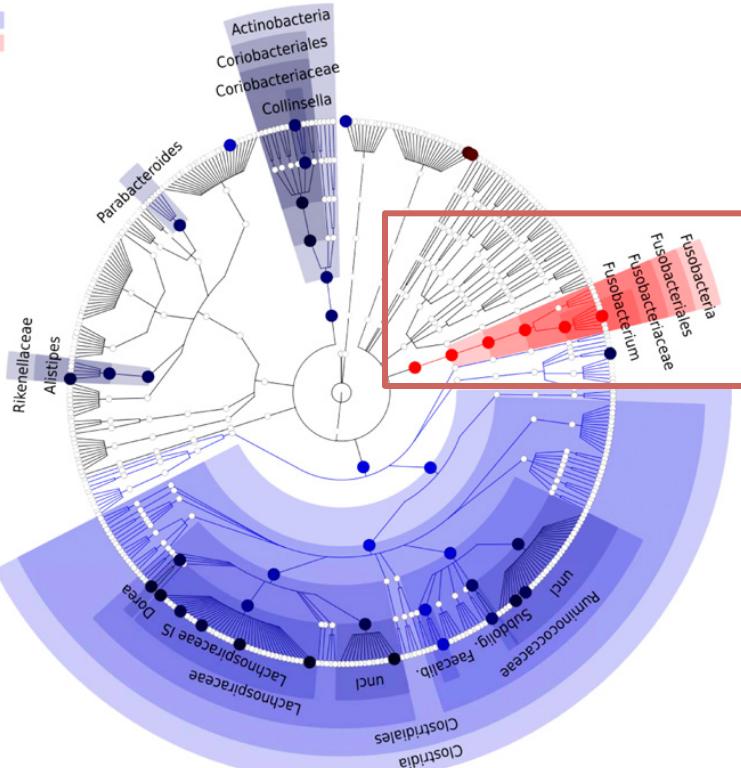
The NEW ENGLAND  
JOURNAL of MEDICINE

# Bacteria-associated inflammation in cancer

## Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma

Aleksandar D. Kostic, Dirk Gevers, Chandra Sekhar Pedamallu, et al.

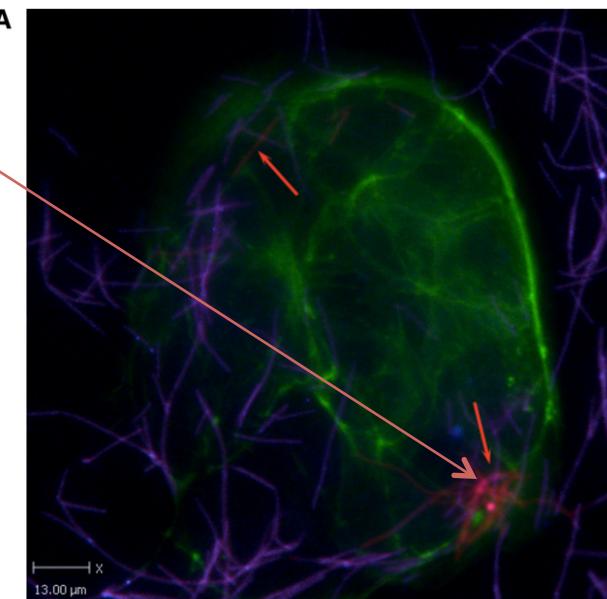
Genome Res. 2012 22: 292-298 originally published online October 18, 2011  
Access the most recent version at doi:[10.1101/gr.126573.111](https://doi.org/10.1101/gr.126573.111)



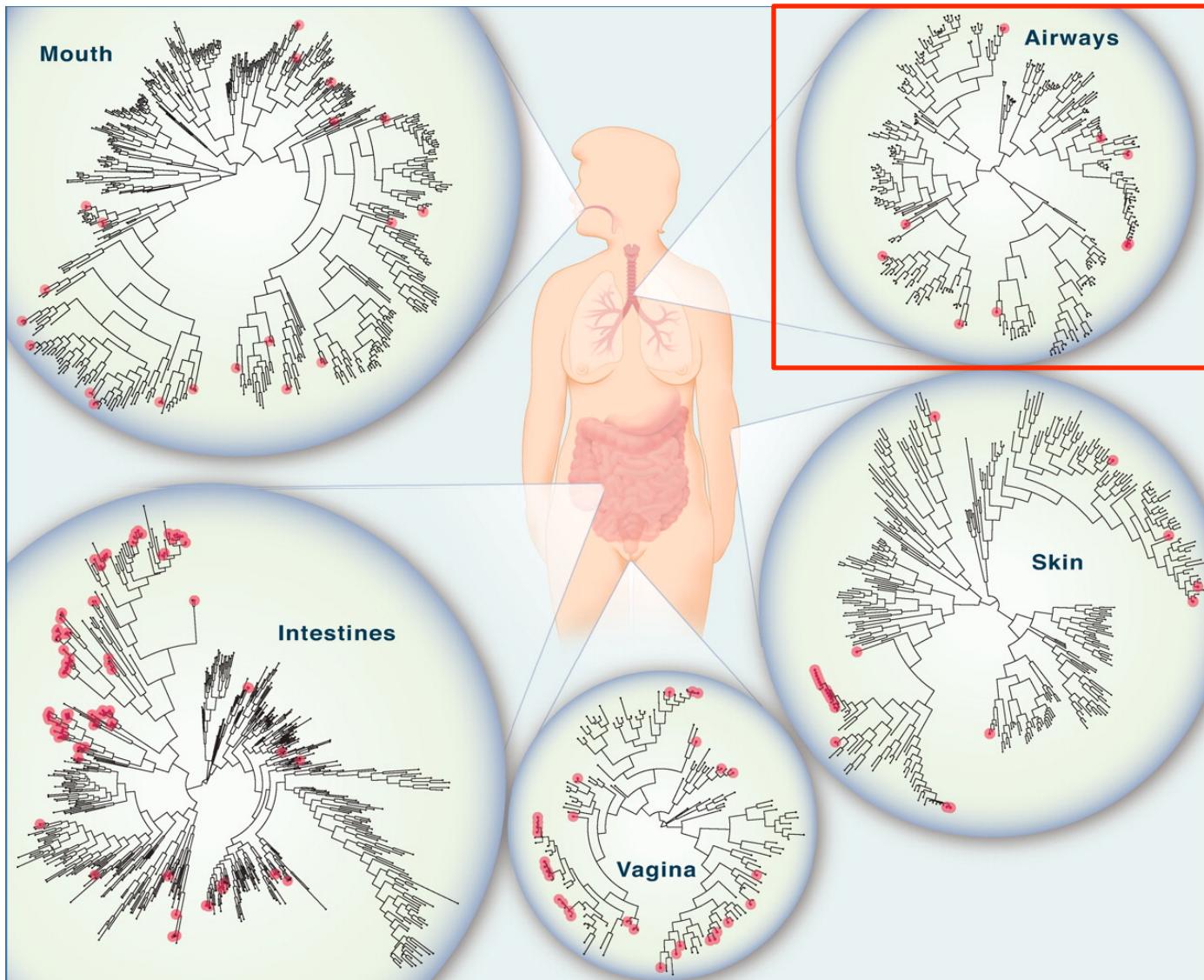
## *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma

Mauro Castellarin, René L. Warren, J. Douglas Freeman, et al.

Genome Res. 2012 22: 299-306 originally published online October 18, 2011  
Access the most recent version at doi:[10.1101/gr.126516.111](https://doi.org/10.1101/gr.126516.111)



# The Lung Cancer Microbiome



# Lung Cancer Microbiome Study Design

## Study Populations:

### *NCI-MD Case-Control Study*

- 398 frozen tumor (T) and non-tumor adjacent (NT) tissues (121 T/NT pairs)
- 41 frozen lung tissue from immediate autopsy (IA) - control
- 16 frozen lung tissue biopsies (non-cancerous) from the Lung Cancer Biorepository Research Network (LCBRN) – control
- Negative controls
  - 11 different swabs taken from pathology to assess possible contamination
  - no-template water controls for sequencing and DNA extraction reagents
- Positive control – mock community (High Even) of known bacteria and known concentrations
- Data from interview taking at time of study entry used for statistical analysis

# Lung Cancer Microbiome Study Design

## Study Populations:

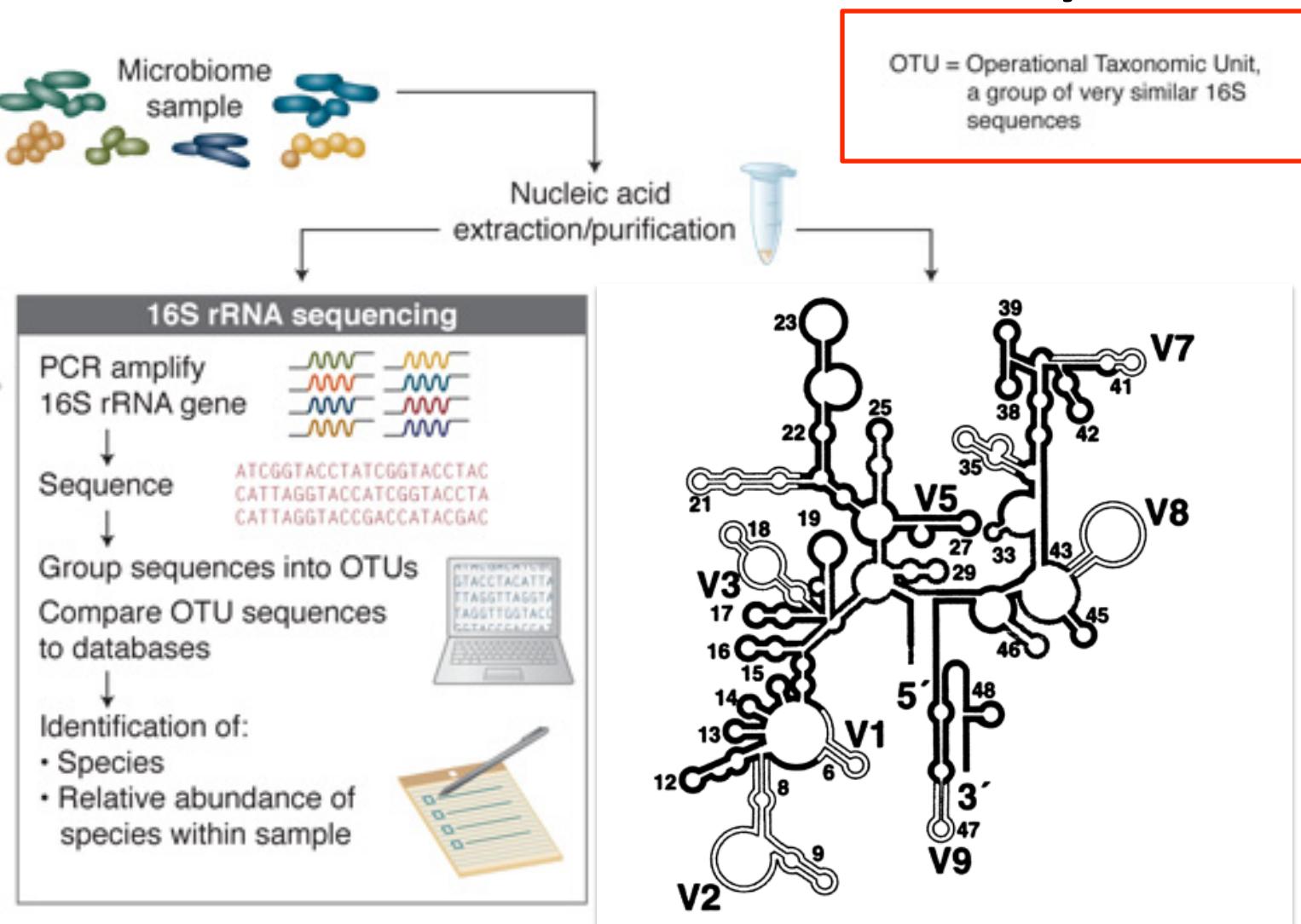
### *NCI-MD Case-Control Study*

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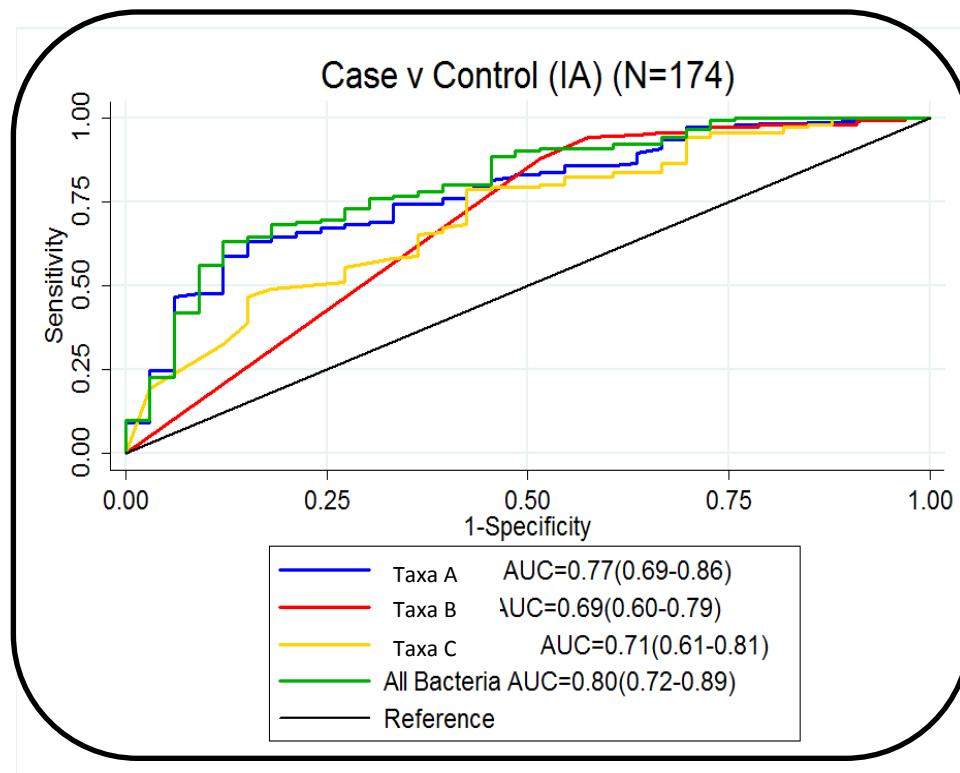
## The Cancer Genome Atlas (TCGA) – Validation cohort

- 1,112 Lung T and NT (98 paired T/NT) RNAseq samples
- Used unmapped reads for metagenomic analysis (fasta files)
- Clinical data deposited to TCGA database
- Used mutation analysis (Nature 2014) on 195 squamous cell carcinoma sample

# Tools for Microbiome Analysis

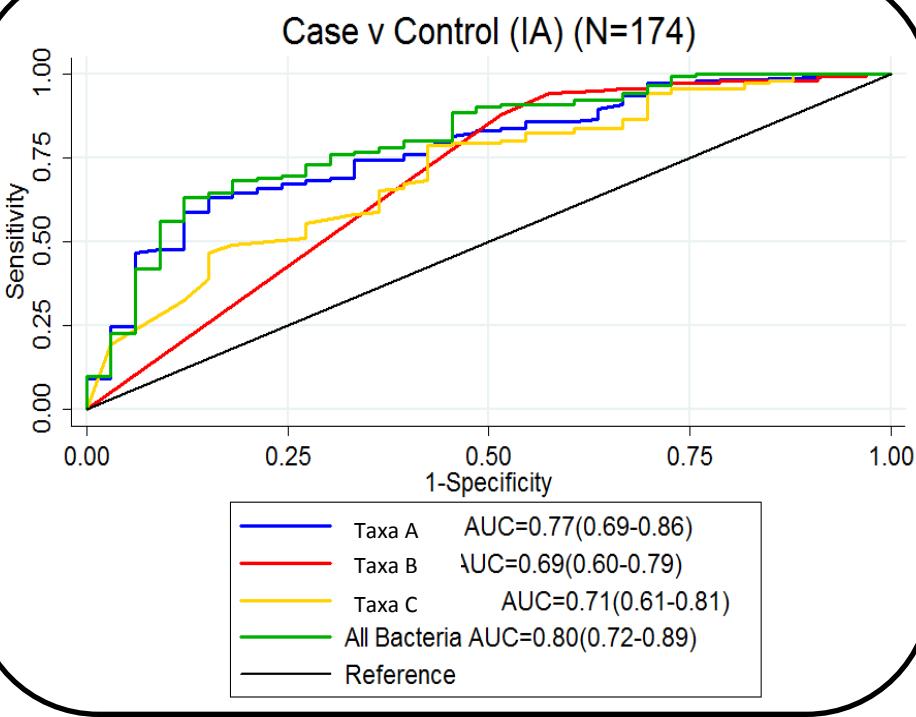


## Specific taxa can distinguish lung cancer from healthy lung and classify disease state

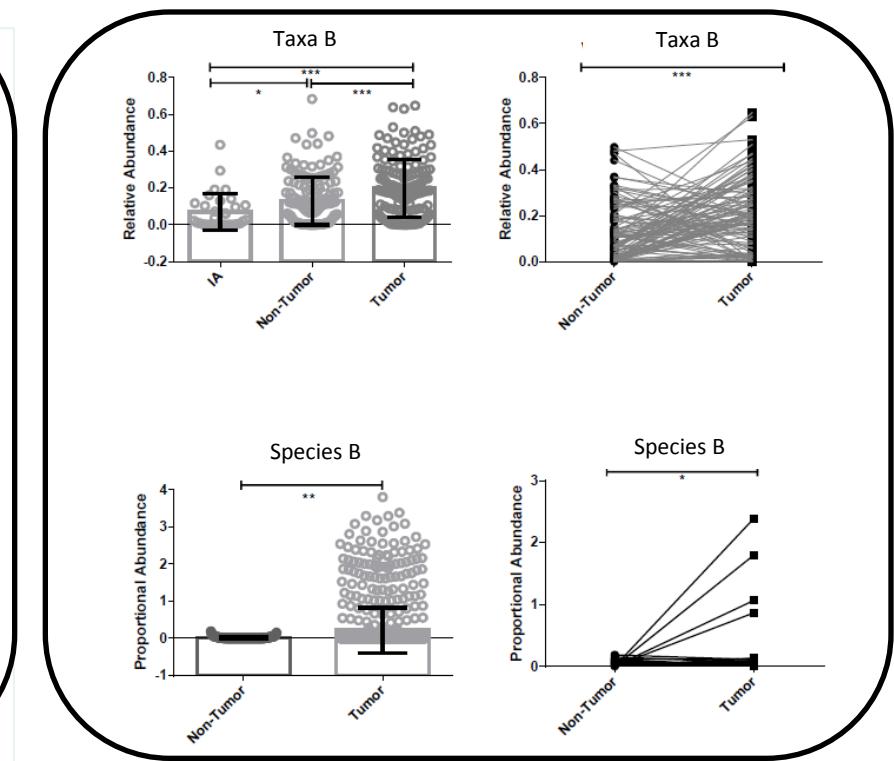


Pulmonary bacteria can differentiate lung cancer from healthy lung. ROC analysis of the combination of bacteria (*Taxa A*, *Taxa B*, *Taxa C*) from 5-fold cross validation using random forest between lung cancer cases (n=148) and healthy controls (IA) (n=34).

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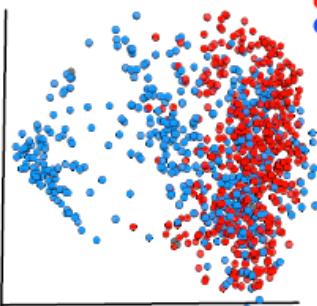


Relative abundance of *Taxa B* from 16S paired tumor/non-tumor, and **right** – proportional abundance of *Taxa B* from TCGA tumor/non-tumor (RNA-seq) (n=1006/106). Mann-Whitney test or paired t-test.

# Microbiome distinguishes smoking and histological subtype

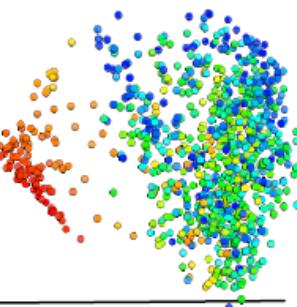
TCGA: AD vs SCC

● AD  
● SCC



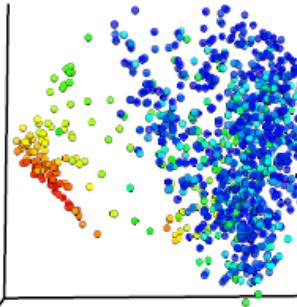
Taxa D

● High Abundance  
● Low Abundance



Taxa A

● High Abundance  
● Low Abundance

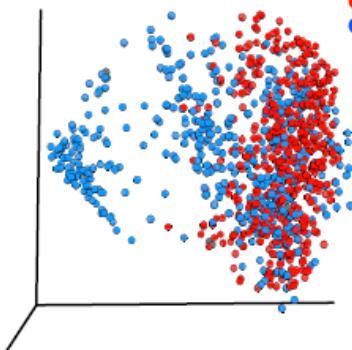


Unpublished data

# Microbiome distinguishes smoking and histological subtype

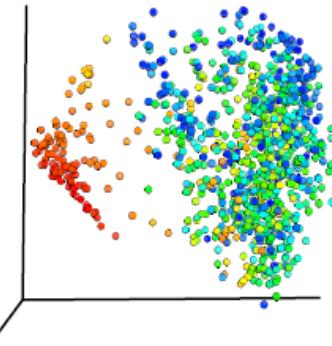
TCGA: AD vs SCC

● AD  
● SCC



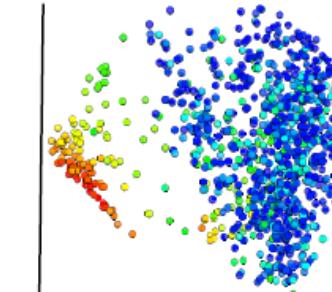
Taxa D

● High Abundance  
● Low Abundance



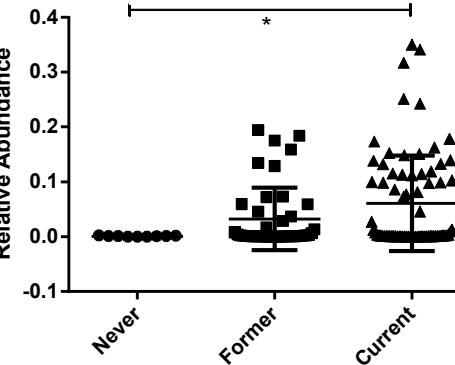
Taxa A

● High Abundance  
● Low Abundance



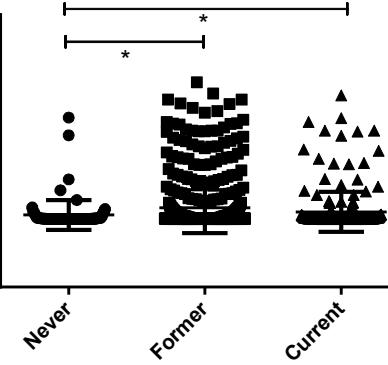
16S: Taxa D

Relative Abundance



TCGA: Species D

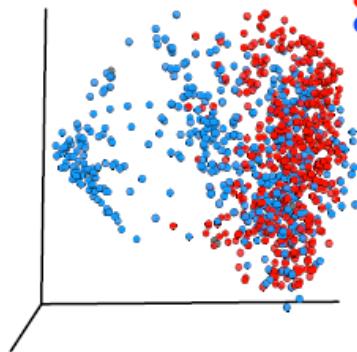
Proportional Abundance



# Microbiome distinguishes smoking and histological subtype

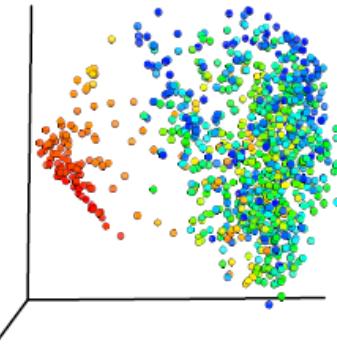
TCGA: AD vs SCC

● AD  
● SCC



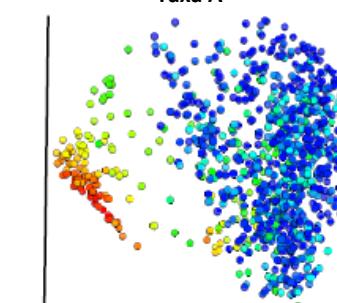
Taxa D

● High Abundance  
● Low Abundance



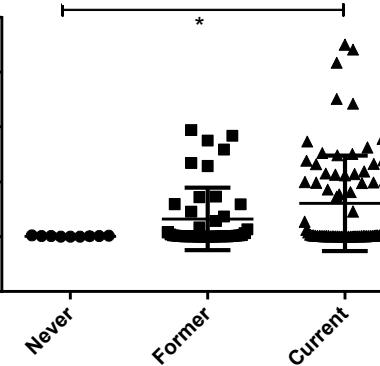
Taxa A

● High Abundance  
● Low Abundance



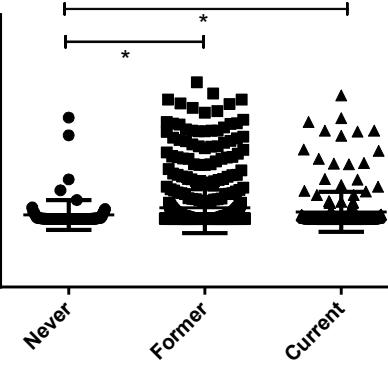
16S: Taxa D

Relative Abundance



TCGA: Species D

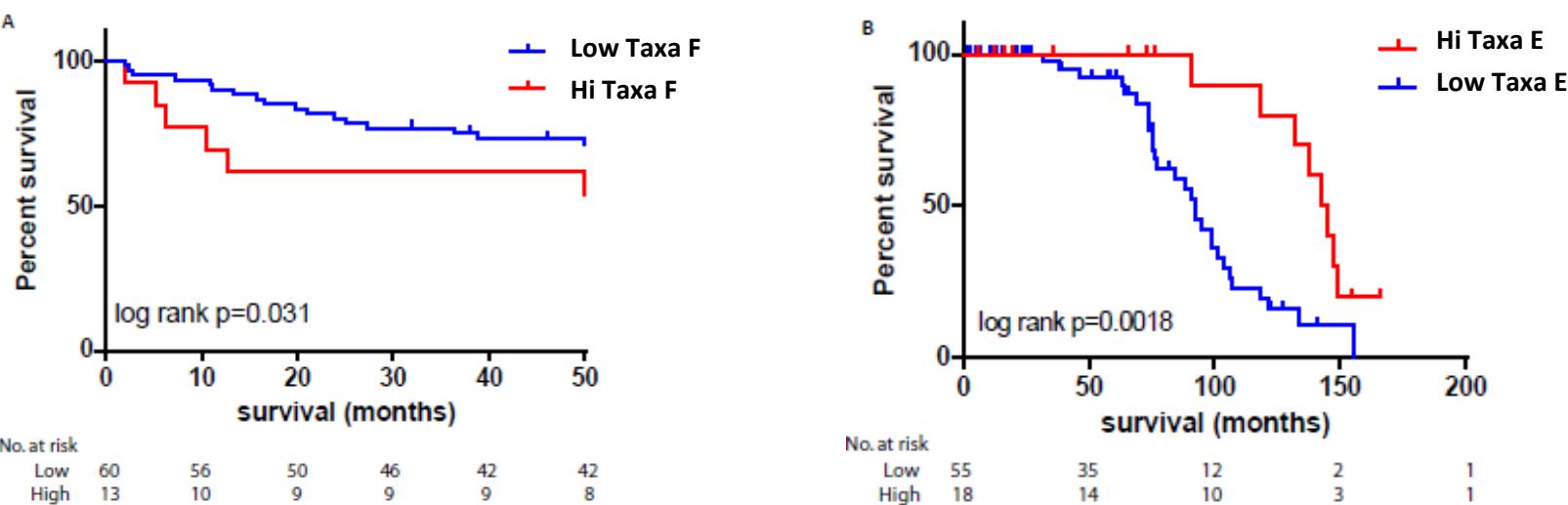
Proportional Abundance



**May explain in part the etiology of squamous cell carcinoma (SCC) lung cancer**

**Possible link between smoking and development of a subset of SCC lung cancer**

# The lung cancer microbiome as a prognostic biomarker



Taxa	Model 1			Model 2*		
	HR	95% CI	p	HR	95% CI	p
Low Taxa F	1	ref		1	ref	
Hi Taxa F	2.67	1.06-6.74	0.038	6.19	1.67-22.84	0.006
Low Taxa E	1	ref		1	ref	
Hi Taxa E	0.28	0.12-0.64	0.003	0.1	0.02-0.41	0.002

\* adjusted for age, gender, race, stage, smoking, lung location

# Why is this important for cancer prevention and therapy?

Primary

- Identify prebiotics/probiotics that can prevent loss of diversity and maintain gut barrier function
- Identify microbiota or microbial metabolites that can improve diagnostic tools for screening and early prediction of cancer

Secondary

- Improve response to therapy by identification of individuals who may benefit
- Reduce the use of broad spectrum antibiotics that evicts both commensal and pathogenic bacteria to improve response to therapy

Tertiary

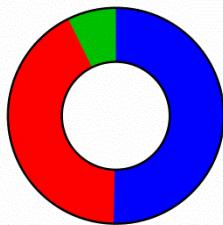
- Identification of microbiota and metabolites associated with cancer recurrence

# Inflammatory-associated cancers

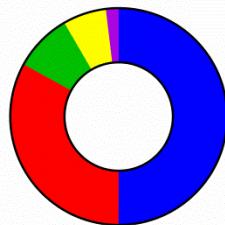
Inflammation that causes insults or pathological conditions	Associated malignancy
Silica, asbestos, smoking-associated silicosis and bronchitis	Lung carcinoma
Pelvic inflammatory disease	Ovarian carcinoma
Chronic indwelling urinary catheter	Bladder carcinoma
TRYP1 mutation-associated pancreatitis and alcoholism-associated pancreatitis	Pancreatic carcinoma
UV irradiation-associated skin inflammation	Melanoma
Asbestos	Mesothelioma
Bile acids	Cholangiosarcoma and colorectal carcinoma
Gastric acid-associated Barrett's metaplasia and reflux oesophagitis	Oesophageal carcinoma
Gall bladder stone-associated cholecystitis	Gall bladder carcinoma
Lichen sclerosus (a skin condition)	Vulvar carcinoma
Inflammatory bowel disease	Colorectal carcinoma

# Diversity alterations in lung cancer

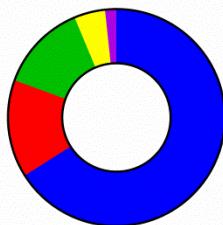
Healthy (IA)



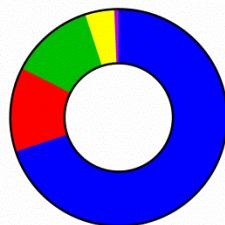
Healthy (NL)



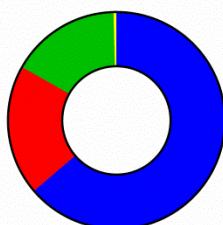
Non-Tumor



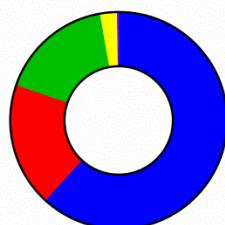
Tumor



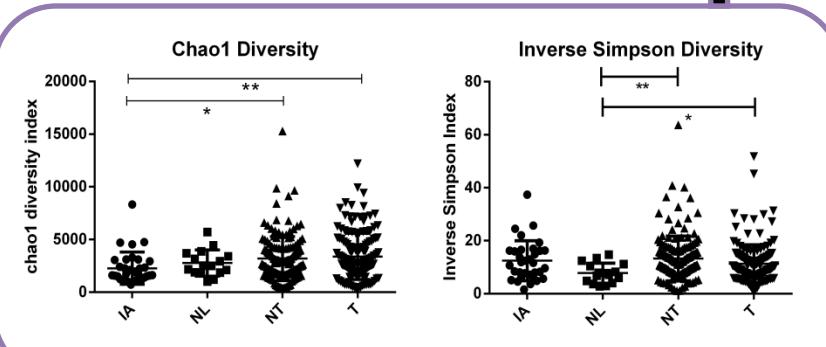
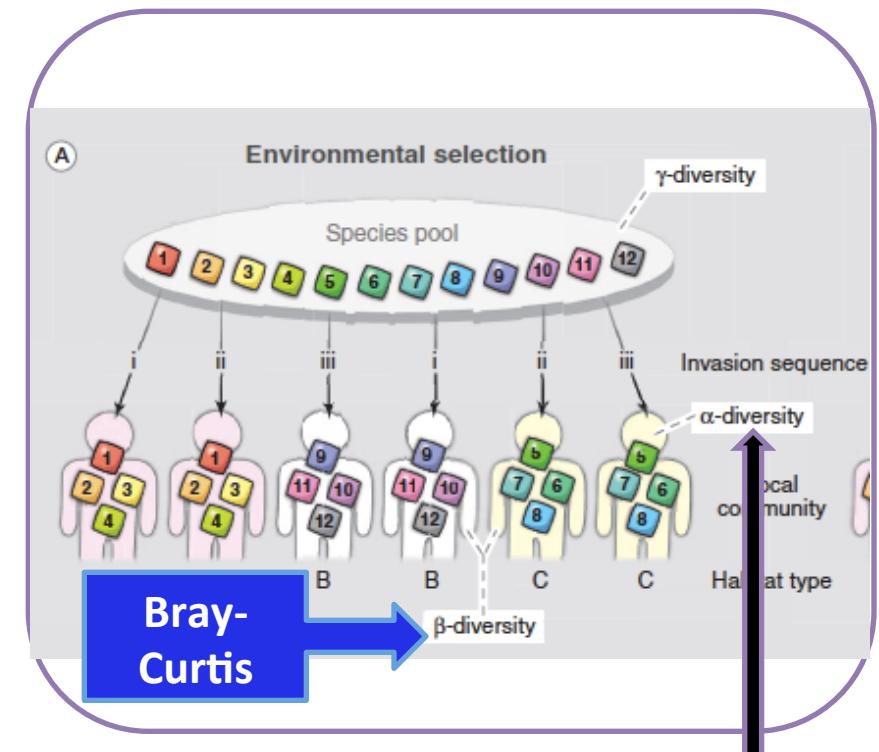
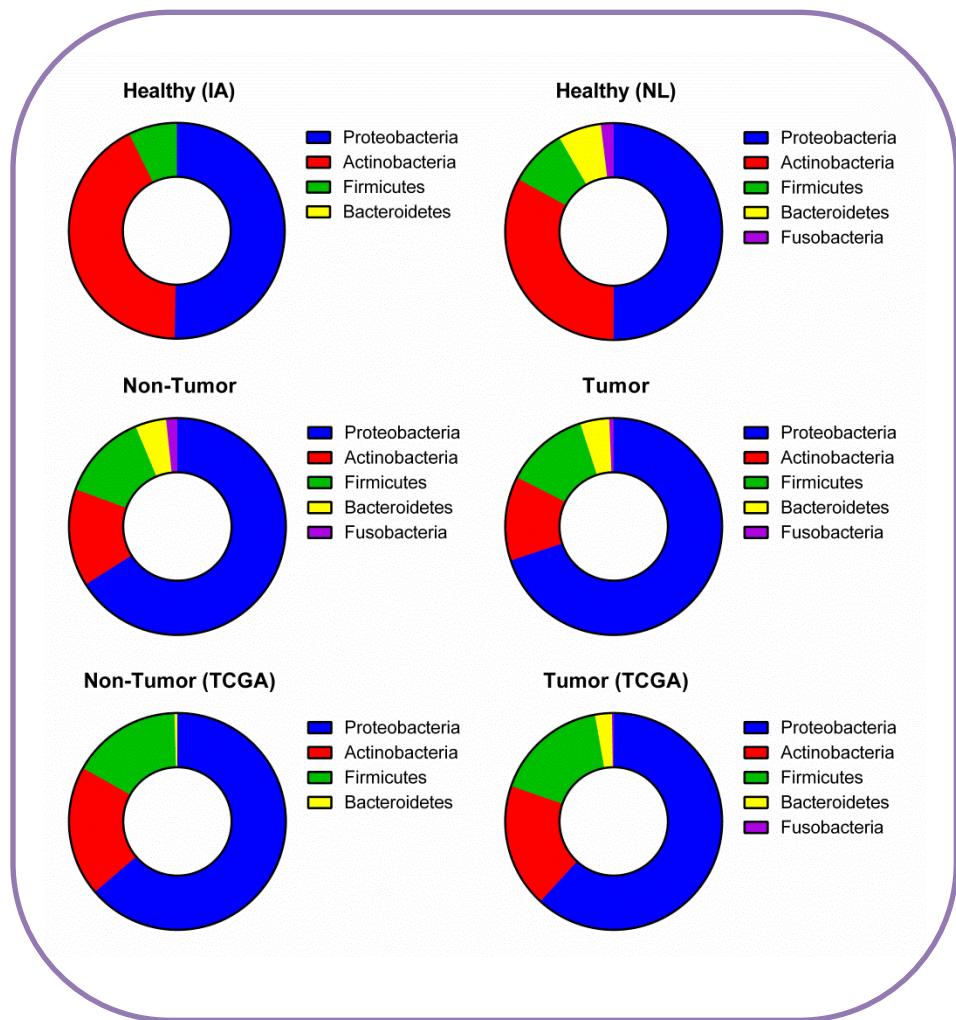
Non-Tumor (TCGA)



Tumor (TCGA)



# Diversity alterations in lung cancer



Unpublished data

# A primer on microbial ecology

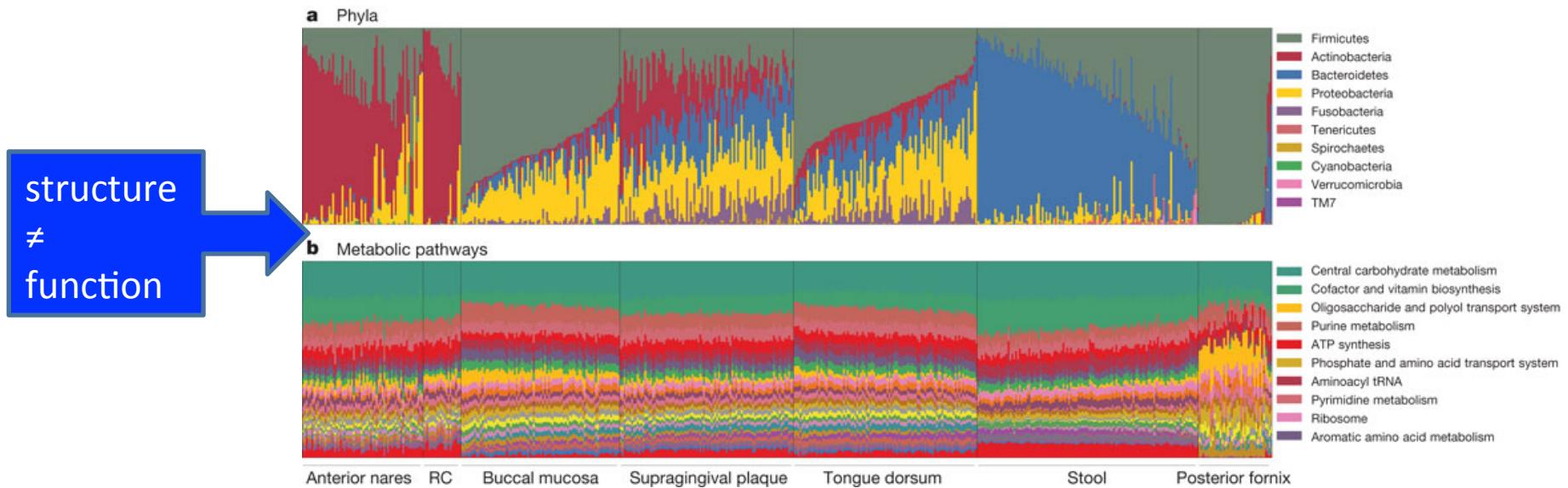
Disclosure: I am not a microbiologist

**The human microbiome** - the collection of all of the microbes and their genes

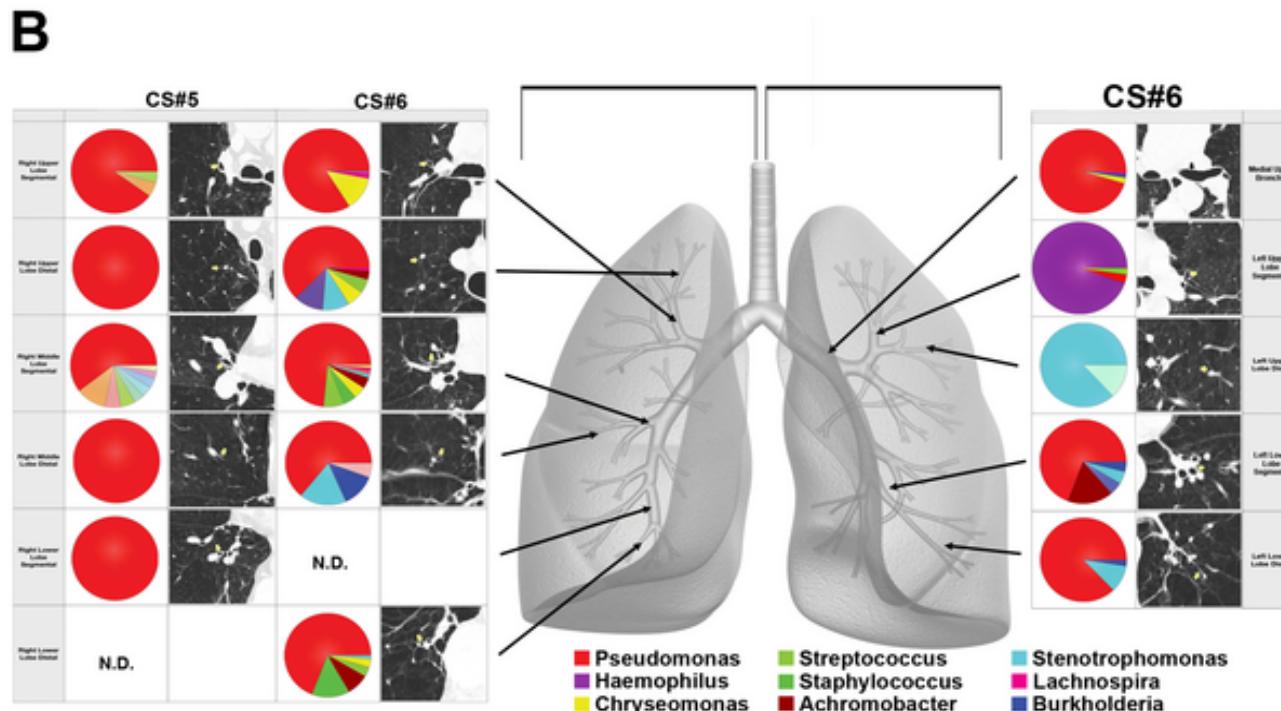
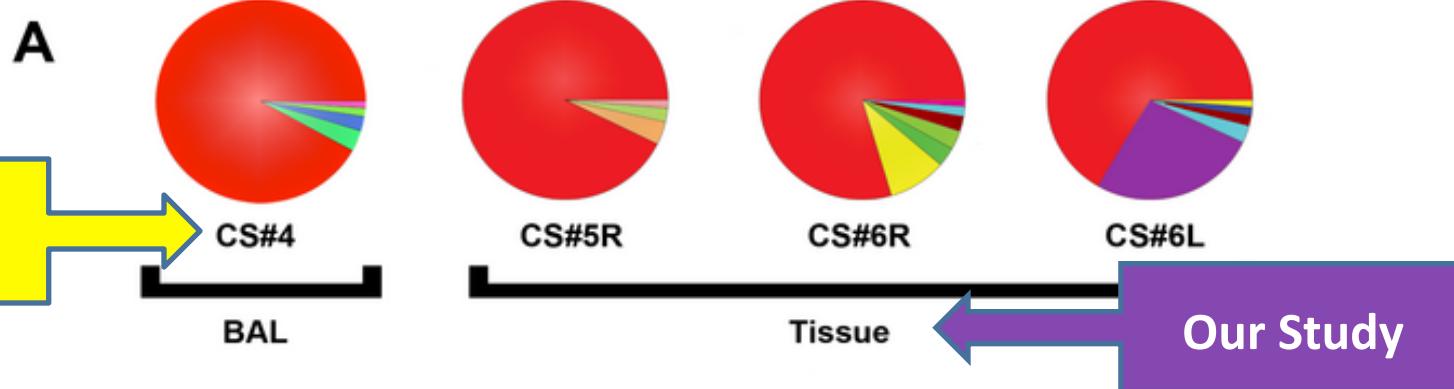
**The human microbiota** - communities of microbes found in or on humans (viruses, bacteria, archaea, eukarya)

**Metagenome** – the genetic material derived from the microbiota (environmental samples)

**Metatranscriptome** - the genes transcribed from the microbes inhabiting our body



# Bacterial Communities Present in Individual Lung Airways



Erb-Downward JR, Thompson DL, Han MK, Freeman CM, et al. (2011) Analysis of the Lung Microbiome in the “Healthy” Smoker and in COPD. PLoS ONE 6(2): e16384. doi:10.1371/journal.pone.0016384

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0016384>