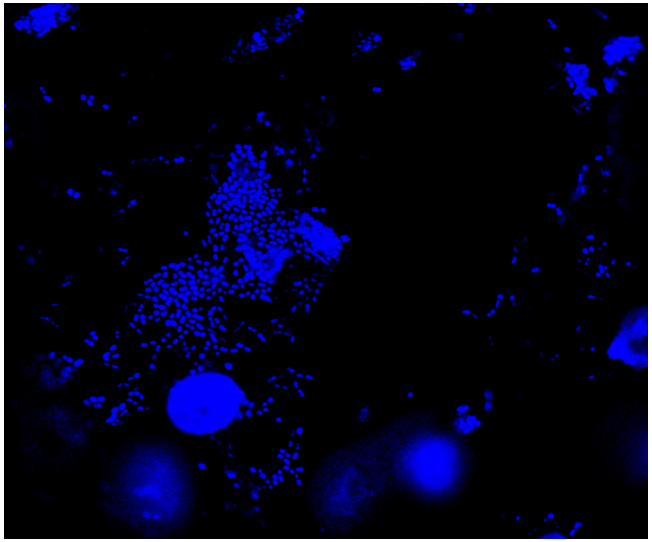
September 13, 2018
Border Public Health Interest Group Meeting
City of El Paso Department of Public Health

Profiling Oral Bacteria by Next Generation DNA Sequencing



Oral bacteria on cheek epithelium.

DAPI dye (Blue fluorescence on DNA binding)

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70 samples from dental patients collected by
Daniel Terreros, Danielle Liss (Sub-project 1)

Children and adults with various levels of dental caries.

Collect oral microbes from saliva. Saliva has many of the same species that form dental plaque.

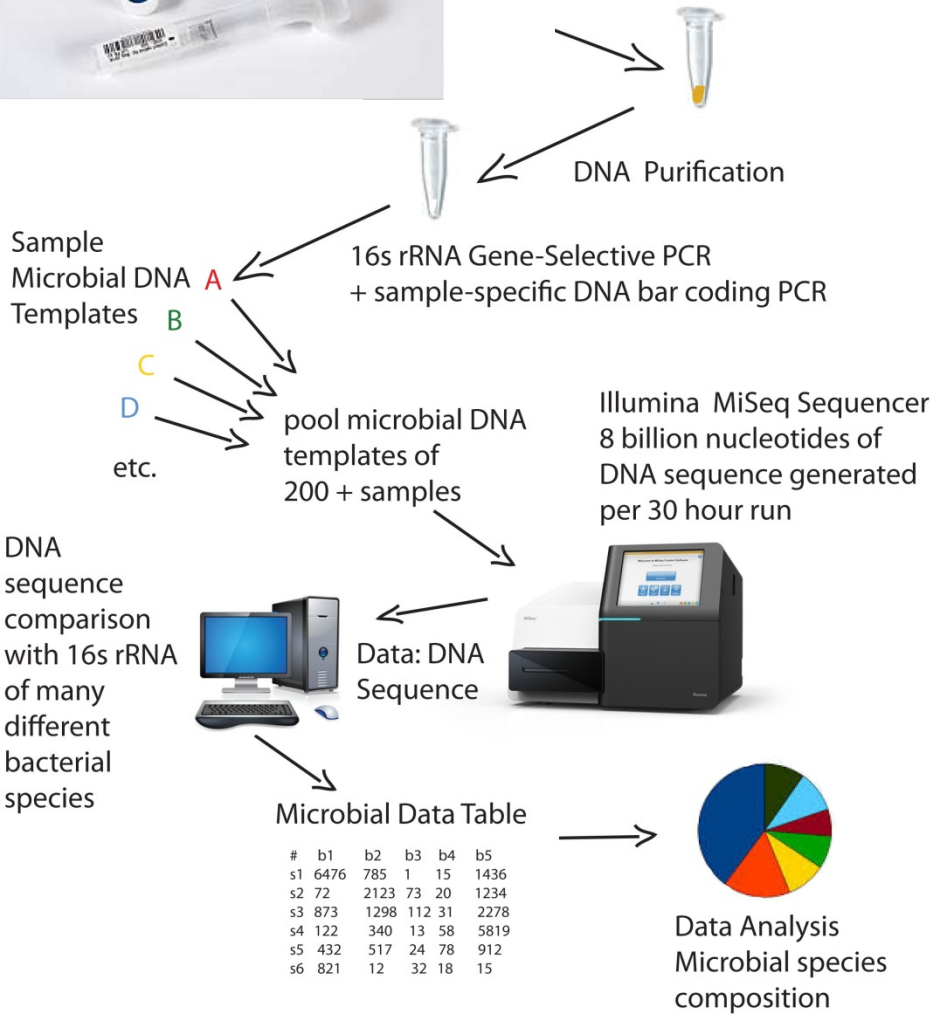
Explore bacterial species distribution, and correlate with dental health and disease:
Caries (cavities), abcesses (toothache), gum disease.

We are currently using the Illumina MiSeq 16s rRNA gene bacterial identification system in a study of chronic upper gut infections in the Department of Medicine.

The method is able to identify over 800 different bacterial species in a sample. The data also provides information on the relative abundance of each species of bacteria in the mixture.

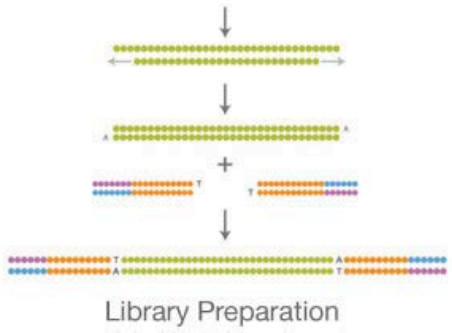
Test samples have been run with saliva and dental plaque using the ORAGENE saliva DNA collection kit. Samples stable for 1 year at room temperature.

Images from Oragene and Illumina Inc.



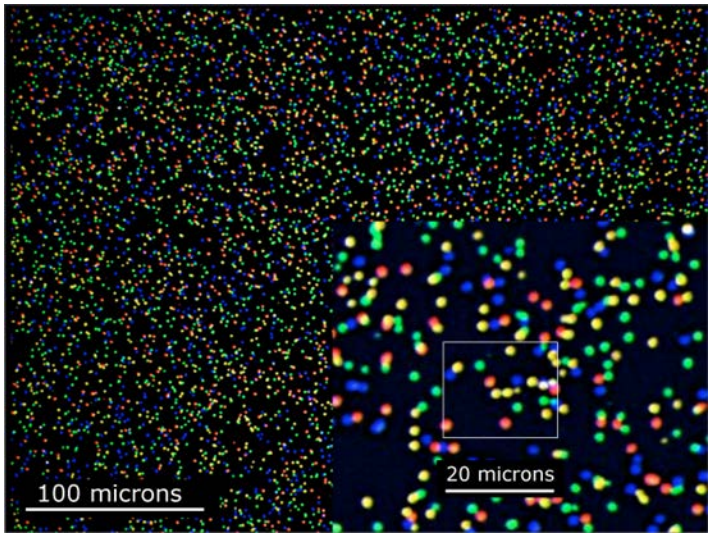
A single run can generate ~ 10^{10} nucleotides of DNA sequence from ~ 2 x 10^{7} individual DNA template molecules. Each molecule = 1 dot

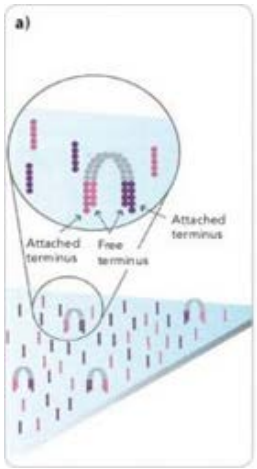
All images from Illumina Inc.

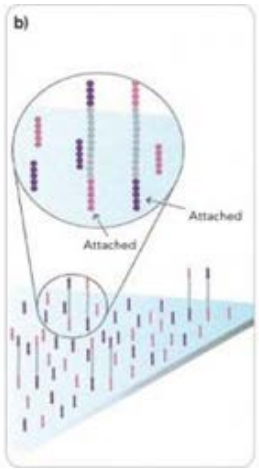


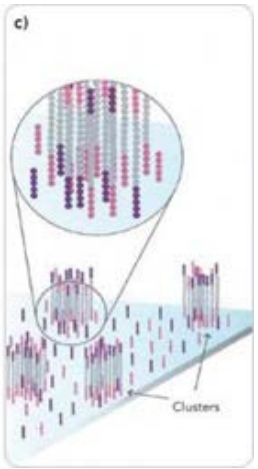
~ 450 bp sequenced per template, combined forward and reverse strands.

~ 500,000 templates sequenced per sample.

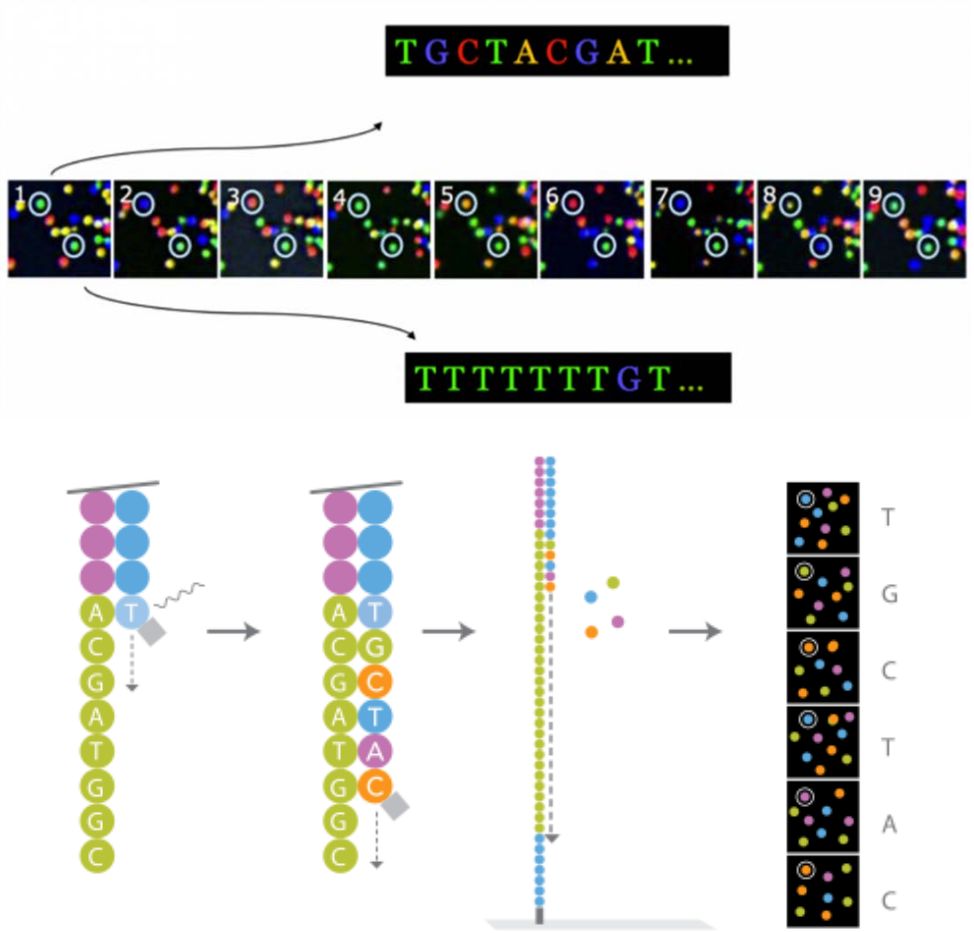








A primer on each template is extended by DNA polymerase in a stepwise manner. For each step, the terminal fluorescent nucleotide is recorded for all template-clusters (microscopic dots).



All images from Illumina Inc.

Summary of preliminary results from Dental Plaque (test runs of saliva and plaque).

Major Phyla and Species in Dental Plaque, with % of Total Bacterial 16s rRNA Sequences

	% All Bacteria	Top 4 Species in Phylum	% All Bacteria
Fusobacteria	34.2 %	*Fusobacterium naviforme*	8.53 %
		Fusobacterium nucleatum	5.13 %
		Leptotrichia hofstadii	4.74 %
		Leptotrichia shahii	1.60 %
Firmicutes	23.7 %	*Veillonella atypica*	3.05 %
		Veillonella dispar	2.69 %
		Selenomonas artemidis	2.19 %
		Veillonella montpellierensis	1.29 %
Bacteroidetes	22.5 %	*Prevotella oris*	3.27 %
		Prevotella tannerae	2.39 %
		Prevotella nigrescens	1.90 %
		Prevotella multiformis	1.72 %
Proteobacteria	13.8 %	*Neisseria elongata*	1.98 %
		Campylobacter gracilis	1.30 %
		Campylobacter showae	1.07 %
		Campylobacter concisus	0.98 %
Actinobacteria	4.7 %	*Corynebacterium matruchotii*	1.10 %
		Actinomyces meyeri	0.24 %
		Actinomyces lingnae	0.22 %
		Actinomyces naturae	0.18 %
Spirochaetes	0.80 %	*Treponema socranskii*	0.40 %
		Treponema denticola	0.27 %
		Treponema succinifaciens	0.04 %
		Treponema porcinum	0.02 %
Other phyla	0.29 %		

Other Firmicutes:

Streptococcus tigurinus	0.61 %
Streptococcus milleri	0.41 %
Streptococcus mutans	0.001%

S. mutans is traditionally considered the “main cause” of dental caries. Given its rarity in this case, other bacterial species may be involved in cariogenic dental plaque.

Actinobacteria form a structural scaffold for dental plaque biofilms.

Spirochaetes are often involved in gum disease.
(note- we did not find S. pallidum !)

Dental Plaque vs Saliva : Some bacteria are enriched in plaque

Species of Bacteria	Phylum	% of Dental Plaque	% of Saliva Bacteria	Ratio
Fusobacterium naviforme	Fusobacteria	8.534	8.445	**1.01**
Fusobacterium nucleatum	Fusobacteria	5.133	7.549	**0.68**
Leptotrichia,hofstadii	Fusobacteria	4.747	0.37	**12.8**
Prevotella oris	Bacteroidetes	3.274	2.137	**1.53**
Veillonella atypica	Firmicutes	3.054	2.553	**1.19**
Neisseria mucosa	Proteobacteria	0.556	4.081	**0.14**
Mannheimia caviae	Proteobacteria	0.518	3.478	**0.15**
Treponema socranskii	Spirochaetes	0.398	0	**> 398**
Streptococcus mutans	Firmicutes	0.001	0.007	**0.14**

Dental Plaque vs Saliva: results by bacterial genus

Oral Bacteria: Comparison of Dental Plaque and Saliva

Rank	Genus and Species	Phylum	% Plaque	% Saliva	Ratio P/S
1	*Fusobacterium naviforme*	Fusobacteria	8.53	8.45	1.01
2	*Fusobacterium nucleatum*	Fusobacteria	5.13	7.55	0.68
3	*Leptotrichia hofstadii*	Fusobacteria	**4.75**	**0.37**	**12.84**
4	*Prevotella oris*	Bacteroidetes	3.27	2.14	1.53
5	*Veillonella atypica*	Firmicutes	3.05	2.55	1.19
6	*Neisseria mucosa*	Proteobacteria	0.556	4.08	0.14
7	*Mannheimia caviae*	Proteobacteria	0.518	3.48	0.15
8	*Treponema socranskii*	Spirochaetes	**0.398**	**0.001**	**398**
28	*Streptococcus mutans* (dental caries - associated)	Firmicutes	0.001	0.007	0.14

Genus	Phylum	% of total bacteria
Streptococcus	Firmicutes	15.469
Prevotella	Bacteroidetes	14.054
Veillonella	Firmicutes	12.350
Rothia	Actinobacteria	5.181
Neisseria	Proteobacteria	4.539
Fusobacterium	Fusobacteria	3.894
Haemophilus	Proteobacteria	3.051
Actinomyces	Actinobacteria	2.595
Staphylococcus	Firmicutes	2.112
Porphyromonas	Bacteroidetes	2.037
Gemella	Firmicutes	1.424
Oribacterium	Firmicutes	1.219
Cohnella	Firmicutes	1.155
Bacteroides	Bacteroidetes	1.119
Atopobium	Actinobacteria	1.083
Leptotrichia	Fusobacteria	1.021
Corynebacterium	Actinobacteria	0.989
Granulicatella	Firmicutes	0.931
Propionibacterium	Actinobacteria	0.916
Selenomonas	Firmicutes	0.854
Megasphaera	Firmicutes	0.850
Mannheimia	Proteobacteria	0.733
Peptostreptococcus	Firmicutes	0.622
Campylobacter	Proteobacteria	0.612
Calothrix	Cyanobacteria	0.120
Treponema	Spirochaetes	0.076

Combined Results for First 31 dental patients:

Top 24 Genera

Summary:

1. Salivary bacterial population is similar to that reported by other laboratories using 16S rRNA metagenomic methods.

2. Saliva contains many of the same bacteria as dental plaque, but is not the same. Cheek, gum and tongue have different populations.

3. Bacteria that have been the focus of past work on dental cavities (caries) such as *Streptococcus mutans*, are only a minor part of the oral population. Other bacteria likely contribute more to dental disease risk.

4. Minor genera, such as Treponema (Spirochaetes) are involved in gum disease. None of the patients had Treponema pallidum (syphilis microbe).

5. Cyanobacteria were found at significant levels (4 %) in one out of 31 patients. The genus was Calothrix, which is one of the blue-green algae.

6. With more samples, the analysis will proceed to examine correlations with age, gender, and dental health.

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