***The Garey Lab: Technologies and Skills***

University of Houston College of Pharmacy

Below you will find a list of some of the skills and expertise we offer at the Garey Lab. **Although we specialize in *C. difficile*, many of the options listed below are available for other organisms as well**. Note: for a full list of the equipment we have available, please visit the “Microbiology” page of our website.

“Deliverables” below refer to the product we provide in the event of a collaboration with the Garey Lab. Pricing for each service is dependent on several factors, including volume and level of data interpretation. Please reach out to us by email ([kgarey@uh.edu](mailto:kgarey@uh.edu)) if you are interested in a partnership with us or have more questions regarding our services!

**1. *C. difficile* growth, toxin characterization, and ribotyping.**

These experiments provide data on presence of toxigenic C. difficile including the genes for toxins A, B, and the binary toxin. Ribotyping is considered a moderately discriminatory typing method which includes identification of the recent epidemic 027 strains and emerging strains such as the 078 and 106 ribotypes. Our library is matched to the European library allowing for worldwide comparison of strains.

Deliverable: For each sample, growth of C. difficile (yes/no), toxin test results (presence or absence of toxin A, toxin B, and binary toxin genes) and ribotype are provided.

**2. *C. difficile* whole genome sequencing (WGS) analysis**

WGS typing is the most discriminatory typing method available for *C. difficile* studies. After growth, C. difficile DNA is extracted, sequenced, and fastq files generated.

Deliverable: The fastQ and quality data is provided for each sample. Phylogenetic analyses in the overall population and per intervention analysis is performed as appropriate. Presence of other known antimicrobial resistance genes in the *C. difficile* genome is also analyzed based on treatment group.

**3. 16S rRNA microbiome analysis**

16S rRNA analysis is a cost-effective sequencing method to understand microbial diversity at the phylum level. Stool DNA is extracted, sequenced, and fastq files are generated.

Deliverable: The fastQ and quality data is provided for each sample. OTU tables are generated for species diversity to the phylum and genera level. Analyses include diversity differences between groups, alpha and beta diversity among groups, and appropriate statistical tests for inference between treatment groups (PERMANOVA).

**4. Shot gun sequencing analysis**

Shot gun sequencing is the most discriminatory microbiome typing method allowing understanding of microbial diversity at the bacterial species level. Procedures and analysis are similar to 16S rRNA but the deeper sequencing of the entire genome increases sequencing costs. Note: if shot gun sequencing is done, there is no need for 16S analysis.

Deliverable: The fastQ and quality data is provided for each sample. OTU tables are generated for species diversity to the phylum, genera, and species level. Analyses include phylogentic analysis and diversity differences between groups, alpha and beta diversity among groups, and appropriate statistical tests for inference between treatment groups.

**5. Quantitation of microbiota changes using qPCR**

16S rRNA and shot gun sequencing allows for analysis of diversity changes among treatment groups. To assess quantitative differences in microbiota composition, qPCR is used to assess quantities of appropriate bacterial groups (Table 1).

Deliverable: Quantitative measurement of each bacterial group is provided per sample. Analysis includes comparison of bacterial group median quantities between time points and treatment groups with appropriate statistics.

**6. *C. difficile* MIC susceptibility testing**

*C. difficile* MIC susceptibility is performed by broth microdilution. MIC determinations are generally done for antibiotics used to treat *C. difficile* (metronidazole, vancomycin, and fidaxomicin) or antibiotics that may be causative for CDI (usually ampicillin, cefotaxime, levofloxacin, meropenem, and others)

Deliverable: The MIC for selected antimicrobials is provided for each sample. Analysis includes a summary table of MIC50, MIC90, and geometric mean MIC.

**7. Culture for MDROs from stool**

Stool samples are screened for MDROs including MRSA, VRE, ESBL-E/CRE and Candida. After enrichment in brain heart infusion (BHI), samples are sub-cultured onto highly selective agar plates (usually HardyCHROM plate). Species and resistant determinants are then confirmed by PCR and/or MALDI-TOF. Other species than the ones names above can be grown. All isolates identified can also be further analyzed using whole genome sequencing.

Deliverable: For each sample, presence or absence of each MDRO requested along with confirmation of species and resistant determinant.