Ant Microbe Protocols

Corrie S. Moreau – Field Museum (January 2012)

- Ant specimen preservation: To insure you are starting with high quality material, collecting in 100% ethanol (EtOH) is preferred (although 95-100% EtOH is acceptable). If you need to also preserve material for RNA or other sensitive work use RNAlater or Lifeguard Soil Preservation Solution: (http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/rnalater.html); (http://www.mobio.com/soil-rna-isolation/lifeguard-soil-preservation-solution--10ml.html).

- Surface sterilization of insect material: Whole ants, pupae, larvae, or eggs should be surface sterilized before whole body or tissue specific microbial characterization by dipping whole (undissected) specimen(s) in 95% ethanol, followed by a one minute soak in 5% bleach (sodium hypochlorite), and a final rinse in sterilized water.

- DNA extractions: If you are only interested in microbes (we realize sometimes you may be more interested in the host and may want to use another kit in those cases) DNA extraction should be done with the MoBio PowerSoil DNA isolation kit (http://www.mobio.com/soil-dna-isolation/powersoil-htp-96-well-soil-dna-isolation-kit.html -or- http://www.mobio.com/soil-dna-isolation/powersoil-dna-isolation-kit.html) following the modifications on the Earth Microbiome Project website: http://www.earthmicrobiome.org/emp-standard-protocols/.

- Ant tissues for DNA extraction: If you want to definitely say that you are investigating *ant gut microbe communities, you will have to do gut dissections*. If you are more broadly interested in *ant associated microbes, then you can use the whole gaster*. If you want to compare external microbes with tissue specific microbes then you may have to do a series of extractions from a single specimen (i.e. one leg, head, and gut in separate extractions). You must be clear which you are doing and why and clearly state this in communications and publications. We have decided based on difficulty of gut dissections that we will target the midgut and large intestine (excluding the crop and rectum, but it is fine to include some of the malpighian tubules). Please state in publications which gut sections were included in case someone is interested in other gut sections and would like to include these. Please follow the nomenclature of gut sections as outlined under the "Resources" tab on the Moreau Lab website and scroll down to "Ant gut dissection for microbial research" section: www.moreaulab.org

- Bacteria 16S primers: For Illumina sequencing of bacteria we advocate the V3/V4 segment of 16S rRNA using the following primers 515F/806R (see EMP website above for primer sequences and amplification protocol). For other bacterial 16S sequencing, any primer pairs are acceptable as long as they cover the V3/V4 regions so future results are comparable with all data.

- Bacteria sequencing: If you are not planning to sequence the data in your own lab, then working with the Earth Microbiome Project (EMP) is the preferred group (http://www.earthmicrobiome.org/). They are currently sequencing about a 125bp fragment of 16S V3/V4 using Illumina. Please contact them directly if you have questions regarding their project or protocols.