



# Analyzing the Gut Microbiome of Human Populations in Crawford County

Tayita Abudu, Megan Peters, Mandy M. Peak  
Department of Biology, Pittsburg State University, Pittsburg, Kansas



## Research Question: What is the gut microbiome diversity of the residents in Southeast Kansas?

### Abstract

Human microbiota is the collection of microbes living in and on our body. Microbial cells outnumber human cells by 10 to 1 in a healthy human body. Previous studies demonstrated the gut microbiome from human samples correlate with disease, diet, and other human characteristics. Three microbe families that are prevalent are *Bacteroidaceae*, *Prevotellaceae*, and *Lachnospiraceae/Ruminococcaceae*. Even though studies have found a link between microbiome and health, the role in chronic diseases has yet to be elucidated.

To determine the type of bacteria in the human microbiome, 16S rRNA gene sequence data is isolated and examined from human DNA. Wastewater serves as a source for human microorganisms. DNA is extracted from the microorganisms and isolated so the 16S rRNA is tagged and fragmented. Bacterial genome libraries are available to provide descriptions to match laboratory results of the unidentified sequenced DNA to known labeled bacterial sequences.

Studies have been conducted in many cities and have identified diseases that correlate with human samples of microorganisms. There is interest in the gut microbiome of the residents in Crawford County, the role of the microbiome in chronic diseases, and the significant impact on human health. In our study, we will collect the influent wastewater from Pittsburg, KS and surrounding communities, and analyze the gut microbiome.

### Introduction

16S rRNA are components of genes that are highly conserved among different species of microorganisms. Researchers have identified numerous bacterial genes. This allows unknown bacterial DNA to be identified through the examination of sequences. The human gut microbiome has a diverse microbial community that varies among human populations. Even with a large variety of microorganisms existing in the gut communities, the human microbiome catalogue contains known data sets that correspond with specific functional gene profiles of bacteria. Identifying the 16S rRNA gene sequences in particular human gut communities simplifies the task of identifying species that exist within the gut.

There are numerous methods in identifying DNA. A common method is creating a genomic library via DNA sequencing. DNA is first extracted from microorganisms and isolated. The gene sequence is tagged and fragmented. The sequence is amplified through polymerase chain reaction (PCR), which creates the genomic library of the microbial community. Sequence length and base pair composition are important components of the genomic library that is provided. The DNA sequences can then be identified through matching unknown sample sequences with identified sequences provided by gene databases.

Researches have conducted experiments to evaluate the human gut microbiome in selected cities. It is found that bacterial families are shared and prevalent in certain communities. The bacterial families that are present also correlate with the diet and health. One critical finding is that cities with higher populations of obesity (consume high proportions of animal fat) have a larger representation of *Bacteriodes* and a decreased representation of *Faecalibacterium* in the gut community. *Bacteriodes* are associated with proinflammatory gut communities while *Faecalibacterium* increases with anti-inflammatory gut communities. Our study focuses on Crawford County of Southeast Kansas. Expectations are that among the samples of bacterial DNA, there will be the following species of bacteria: *Bacteroidaceae*, *Prevotellaceae*, and *Lachnospiraceae/Ruminococcaceae*.

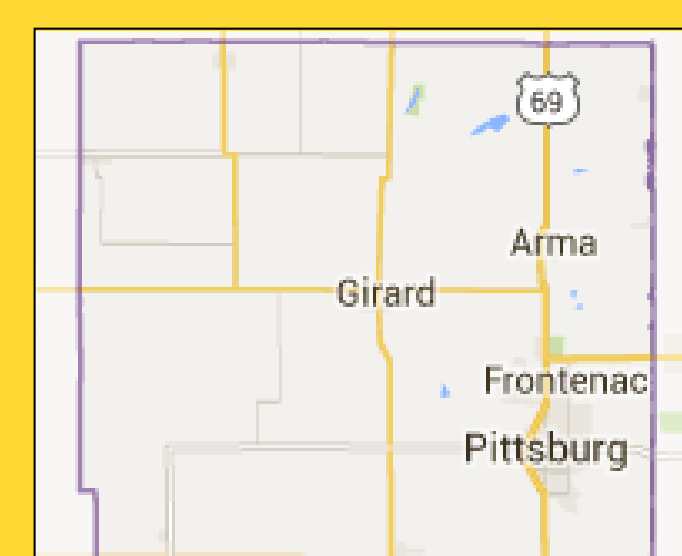


Figure 1: Crawford County

### Experimental Approach

Human DNA is extracted from microorganisms, filtered from influent wastewater. Utilizing a DNA Library Kit, DNA is isolated and the 16S rRNA is simultaneously tagged and fragmented at the V4-V5 region of the gene. The fragmented gene is amplified through PCR to produce a sequencing-ready genomic library. The genomic library will then be sequenced for identification through the use of the MiSeq Sequencing System.

With the 16S rRNA now identifiable, the results will be organized and interpreted with known 16S rRNA through the use of QIIME (Quantitative Insights Into Microbial Ecology). The QIIME is a software program with a library of known bacterial 16S rRNA. This allows comparative studies for accurate identification.

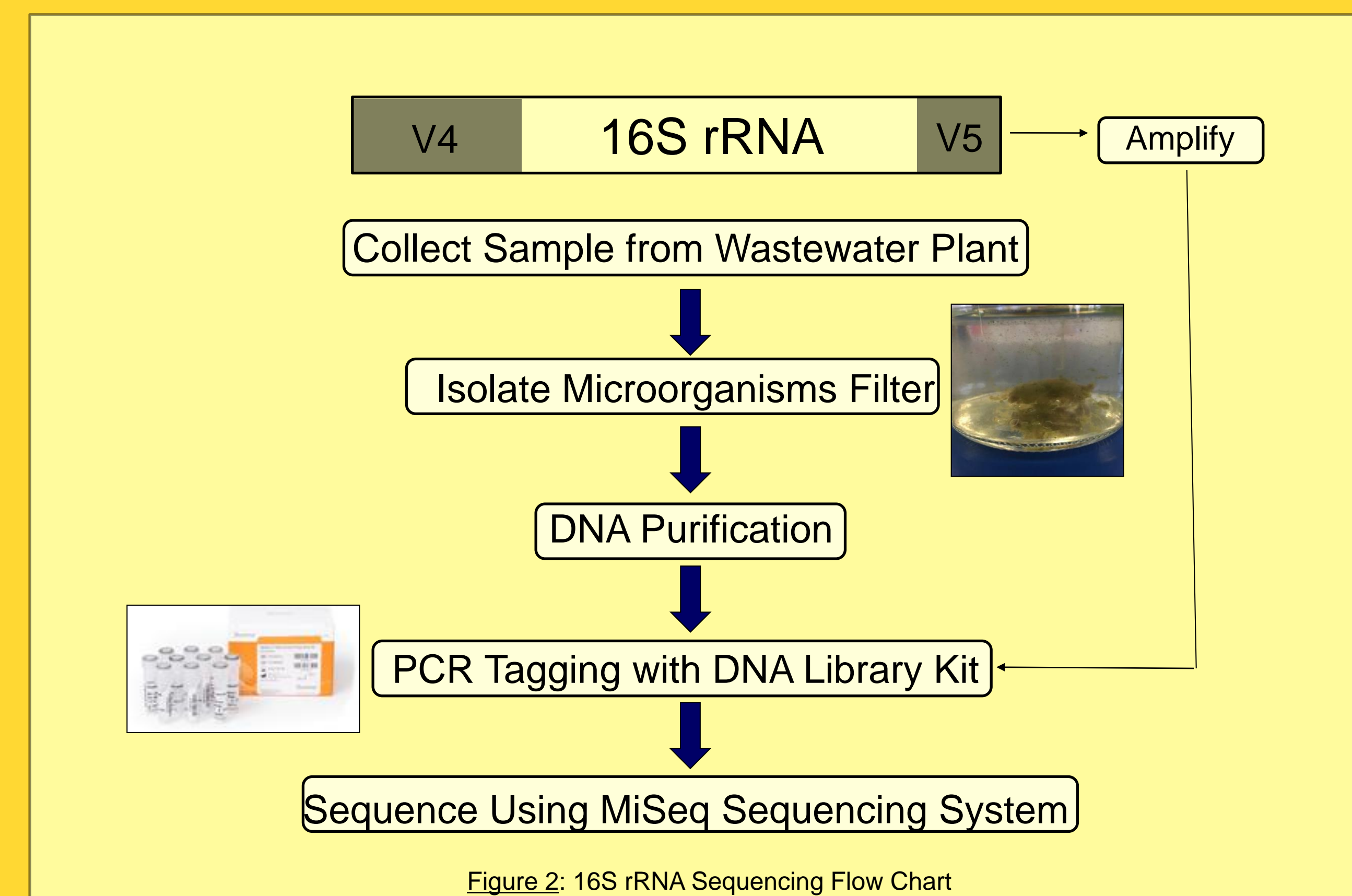


Figure 2: 16S rRNA Sequencing Flow Chart

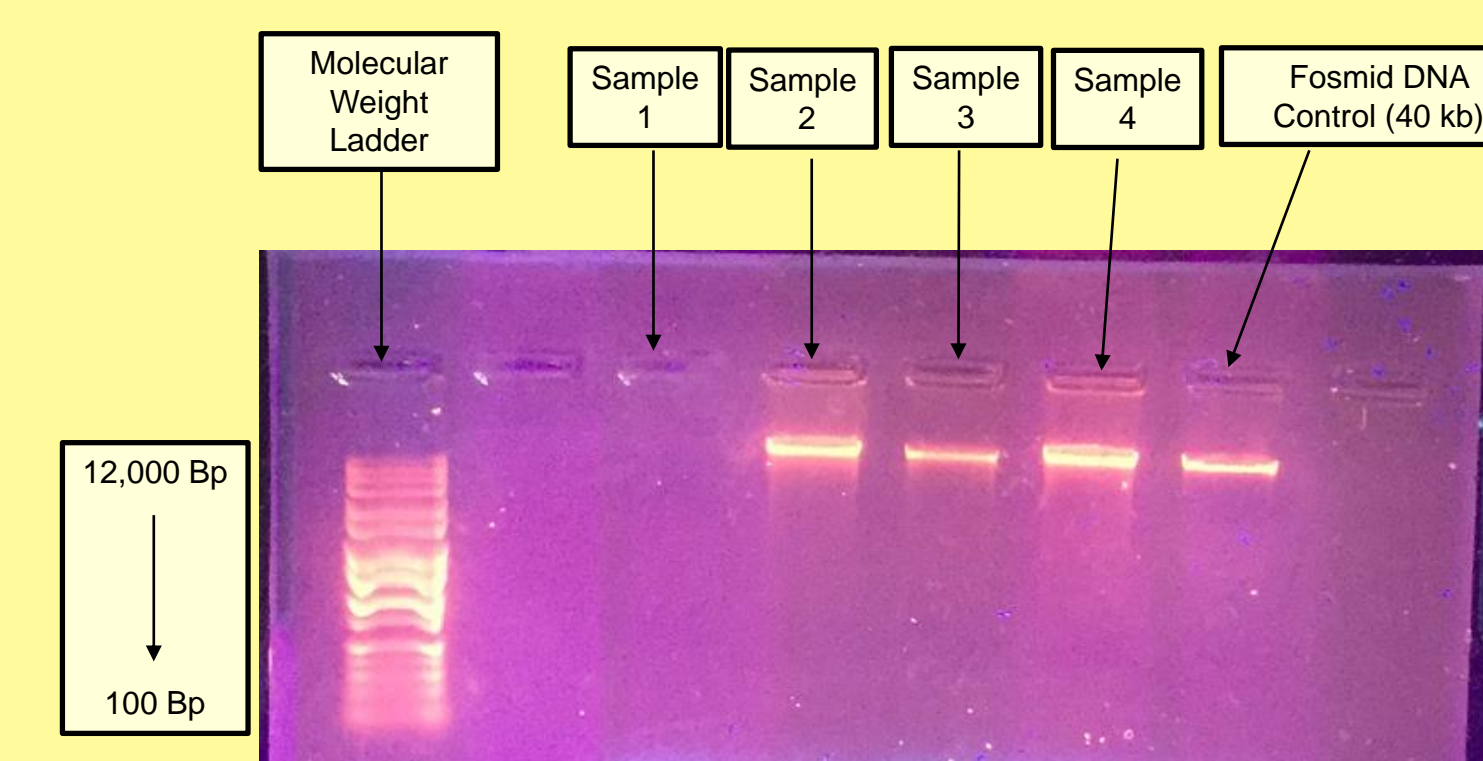


Figure 3A: Pittsburg DNA Results

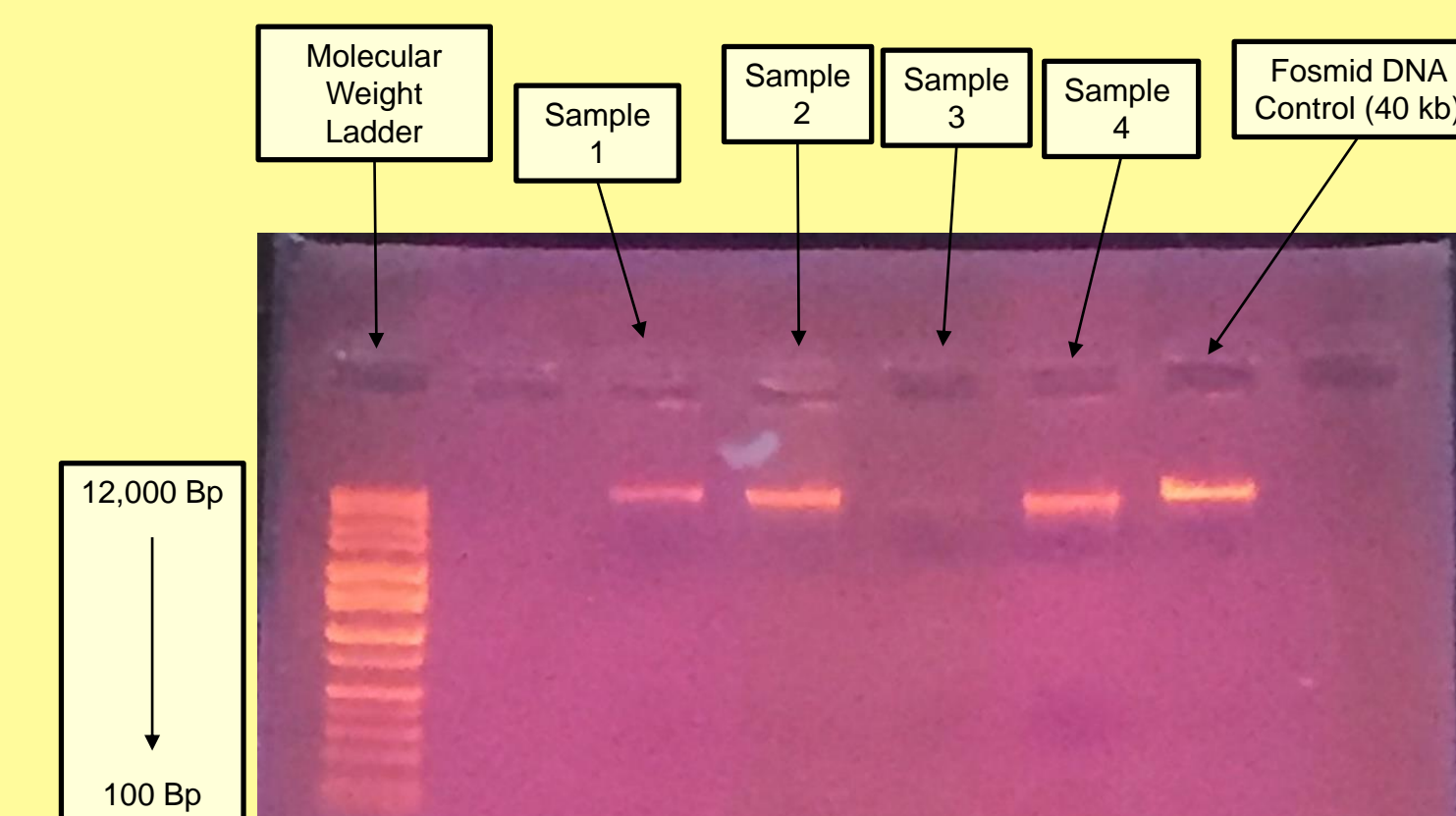


Figure 3B: Arma DNA Results

### Previous Research

- Obesity rate of Kansas is 31.3%, with the median age of 36
- 14.2% of children between 10 and 17 in Kansas are obese
- The gut microbiota serves as an important function in healthy humans
- Human fecal microbial community is a proxy for the human gut community

Kansas and Oklahoma Counties' Obesity Rate and Age Average			
State	County	Obesity %	Median Age
Kansas	Crawford	36.9	32
	Saline	34.2	37.8
	Geary	29.3	27.7
Oklahoma	Le Flore	32.1	38.6
	Canadian	31.9	35.7
	Cleveland	29.5	32.7

Table 1: Obesity Rate and Age Median of Kansas and Oklahoma. Note that Crawford County has the highest obesity rate of the selected counties. It is expected to find a higher percentage of *bacteroidaceae* in Crawford County.

### Objectives

- Sequence the human population in Crawford County
- Identify the prevalent microorganisms in the human gut microbiome in Crawford County
- Compare gut microbiome bacterial families in three communities in Crawford County to national and regional data
- Correlate our results with obesity health studies

### References

"Adult Obesity in the United States." : *The State of Obesity*. Trust for America's Health and Robert Wood Johnson Foundation, 21 Sept. 2015. Web. 12 Jan. 2016. <<http://stateofobesity.org/adult-obesity/>>.

"County Profile: Crawford County, Kansas." *SpringerReference* (n.d.): n. pag. Institute for Health Metrics and Evaluation. Web. <[http://www.healthdata.org/sites/default/files/files/county\\_profiles/US/County\\_Report\\_Crawford\\_County\\_Kansas.pdf](http://www.healthdata.org/sites/default/files/files/county_profiles/US/County_Report_Crawford_County_Kansas.pdf)>.

Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207-214.10.1038/nature11234.

Newton RJ, McLellan SL, Dila DK, Vineis JH, Morrison HG, Eren AM, Sogin ML. 2015. Sewage reflects the microbiomes of human populations. *mBio* 6(2):e02574-14. doi:10.1128/mBio.02574-14.

"Study of Children Ages 10 to 17 (2011)." : *The State of Obesity*. Trust for America's Health and Robert Wood Johnson Foundation, 2011. Web. 12 Jan. 2016. <<http://stateofobesity.org/children1017/>>.