

# Global Mosquito Microbiome- Sampling Protocol

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## Mosquito Collection:

**Adult mosquitoes may be caught with any standard method** (i.e. light traps, gravid traps, scent lure traps, etc), but please record the method of capture.

- Please be sure to include date of capture, GPS coordinates, ambient temperature in Celcius, and notes on general weather conditions.

Mosquitoes should be handled using gloves when possible or handled directly as little as possible.

Please be sure to wear appropriate PPE when collecting specimens.

## Mosquito Pre-Processing:

Once mosquitoes have been caught, they should be killed via freezing, optimally in sterile falcon-style tubes (size does not matter, but smaller may be easier to handle).

If you are processing the mosquitoes within one week of sampling and initial freezing, **PROCEED TO PROCESSING STEPS.**

- *Do not add DNA/RNA Shield.* Be sure that the frozen mosquitoes remain that way (i.e. do not freeze-thaw-freeze- thaw, as this will degrade their DNA and may alter microbial communities).

If not proceeding to processing within **ONE WEEK** of sampling:

Zymo Research DNA/RNA Shield should be used to cover and preserve the mosquito, once it has been frozen and killed. Typically between 50 and 100ul is sufficient. Be sure to use gloves and a sterile pipettor (sterile transfer pipettors are fine here).

Samples should immediately be returned to the freezer until they are ready to be processed.

## Processing:

When ready to process samples, be sure to clean the working area with 10% bleach and then 70% ethanol. Clean all working instruments with either 70% ethanol or 95/100% ethanol and flame sterilization.

Mosquito heads, wings, and legs should be removed using a sterile scalpel and either changing blades or flame sterilizing between individuals.

Remaining mosquito carcasses should not be surface sterilized (per Hammer et al. 2017) and should be placed and crushed into an aliquot of about 50-100ul of Zymo Research DNA RNA Shield for freezer storage.

Samples should be shipped on ice to:

Attn: Alanna Boyle  
Biology Department  
University of Massachusetts Boston  
100 Morrissey Blvd  
Boston, MA 02125 USA  
720-245-5828

Questions about sampling should be directed to:

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