**MAWDC DNA extraction, PCR and electrophoresis protocol**

**Protocol for Sigma Extract N Amp DNA extraction**

Equipment needed: See checklist. Make sure all items are accounted for.

1. DNA extraction (same day sample processing)
* Designate a pipettor. That person will follow the instructions for the designated pipettor. All other participants will follow the instructions for other participants.
* Fill out the DNA extraction sheet and PCR log sheets and make sure everyone knows the numbers corresponding to each of their samples.
* Set up each sample processing site according to the schematic in figure X.

|  |  |
| --- | --- |
| Designated pipettor1. With gloved hands, remove as many collection tubes as samples to process.
2. Place the collection tubes in a tube rack and label them 1,2,3,4 etc. as per PCR worksheet.
3. Add 100 µl Extraction solution to each tube. Cap the tubes
 | Other participants1. Use one tile for each sample you are processing. Wipe each tile with alcohol. Once alcohol is dried, with a Sharpie, write the number from the DNA extraction sheet corresponding to each sample on each tile.
2. Remove a small amount of tissue from each sample using sterilized forceps and razor blades (as needed). See sample processing guidelines.

Briefly:* 1. Gilled mushrooms—remove a 0.3 to 0.5 mm piece of gill using sterilized forceps
	2. Pored mushrooms—remove a 0.3 to 0.5 mm3 section of the upper pore surface (part closest to the trama)
 |
| Cap the tubes and distribute among the other participants according to sample number. | Using a separate sterilized forceps for each sample, place the gill/pores/other tissue in the Extraction solution according to sample number. |
| If mushroom tissue was previously placed in microcentrifuge tubes and frozen:1. Organize tubes in a tube rack according to DNA extraction worksheet and label 1,2,3,4, etc.
2. Add 100 µl Extraction solution to each tube, taking care to not touch tube or contents with pipette tip. Replace tip as needed to avoid contamination.
3. Cap the tubes and proceed with heat bath.
 |
| Place tubes in 95 C water bath for 10 minutes |
| 1. While tubes are incubating, prepare PCR master mix. Refer to the PCR log sheet for amounts to include of each reagent.
2. Label PCR tubes (strips of small tubes) according to sample log and aliquot 16 µl of master mix in each tube.
3. Provide PCR tubes with master mix to each participant according to sample numbers.
4. Add water to one tube for the negative control.
 |  |
| Remove DNA extraction tubes from 95 C water bath  |
| 1. Add 100 µl Dilution solution to each DNA extraction tube using a new tip for each tube. Gently mix the dilution solution as it is added by pipetting up and down.
2. Provide participants with their corresponding DNA extractions to add to PCR reactions.
 |  |
|  | Using a new tip for each sample, add 4 µl DNA extract to the corresponding PCR master mix and gently mix by pipetting up and down. |
| Place tubes in thermal cycler. |

1. PCR

Gently close the thermal cycler lid and latch. Turn on the machine and wait 5 seconds, then connect to machine via Bluetooth using the MiniOne app. Refer to the cycling conditions sheet and enter the appropriate cycling conditions for the targeted gene region. Progress of the run can be monitored in the app.

While waiting for the PCR to complete, prepare the gels for electrophoresis.

1. Electrophoresis

Prepare the number of gels needed for the number of samples processed.

For each gel

1. Prepare 1X TBE buffer. Use an orange cap (50ml) conical tube to measure out 2.5ml of 20X TBE buffer concentrate and add deionized water to 50ml.
2. Dissolve the agarose. Use a 15ml conical tube to mix agarose and TBE buffer for each gel. Add the contents of a microfuge tube labeled “agarose” to the tube and fill to 15ml with 1X TBE. Mix well. (Double check on if this works. Fill to 10, mix, pour into bottle and then fill to 5 to remove any remaining agarose?) Place bottle in 100 C water bath to dissolve agarose. Add GelGreen stain (how much?)
3. Pour the gels. Use approximately 10 ml per casting tray.
4. Once a gel is set, place in electrophoresis chamber. Add 1XTBE to chamber, using enough to cover the gel by about 1mm.

Once PCR cycles are complete

1. Prepare a piece of parchment paper/wax paper with loading dye for each sample. Each participant will load their samples in the gels.
2. For each sample, mix 2 ul loading dye with 8 ml PCR product. Load in the corresponding well on each gel.
3. Run the gel for about 20 minutes, or until DNA marker is well separated.
4. Once the bands and marker are easily viewed, take a picture of the gel for submission with the PCR products for sequencing.