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Sequencing for Bark beetles and associated fungi

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Bark Beetle Mycobiome Research Coordination Network Bark
Beetle

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Abstract

The purpose of this protocol is to describe the submission for sequencing at the UF Forest Entomology Lab.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

Currently, GENEWIZ offers the best rate for sequencing 48 or fewer reactions with clean up. If you are submitting 48 or less, please follow the GENEWIZ protocol below, using 8 strip cap tubes. If you have greater than 48 samples, please submit using a prepaid plate through Eurofins, following the Eurofins protocol below.

You may prefer to use one company over the other for a multitude of reasons. For submitting larger numbers of samples to GENEWIZ, the protocol is roughly the same, but you use semi skirted plates and strip caps. For submitting fewer samples to Eurofins, we have barcodes (used for loose 1.5mL vials, which they use for orders of less than 24 samples)and packaging material available. Please follow the guidelines for sample submission on the Eurofins Genomics website.

GENEWIZ
In general, we will be using the “Custom” option for GENEWIZ. This entails sending 10uL of diluted un-purified PCR product per sample, with 5uL of 5uM primer per sample. For most cases, we will opt to have clean up performed at the facility.

Primers: Send 5uL of 5uM primer per sample to be sequenced. To make a solution of 5uM in 5uL, mix 2.5uL of your 10x primer stock with 2.5uL PCR water. Multiply these values by your number of samples + 2 and send in one Eppendorf tube per primer. Label with the primer name and concentration (5uM).

Template: Send in 10uL of your un-purified PCR product per well. This can be diluted with water, 0.5uL PCR product mixed with 0.5uL water tends to work well for most. If your band is strong and you know your concentration is high, you can increase the dilution to conserve PCR product. If it is weak or you know the concentration is low, you can send in less dilute product.

Submitting samples:
1) Create or log in to your GENEWIZ account. If this is your first time creating it, use 11575668 as the PI/Institution ID. Contact Zach with your account email to be added to the Lab Group to share payment info and sequence results.
2) Create a sequencing order from your account page. Choose the options for “Same Day” and “Upload Excel Form.” Choose and download the “Custom Form.”
3) Fill out and upload the Excel form by typing in your sample names, primer names, and Primer Concentrations. Select your DNA Type, DNA Length, and Special Protocols from their drop down menus. You can skip DNA Concentration, GENEWIZ Primer, and Notes.
4) Select payment method. You may use your own P-card if you have one. If not, access to our Lab Group should allow you to use Zach’s.
5) Select the Newins-Ziegler Hall Mailroom as your sample pickup location.
6) Submit your request, here you can print off the form you will include with your samples. This will also give you vial labels if you are sending in samples in strip tubes, label these values on the sides. Plates do not need labels as the excel file assigns well positions. Use 8 strip domed caps to seal vials and plates. You can wrap in parafilm for extra security.
7) Place samples in ziploc sample bag with primers and the provided order form. Drop off your sample in the GENEWIZ box in the NZH mail room before 4pm on the day of submission for pickup. If you cannot make it by this

time, send an email to DNASeq@genewiz.com on the day you drop it off to notify them so it will be picked up.
Pricing: For custom orders the cost is $4 per sample. Clean up is an additional $1. You can perform clean up yourself, however this saves us little money given the cost of ExoSAP.