

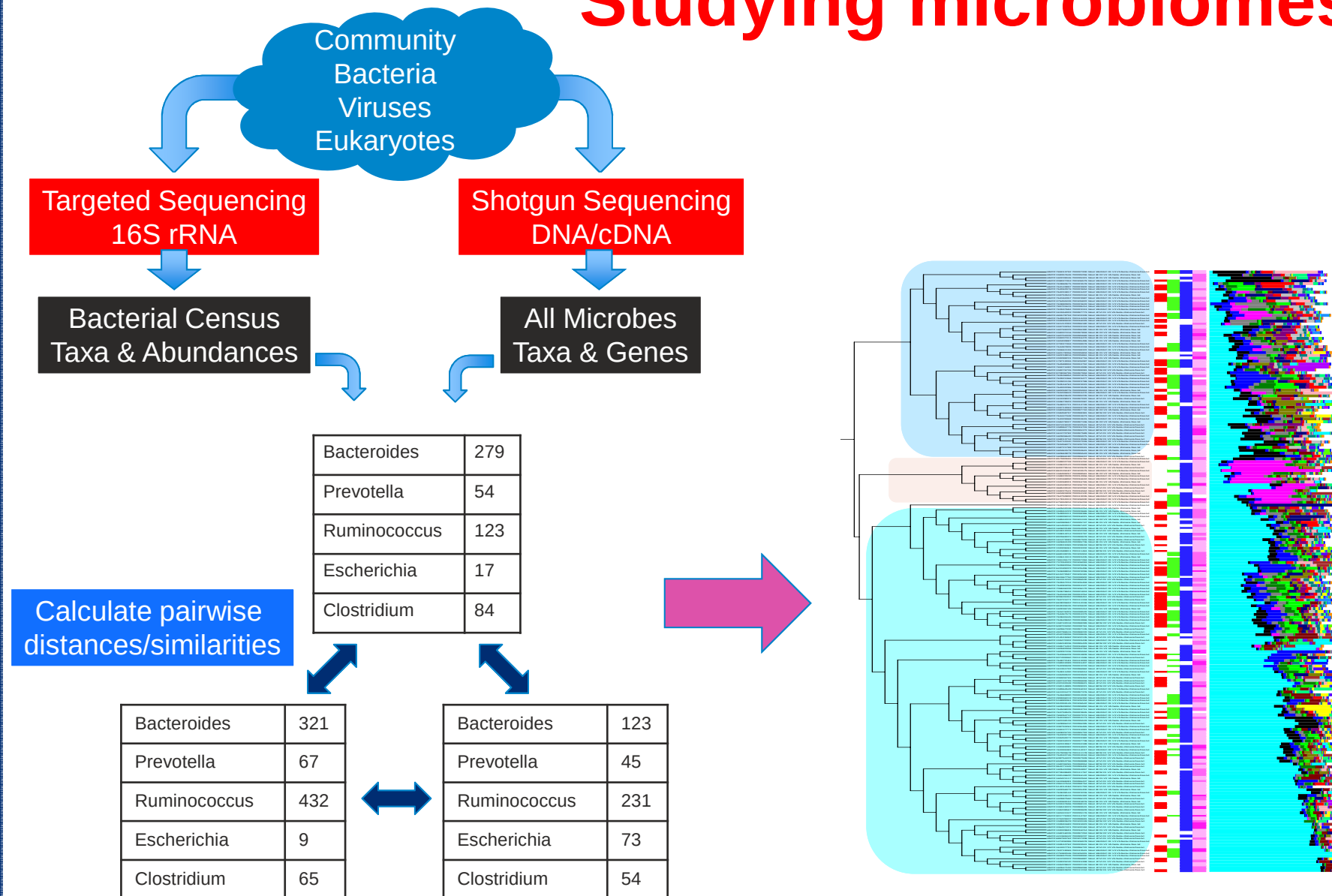
Probing the Medical Microbiome

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International Conference on Clinical Metagenomics
Geneva
October 13, 2016

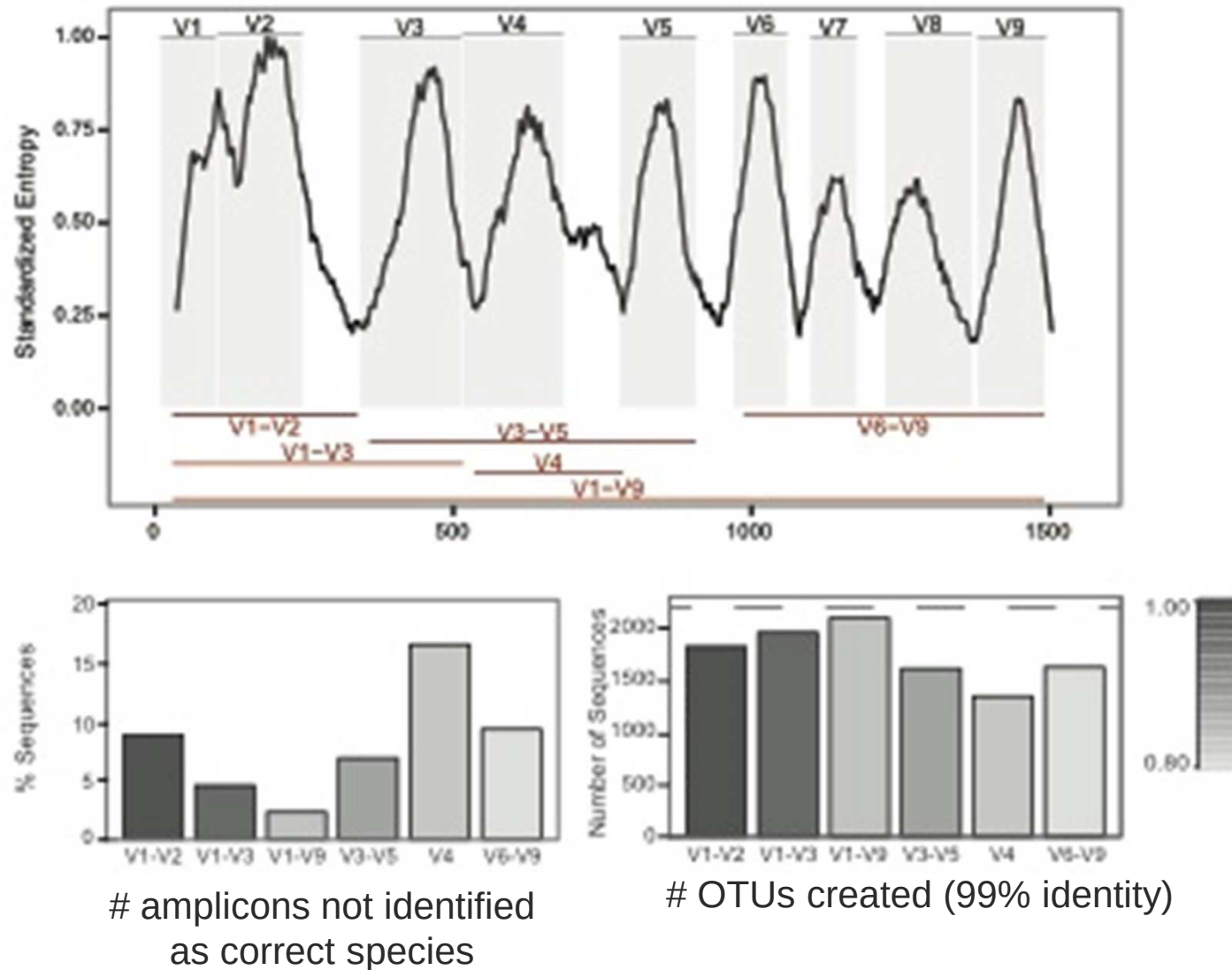


Studying microbiomes

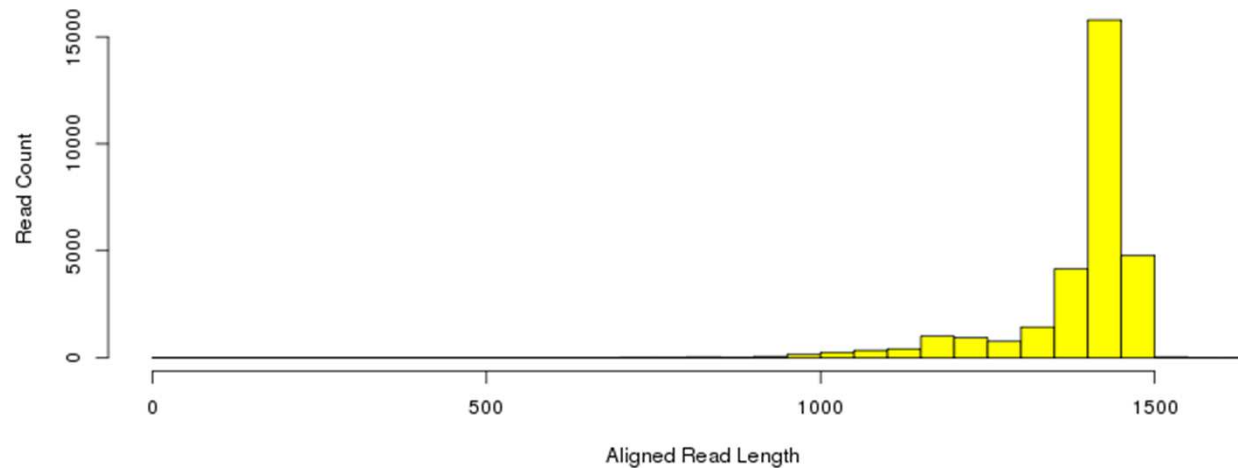




16S rRNA Targets: Full-length is better



PacBio full-length 16S rRNA sequencing

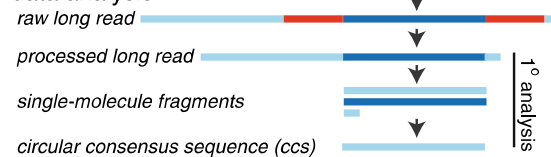


1. generate amplicon

2. ligate adaptors

3. sequence

4. data analysis

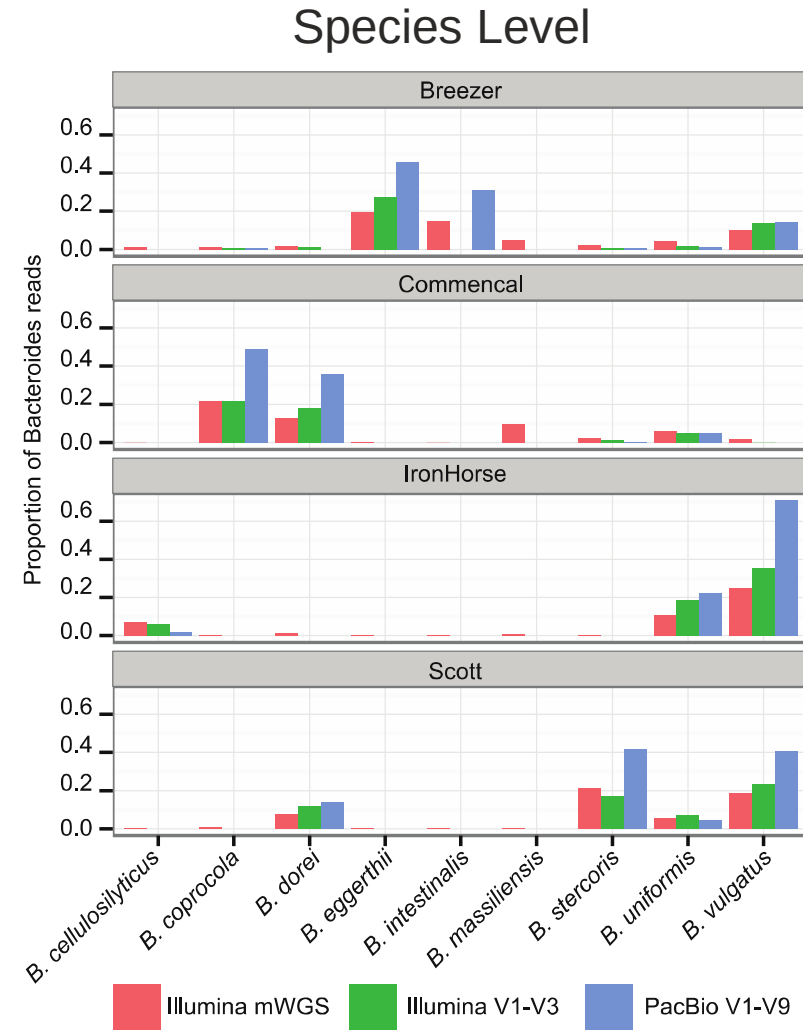
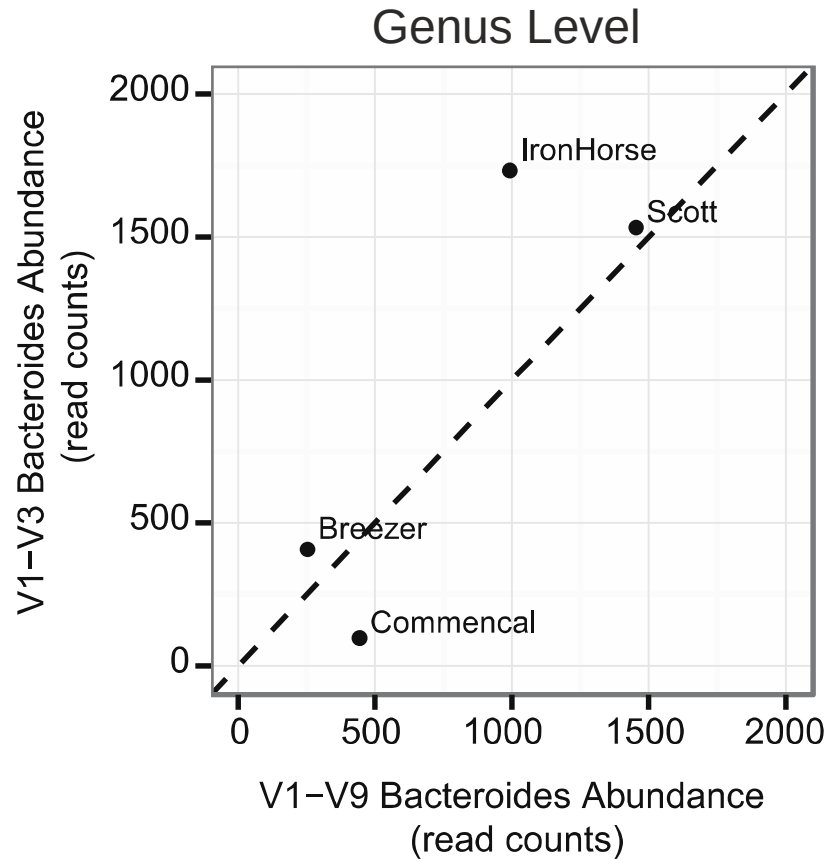


- Averaging 10x coverage
- 5x around the “circle”
- 5x each strand



Cartoon from Fichot and Norman,
Microbiome 2013,1:10

Species level is very important



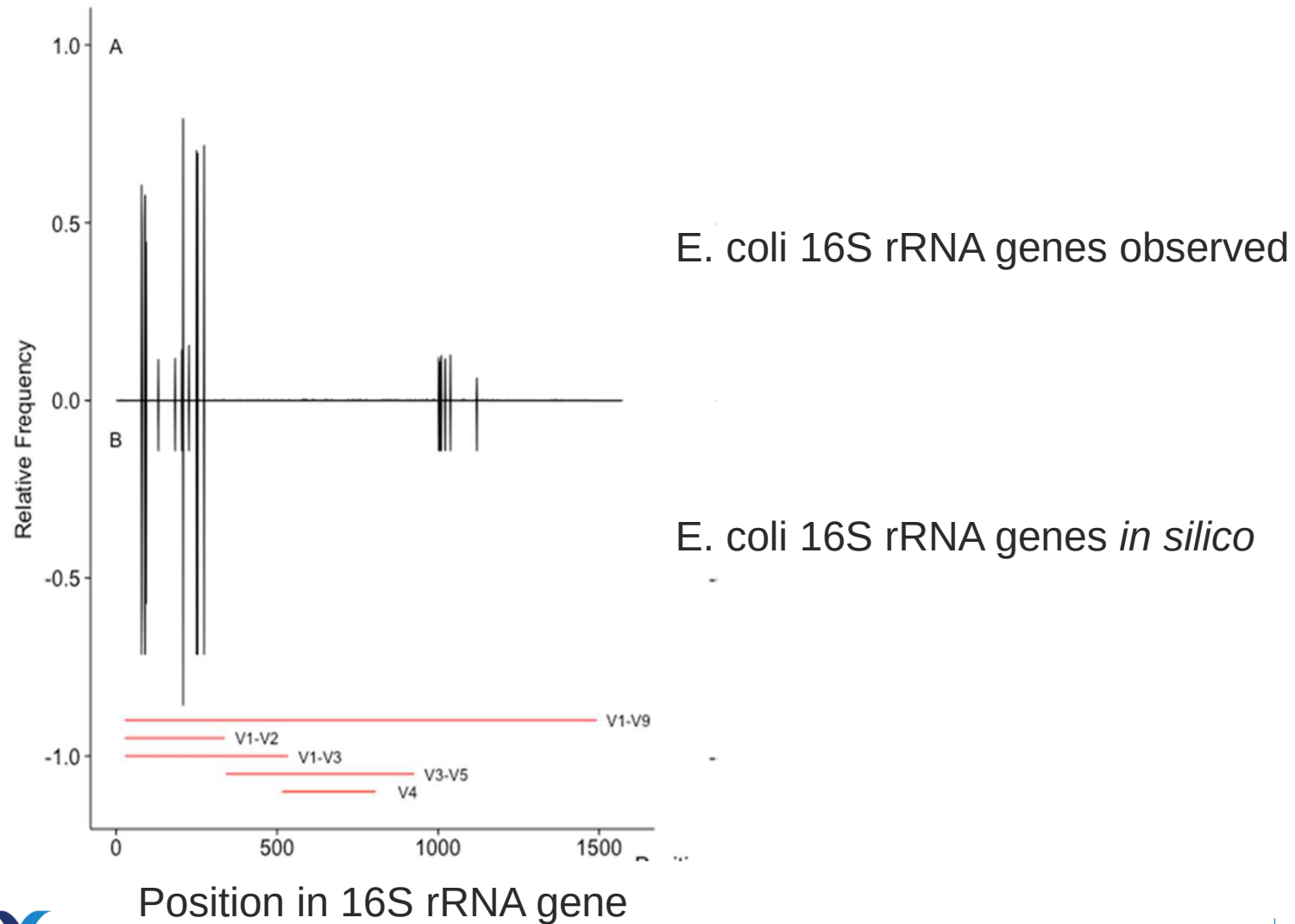
Top 10 *P. acnes* ribotypes: Subspecies level is very important

Full-length Sanger sequencing of >31,000 16S rRNA gene clones to define ribotypes

Ribotype	Nucleotide changes from RT1	Number of subjects	Number of clones	Percentage of clones from acne patients ^a	Percentage of clones from normal individuals ^b	<i>p</i> -value ^c
RT1	-	90	5536	48%	52%	0.84
RT2	T854C	48	1213	51%	49%	0.36
RT3	T1007C	60	2104	40%	60%	0.092
RT4	G1058C, A1201C	23	275	84%	16%	0.049
RT5	G1058C	15	205	99%	1%	0.00050
RT6	T854C, C1336T	11	262	1%	99%	0.025
RT7	G529A	10	188	99%	1%	0.12
RT8	G1004A, T1007C	5	239	100%	0%	0.024
RT9	G1268A	4	68	99%	1%	0.29
RT10	T554C, G1058C	5	61	100%	0%	0.024



Detection of single nucleotide variation using PacBio CCS



Detecting organisms of interest with mouse models



C. difficile mouse model

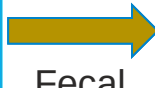
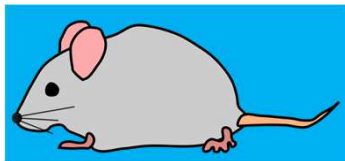
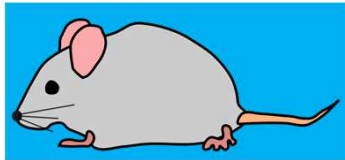
WUSTL C57BL/6J
Resistant



JAX C57BL/6J
Sensitive



Clostridium difficile



Fecal
transplant

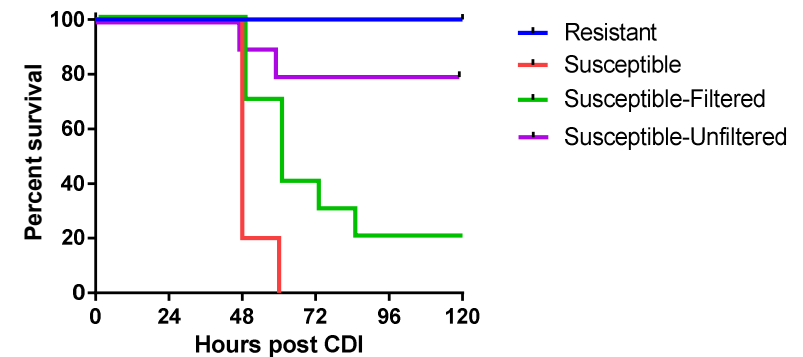
Compare Microbiomes

Identify Differences

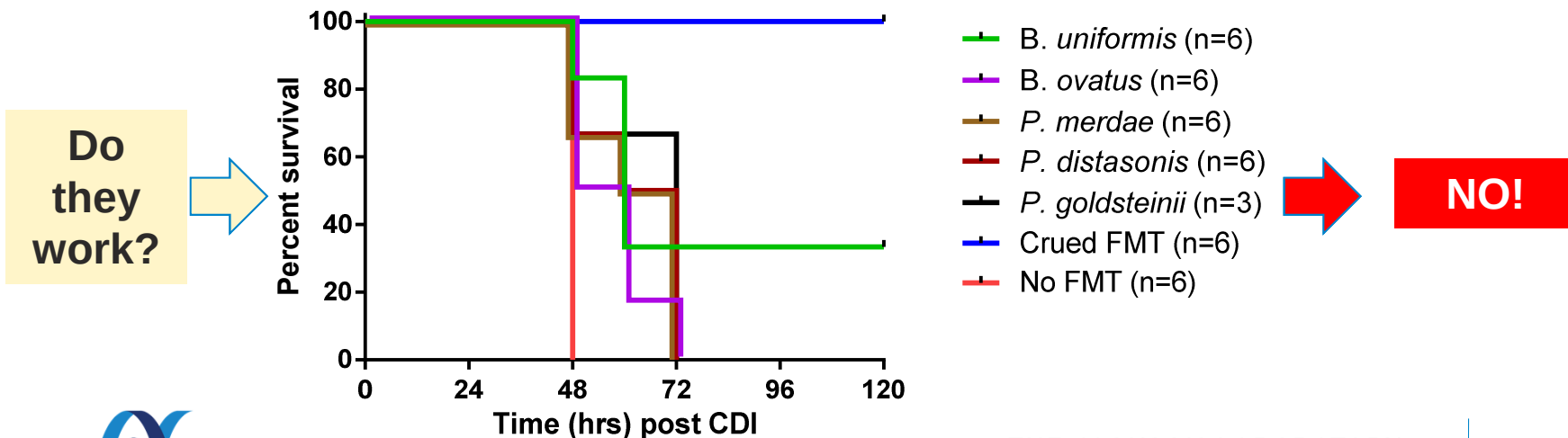
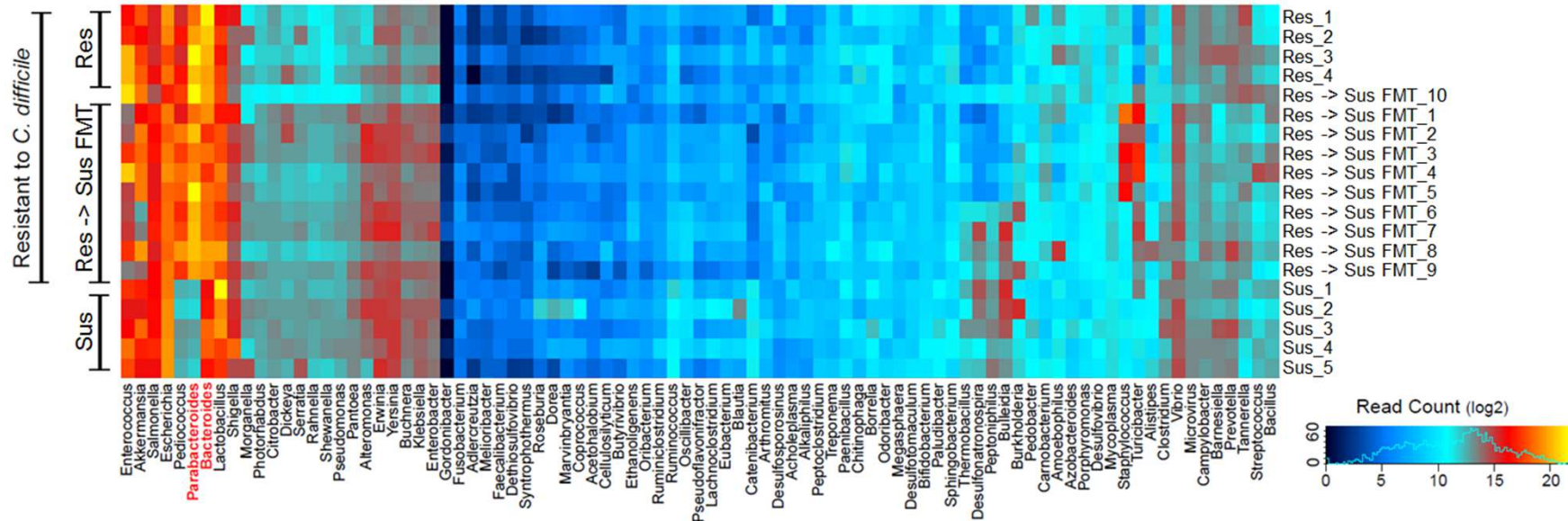
Test



David Haslam, MD
Cincinnati Children's
Hospital Medical Center

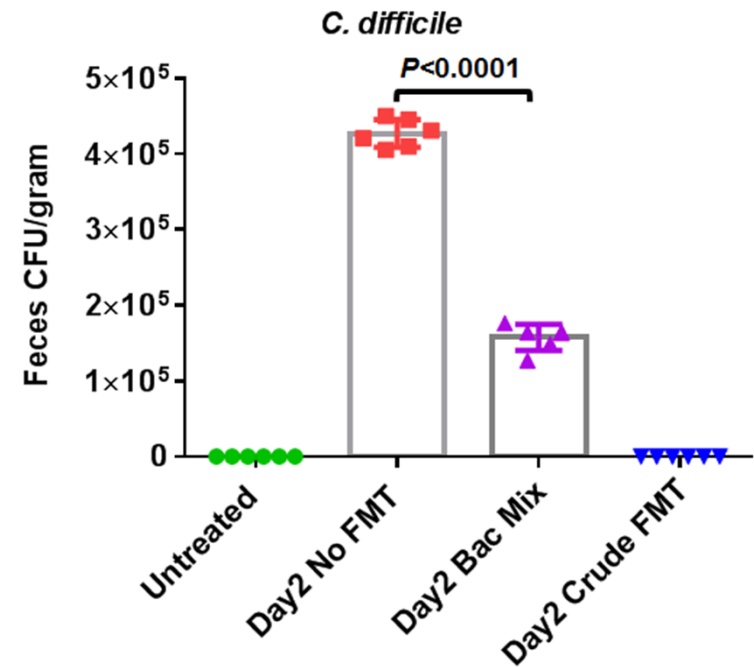
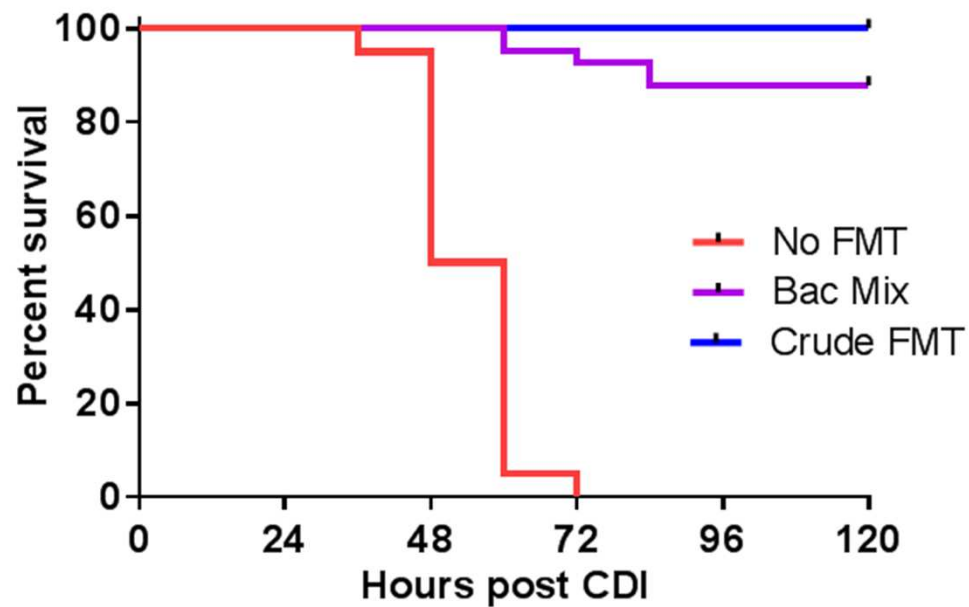


Identification of Protective Organisms

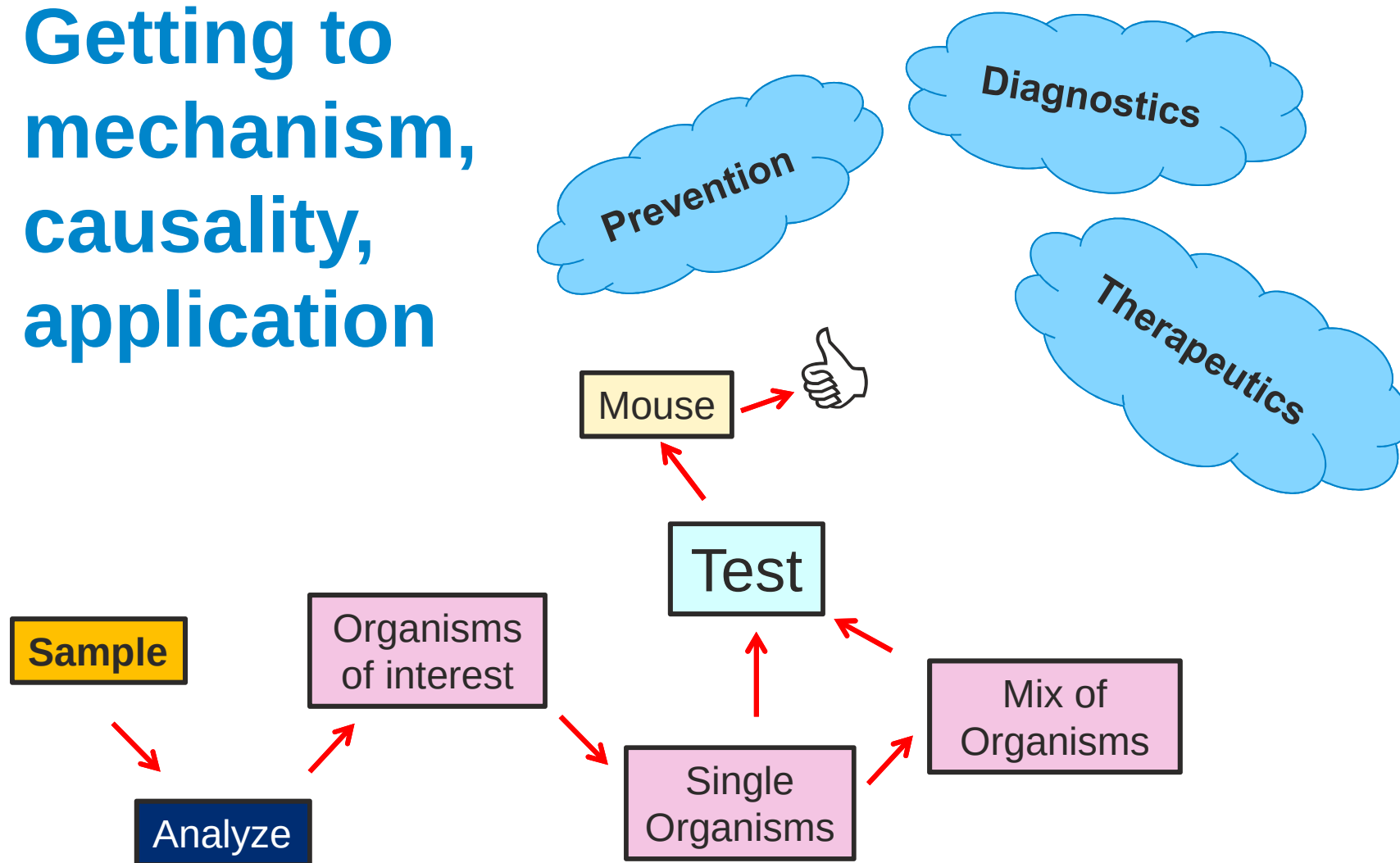


C. difficile mouse model

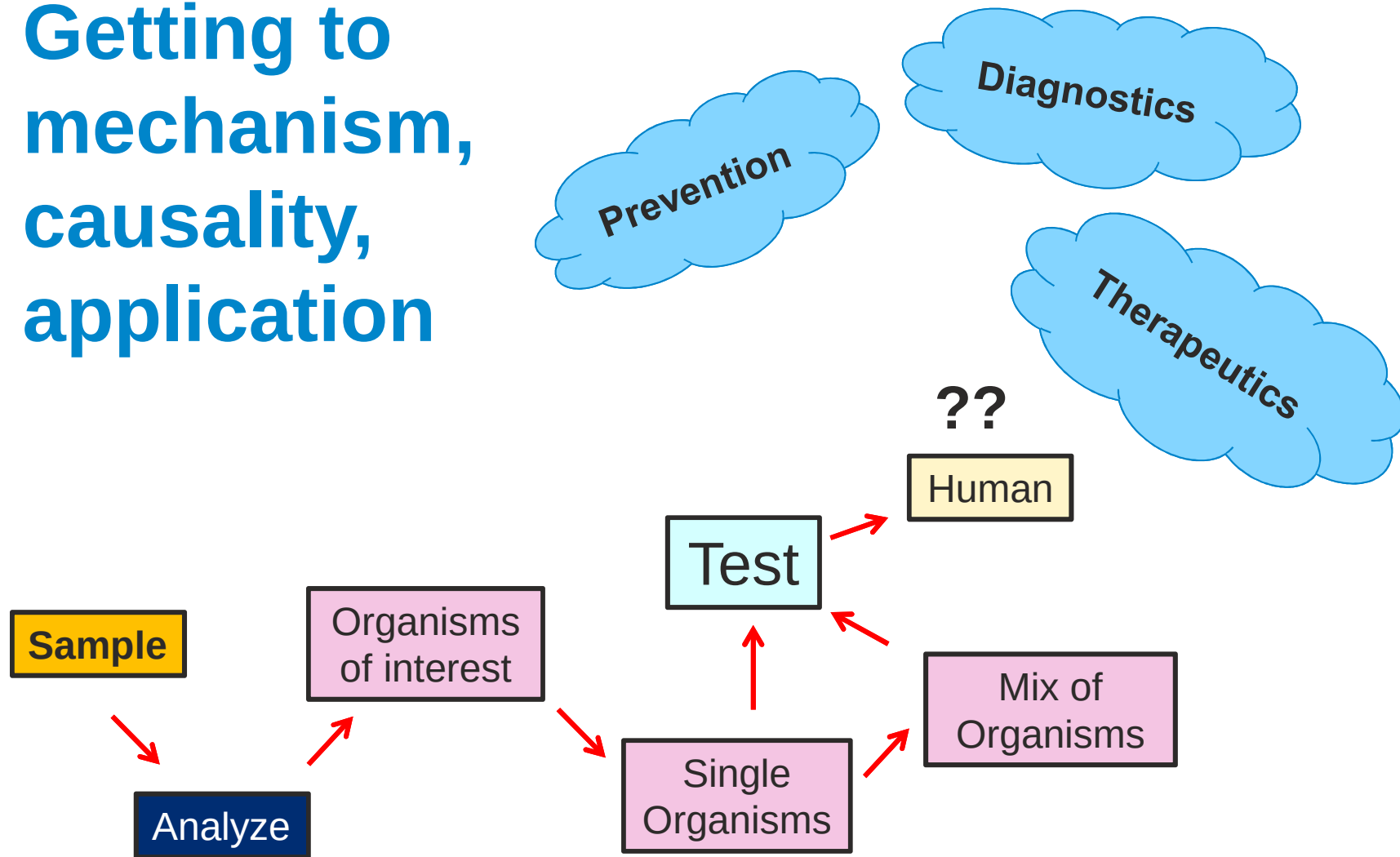
Treatment by fecal microbiota transplant (FMT)
or a mix of 4 gut bacteria



Getting to mechanism, causality, application



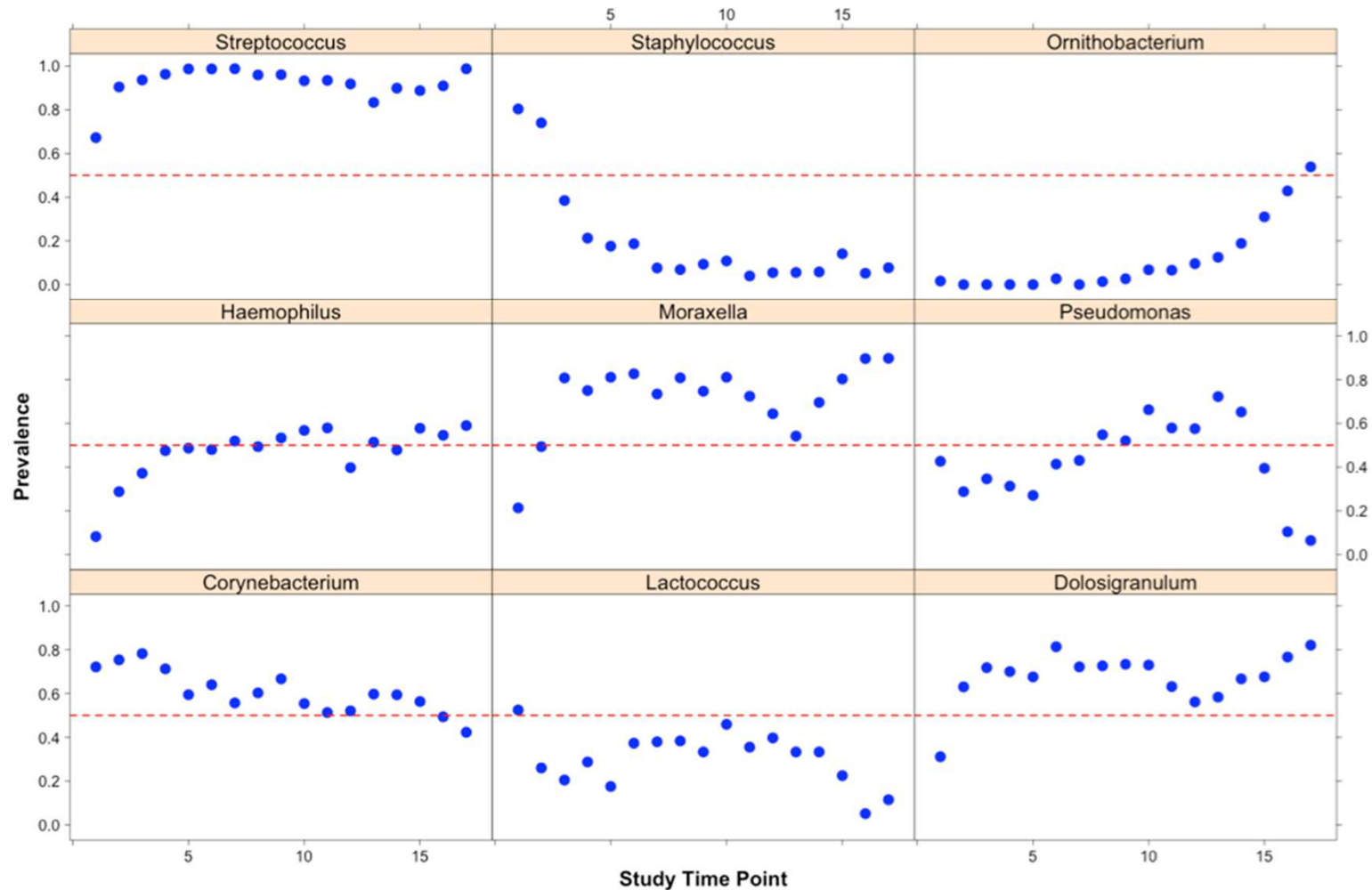
Getting to mechanism, causality, application



Humans: What's Normal?



Prevalence of nasopharyngeal bacteria from birth



Don't Forget the Virome

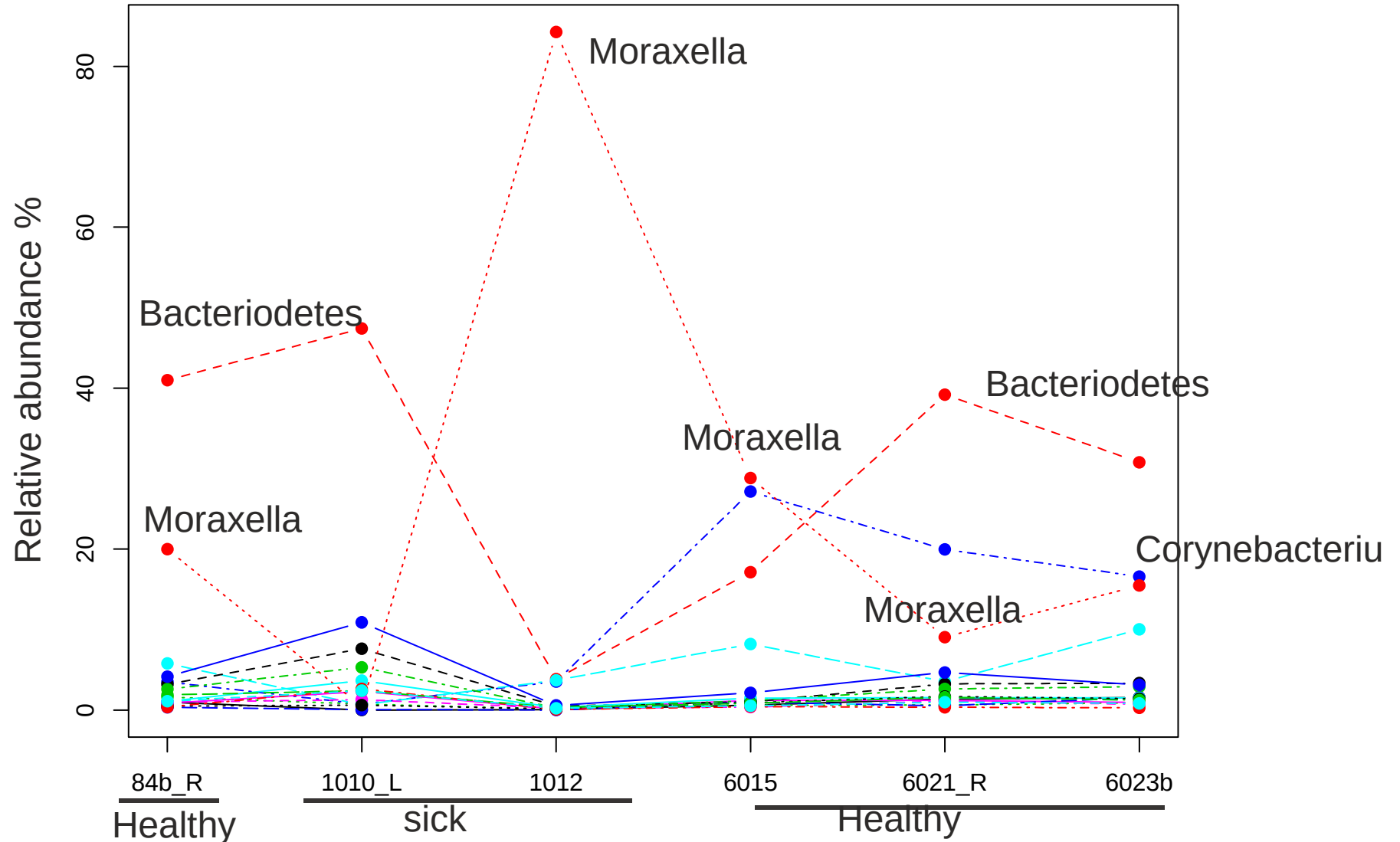


Viruses detected in nasal RNA samples

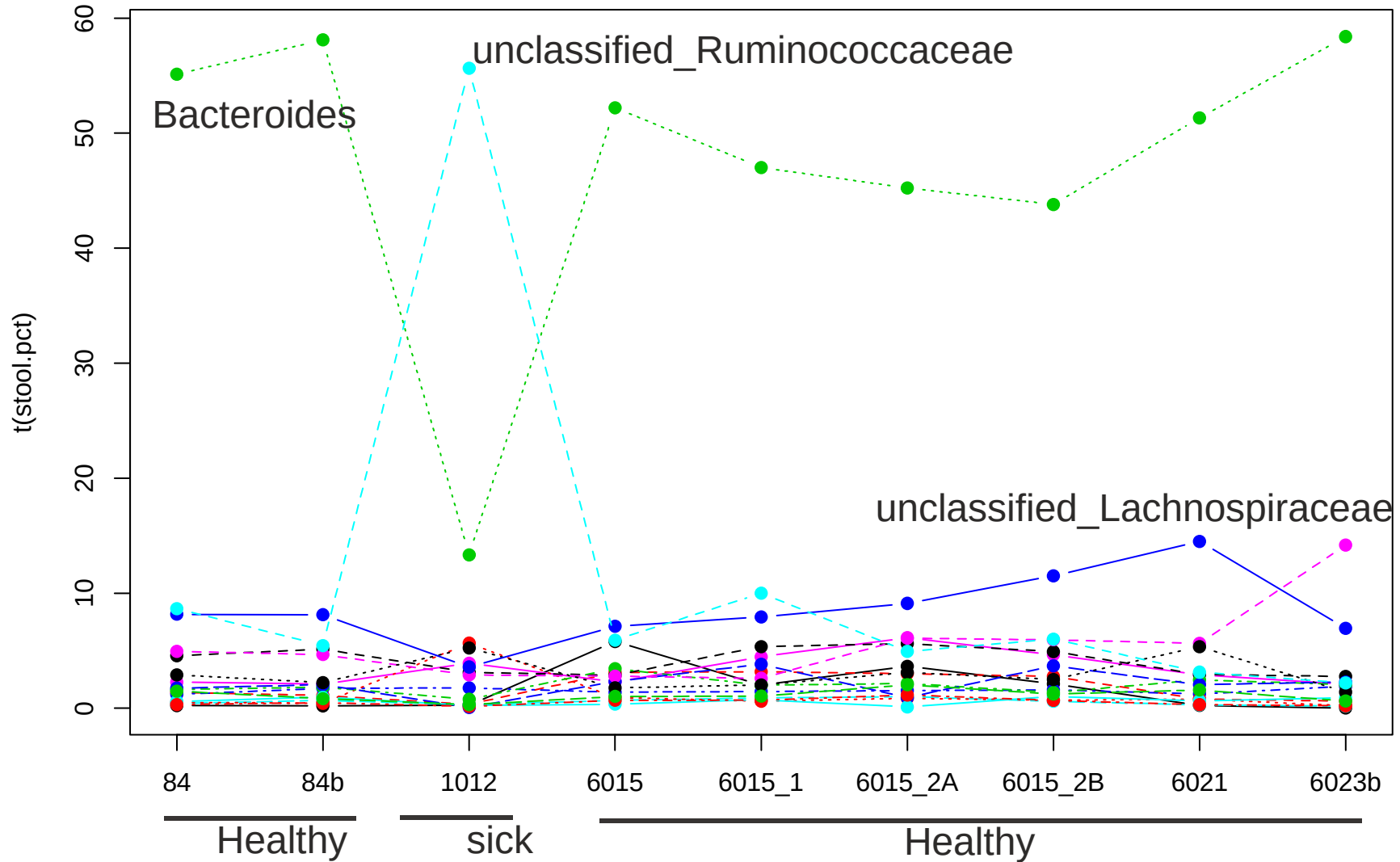
69_001_64_A	69_001_64_B	69_001_84_L	69_001_84_R	69_001_1010_L	69_001_1012_L	69_001_1012_R	69_001_1013_L	69_001_1013_R	69_001_6015_L	69_001_6015_R	69_001_6021_L	69_001_6021_R	69_001_6023b_L	69_001_6023b_R	
0	0	3	2	11	0	0	5	0	0	8	0	4	1	11	Agaricus bisporus virus
0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	Betapapillomavirus 2
0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Fujinami sarcoma virus
0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	Hepatitis C virus
0	1	0	3	0	5	0	2	0	0	0	0	0	0	0	Human immunodeficiency virus
0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	Murine astrovirus; virus
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Porcine reproductive and respiratory syndrome virus
1	2	4	6	49	430887	122120	106170	98961	35	783	0	0	2	0	Human rhinovirus
5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	Semliki Forest virus
0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Simian virus 40
0	0	0	2	0	10	0	0	0	0	0	7	13	0	0	Tomato mosaic virus
14	0	0	0	1	0	0	12	4	19	12	1	0	0	0	uncultured marine virus
31	53	9	13	61	430902	122123	106193	98965	54	805	8	17	3	11	Total Virus
963242	907600	550172	578187	850450	2212367	6176431	918368	1290334	1490989	2567199	1071595	846700	847331	1344433	Total Reads
Healthy				Sick					Healthy						



Nasal bacterial changes during viral infection



Stool bacterial changes during viral infection



Back to Technology: Solving the Gnarly Genomes



Piperacillin-Tazobactam (TZP)

- TZP broth microdilution and agar dilution produced different results
 - Piperacillin: β -lactam antibiotic, inhibits cell wall synthesis.
 - Tazobactam: inhibits bacterial β -lactamase (encoded by *bla*)
- TZP-resistant *E. coli* have a *bla*_{TEM-1} gene
 - Tazobactam should inhibit the β -lactamase.
 - Why not?
 - Over-expression of *bla* gene?
 - High plasmid copy number leads to high *bla* copy number?
 - Efflux or porin alterations?



Shubert C, et al., *Antimicrobial Agents and Chemotherapy*, March 2014

THE JACKSON LABORATORY

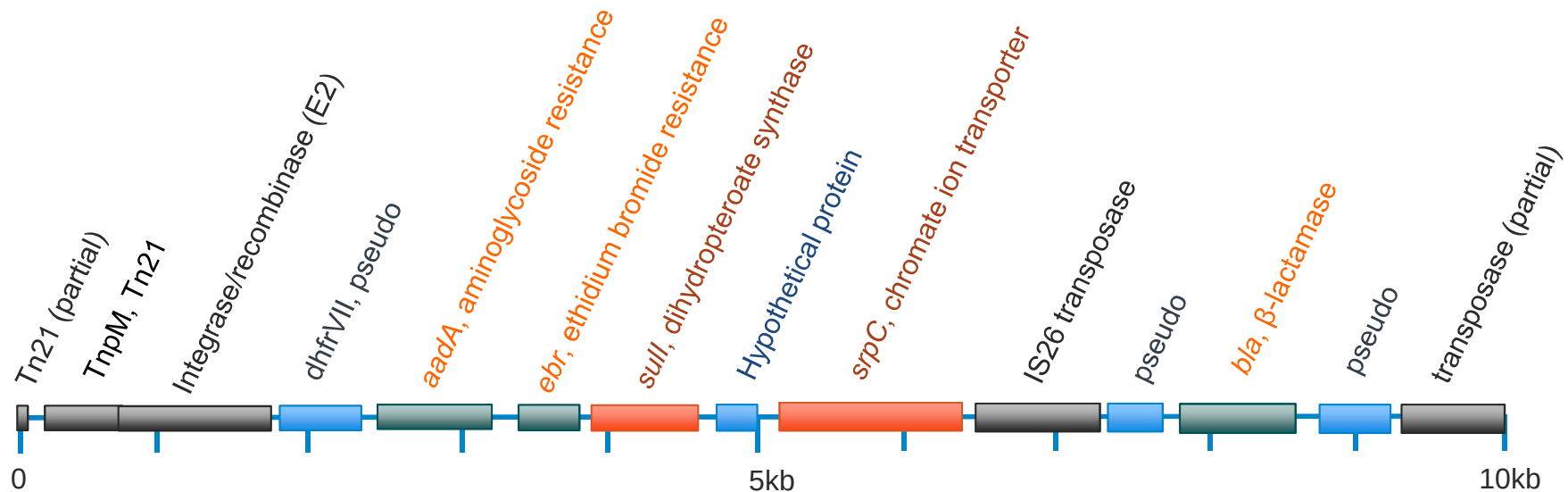
Estimate bla and plasmid copy

Copy # (Estimated compared to MLST coverage)

JAX ID	BMX ID	Antibiotic Conditions	bla (Illumina)	bla (PacBio)	Tral
J05301	907355	None	10.61	11.98	1.01
J05302	907355	TZP (4/4 µg/ml)	53.91	46.13	1.50
J05303	907355	TZP (8/8 µg/ml)	96.78	90.42	1.15
J05304	907357	None	7.94	9.30	0.66
J05305	907357	TZP (4/4 µg/ml)	64.67	55.23	1.09
J05306	907357	TZP (8/8 µg/ml)	59.83	41.23	1.13
J05307	907402	None	12.15	11.60	1.14
J05308	907402	TZP (4/4 µg/ml)	44.85	58.01	1.50
J05309	907402	TZP (8/8 µg/ml)	79.98	47.23	1.63
J05310	907700	None	1.80	2.63	0.77
J05311	907700	TZP (4/4 µg/ml)	34.48	45.22	1.61
J05312	907700	TZP (8/8 µg/ml)	76.06	39.92	1.62
J05313	907701	None	0.83	1.58	0.73
J05314	907701	TZP (4/4 µg/ml)	4.47	6.13	4.19
J05315	907701	TZP (8/8 µg/ml)	6.13	7.75	5.72
J05316	908524	None	1.13	0.39	0.00
J05317	908524	TZP (4/4 µg/ml)	8.48	1.92	0.00
J05318	908524	TZP (8/8 µg/ml)	5.42	2.53	0.00



907355, 0 ug/ml TZP, Illumina data: *bla*-containing transposable element



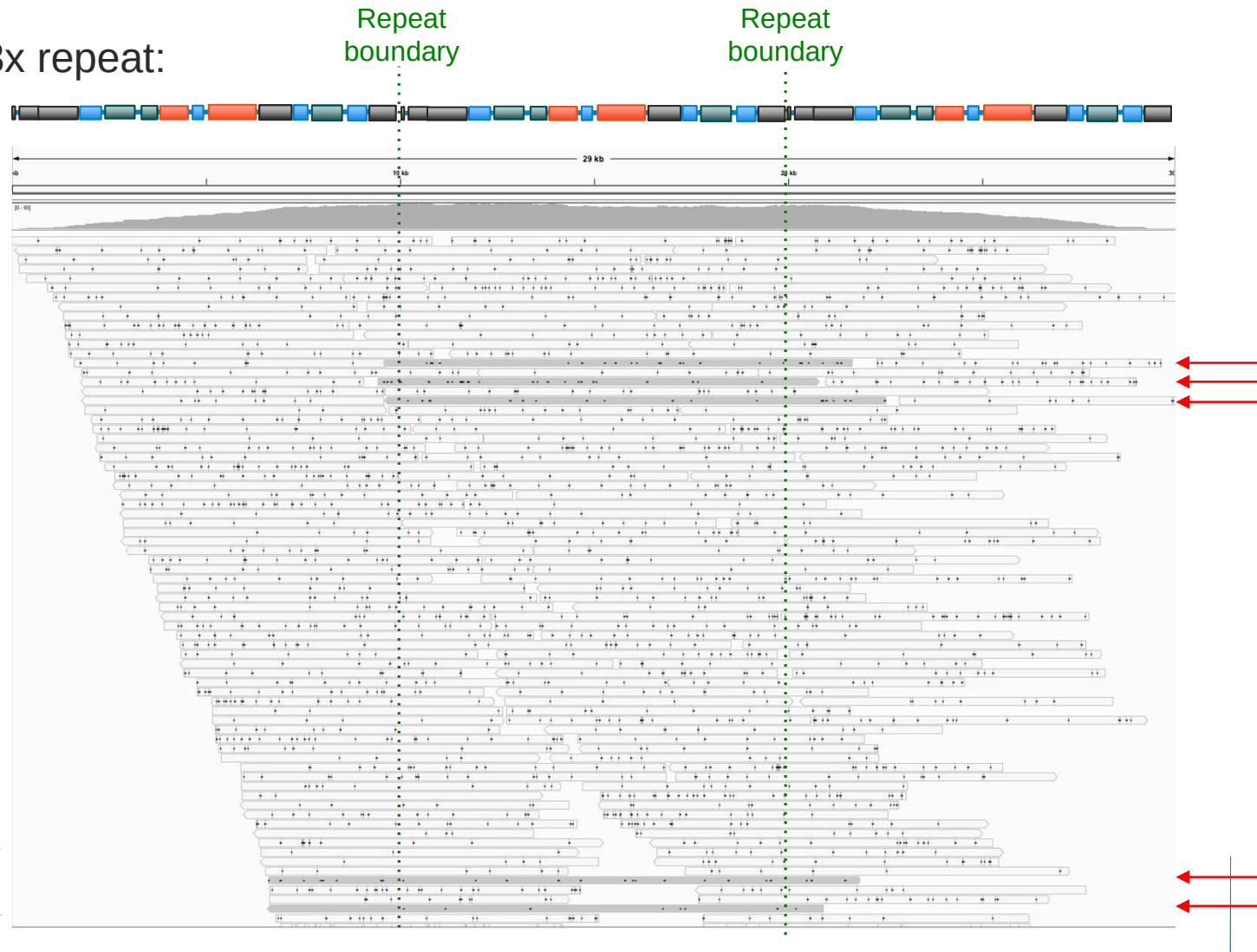
Annotation based on *E. coli* UMN026 homology

Illumina reads suggest tandem-repeats



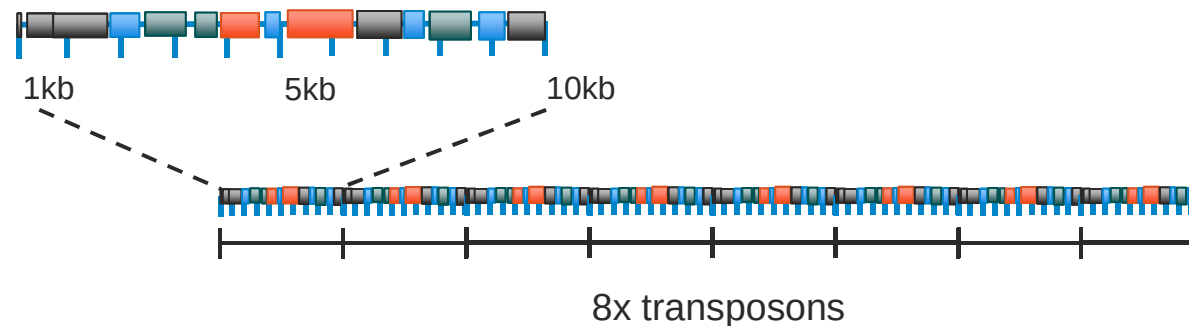
PacBio reads supporting tandem resistance transposon

in silico 3x repeat:



Oxford Nanopore finds longer tandem repeats: but is that all?

Align MinION reads to 8x tandem repeat transposon sequence

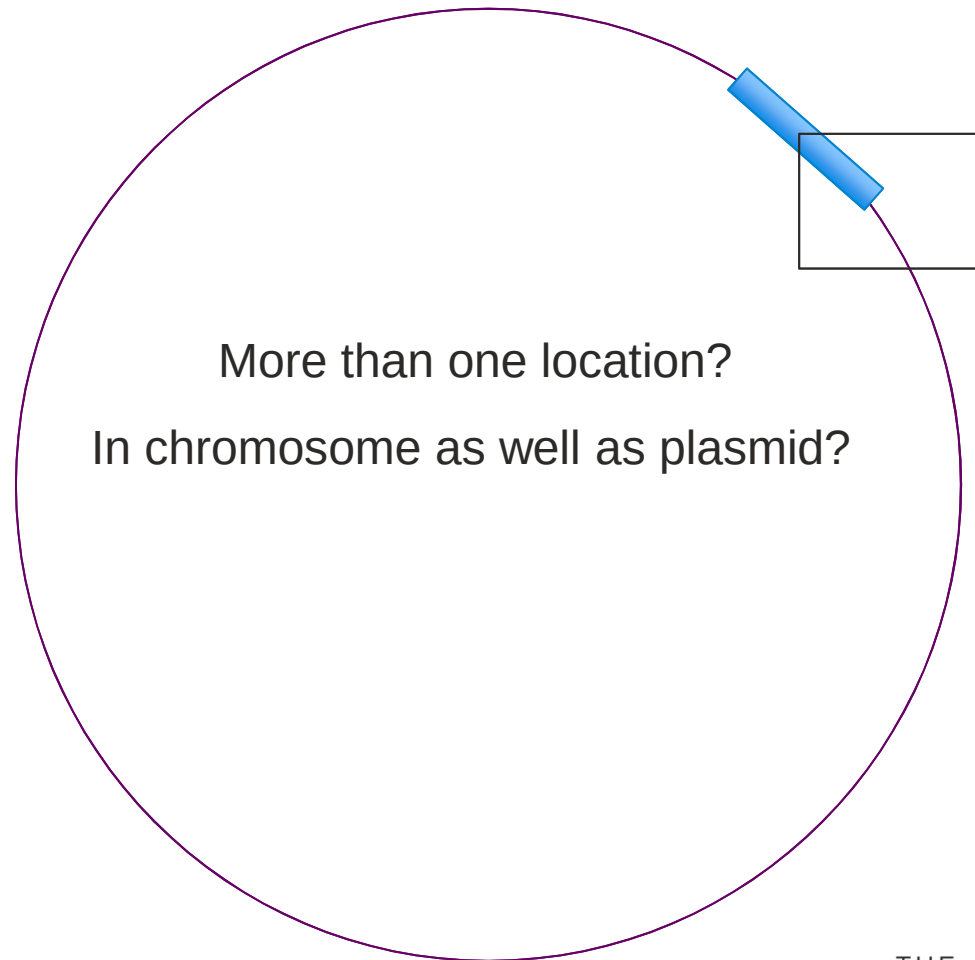


- Longest tandem repeat:
 - No Antibiotic strain = 2
 - 8 ug/mL TZP strain = 5



Where is the transposon located?

Finding junction fragments (or SVs)



More than one location?
In chromosome as well as plasmid?

“junction fragments”

Algorithms
evolving for
ONT, PB, ILMN



Population Heterogeneity

- Transposon- interrupted genes
 - *tral*
 - *astB*
 - *araJ*
- Whole plasmid inserted in genes
 - *waaP*
 - *Mer operon*
- Illumina: can find examples of these genes without apparent breaks => heterogeneity



Acknowledgments

Jackson Laboratory for Genomic Medicine

- Erica Sodergren
- Shana Leopold
- Yanjiao Zhou
- Yair Dorsett
- Benjamin Leopold
- Blake Hanson
- Yanfei Chen
- Purva Vats
- Sai Lek
- Lei Chen
- Lauren Petersen
- Samantha Smith
- Hoan Nguyen
- Eddy Bautista
- Dan Spakowicz
- Jethro Johnson
- Laura Rojas
- Xin Zhou
- Dan Philips
- Joe Brown
- Kristi Kearney
- Yini Lek
- Patrick Bergamo



U Conn

- George Kuchel
- Patricia Diaz
- Bo-Young Hong
- Anilei Hoare

IPOP/iHMP

- Michael Snyder
- Wenyu Zhou
- many others

**Jackson Laboratory Bar Harbor
Nathan Shock Center
Gary Churchill, Ron Korstanje,
Luanne Peters, and many more**

Clinical

- Greg Storch, WU
- Phil Tarr, WU
- Barb Warner, WU
- J. Dennis Fortenberry, Indiana U
- Katherine Gregory, B&W Hospital
- Homer Twigg, Indiana U

**Thank you to the
subjects and their
families**

**Funding:
NIH
Gates Foundation**

Washington University

- Makedonka Mitreva
- Kristine Wylie
- Kathie Mihindukulasuriya
- Todd Wylie
- Bill Shannon
- Patricio La Rosa

- Scott Weiss, Harvard
- Diane Gold, Harvard
- Joanne Sordillo, Harvard
- Martin Blaser, NYU
- Huiying Li, UCLA
- Martin Antonio, MRC-Gambia