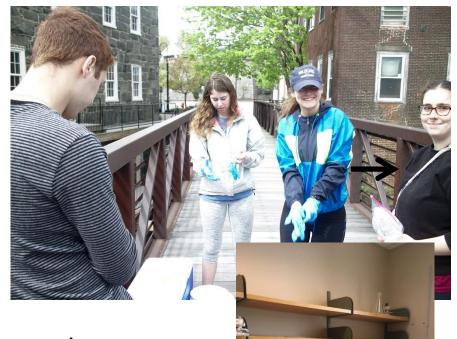
#### **eDNA 101**

Collect it

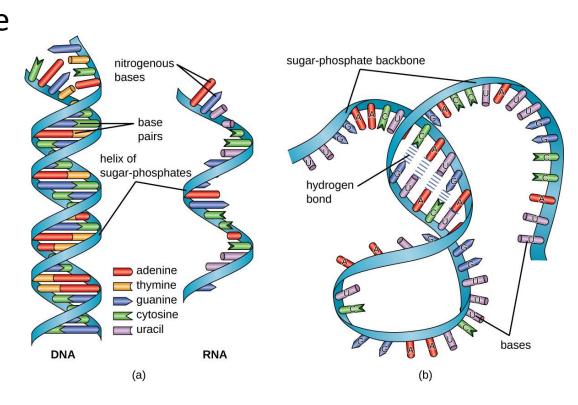
Sometimes filter or other post-processing

 Concentrate, extract, amplify, analyze and Interpret (Bioinformatics)



### Environmental DNA

16S ribosomal RNA is the standard gene used to reconstruct phylogenies for bacterial communities. It can be used to characterize the diversity and phylogenic composition of environmental samples. (Shokralla et al. 2012)

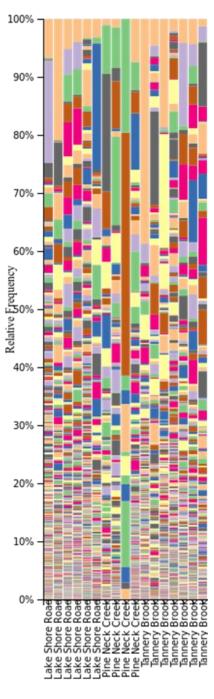


## Dry and Wet Weather Sampling

The amplicon sequencing method on the Amplicon sequencing of the 16S RNA gene was performed and the results classified by most common genus and species.

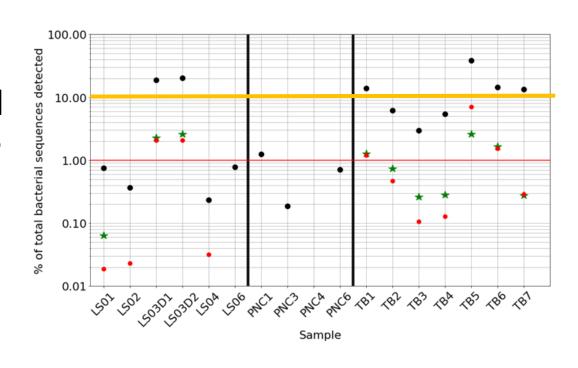
Sample Number	Site Name	Sample ID	Collection Date	Municipality
#	-	-	dd/mm/yyyy	1
1	Pine Neck Creek	PNC1	7/7/2016	Neponset
2	Tannery Brook	TB1	7/21/2016	Corey Farm
3	Pine Neck Creek	PNC2	8/9/2016	Neponset
4	Tannery Brook	TB2	9/8/2016	Corey Farm
5	Tannery Brook	TB3	11/8/2016	Corey Farm
6	Pine Neck Creek	PNC3	11/8/2016	Neponset
7	Lake Shore Road	LSO1	12/1/2016	Brighton
8	Tannery Brook	TB4	4/4/2017	Corey Farm
9	Tannery Brook	TB5	4/18/2017	Corey Farm
10	Pine Neck Creek	PNC4	4/19/2017	Neponset
11	Lake Shore Road	LSO2	4/25/2017	Brighton
12	Tannery Brook	TB6	5/18/2017	Corey Farm
13	Pine Neck Creek	PNC5	5/19/2017	Neponset
14	Lake Shore Road	LSO3	5/25/2017	Brighton
15	Lake Shore Road	LSO4	6/6/2017	Brighton
16	Pine Neck Creek	PNC6	6/27/2017	Neponset
17	Tannery Brook	TB7	6/29/2017	Corey Farm
18	Lake Shore Road	LSO5	7/13/2017	Brighton
19	Lake Shore Road	LSO6	7/23/2017	Brighton

Species level taxonomic composition



### Final Results

when fecal bacterial sequences are detected at 10 - 15% of all of the microorganisms human waste is most likely present in the sample.



- fecal bacterial sequences detected
- ★ Faecalibacterium sequences detected
- Bacteroides sequences detected

### Outfall Monitoring

Monitor and sample its outfalls through sampling and testing at the frequency and locations required in connection with IDDE screening

- i. IDDE screening shall include collection of grab samples and analysis for E. coli or enterococcus
- ii. Bacteria analysis shall be conducted using the analytical methods found in 40 CFR §136, or alternative methods approved by EPA in accordance with the procedures in 40 CFR §136.
- iii. Other IDDE screening parameters shall be considered field screening and are not subject to 40 CFR §136 requirements:

### Other IDDE screening parameters

- Ammonia
- Chlorine
- Conductivity
- Salinity
- Surfactants (such as MBAS)
- Temperature
- Pollutants of Concern

# Approach