Finding Biology in the Human Microbiome

George Weinstock

What's next for the Human Microbiome?

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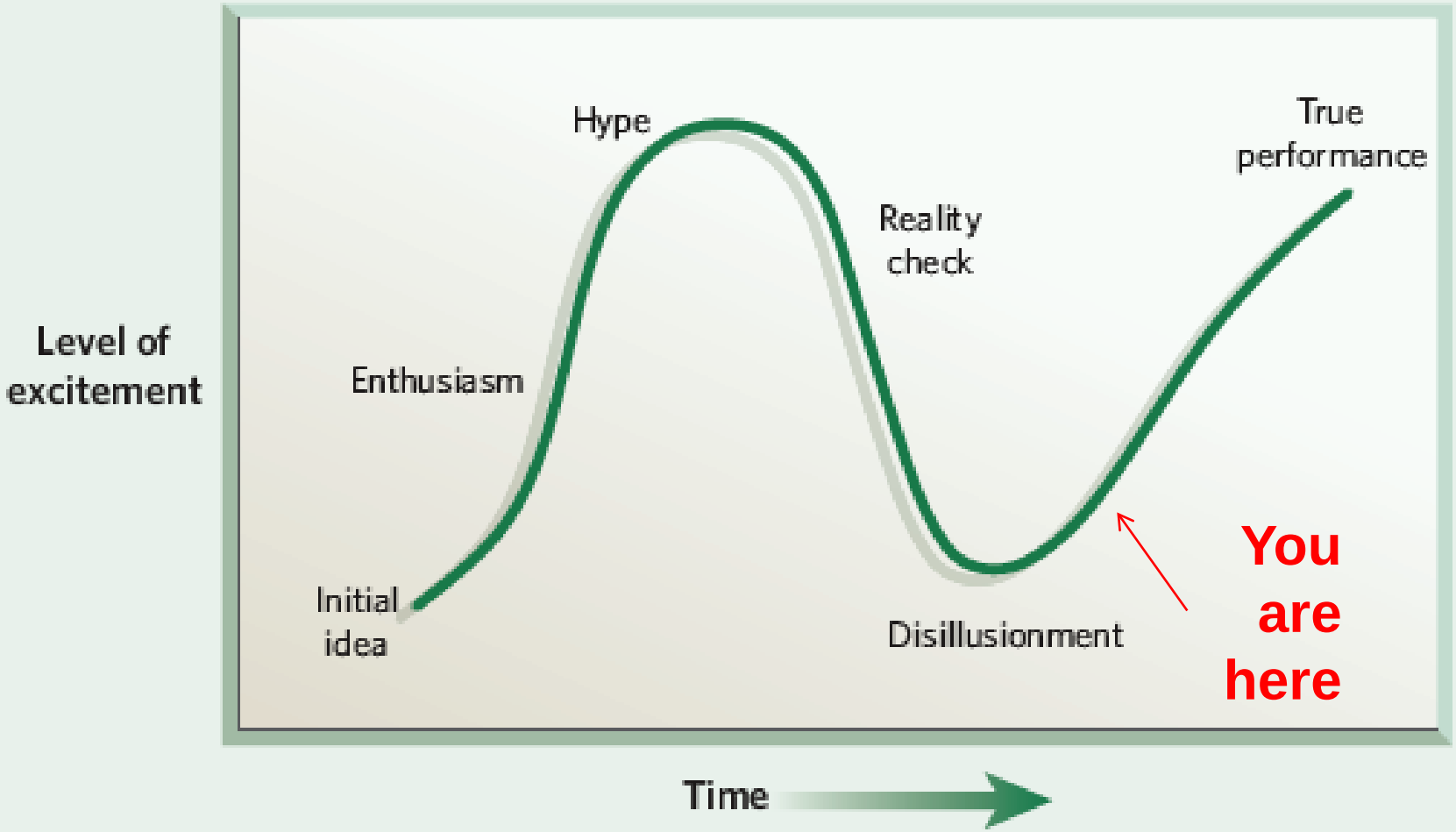
Metagenomics Unfolds

Setting Up → Descriptive Phase → Hypothesis Testing

Metagenomics Unfolds



Setting Up → Descriptive Phase → Hypothesis Testing

Human Microbiome Research Thoughts

- St. Louis (6 months ago) showed a wide-ranging field
- Vancouver shows momentum and maturation continues
- Analogy to Human Genome Project?
  - Probably not a "reference" microbiome
- Distinguish a "healthy" from a "perturbed" microbiome?
  - Genomic, Transcriptomic, Proteomic, Metabolomic features
  - Ecological concepts apply to define disease?
- Involvement of clinical concepts early in the project
  - Close interactions between clinicians and basic scientists
  - Massive amount of clinical samples in play; many clinical studies
  - *Opportunities for new strategic and funding models early on*?

Sequencing Technology

- HMP
  - 3000 reference genomes, euks and viruses too
  - Metagenomic 16S: 50 million reads at first data freeze
  - Metagenomic shotgun: >7 , ~10^{11} reads at first data freeze
- 454 for 16S, 200 samples/run
- Illumina for shotgun
  - Then: GAIIx @ 40 Gb/run
  - Now: HiSeq @ 500 Gb/run and increasing
- Coming: PacBio, 454 Junior, Ion Torrent, MiSeq, SOLiD

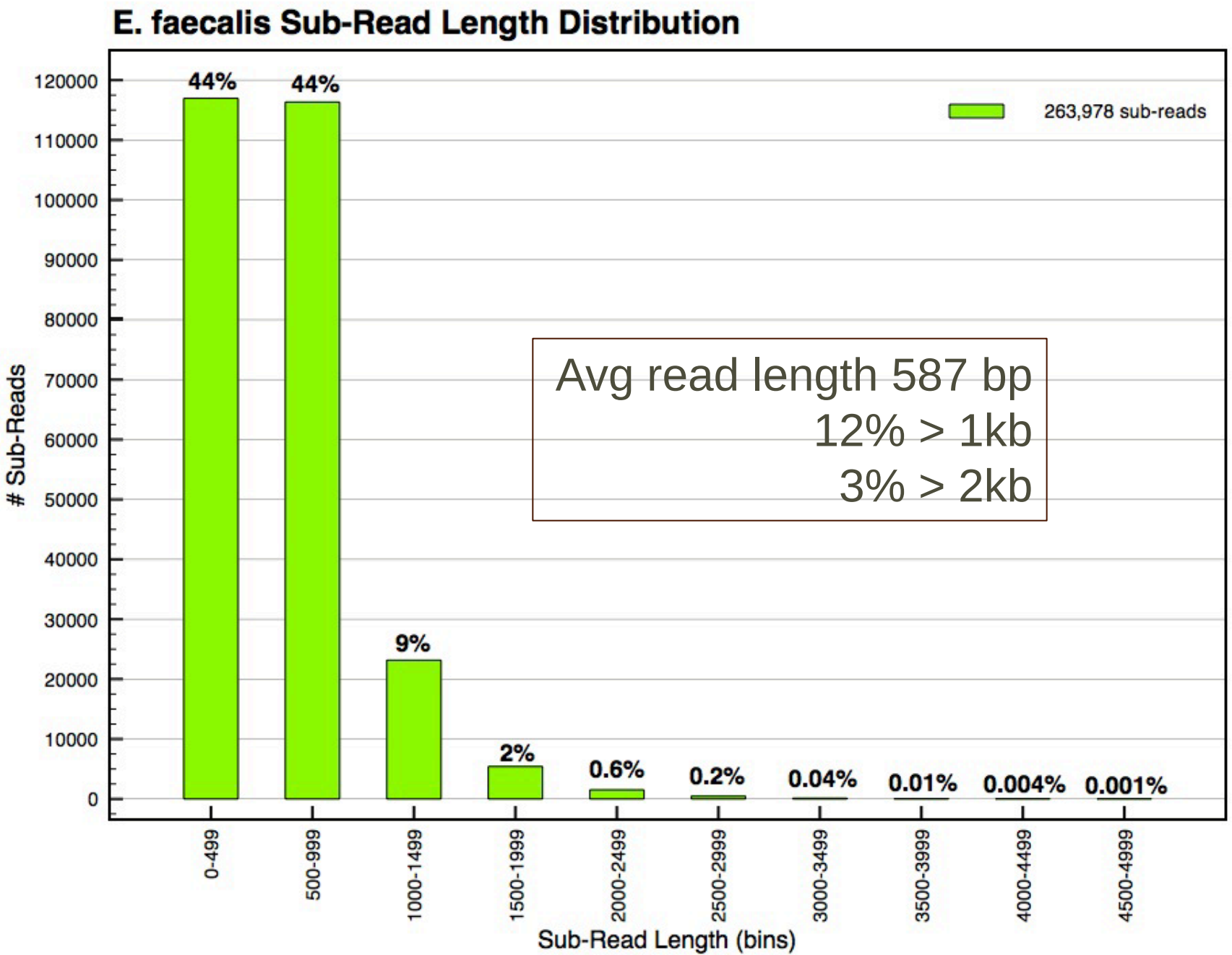
Early Pac Bio Experiences

- What's special?
  - Longer reads: assembly, novel organism/virus detection, 16S
  - Shorter run time: flexible throughput, match experiment better
- Whole genome reference sequencing
  - Enterococcus faecalis example
- 16S sequencing
  - Whole 16S gene

E. faecalis genome sequencing with Pac Bio

Alignment All Reads

Percent of Reference Bases Covered	**93% (Ref = V583)**
Average Coverage Depth	48x
Number of Gaps	50
Average Gap Length	4464


E. faecalis Sub-Read Length Distribution
263,978 sub-reads
Sub-Reads
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
44%
44%
9%
2%
0.6%
0.2%
0.04%
0.01%
0.004%
0.001%
0-499
500-999
1000-1499
1500-1999
2000-2499
2500-2999
3000-3499
3500-3999
4000-4499
4500-4999
Sub-Read Length (bins)
Avg read length 587 bp
12% > 1kb
3% > 2kb

E. faecalis genome sequencing with Pac Bio

Alignment All Reads

Percent of Reference Bases Covered	**93% (Ref = V583)**
Average Coverage Depth	48x
Number of Gaps	50
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Alignment Reads >2kb

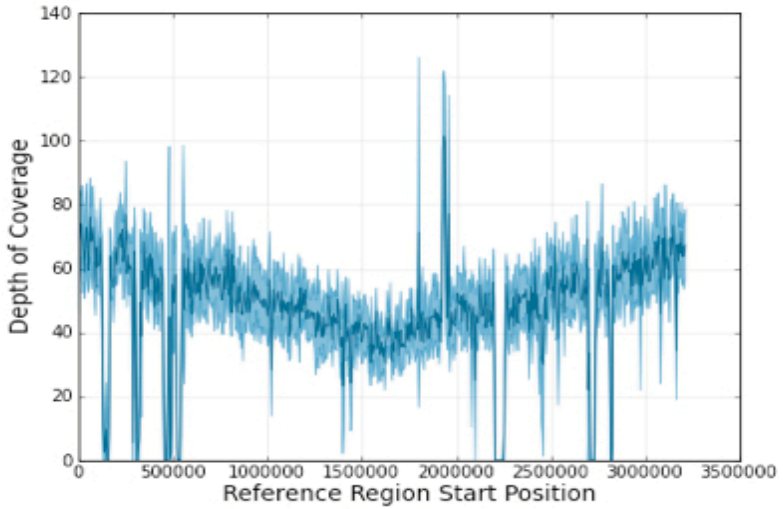
Percent of Reference Bases Covered	**75%**
Average Coverage Depth	1.6x
Number of Gaps	380
Average Gap Length	2144

Coverage Metrics

Report Summary

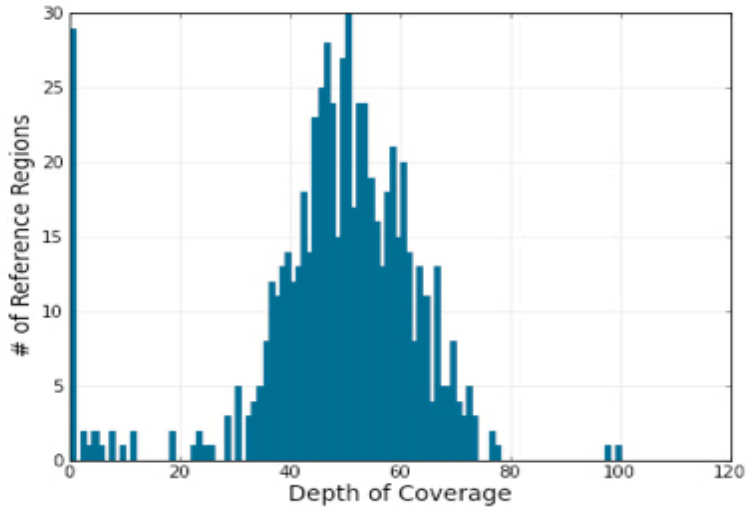
Mean Depth of Coverage	48.15
% Missing Bases	6.94

Depth Of Coverage Across Reference



Observed depth of coverage across E_faecalis_V583_NC_004668_1 (window size = 5004bp).

Depth Of Coverage Histogram



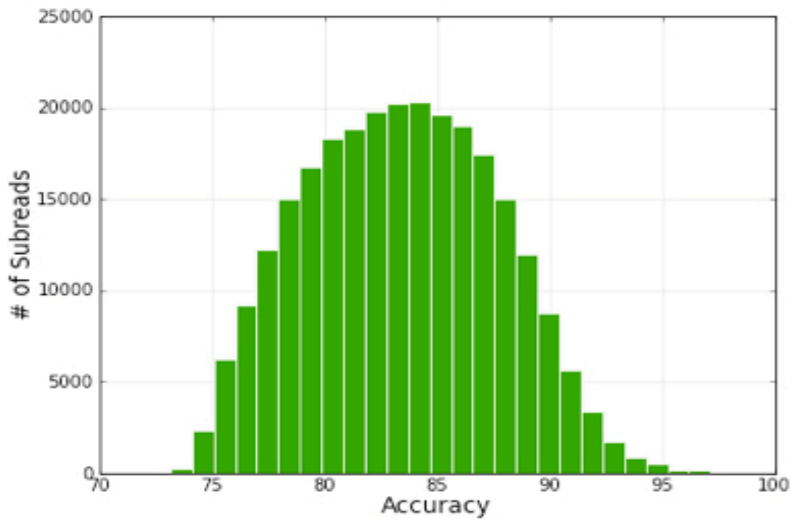
Depth of Coverage Distribution

Read Length & Accuracy

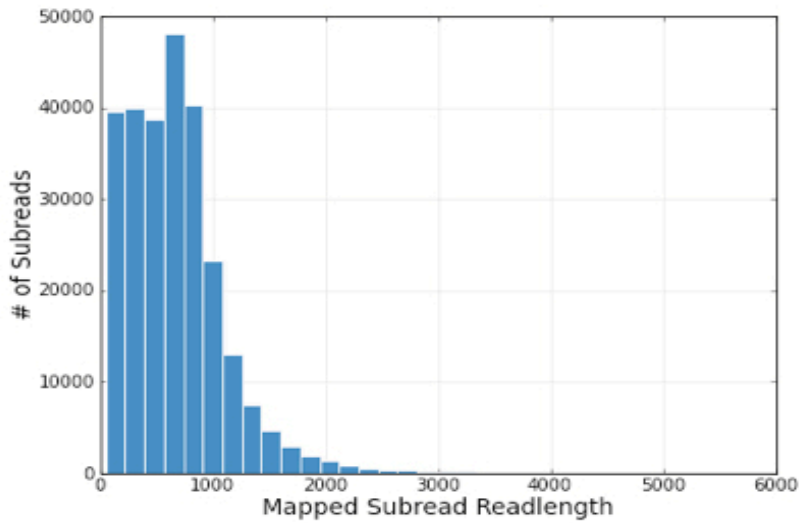
Report Summary

# of Post-Filter Reads	266730	# of Mapped Reads	151107
# of Mapped Bases	213650285 bp	95th Percentile Mapped Readlength	3616 bp
Maximum Mapped Readlength	6503 bp	Mean Mapped Readlength	1414 bp
# of Mapped Subreads	263978	Mean Mapped Subread Readlength	657 bp
Mean Mapped Subread Accuracy	83.36%		

Accuracy Histogram



Mapped Subread Readlength Histogram



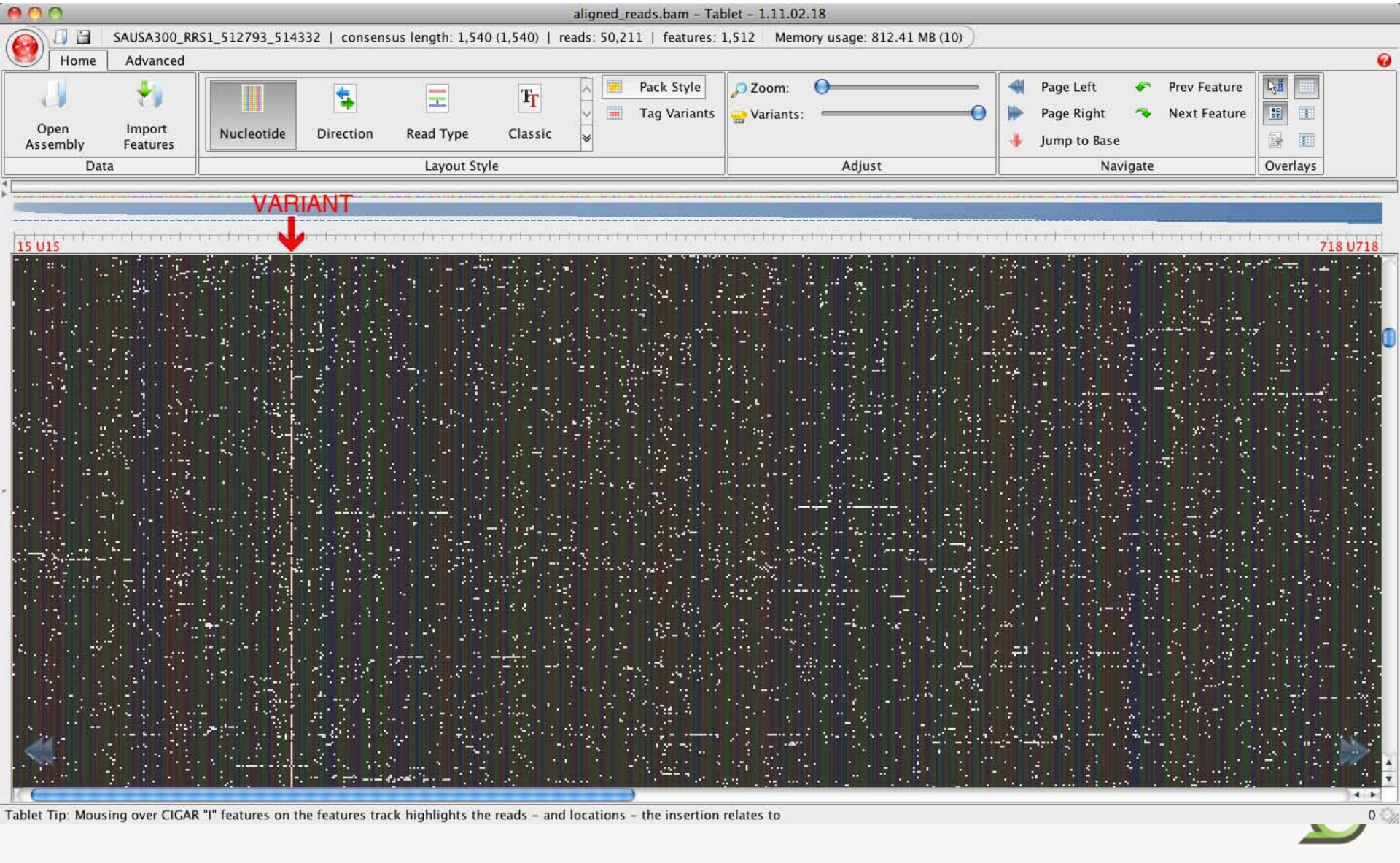
Allora Assembly of *E. faecalis*

- Velvet, Illumina: 162 contigs/scaffolds, N50 19kb
- Allora, Pac Bio: 622 contigs/scaffolds, N50 7.8kb
- Allora, Illumina + Pac Bio: 49 contigs/scaffolds, N50 243kb

16S sequencing on Pac Bio

- Full length sequences possible due to read length
- High error rate (85%) challenging:
  - Different ribotypes vs sequencing errors
  - High coverage needed for each fragment sequenced
- Alignment algorithm critical:
  - Conserved regions low information for clustering
  - Emphasize variable regions

16S genes of *S. aureus* on Pac Bio



Computational Technology Evolving

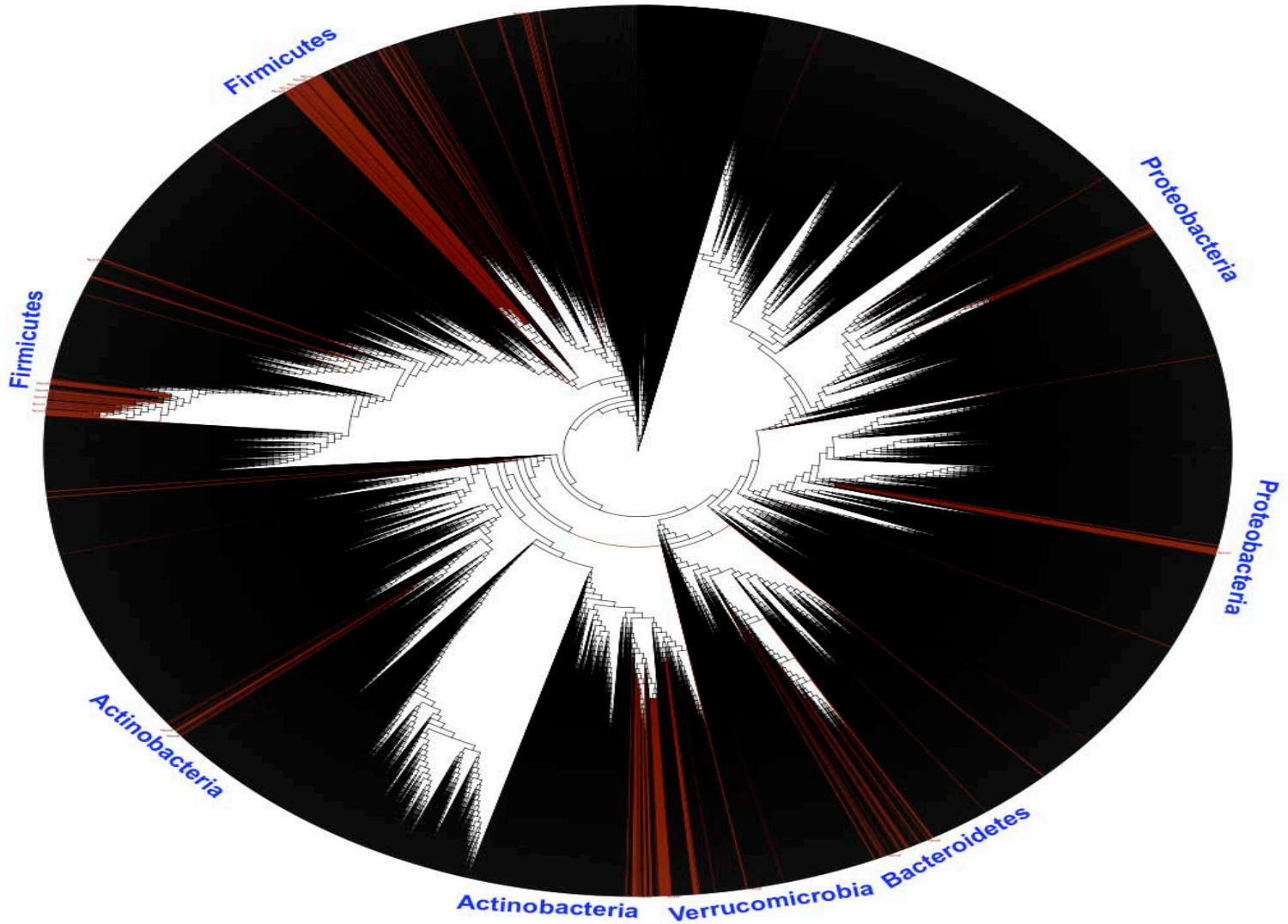
- Continued development of methods for community comparisons (16S)
  - What's a person to do?
  - Qiime (Rob Knight)
- Large-scale shotgun metagenomic data
  - HUMAnN pipeline (Curtis Huttenhower)
  - HMP (Makedonka Mitreva)
    - MulticoreWare, Real Time Genomics accelerated Blast and more
- Power calculations (Bill Shannon)

Novel organism discovery

- Need to know who's there to understand phenotypes
- Phylum level analysis
  - 454 16S reads, Illumina shotgun reads (Erica Sodergren et al)
- OTU/Species level analysis
  - Mothur (Sue Huse et al)
  - PhylOTU (Katie Pollard et al)
- Assembly of shotgun metagenomic data (HMP)
- Technologies for sequencing uncultured organisms

Novel Phyla?

1200 reads in initial HMP 16S set



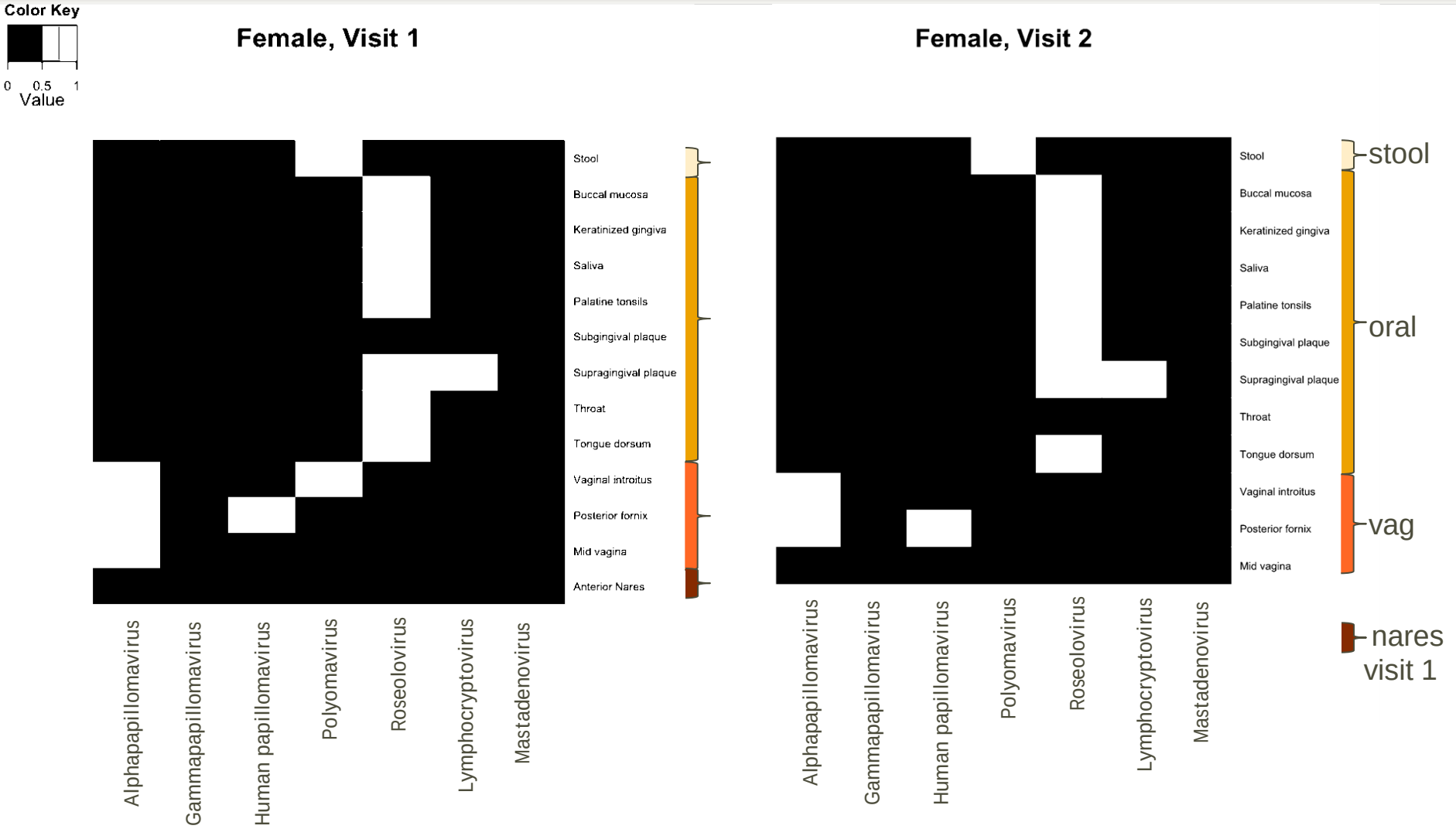
Load of deleterious organisms/genes

- Virulence factors
- Antibiotic resistances
- Found in all body sites
  - Low levels but easily detectable with shotgun sequencing

The Virome

- Growing activity in this area
- Healthy people have many viruses, sick people have more
- Animal viruses and Bacteriophages (and hybrids!)
- Methodology for detection of novel viruses improving
  - Known taxa but highly diverged
  - New taxa
- Relation to prokaryotic microbiome?

Virome of an individual over two visits



Presence/absence
White=virus present

Challenges to the field

- Minor organisms
  - Long tail in abundance distributions
  - Mainly unimportant?
  - Mix of communities?
- New types of data being included
  - Transcriptome
  - Proteome
  - Metabolome
- Host genotype
  - Enough said!!

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