NGS Interpretation

Next generation sequencing (NGS) is a powerful molecular biological tool designed to answer the question: *What microorganisms are present?* With the wealth of data provided in an NGS report however, data interpretation can be challenging particularly at larger sites with more samples. Below are some guidelines and suggestions to aid in interpreting NGS results.

1.0 Focus on Comparisons between Samples or Sample Groups

The key to interpretation of NGS results lies in *making comparisons* between samples or groups of samples. For many projects, *background* samples or *baseline* samples serve as the basis of comparison so their importance in data interpretation cannot be overstated.

For example, site managers may ask:

Questions	Sample Groups for Comparisons
• How did the microbial community composition change in response to the remediation approach? • Which microbes were more abundant after treatment? • Which were less abundant after treatment?	Pre-Treatment (Baseline) vs Post-Treatment
• What are the differences in the microbial community composition along a dissolved contaminant plume? • What biogeochemical processes are likely in the source zone? • Are microbial communities different in the downgradient areas?	Background vs Source Area vs Downgradient

2.0 Use the Statistical Analyses Provided to Identify Sample Groups for Comparisons

The **Hierarchical Clustering Dendrogram** and **Principal Coordinates Analysis** provided in standard NGS reports are excellent tools for visualizing similarities and differences in microbial communities between samples and may aid in identifying sample groups for additional comparisons.

The key to using Hierarchical Clustering Dendrograms (HCD) to identify sample groups for comparisons is that samples that cluster “on the same branch” have more similar microbial communities while samples “on different branches” are less similar.

While a different statistical method, using Principal Coordinates Analysis (PCoA) to visualize NGS results and identify groups of samples for comparisons is analogous to that described above for HCD. Samples that “group together” in a PCoA plot have more similar microbial communities while samples that are “separated” are less similar.

3.0 Compare Site Specific Data between Sample Groups

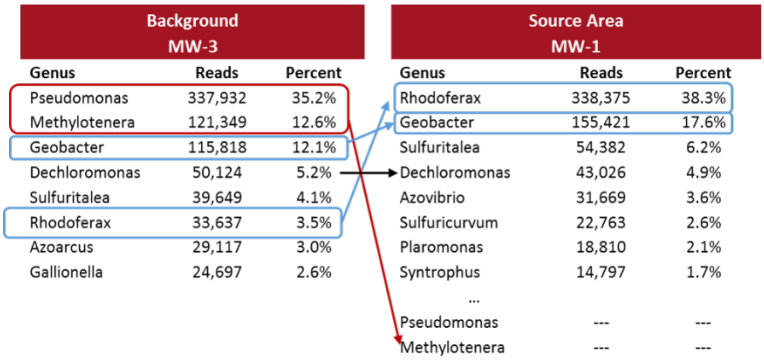
The next step is to examine groundwater monitoring data for site specific factors (e.g. well locations, contaminant concentrations, geochemistry, depth, treatment) that may explain the observed differences in microbial communities between sample groups.

4.0 Compare Top Genera Classification Results to Identify Populations Differences or Shifts

The **Top Genera Classification** table includes the number of reads and percent of the reads that were classified as belonging to a specific genus and a generalized description of some common characteristics of that genus. Sequencing is not quantitative, but the percent of total reads has been used for comparison purposes as an indicator of relative abundance within the sample. As a first step, site managers will typically compare percent reads for top genera between samples or groups of samples to identify key differences/shifts in microbial populations.

In the abbreviated table shown, major differences in microbial community composition between the two samples included the relative abundances of *Pseudomonas*, *Methylotenera*, Rhodoferax, and Geobacter while the relative abundance of *Dechloromonas* were similar between the two samples.

Figure 1: Identifying Differences/Shifts in Top Genera



5.0 Use the Genus Descriptions

With differences/shifts in top genera identified, site managers will then examine the genera descriptions more closely and relate the differences in microbial communities to site specific factors, if possible. The genus descriptions are *generalizations* about of general physiological and metabolic traits that are common among the characterized isolates of that genus described in the literature.

Genus	Reads	Percent	Description
Rhodoferax	338,375	38.3%	Metabolically versatile genus of bacteria with species capable of growth via photosynthesis, aerobic respiration, anaerobic respiration and fermentation. *Rhodoferax* utilize simple organic compounds as carbon and energy sources such as lactate, acetate, and ethanol. *Rhodoferax ferrireducens* is a facultative anaerobe utilizing iron and other metal oxides as electron acceptors.
Geobacter	155,421	17.6%	This anaerobic genus of iron-reducing bacteria grows chemoorganotrophically, utilizing Fe(III) as an electron acceptor and acetate as an electron donor. Several *Geobacter* species also utilize hydrogen as an electron donor. While *Geobacter* spp. are known as iron-reducing bacteria, some species can also utilize a variety of other growth supporting electron acceptors such as elemental sulfur, Mn(IV), and U(VI). Some species have been shown to degrade toluene and benzene.

In the example shown, the differences in the Top Genera such as the increase in relative abundance of *Geobacter* and *Rhodoferax* in the source area well MW-1 compared to background well MW-3 are consistent with the differences in subsurface conditions. *Geobacter* are anaerobic iron-reducing bacteria and several characterized *Rhodoferax* species are capable of anaerobic respiration or fermentation. Also, both genera utilize the volatile fatty acids and/or hydrogen typically produced in anaerobic environments. Thus, *Rhodoferax* and *Geobacter* are likely to thrive under the prevailing subsurface

conditions within the petroleum hydrocarbon plume. Conversely, relative abundances of aerobic microorganisms like *Methylotenera* would generally be expected to decrease in the plume as dissolved oxygen was consumed.

6.0 Identify Needs for Additional Analysis to Demonstrate Biodegradation Potential

As mentioned previously, the genus descriptions are generalizations about the physiological and metabolic traits that are common among the characterized isolates of that genus described in the literature. However, the genus description may also include interesting abilities characterized *in some strains* of that genus and species. These abilities such as the ability to biodegrade a specific contaminant are noted but are *not universal to the genus*. Actually, quite the opposite — those metabolic abilities are often fairly rare within the genus.

For example, strains of some *Geobacter* species are capable anaerobic BTEX biodegradation. Likewise, two known *Sulfurospirillum* species are capable of reductive dechlorination of tetrachlorethene (PCE).

Genus	Reads	Percent	Description
Geobacter	155,421	17.6%	This anaerobic genus of iron-reducing bacteria grows chemoorgano-trophically, utilizing Fe(III) as an electron acceptor and acetate as an electron donor. Several *Geobacter* species also utilize hydrogen as an electron donor. While *Geobacter* spp. are known as iron reducing bacteria, some species can also utilize a variety of other growth supporting electron acceptors such as elemental sulfur, Mn(IV), and U(VI). Some species have been shown to degrade toluene and benzene.

Genus	Reads	Percent	Description
Sulfurospirillum	454,997	36%	This genus of anaerobic bacteria reduces sulfur and utilizes a variety of electron donors including lactate, succinate and hydrogen. In addition to sulfur, thiosulfate and sulfite may also serve as electron acceptors. Strains of two species, *S. halorespirans* and *S. multivorans*, have been shown to sequentially reduce PCE to cis-DCE.

However, most characterized *Geobacter* species do not biodegrade benzene and most characterized *Sulfurospirillum* species are not able to perform reductive dechlorination of PCE.

Therefore, site managers would not infer the potential for BTEX or PCE biodegradation based solely on the detection of even high relative abundances of *Geobacter* and *Sulfurospirillum*, respectively.

- To assess the potential for anaerobic biodegradation of PCE, site managers would submit samples for **CENSUS® qPCR** or **QuantArray®-Chlor** to quantify concentrations of specific functional genes (e.g. PCE, TCE, and vinyl chloride reductases) as well as obligate halorespiring bacteria such as *Dehalococcoides*.
- To assess the potential for BTEX biodegradation, site managers would submit samples for **CENSUS® qPCR** or **QuantArray®-Petro** to quantify concentrations of specific functional genes (bssA, abcA, gmet) responsible for initiating anaerobic BTEX metabolism.