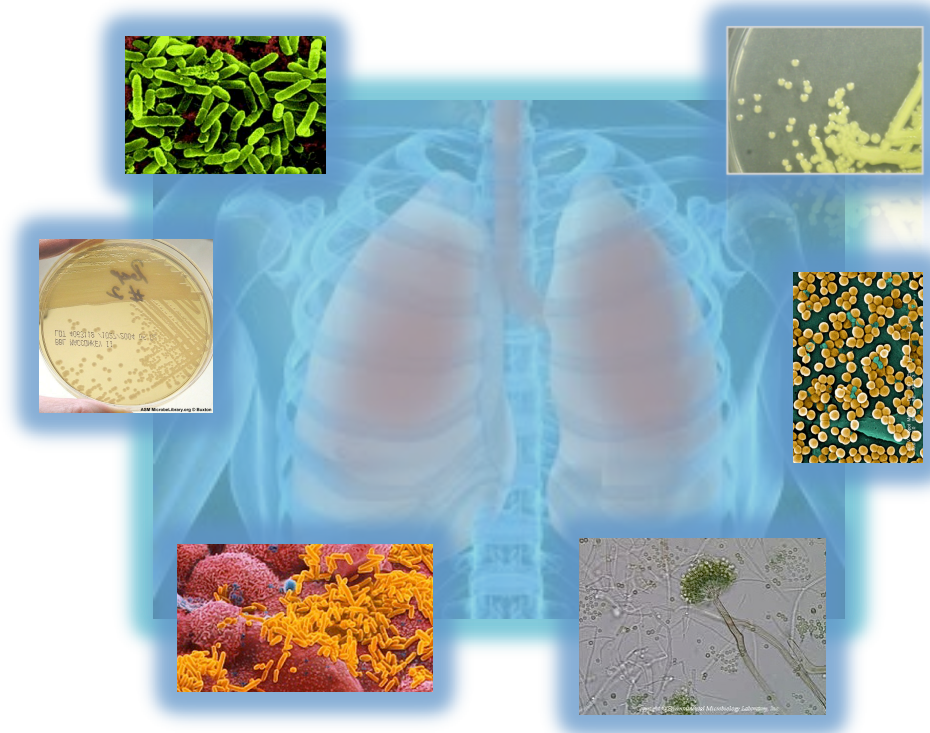


Ecology and evolution of the Cystic Fibrosis lung microbiome



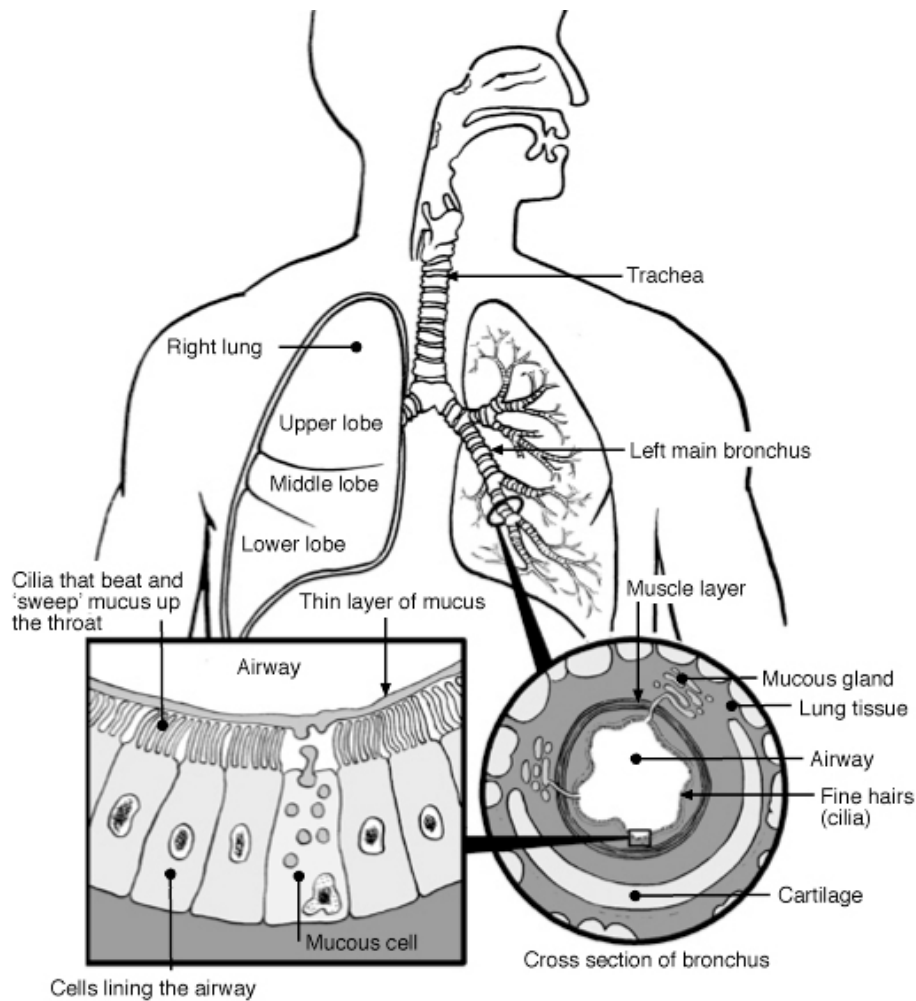
**Heather Maughan¹, Yunchen Gong², Pauline Fung², Pauline Wang²,
David M. Hwang³, David S. Guttman^{1,2}**

¹Cell & Systems Biology

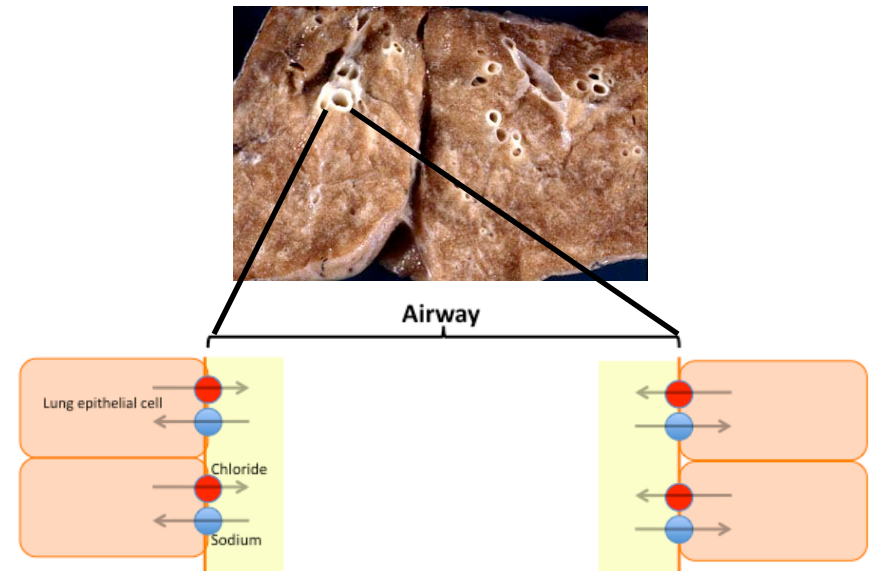
²Centre for Analysis of Genome Evolution and Function

³University Health Network
University of Toronto

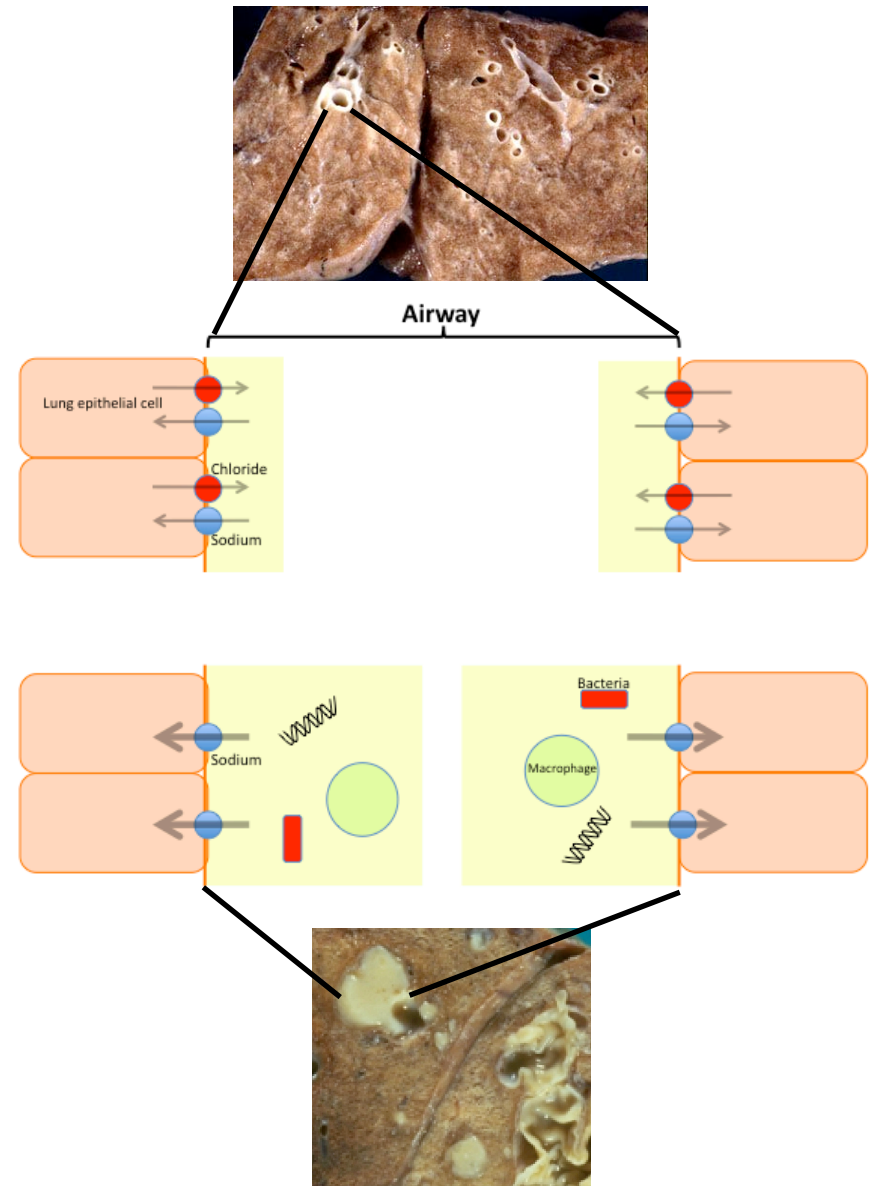
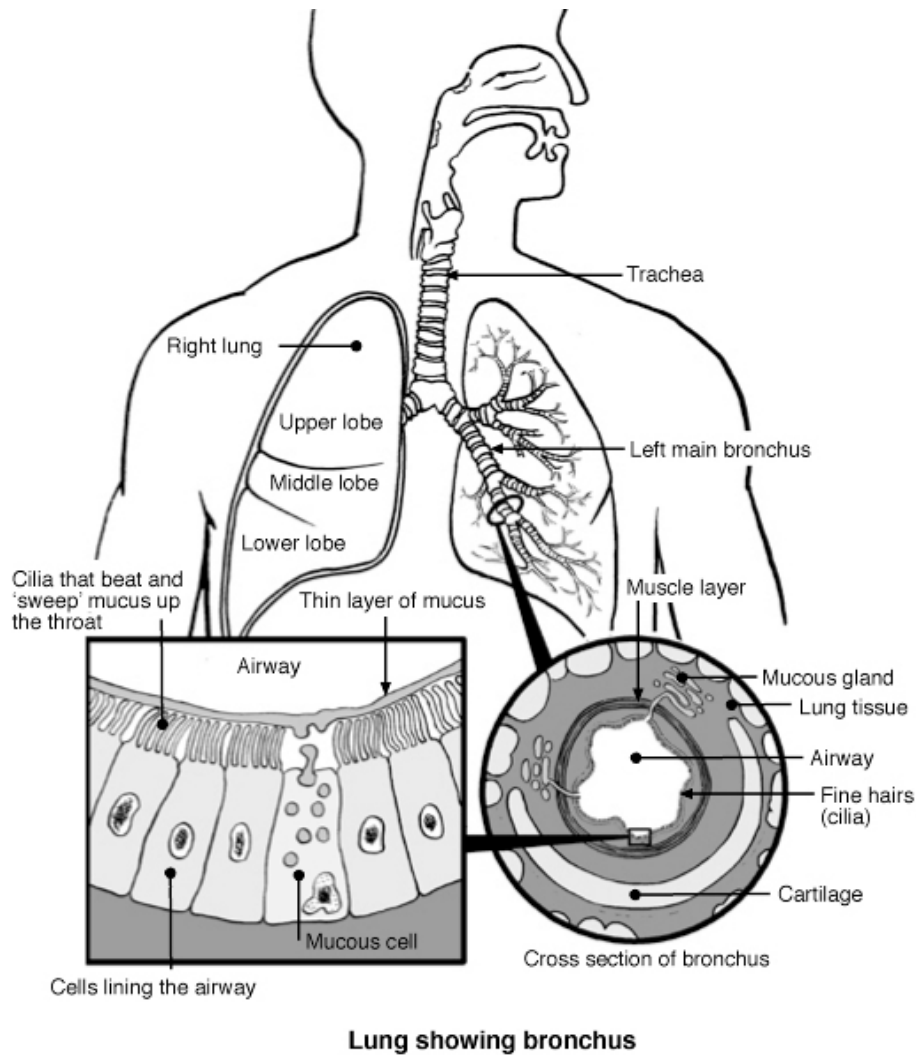
Cystic Fibrosis and lung airways



Lung showing bronchus

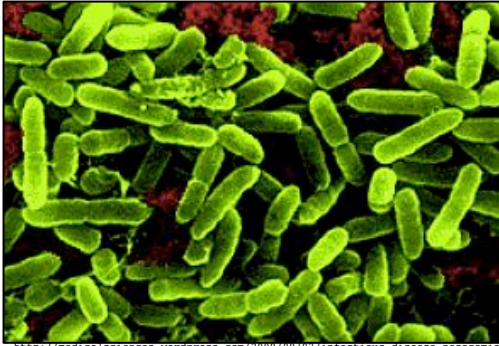


Cystic Fibrosis and lung airways



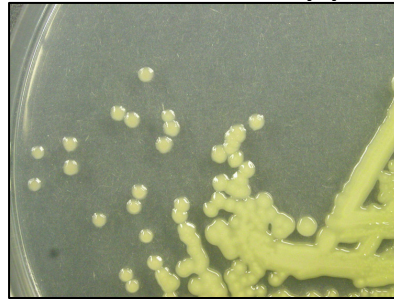
Multiple microbes inhabit CF lungs

Pseudomonas aeruginosa



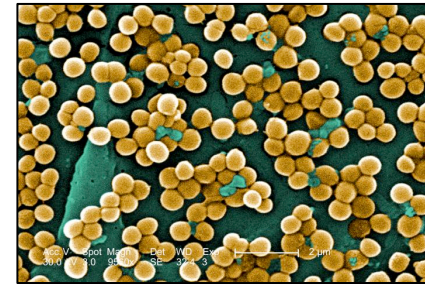
<http://medicalsciences.wordpress.com/2009/09/07/infectious-disease-nosocomial-pathogens/>

Burkholderia spp.



<http://www.getor.4t.com/concurso/bacteriologia/burkholderiafacieapical.jpg>

Staphylococcus aureus



<http://www.healthype.com/staphylococcus-aureus.html>

Stenotrophomonas maltophilia



ASM MicroLibrary.org © Buxton
<http://www.microbelibrary.org/microbelibrary/11165/czimages/Articleimages/Atlas-Mac/Stenotrophomonas%20maltophilia%20fig20.jpg>

Haemophilus influenzae



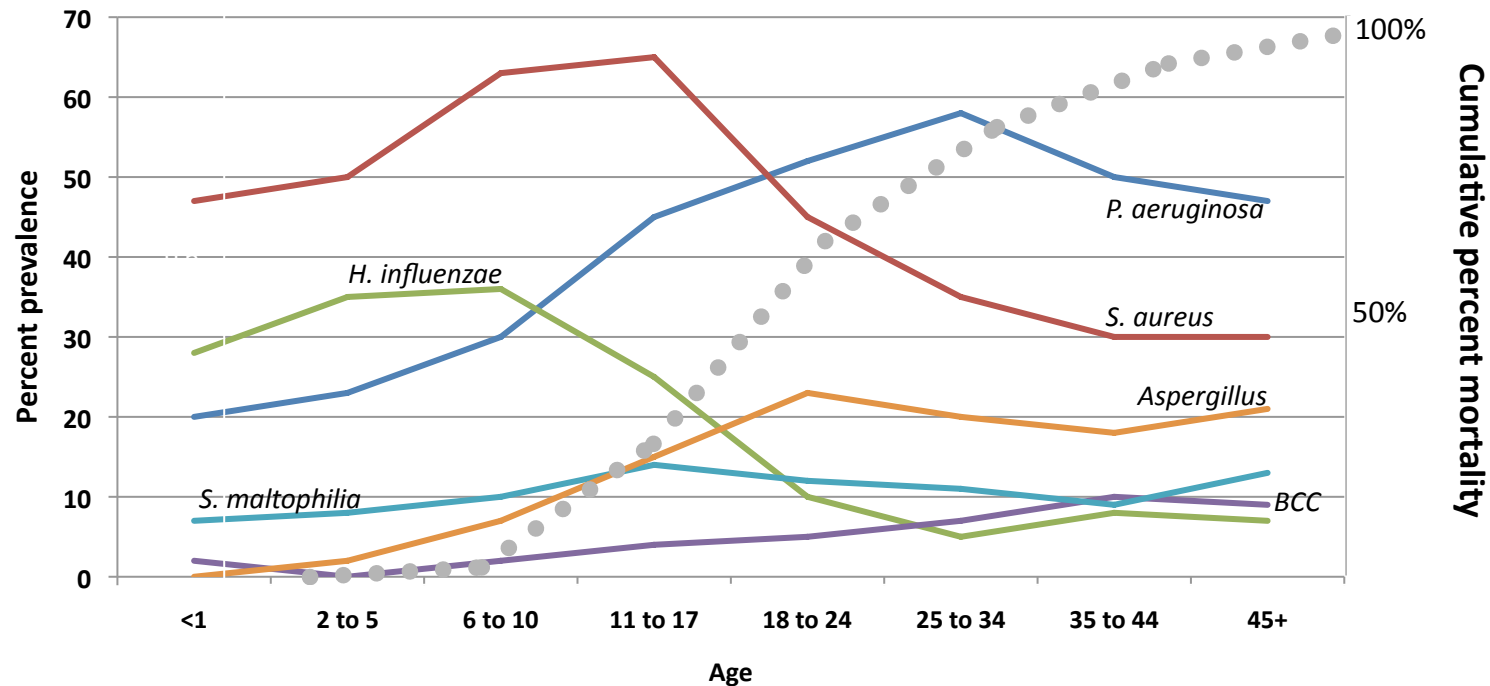
<http://www.nhs.uk/conditions/Hib/pages/introduction.aspx>

Aspergillus



Known from culturing and some molecular characterization

Microbial abundances are dynamic



- Determine how abundances are influenced by clinical treatments
- Identify genes encoding clinically relevant traits (e.g., antibiotic resistance)
- Determine how species interactions influence disease progression

Study

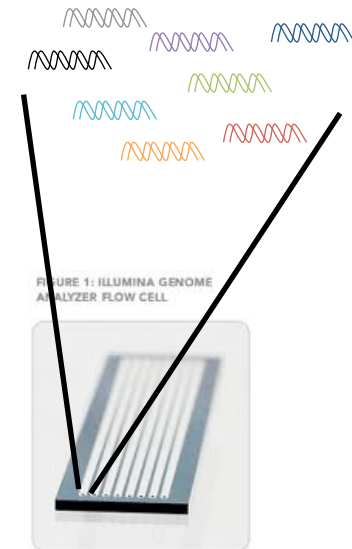
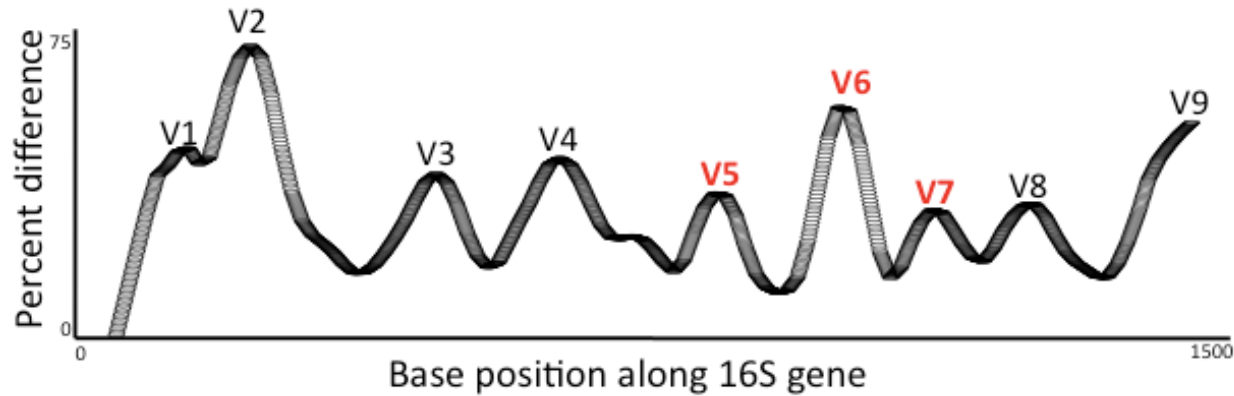
- Longitudinal sampling of sputum and lung explant specimens
 - 15 lung explants per year
 - 3-4 sputum samples from 600 adult and child patients per year
- Characterize diversity of bacterial and fungal communities
 - baseline lung function and acute pulmonary exacerbations
- Characterize fine scale population diversity in major pathogens (*P. aeruginosa*, *Aspergillus*)
- Characterize antibiotic resistance potential of microbial community
- Characterize metabolic potential of microbial community (metatranscriptomics)

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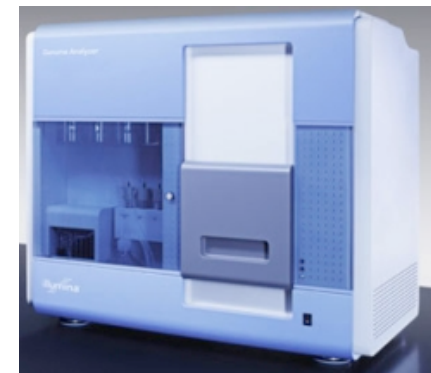
Workflow of 16S rDNA targeted sequencing

- Targeted sequencing of 16S rDNA hypervariable regions

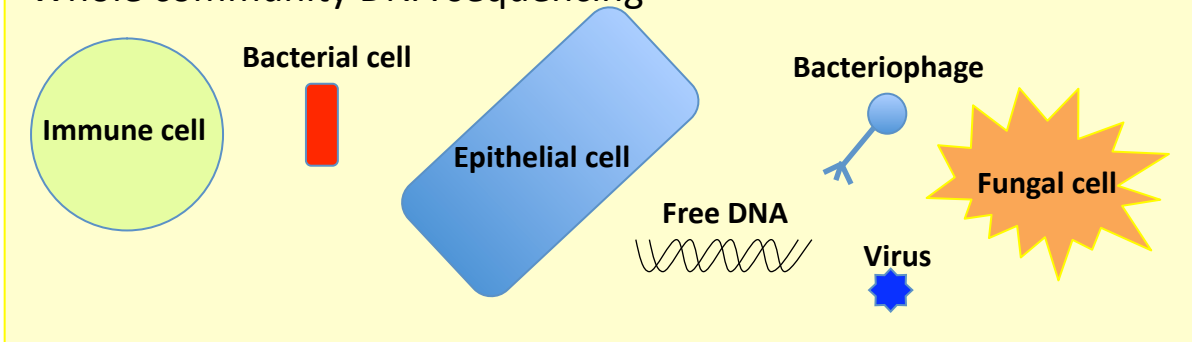


Up to eight samples can be loaded onto the flow cell for simultaneous analysis on the Illumina Genome Analyzer.

8 lanes per run



Whole community DNA sequencing

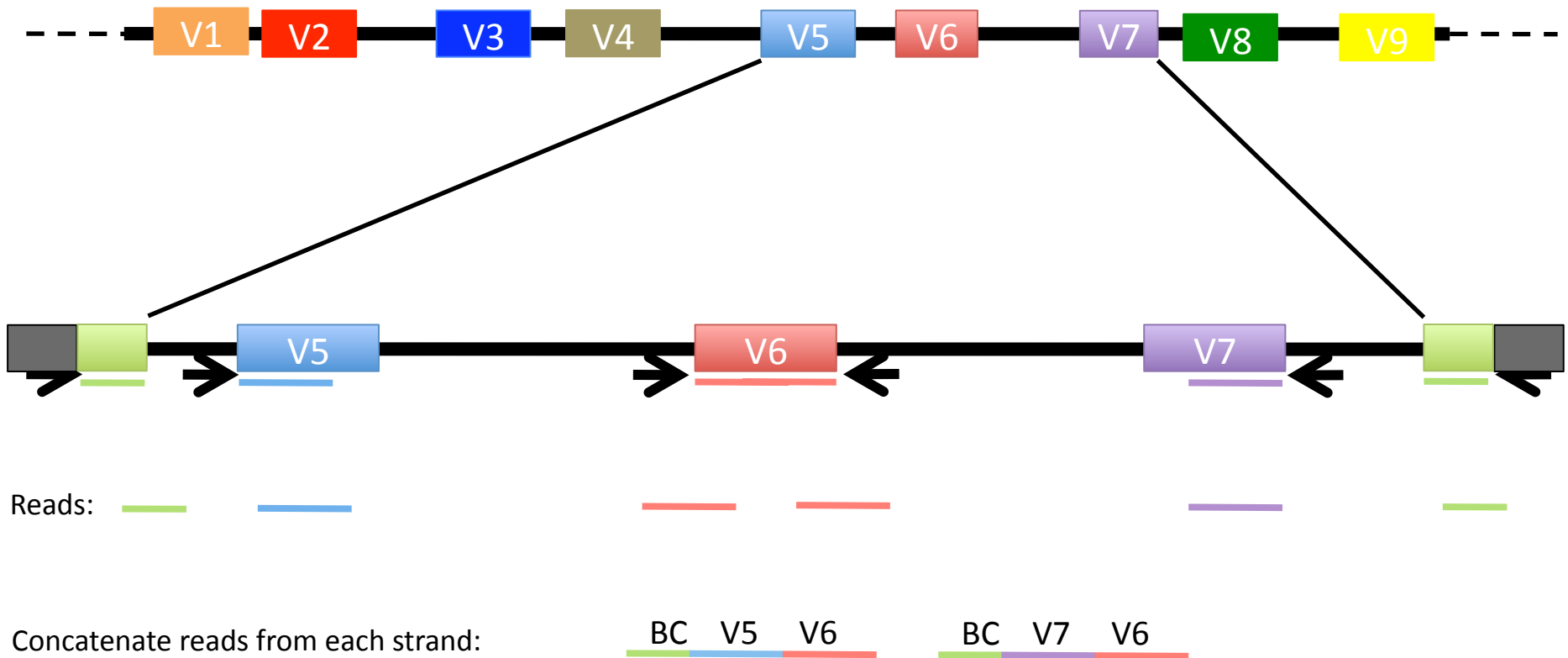


- Classification of reads using RDP Classifier (Wang et al. 2007)
- Comparison between communities
- Associations between community composition and disease



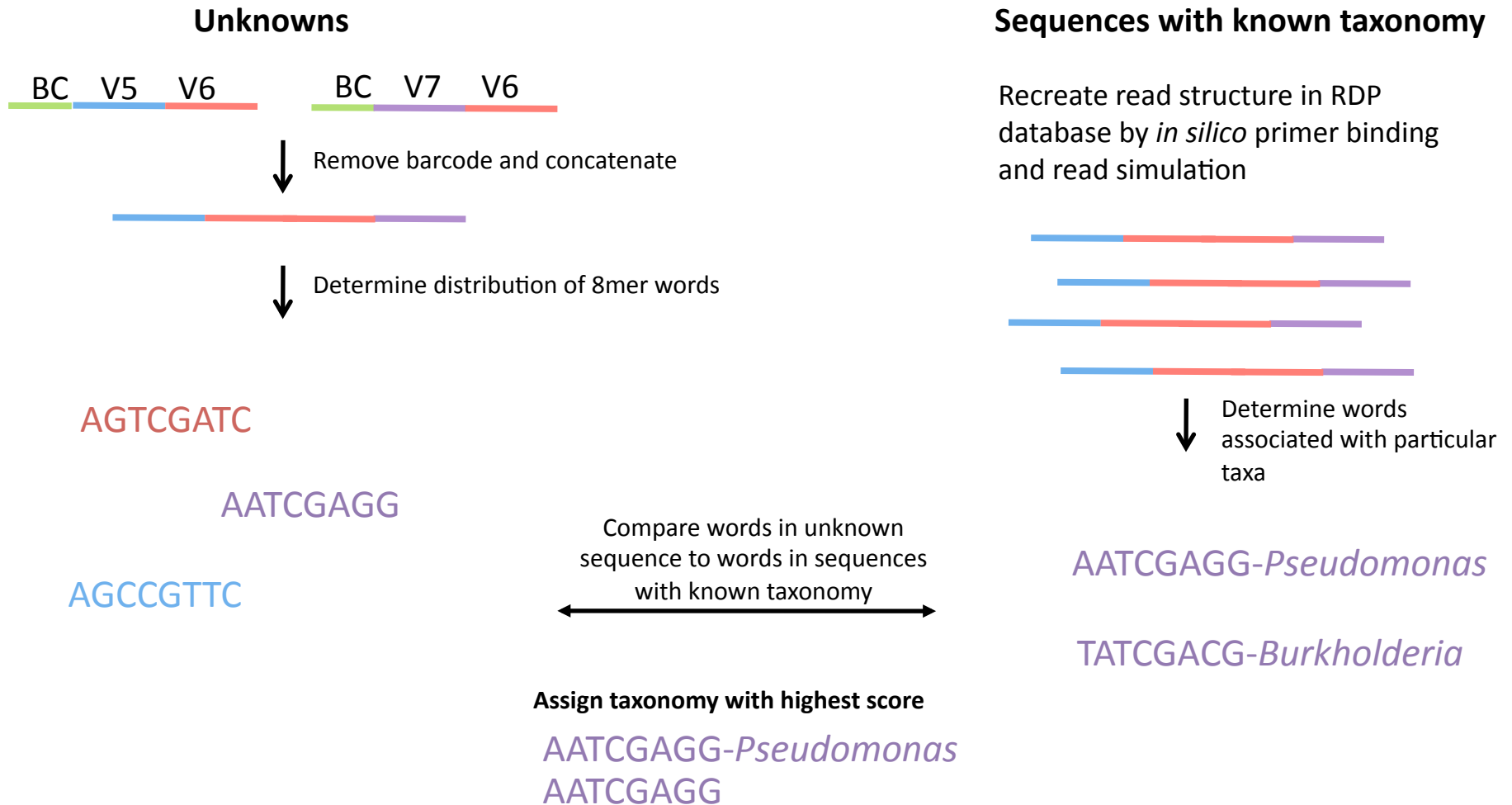
Metagenomic Analysis by Serial Illumina Sequencing

MASIS: targeted sequencing of V5, V6, and V7 hypervariable regions

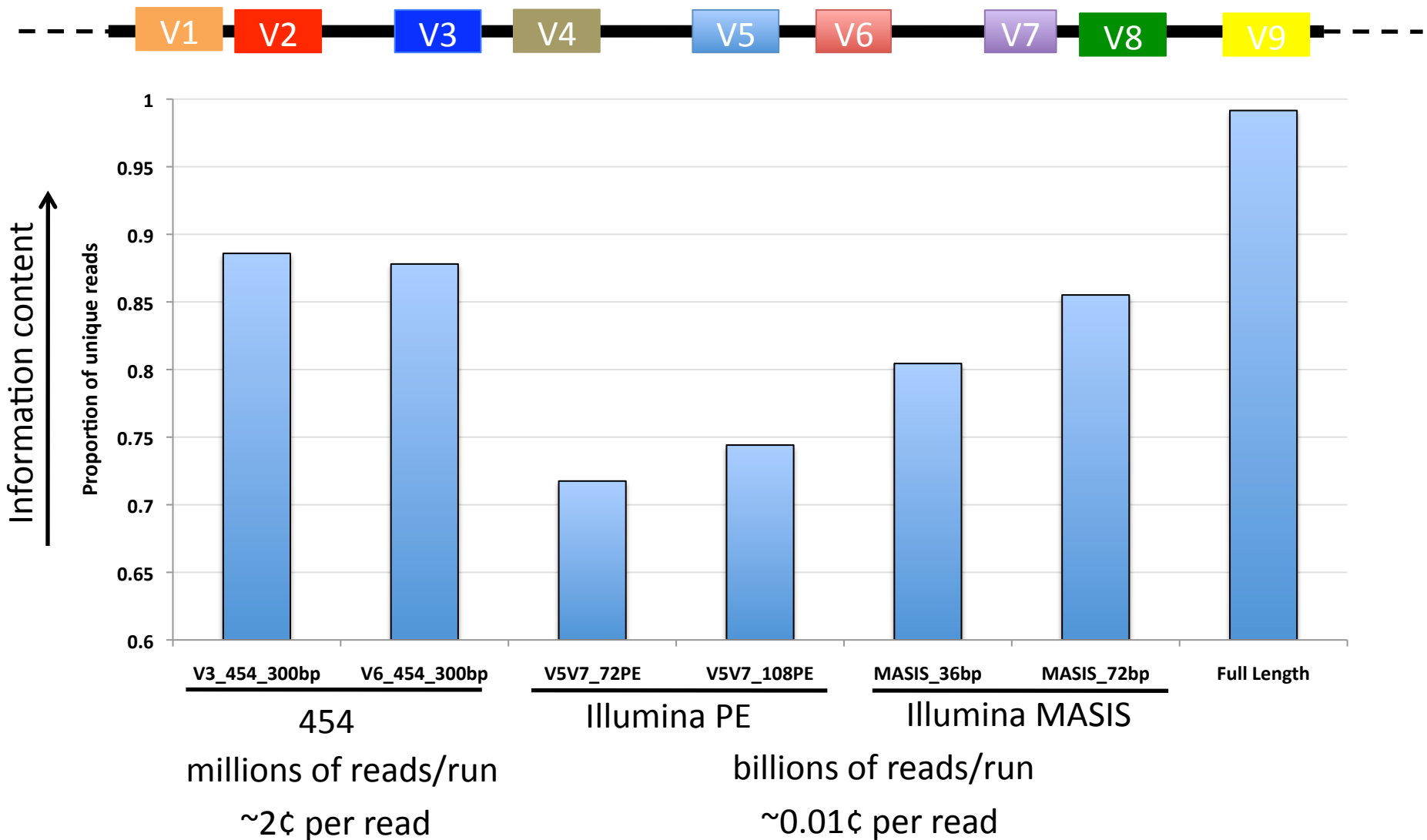


8x multiplexing = 64 patient samples per Illumina GAIIx run

Classification of sequence reads



Information content provided by MASIS



Testing MASIS with known templates

Controls

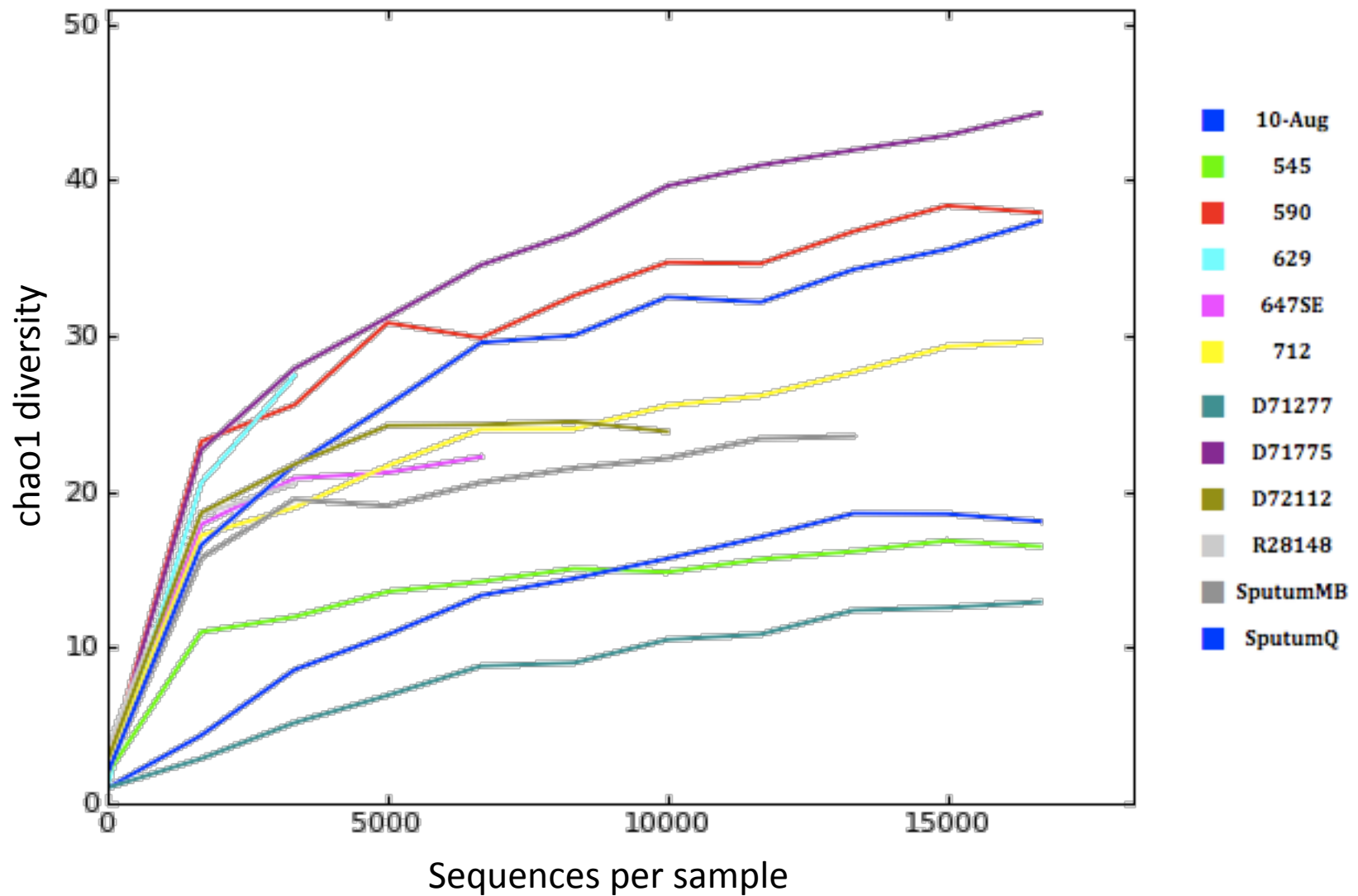
- one known species, total read length = 114bp (36bp × 4)
- laboratory mixed community

Total Reads	Quality cutoff	Quality Reads	Percent Quality	False positive %
17,520,529	5 sites < 30	13,530,371	77%	0.08%
17,520,529	2 sites < 30	9,281,166	53%	0.06%
17,520,529	2 sites < 33	983,819	6%	0.02%
17,520,529	2 sites < 35	45,709	0.3%	0.006%

Characterizing CF bacterial communities

Sample	#reads	#reads to genus (%)
629	8,560	3,975 (46%)
R28148	9,926	4,321 (44%)
Sputum2	17,025	14,949 (88%)
647SE	20,510	8,216 (40%)
D72112	27,036	10,435 (39%)
545	61,854	59,349 (96%)
Sputum2Q	77,055	64,219 (83%)
10-Aug	84,625	82,766 (98%)
D71775	105,668	18,332 (17%)
590	110,307	75,229 (68%)
712	131,375	72,344 (55%)
D71277	295,908	292,933 (99%)

CF samples differ in their bacterial diversities



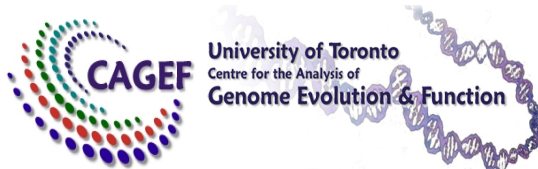
Summary & Future Work

- New MASIS method produces billions of reads per run and sufficient sequence for taxonomic resolution to at least genus level
- Preliminary deep sequencing of CF samples reveals large differences between patients in bacterial community diversity
- Continue 16S targeted sequencing of control communities and more CF samples
- Metatranscriptome sequencing and metabolic reconstruction
- Associations of community composition and function with disease progression

Acknowledgements

THE GUTTMAN LAB

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