

Nearly Finished Genomes Produced using Gel Microdroplet Culturing

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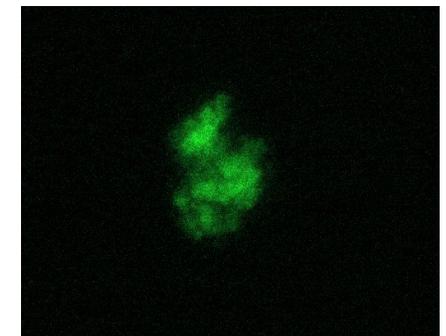
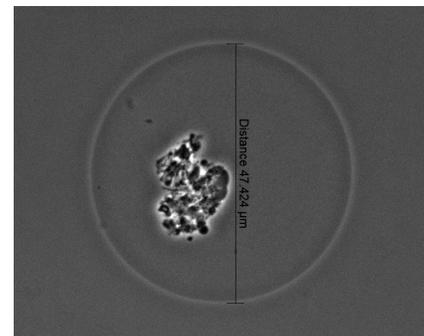
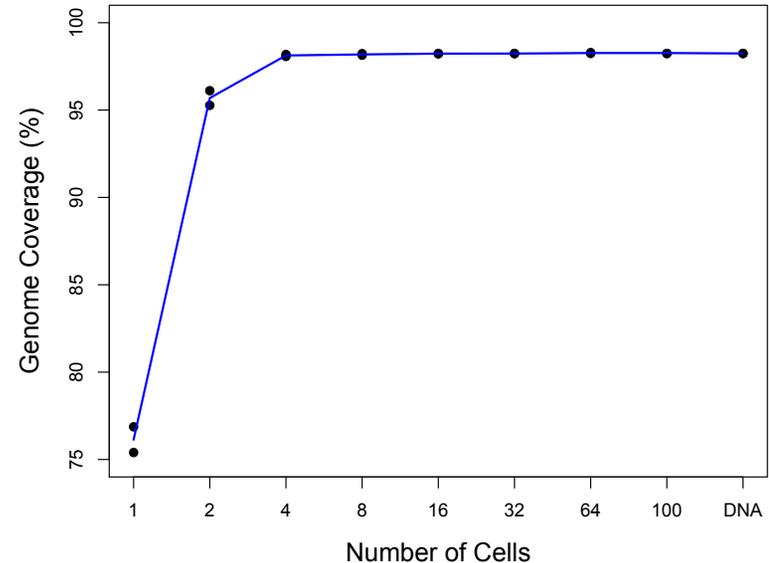
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The Goal: Acquire complete genomes from complex samples

- **Very difficult task**
- **Most genomic diversity is unexplored**
 - Exploring more of it may produce breakthroughs in bioenergy, biotechnology, medicine, and ecology
- **Potential approaches and drawbacks**
 - Shotgun metagenomics produces fragmented results
 - Single cell genomics allows one to assemble fairly large sections of a genome, but will likely never yield complete genomes
 - Traditional culturing is not possible for most microorganisms

Gel microdroplet culturing

- **GMDs are small agarose “cages” created in an emulsion in which one can capture and incubate microorganisms**
- **Porous structure allows cell-to-cell communication**
- **100s to 1000s of cells originating from a single cell can be manipulated via micromanipulation or flow cytometry**
- **Microdroplets have been used for other purposes**
 - Growth and protein secretion assays, chemical “microreactors”, and culturing fastidious microorganisms



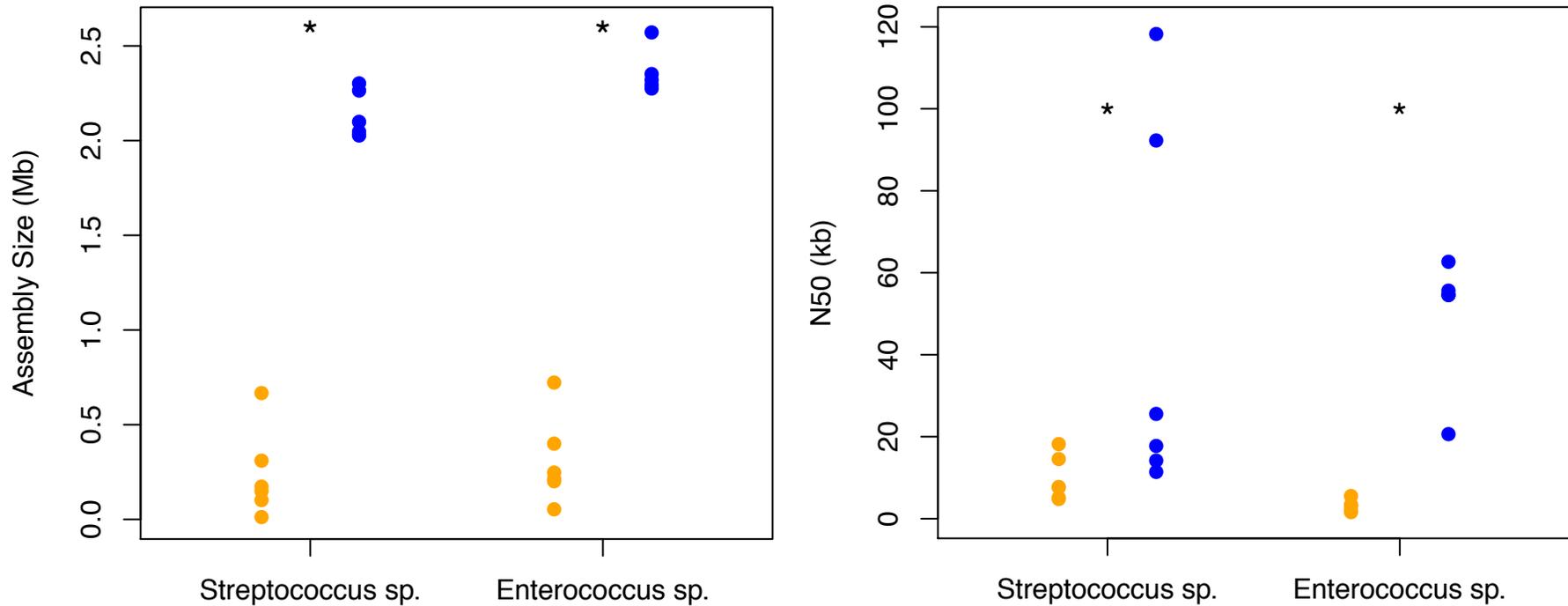
Armand Dichosa

Overview of experiment

- **Collaboration with J. Craig Venter Institute funded by NHGRI**
- **Procedure**
 - Extracted cells from oral and fecal environment
 - Encapsulated, incubated in rich media, and sent to JCVI for micromanipulation
 - GMDs and single cells were amplified via MDA and 16S screened
 - Candidates were sequenced and assembled
 - *Streptococcus oralis* for oral sample
 - *Enterococcus faecium* for fecal sample
 - Both are opportunistic pathogens
 - Compare GMD and single cell results

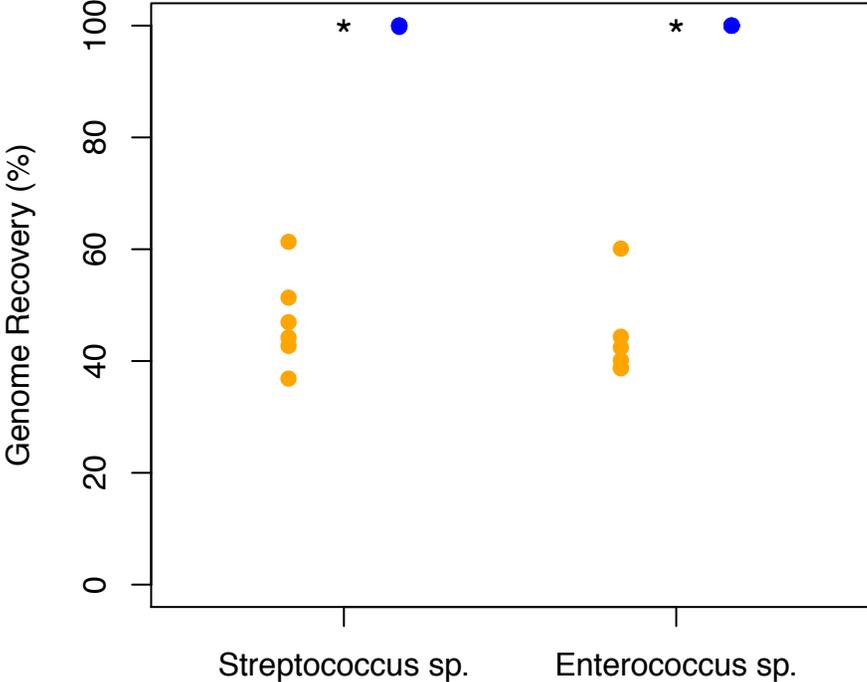


Sequencing stats are much improved



■ GMD
■ Single Cell

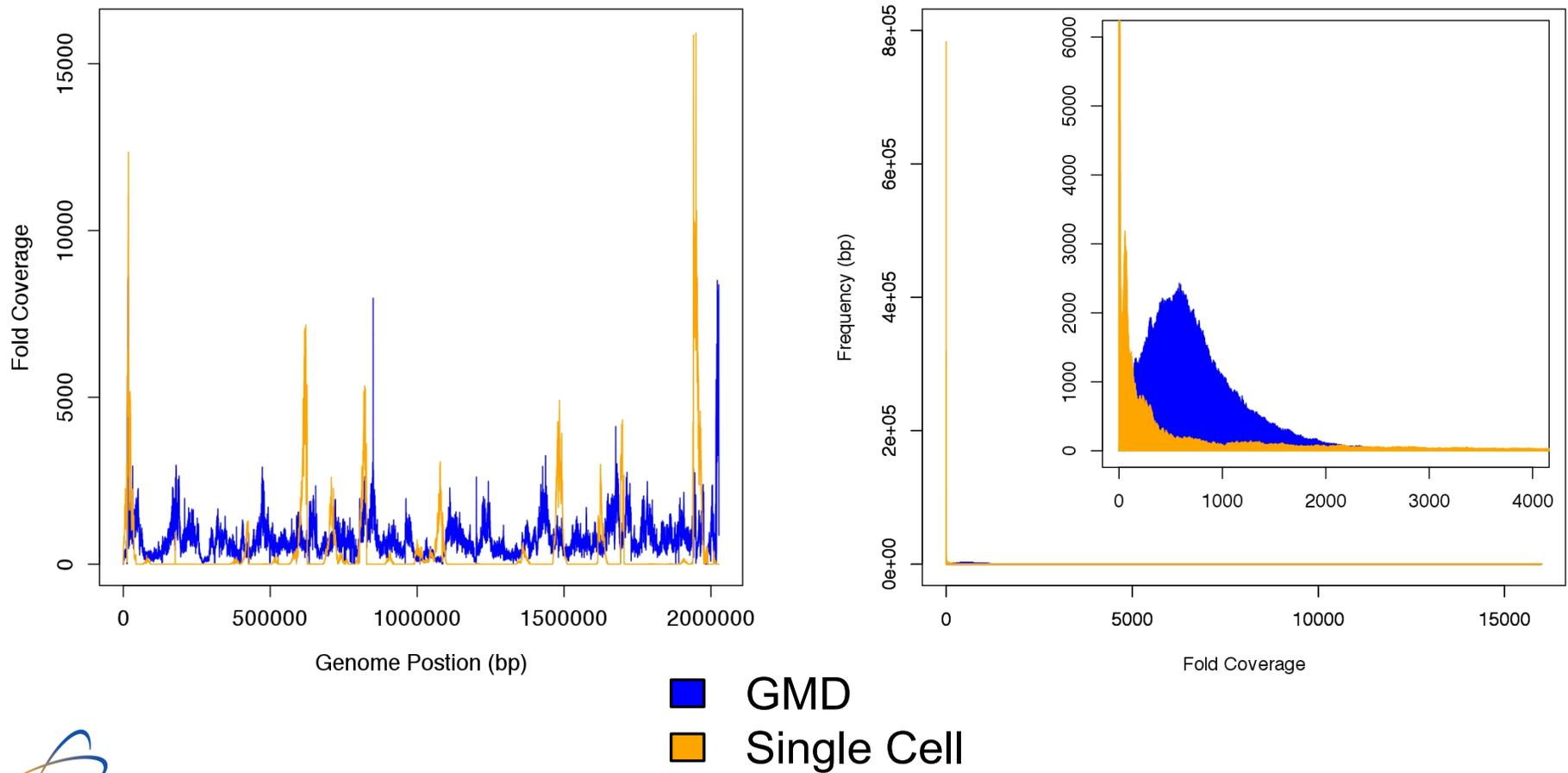
Sequencing stats are much improved



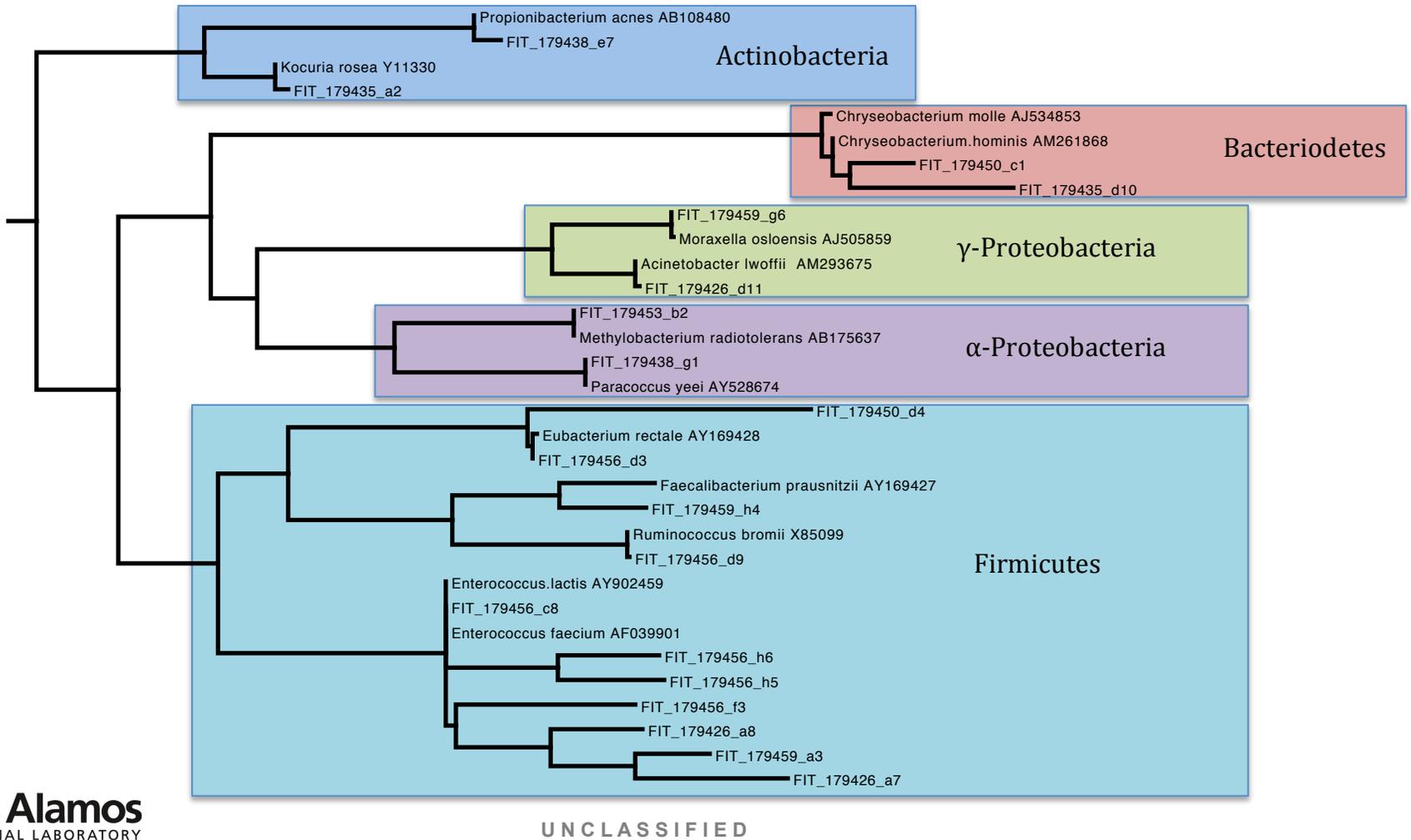
■ GMD
■ Single Cell

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GMDs reduce amplification bias



Diverse microorganisms will grow within GMDs



Variation among genomes

- **What is the species level genomic diversity found within the human microbiome (or in any microbial community)?**
- **Culturing approaches are low throughput and usually involve more than a single individual**
- **Shotgun metagenomics approaches to strain analysis are very complex and cannot give long distance linkage information**
- **GMD derived genomes are sufficiently complete to start exploring this question**

Many Indels and SNPs among Streptococcus genomes

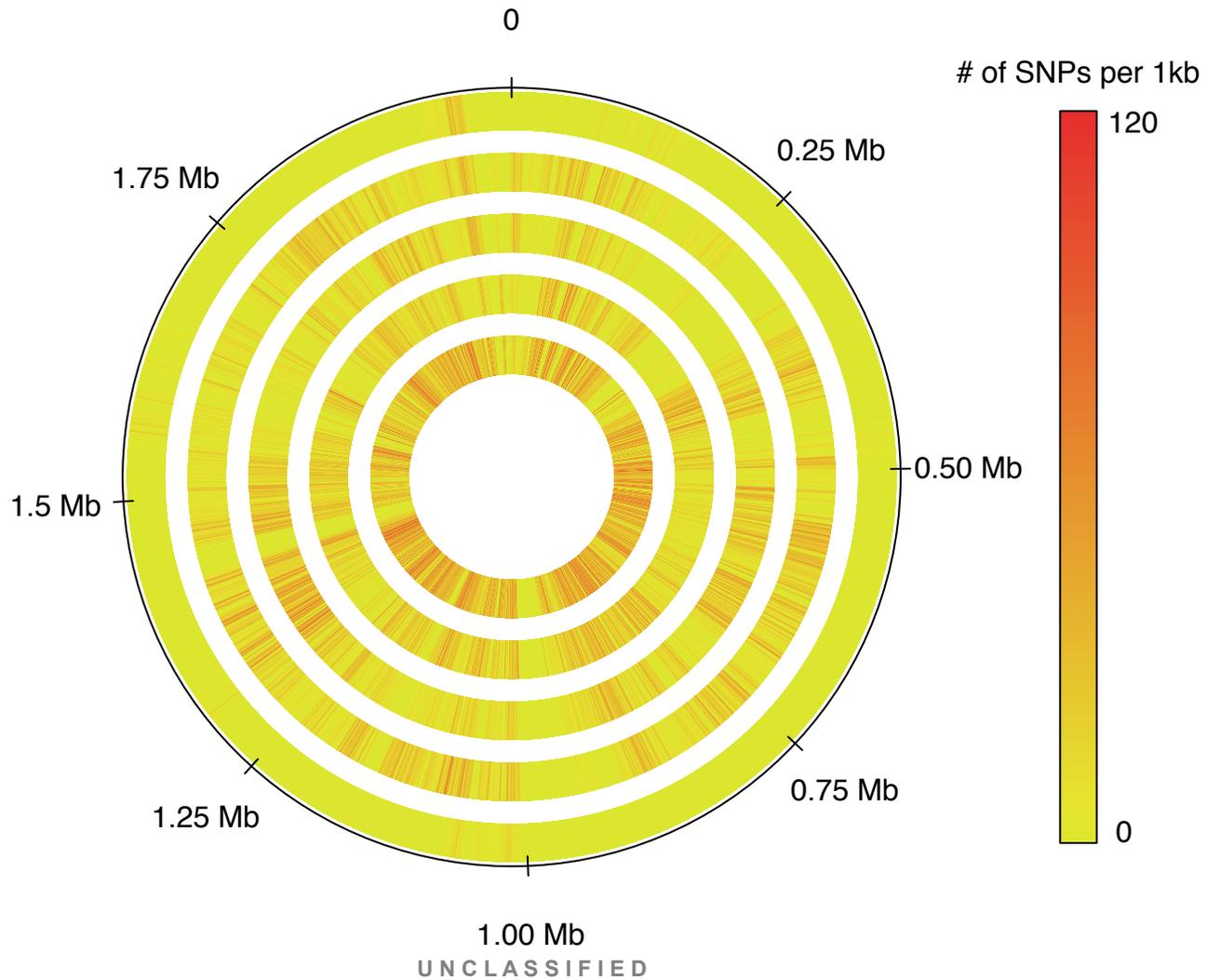
Streptococcus sp.

	Assembly					
Reads	1	2	3	4	5	6
1		45 (2504)	11 (1442)	57 (5708)	46 (24001)	55 (21179)
2	36 (3019)		10 (2384)	45 (6177)	26 (19907)	38 (18026)
3	32 (1984)	45 (2710)		59 (9504)	88 (48449)	80 (41667)
4	53 (9332)	61 (8890)	24 (10071)		55 (21047)	69 (19065)
5	164 (50248)	175 (46259)	178 (64289)	162 (40303)	9 (17)	23 (1058)
6	110 (25858)	129 (24161)	85 (30692)	104 (20541)	11 (1667)	

Enterococcus sp.

	Assembly				
Reads	1	2	3	4	5
1		7 (22)	8 (32)	10 (46)	7 (24)
2	11 (99)		4 (30)	12 (18)	7 (24)
3	15 (106)	8 (27)		7 (20)	7 (26)
4	14 (97)	4 (22)	5 (30)	4 (23)	6 (24)
5	17 (103)	7 (26)	8 (29)	10 (18)	

Location of SNPs



Potential consequences of this variation

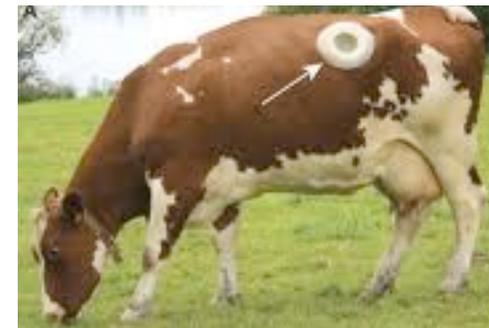
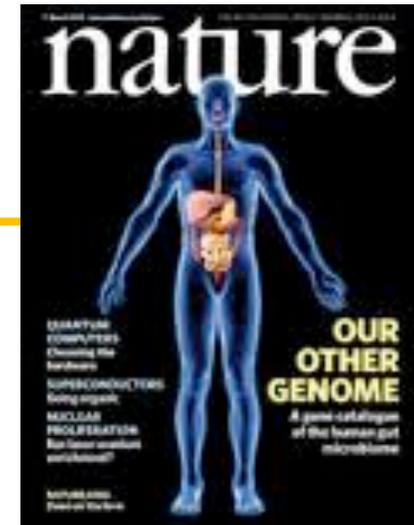
- **Populations of cells have lots of important functional variation**
- **Which genes have indels and non-synonymous SNPs?**
 - Lots. Many related to virulence in *Streptococcus pneumoniae*
 - Examples include IgA1 protease, C5a peptidase, and Choline binding protein A
- **These *Streptococcus sp.* cells were all taken from the base of the same tooth**

Conclusions

- **Gel microdrop culturing can provide extremely high quality genomic data**
 - Provide reference genomes
 - Complementary to shotgun metagenomics and single cell sequencing
- **Will work for a wide diversity of microorganisms**
- **Reveals substantial and likely functional intraspecies genomic diversity within the human microbiome**
 - Possibly more diversity in oral environment than intestine

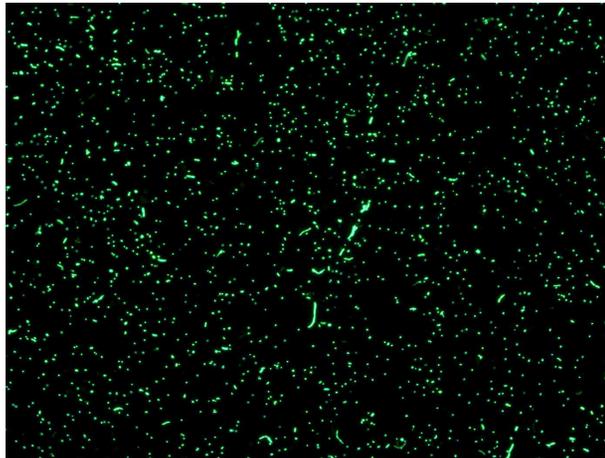
Current and Future Projects at LANL

- ***In situ* incubation of GMDs**
- **Application to various environments**
 - **Freshwater pond**
 - Utah desert crust microbial community
 - Human gut and cow rumen microbiome
 - Corrosion residue of subsurface caves
 - In culture *Microcoleus vaginatus* heterotrophic community
- **Microfluidics approach to GMD formation**
 - Make more robust and include features making them easier to harvest



Future Directions at JGI

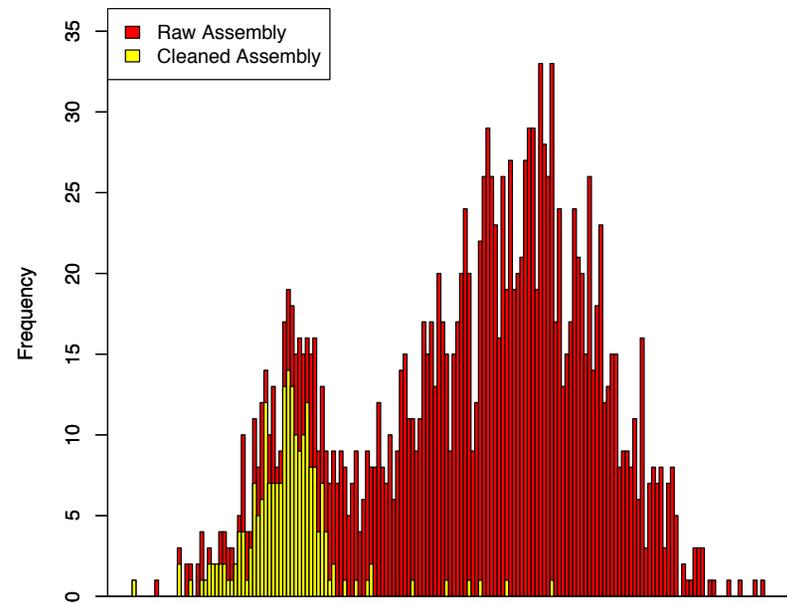
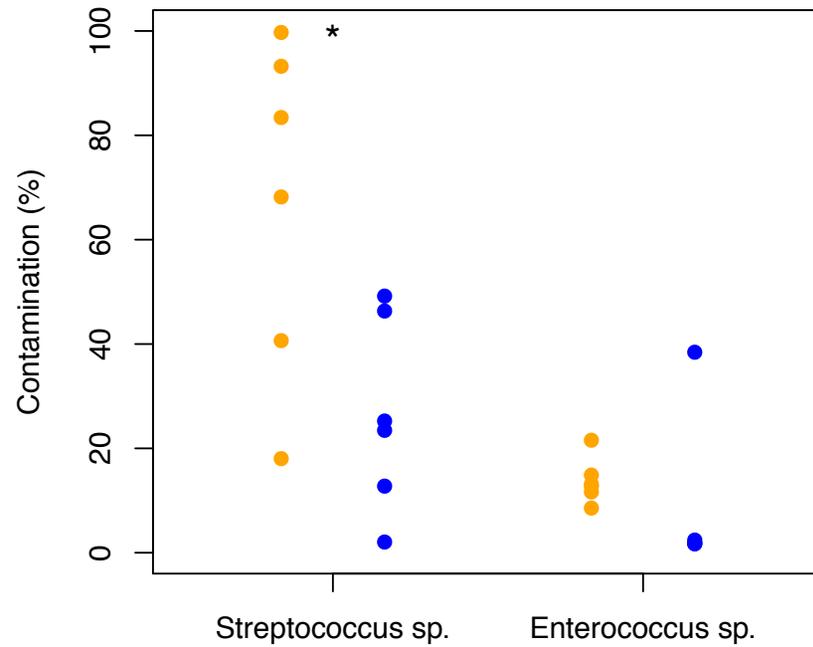
- Small volume MDAs give better results (Marcy *et al.* 2007)
- Develop emulsion MDA, which would have millions of very small reactions (10-100pL)
- Need to put all reagents into microdroplet at once
 - MDA-friendly lysis: Antimicrobial peptides
- Will screen MDA products with fluorescent probes to genes of interest



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Contamination



PCA1 of Tetranucleotide Space