

20 years of sequencing ?-omes

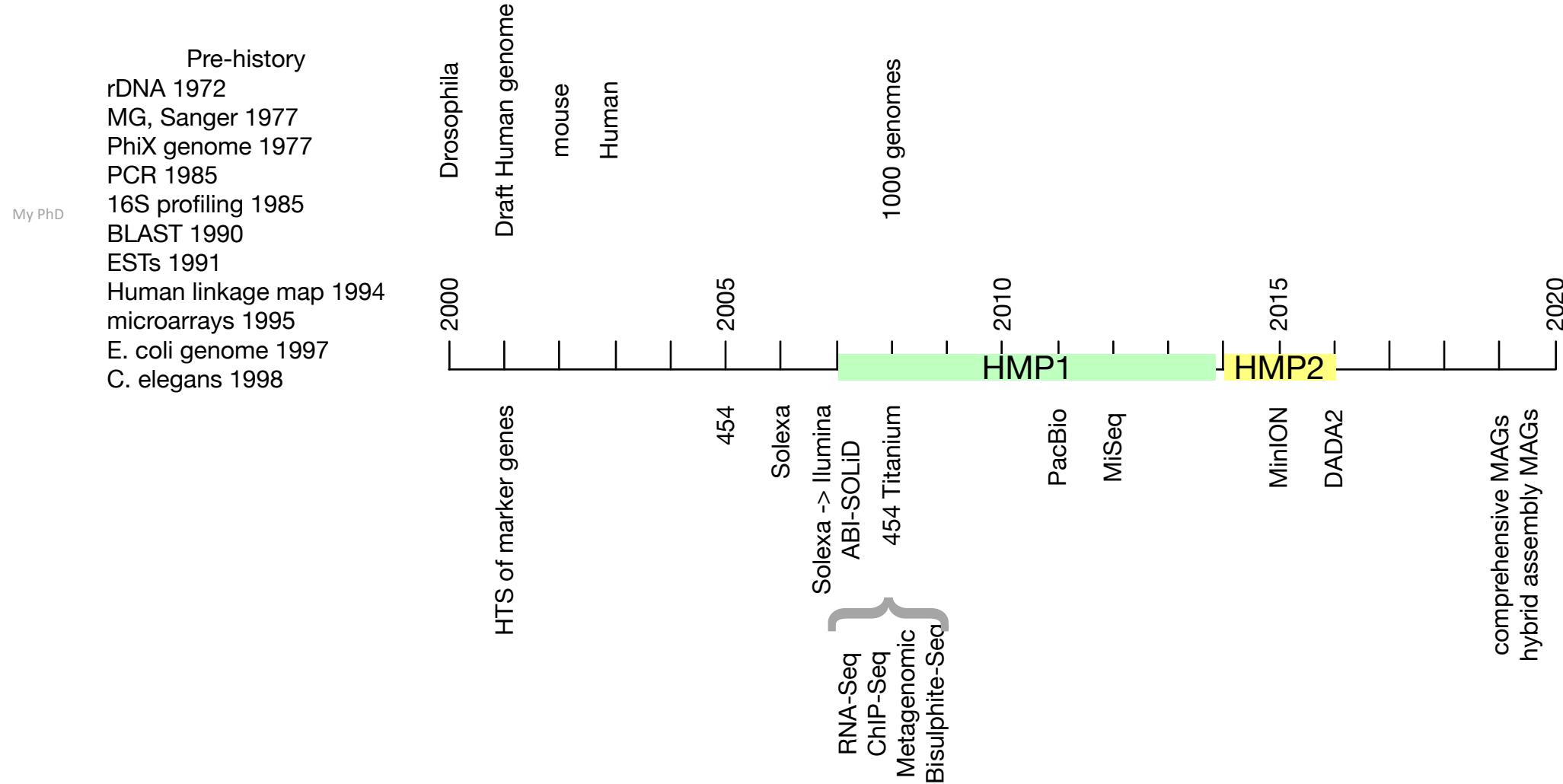


Western

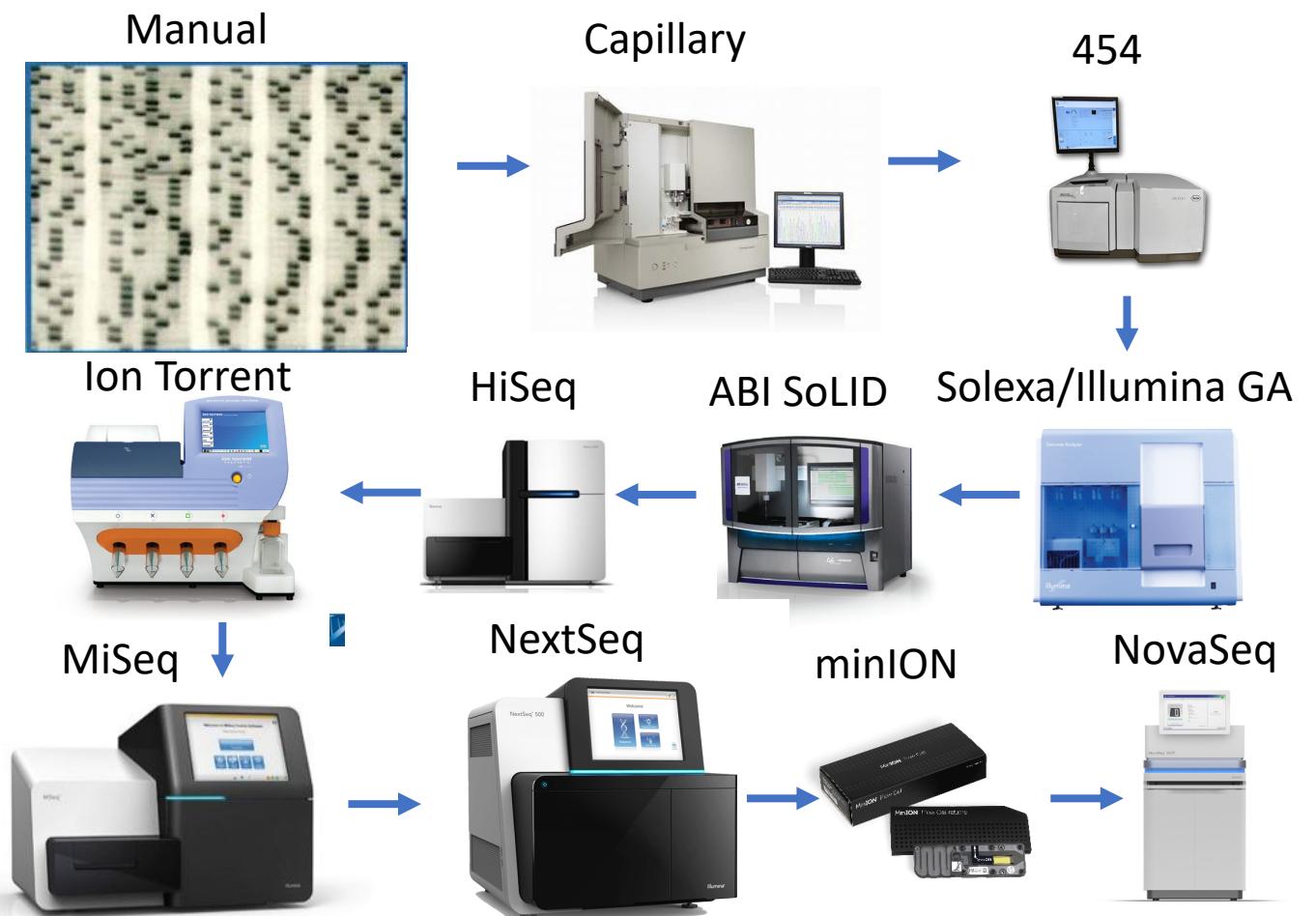
Greg Gloor
Department of Biochemistry
University of Western Ontario
gloor@uwo.ca @gbgloor



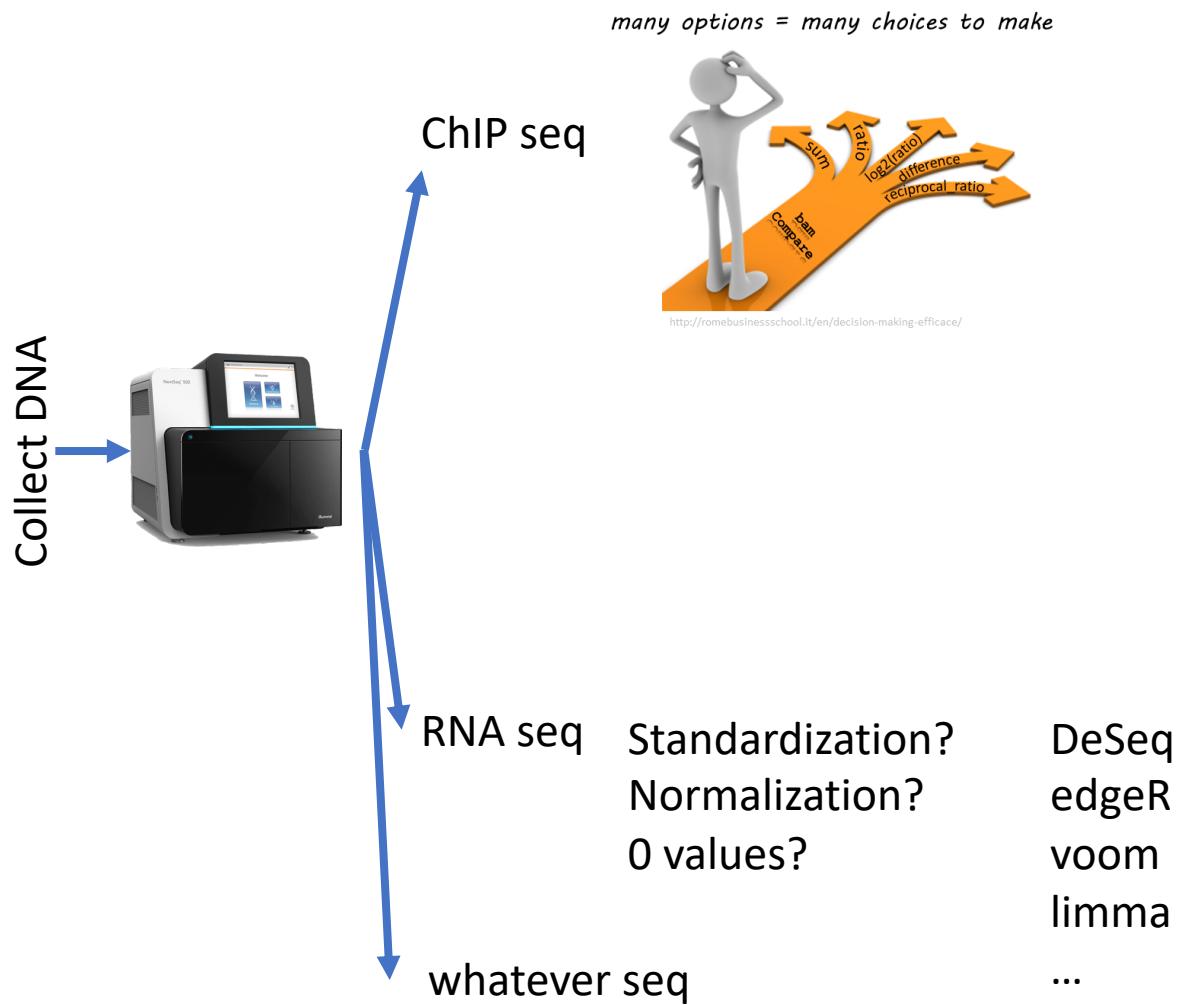
A brief timeline of seq-omics and microbiome



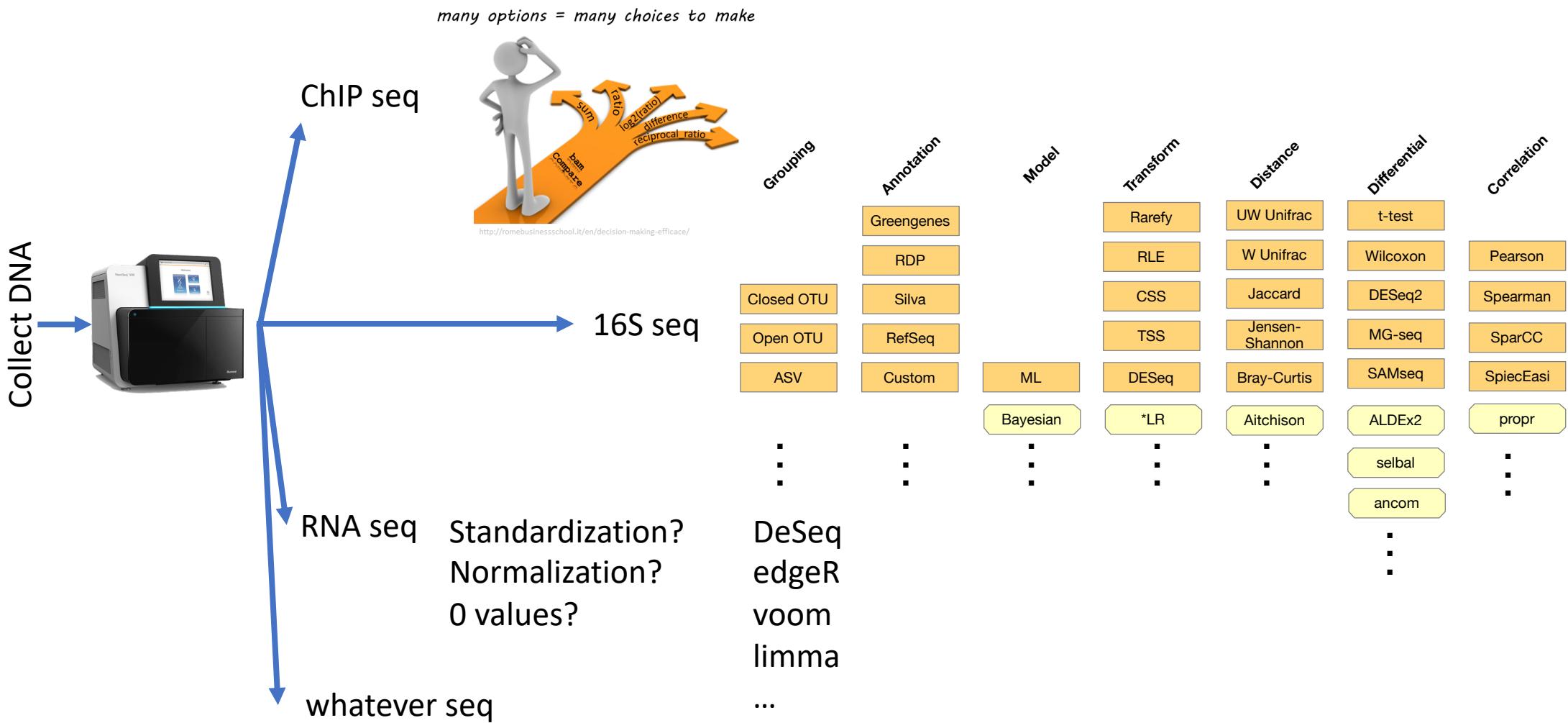
My sequencing journey



It's all just sequencing!

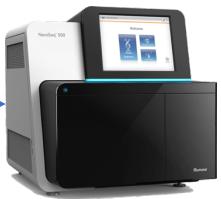


It's all just sequencing!

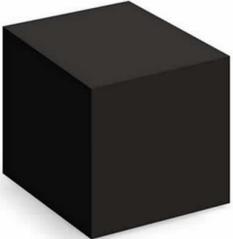


It's all just sequencing!

Collect DNA

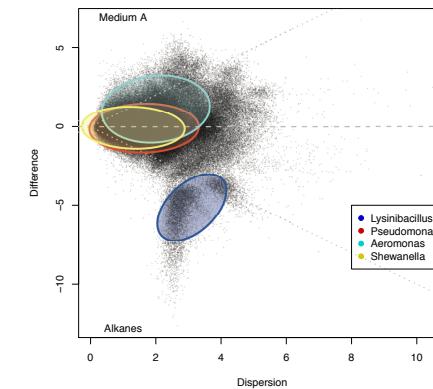
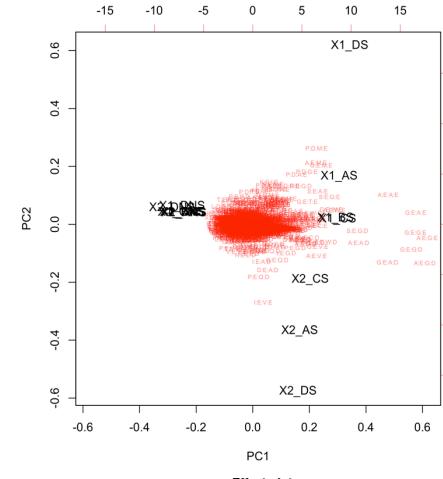


→ whatever seq →

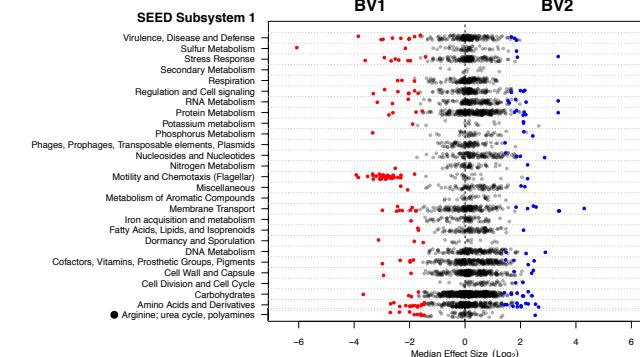


Explore

- Probabilistic random sample
- Ratios not numbers



→ Differential



→ Functionalize

Fernandes et al. 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing {RNA}-seq, 16{S} r{RNA} gene sequencing and selective growth experiments by compositional data analysis. **Microbiome**

Gloor et al. 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. **Frontiers in Microbiology**

Three quick stories

- Low complexity sequencing on the Illumina platform
 - The importance of understanding the platform
 - Error rates
- Microbiomes in the Chinese population
 - Reproducibility and stability
- Hybrid short-long read analysis of microbiomes
 - Ecosystem function

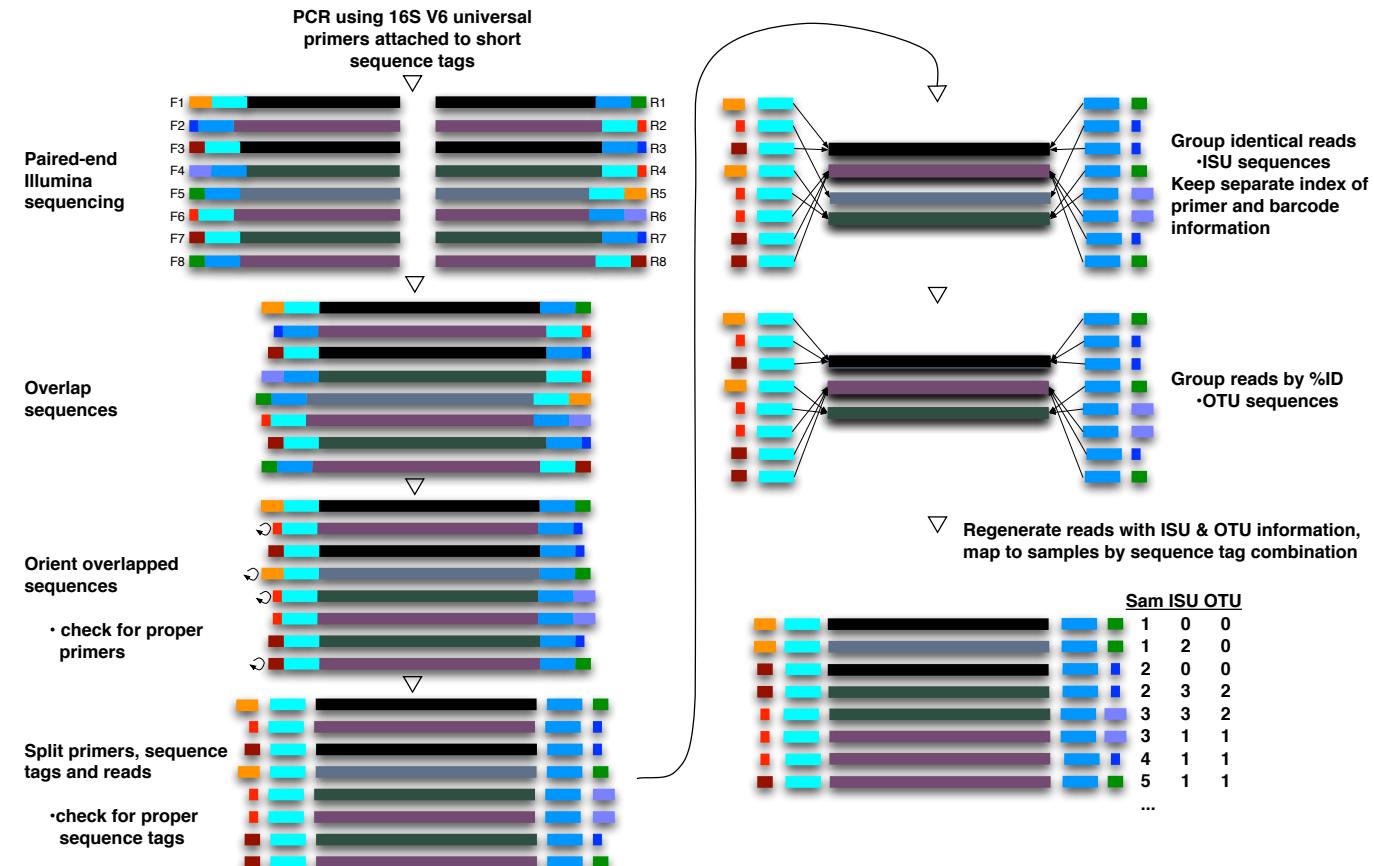
Microbiome Profiling by Illumina Sequencing of Combinatorial Sequence-Tagged PCR Products



PLoS ONE | www.plosone.org

Gregory B. Gloor^{1*}, Ruben Hummelen^{2,3}, Jean M. Macklaim^{1,2}, Russell J. Dickson¹, Andrew D. Fernandes^{1,4}, Roderick MacPhee^{2,5}, Gregor Reid^{1,2,5,6}

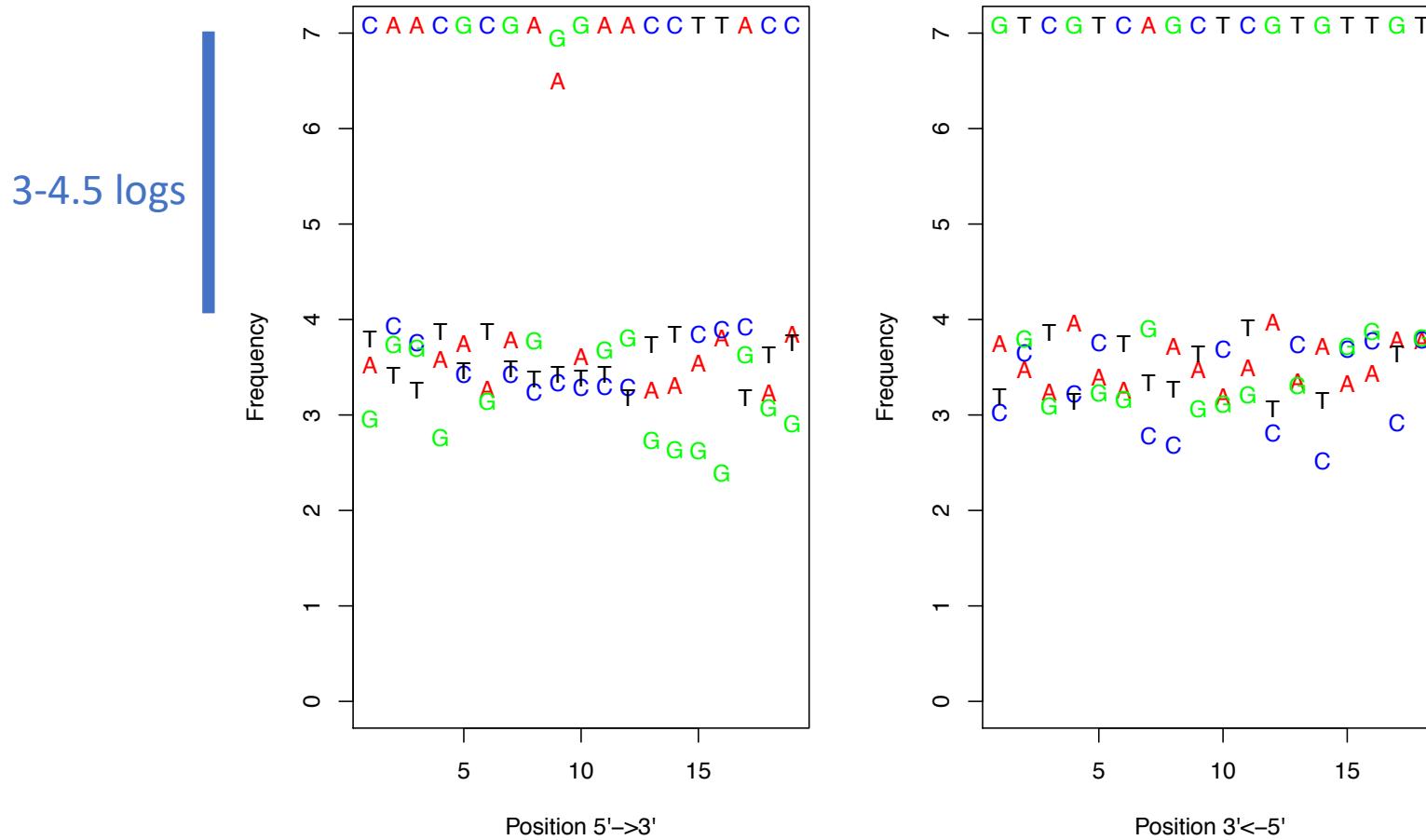
October 2010 | Volume 5 | Issue 10 | e15406



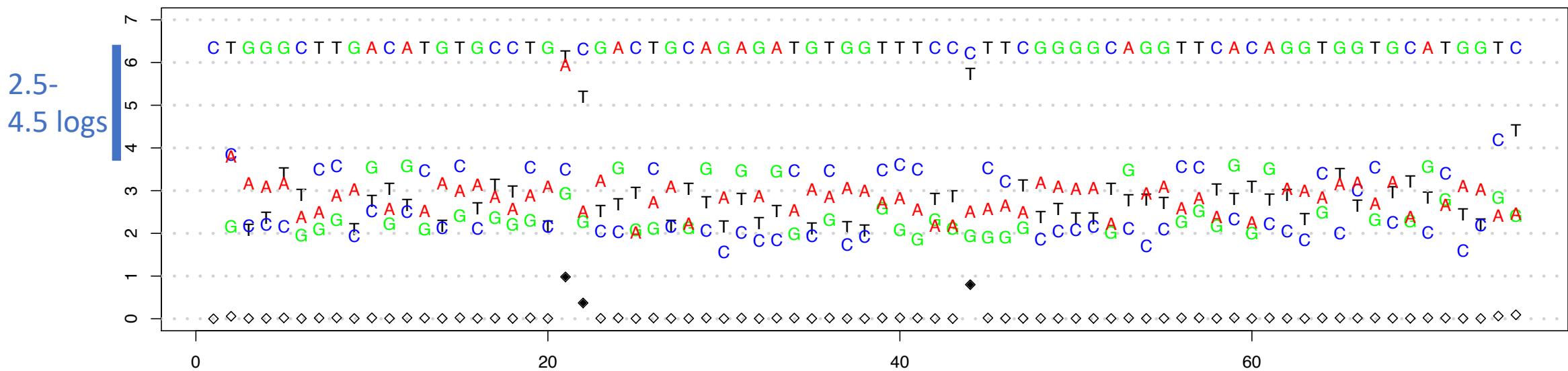
Sequencing error in the primers

Sources of variation

- Mis-synthesis
- PCR error (linear)
- Library prep error
- Sequencing error



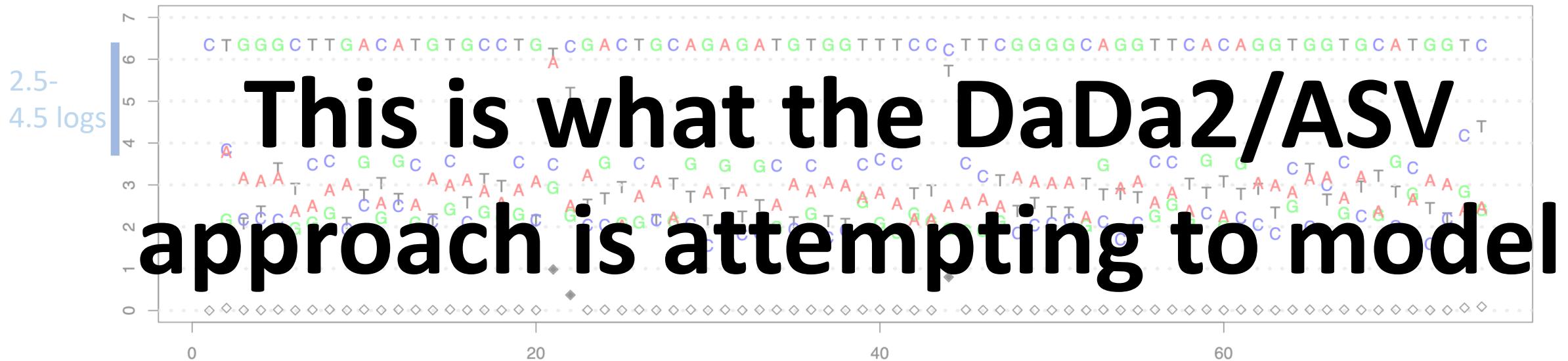
OTU error



About 3-5X greater error rate

- All of the previous but primer synthesis
- PCR error (exponential)
- Natural variation

OTU error



About 3-5X greater error rate. Attributable to

- All of the previous but primer synthesis
- PCR error
- Natural variation

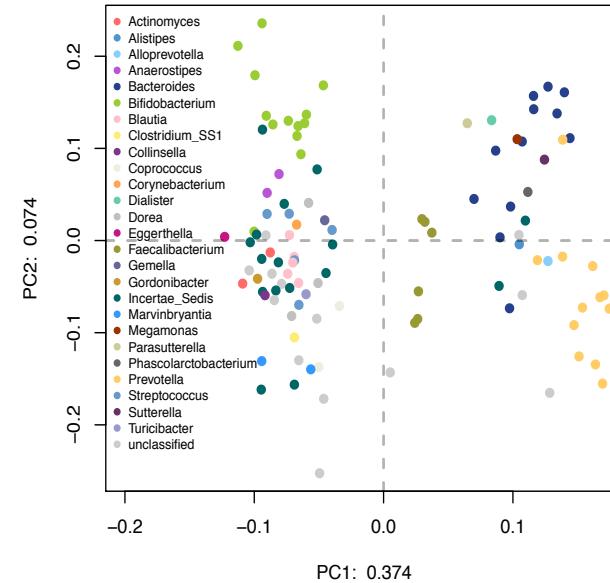
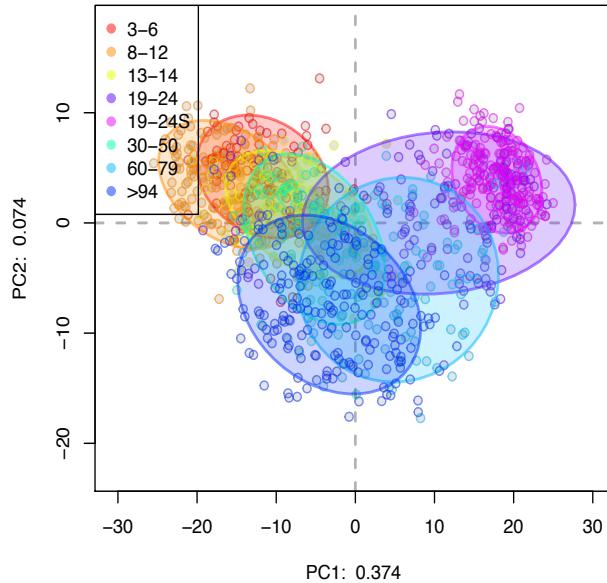


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The Gut Microbiota of Healthy Aged Chinese Is Similar to That of the Healthy Young

Gaorui Bian,^a Gregory B. Gloor,^{a,b,c*} Aihua Gong,^a Changsheng Jia,^a Wei Zhang,^d
Jun Hu,^e Hong Zhang,^f Yumei Zhang,^g Zhenqing Zhou,^h Jiangao Zhang,ⁱ
Jeremy P. Burton,^{a,c,j} Gregor Reid,^{a,c,j} Yongliang Xiao,^a Qiang Zeng,^k
Kaiping Yang,^{a,c,l,m,n} Jiangang Li^a



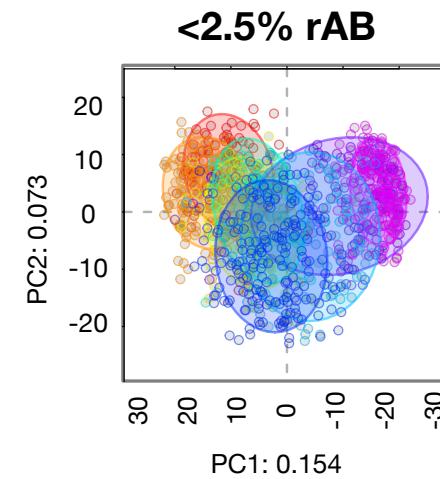
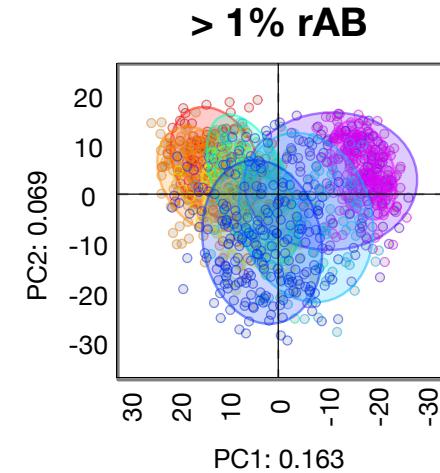
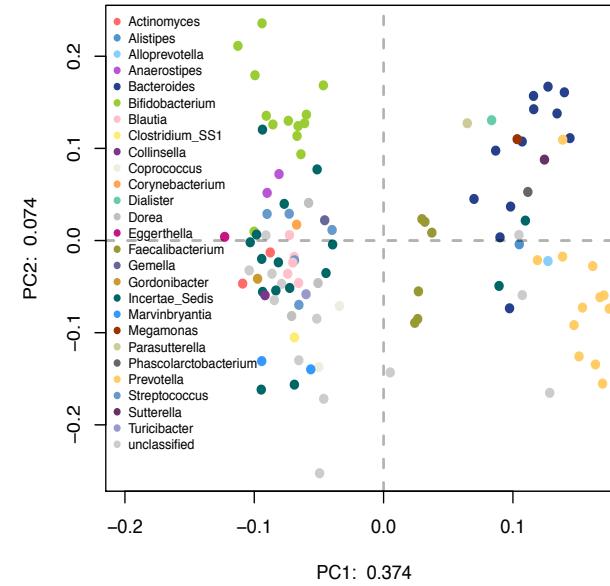
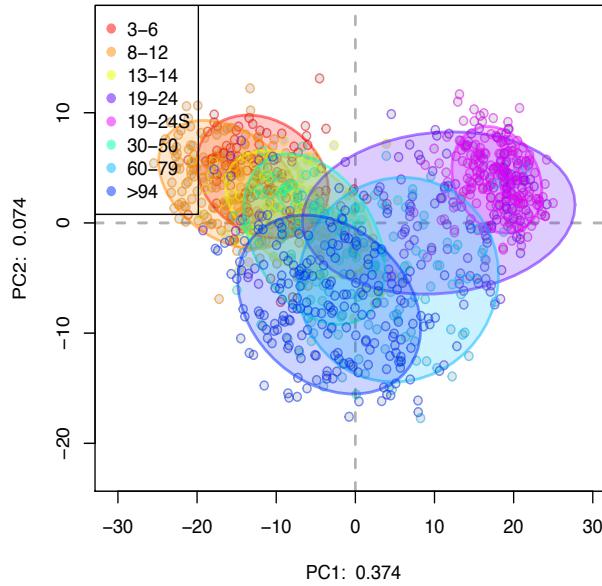


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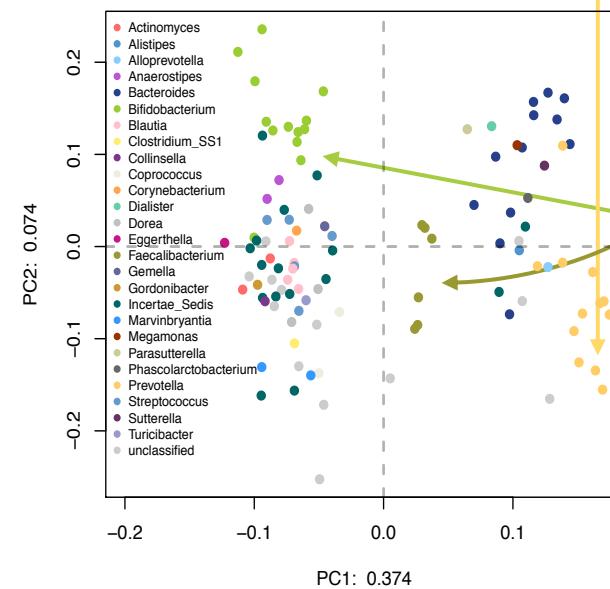
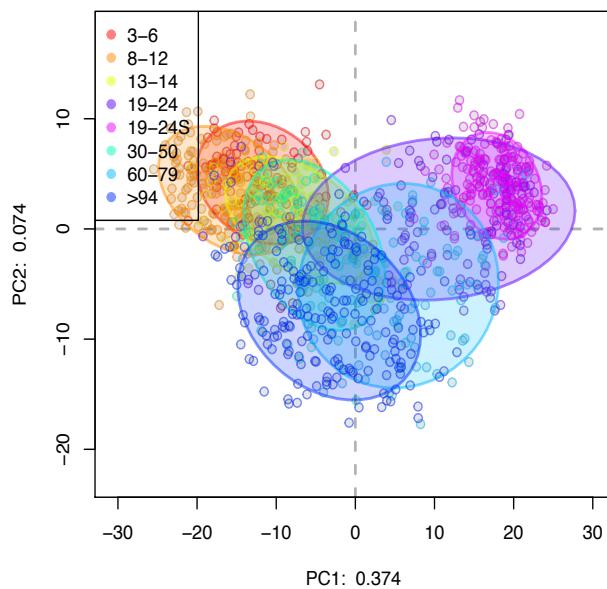
The Gut Microbiota of Healthy Aged Chinese Is Similar to That of the Healthy Young

Stable to sub/supersetting



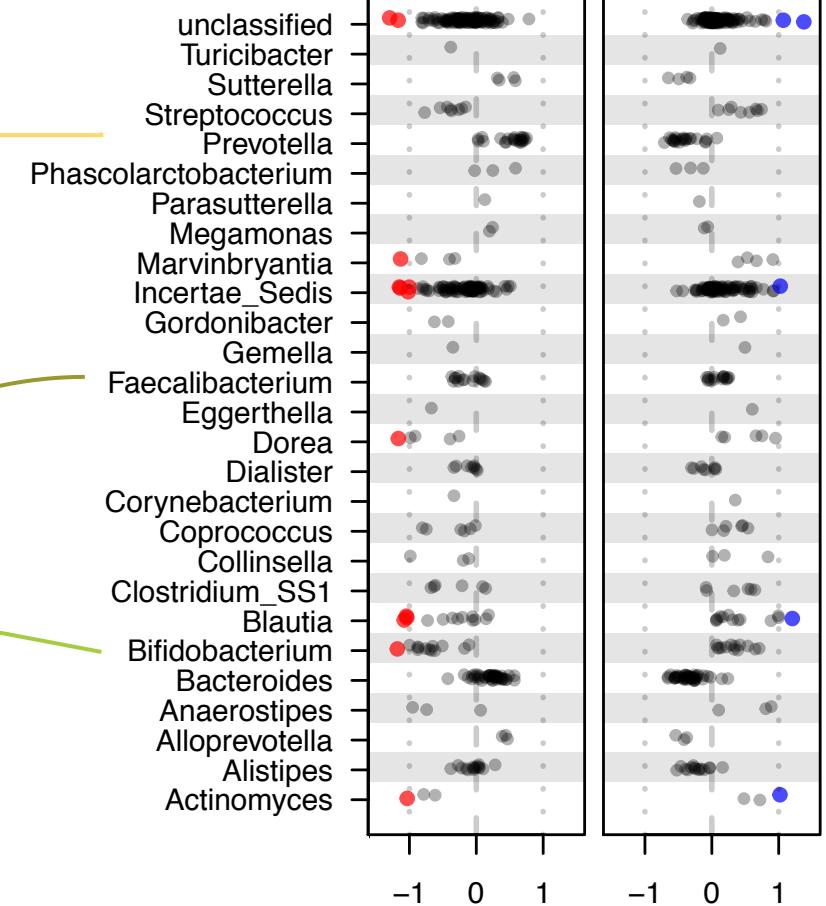
The Gut Microbiota of Healthy Aged Chinese Is Similar to That of the Healthy Young

Internally consistent results

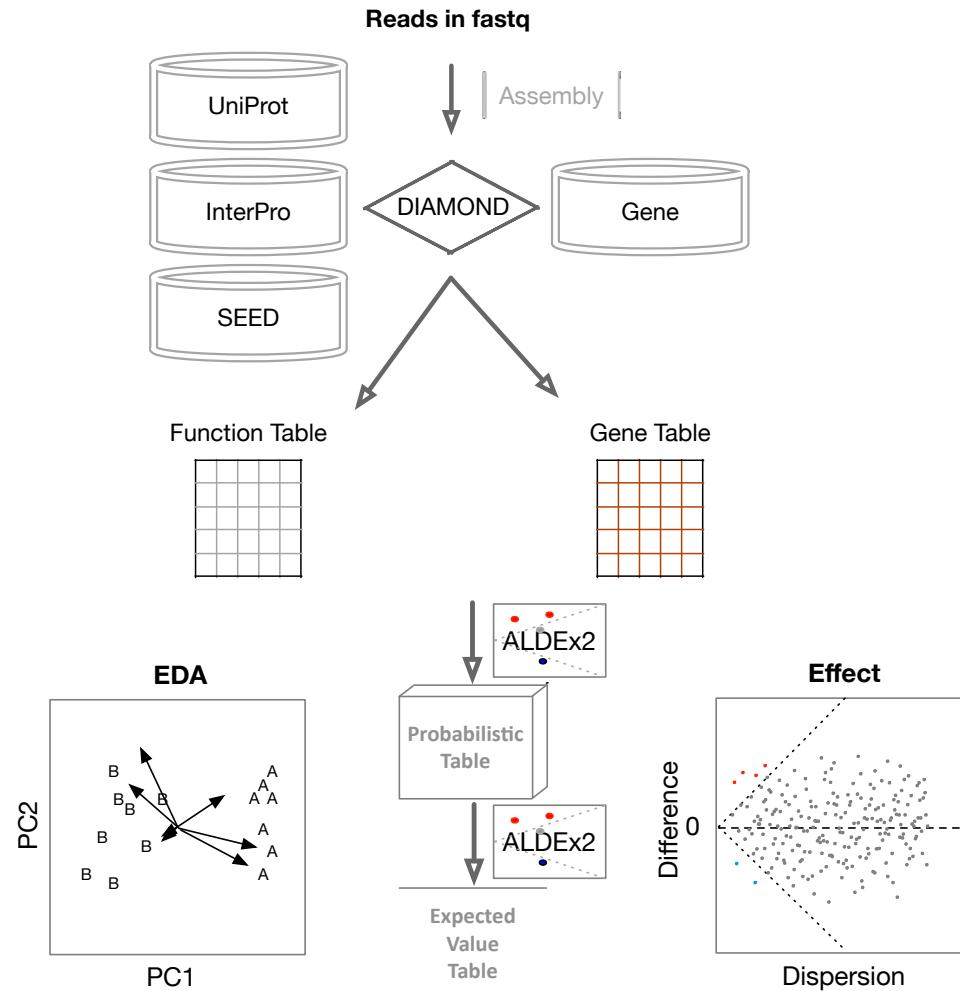


13:14 vs.
19-24

19:24 vs.
30:50



Functionalizing meta-seq 'omes

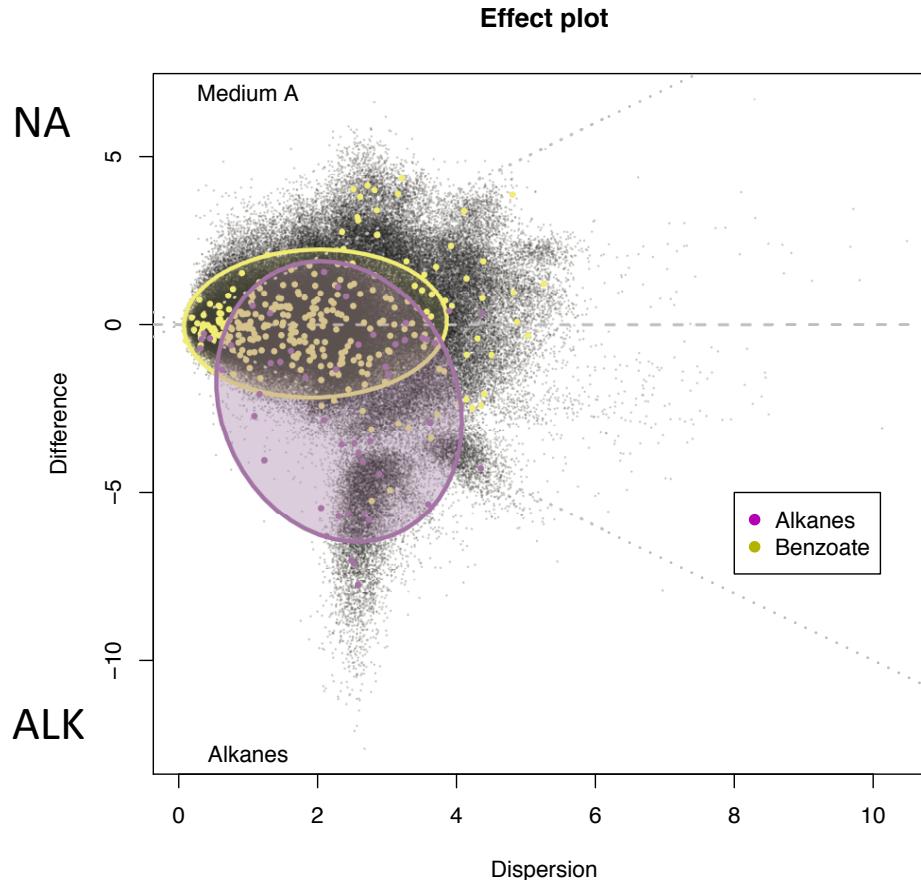


A sequence is a sequence is a sequence

Macklaim & Gloor 2018. From RNA-seq to Biological Inference: Using Compositional Data Analysis in Meta-Transcriptomics. **Meth. Mol. Bio.**
Gloor, Macklaim, Fernandes, 2016. Displaying Variation in Large Datasets: Plotting a Visual Summary of Effect Sizes. **J. of Comp. and Graph. Statistics**

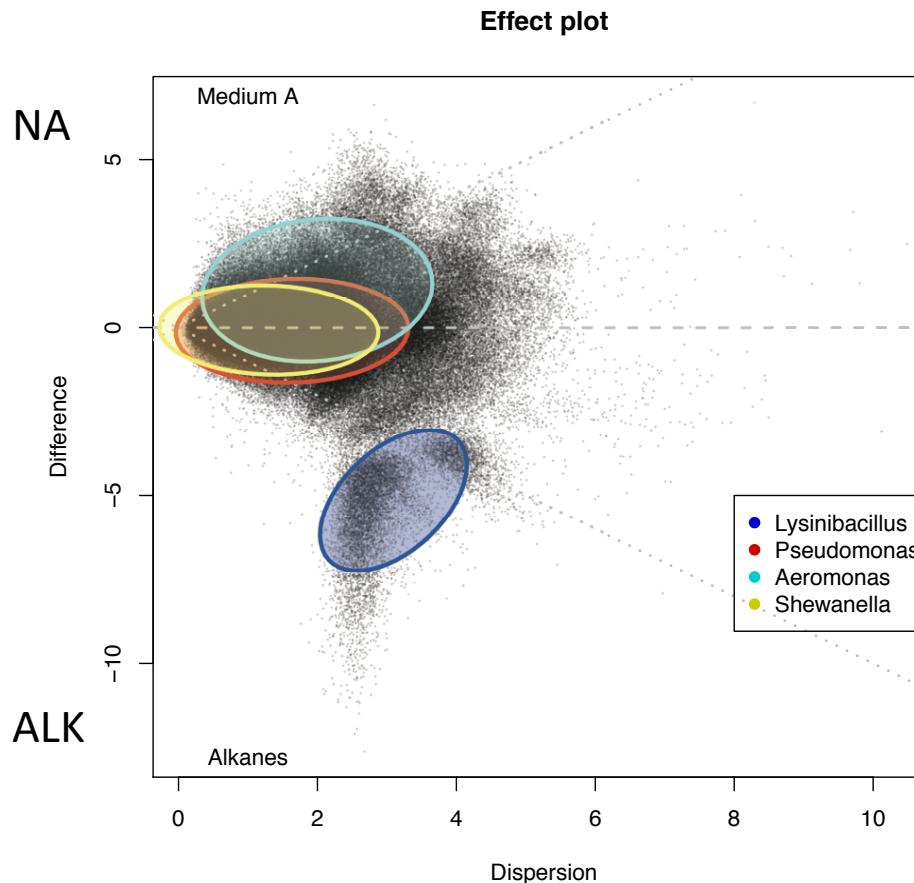
Meta-transcriptome from refinery wastewater treatment plant

- grown in two carbon sources
- Each point is a gene (>250k)
- Y-axis is E(difference) between groups
- X-axis is E(dispersion) within group

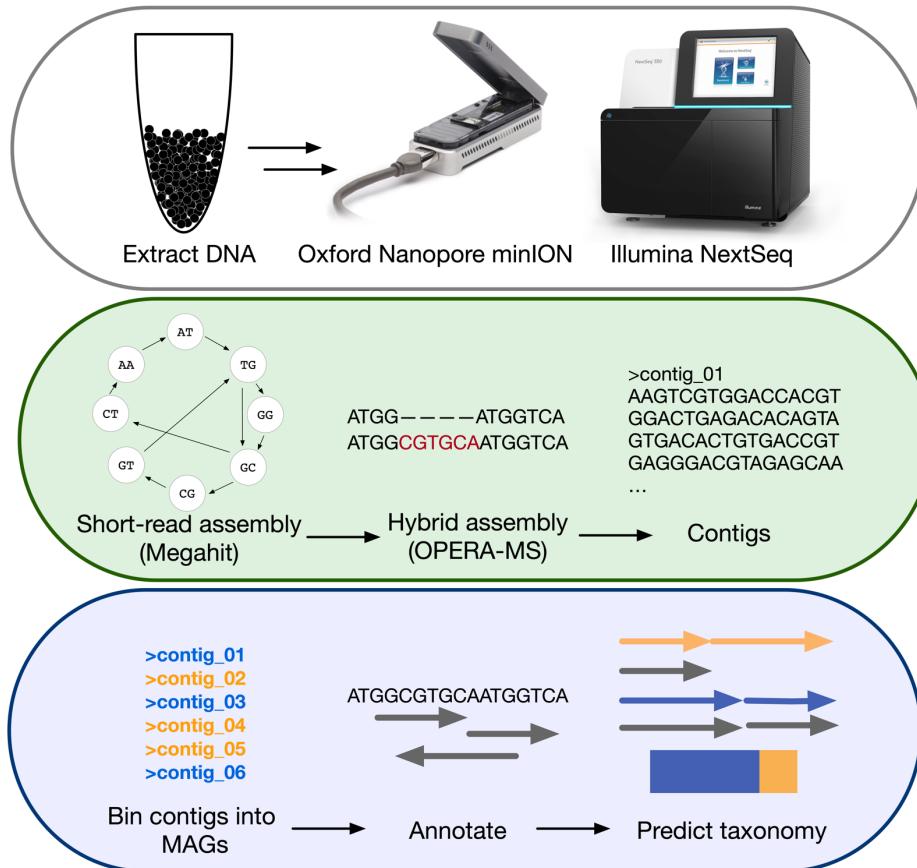


Functional meta-transcriptome of samples from refinery wastewater treatment plant

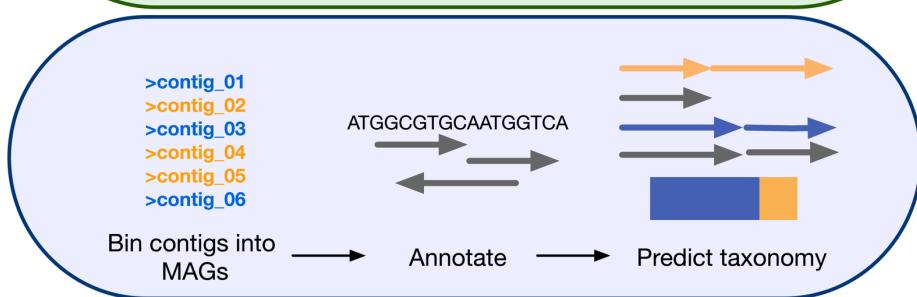
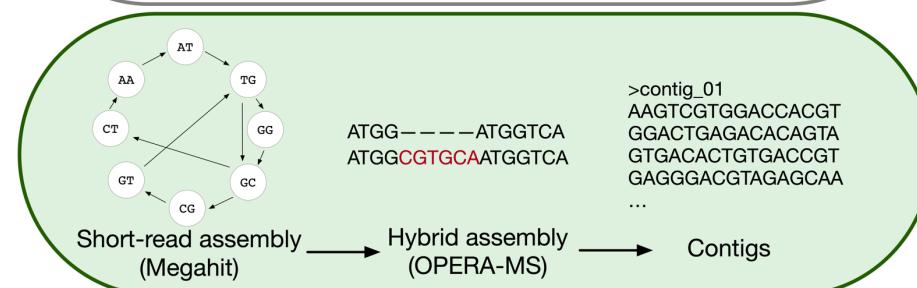
- grown in two carbon sources
- Each point is a gene (>250k)
- Y-axis is E(difference) between groups
- X-axis is E(dispersion) within group
- *Lysinibacillus* prefers alkanes



Metagenomically assembled genomes

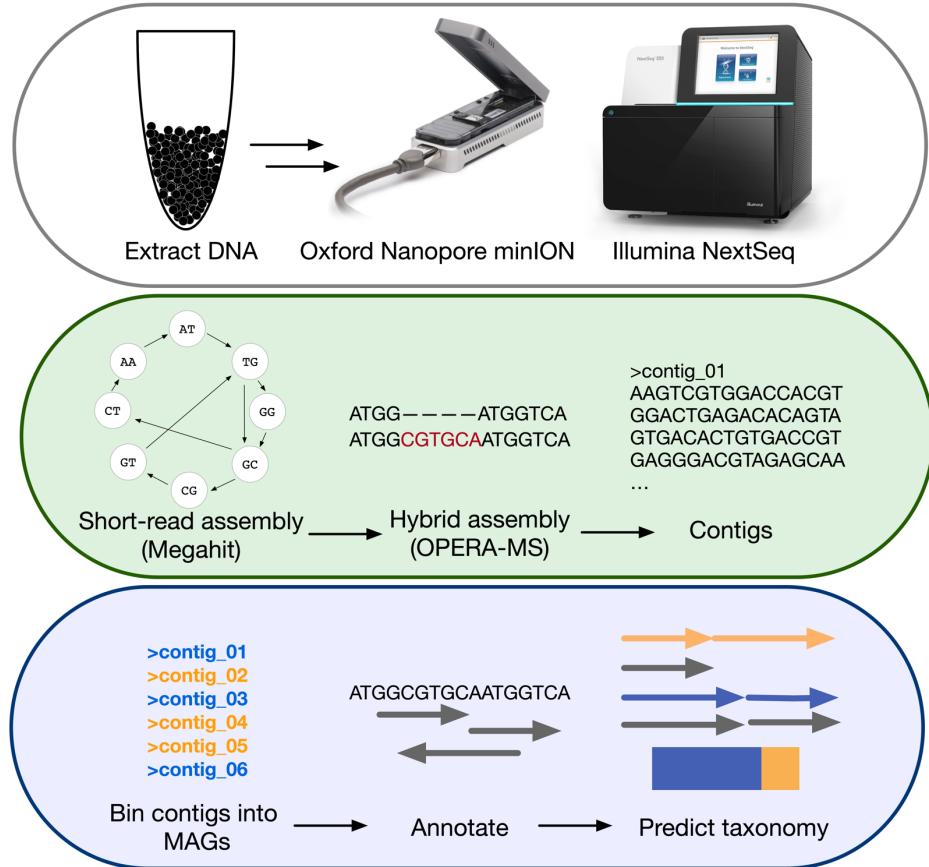


Metagenomically assembled genomes

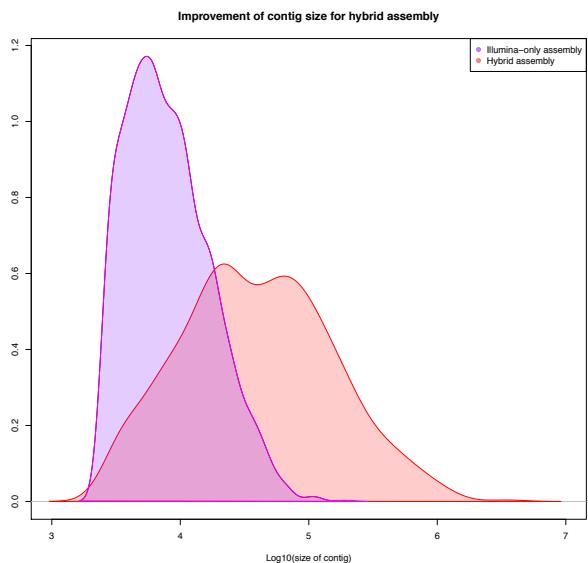


Bin	Source	Taxonomy	Total Size
Bin_22_2	anvi-refine	Unknown_Alphaproteobacteria	2.99 Mb
Bin_5_1	anvi-refine	Unknown_Betaproteobacteria	6.91 Mb
Bin_26_1	anvi-refine	Unknown_Betaproteobacteria	5.36 Mb
Bin_22_1	anvi-refine	Unknown_Alphaproteobacteria	2.97 Mb
Bin_3_1	anvi-refine	Unknown	5.80 Mb
Bin_4_2	anvi-refine	Unknown_Bacteria	4.83 Mb
Bin_12_1	anvi-refine	Unknown_Caulobacteraceae	3.51 Mb
Bin_47_1	anvi-refine	Unknown_Rhodobacteraceae	3.14 Mb
Bin_1_2	anvi-refine	Unknown_Bacteria	4.26 Mb
Bin_12_2	anvi-refine	Unknown_Bacteria	4.25 Mb
Bin_18_1	anvi-refine	Unknown_Gammaproteobacteria	2.83 Mb
Bin_4_1	anvi-refine	Unknown_Bacteria	5.12 Mb
Bin_24	CONCOCT	Unknown_Alphaproteobacteria	5.81 Mb
Bin_32_1	anvi-refine	Unknown_Betaproteobacteria	4.96 Mb
Bin_38_1	anvi-refine	Unknown_Bacteria	3.87 Mb
Bin_33	CONCOCT	Unknown_Bacteria	4.72 Mb
Bin_18_2	anvi-refine	Unknown_Bacteria	2.58 Mb

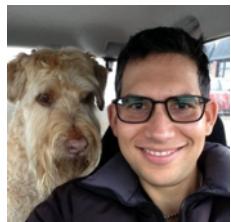
Metagenomically assembled genomes



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Bin_3_1	anvi-refine	Unknown	5.80 Mb
Bin_4_2	anvi-refine	Unknown_Bacteria	4.83 Mb
Bin_12_1	anvi-refine	Unknown_Caulobacteraceae	3.51 Mb
Bin_47_1	anvi-refine	Unknown_Rhodobacteraceae	3.14 Mb
Bin_1_2	anvi-refine	Unknown_Bacteria	4.26 Mb
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Canadian Centre for Human Microbiome
and Probiotic Research



Andrew
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Jean
Macklaim

Gregor Reid
Jeremy Burton
Daniel Giguere



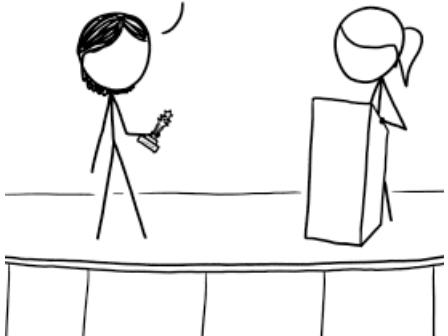
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I'D LIKE TO THANK MY DIRECTOR,
MY FRIENDS AND FAMILY, AND—
OF COURSE—THE WRITHING MASS
OF GUT BACTERIA INSIDE ME.

I MEAN, THERE'S LIKE ONE OR
TWO PINTS OF THEM IN HERE;
THEIR CELLS OUTNUMBER MINE!
ANYWAY, THIS WAS A
REAL TEAM EFFORT.



Government
of Canada Gouvernement du Canada
Agriculture and Agri-Food
Canada

Ontario Genomics

Vogue
vaginal microbiome group initiative
*Advancing Women's Health through
Microbiome Research*

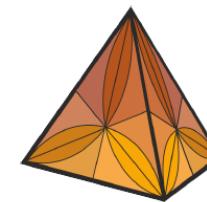


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