miniPCR Guide

Reagents, Supplies & Equipment

- miniPCR Machine
- Device (computer, phone or tablet)
- miniPCR software

The miniPCR machine runs using software on a computer, tablet or phone. Before you can use the miniPCR machine, you will need to install the software.

1. Copy and paste or type the following link into a web browser, then install the software appropriate for your system: http://www.minipcr.com/downloads

2. Connecting the miniPCR machine to the device:
    a. Via USB
        i. Plug one end of the white USB cable into the back of the machine and the other end into the USB port of the computer.
        ii. Plug the black power supply wire into an electrical outlet and the round end into the back of the machine (the circular opening next to the USB port).
        iii. Above the USB port on the back of the machine, flip the power switch to the "ON" setting.
        iv. Once the machine is "ON", the software should launch.
    b. Via Bluetooth *NOTE: make sure Bluetooth is turned on on your device.*
        i. Turn on the mini16 miniPCR machine using the on/off switch on the back of the unit. A flashing blue LED on the front of the mini16 indicates that it is ready to connect to your device.
        ii. Click the "Devices" tab (Win/Mac) or the icon (mobile, top center of the screen). mini16 units within BLE range will be listed. Click on the BLE symbol of the mini16 unit you would like to connect to.
        iii. Successful pairing is indicated by green text "Connected" and by the blue LED staying always on.
3. Importing the PCR protocol *NOTE: The "miniPCR protocol" email includes programs for our standard barcodes. You should not need to change the settings. Check the program before you run it each time to ensure it is correct.*
4. Click the three dots in the top right corner of the miniPCR application.

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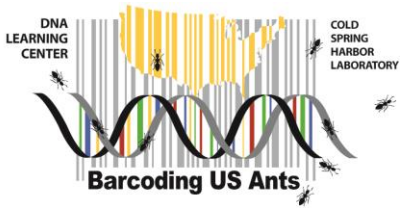

5. Select “Import protocol library” and upload the .plf or .plfx file that was in the “miniPCR protocol” email.
6. The barcoding protocols will now be imported into your miniPCR “Library” tab.
7. Running the PCR program
    a. To begin PCR, select the standard Ant protocol and click the triangle symbol “RUN”
    b. A new window may pop up and ask you to select the machine you should only have one miniPCR to select from. Click on the OK button
    c. This screen shows the status of each cycle of the PCR protocol in real time. We recommend that you pause the program at the beginning of the initial 95°C step, load your PCR tubes from ice directly into the hot PCR machine, and then resume the program. Once the program has started, we recommend that you do not pause or stop the reaction again at any point and let the cycles come to completion.
    d. Under the “Data” tab (as seen in the image above) you will be able to see the duration of the reaction, the cycle number, and a graph depicting the Time (hh:mm:ss) on the X-axis and the Temperature (^{O}C) on the Y-axis. Under the “Protocol” tab you can see the parameters of the protocol that you entered. Under the “Settings” tab you can see details about the miniPCR machine.
    e. Once stored on ice or at -20^{O}C, amplified samples are ready to be analyzed by gel electrophoresis.

For in-depth instructions and troubleshooting see the miniPCR User guide PDF: https://www.minipcr.com/wp-content/uploads/miniPCR-mini16-thermal-cycler-Users-Manual-121119.pdf

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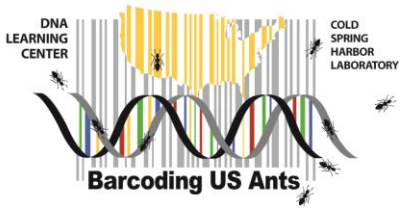

IV. Analyze PCR Products by Gel Electrophoresis Using the blueGel™ Electrophoresis System

Reagents, Supplies & Equipment

- PCR products from part III*
- Container with cracked or crushed ice
- Distilled water
- Plastic cup
- Micropipettes and tips (1–100 µL)
- Microcentrifuge tube rack (or foil over a container)
- Masking tape
- Microwave
- Digital camera
- Permanent marker
- Latex gloves
- blueGel™ Electrophoresis System:
    - blueGel™ base
    - Orange cover
    - Power cord
    - Casting platform
    - 2 gel trays
    - 2 combs (stored under casting platform)
    - Buffer chamber
    - Microfiber cloth
    - ClearView Spray™
    - Fold-a-View™ photo documentation hood
- GelGreen® Agarose Tab™
- 1X TBE buffer (~30 mL per gel)
- 100-bp ladder (5 µL per gel)*
- PCR tube rack (empty yellow tip holder)
- Petri dish (for packing PCR tubes)

*Store on ice

1. Place the 13-well comb in the top slot of gel tray in casting platform (comb is stored under the casting platform).
2. Add one GelGreen® Agarose Tab™ to 20 mL of distilled water in a plastic cup. Microwave 30 seconds and then swirl. Microwave for an additional 10 seconds or until just bubbling and then swirl; repeat until the tab has completely dissolved. Allow the solution to cool for about 3 minutes and then pour the 2% agarose solution into the tray.

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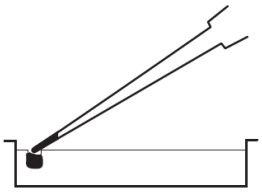
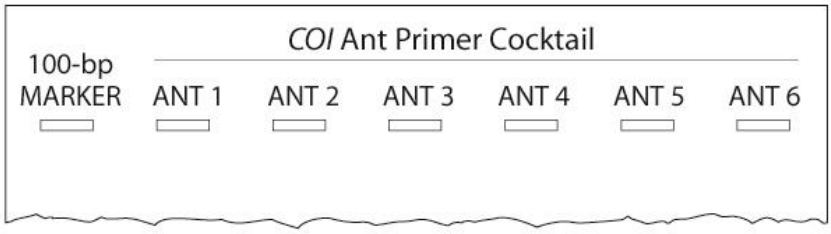
3. Allow the agarose gel to completely solidify; this takes approximately 10 minutes.
4. Carefully remove the comb from the gel by pulling straight upward. Lift the gel tray from the casting platform and wipe off any gel that has formed beneath the tray.
5. Place the gel tray into the buffer chamber and place the buffer chamber into the blueGel™ base. Add just enough 1× TBE buffer to fill in the wells and just cover the gel, creating a smooth buffer surface.
6. Orient the gel according to the diagram below, so the wells are along the top of the gel. Use a micropipette with a fresh tip to load 5 µL of 100-bp marker into the far left well.



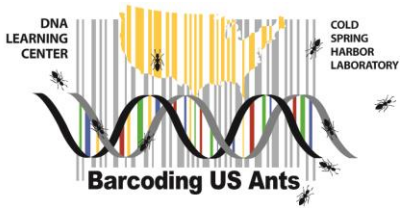
Expel any air from the tip before loading, making sure not to lose any liquid, and be careful not to push the tip of the pipette through the bottom of the sample well.

7. Use a micropipette with a fresh tip to load 5 µL of each PCR sample in your assigned wells, similar to the following diagram:



The samples you load may not be exactly the same as those shown.

8. Store the remaining 20 µL of your PCR product on ice or at -20° C until you are ready to submit your samples for sequencing.
9. Use the microfiber cloth to spread one pump or less of the ClearView Spray™ between the electrodes on the inside of the orange cover to reduce condensation. Place the orange cover on the blueGel™ base. The cover contains the electrodes and will only fit in one direction, with the (+) electrode positioned to attract the negatively charged DNA.
10. Press the power button to start the run. Adequate separation will have occurred when the cresol red dye front has moved at least 40 mm from the wells.
11. You can visualize the DNA at any time by pressing the lightbulb button on the blueGel™ base. The Fold-a-View™ photo documentation hood can be placed over the orange cover to reduce ambient light.



12. Photograph the gel using a digital camera. It may be necessary to use the microfiber cloth to remove excess condensation under the orange cover for a clear photograph.

Extra information on the blueGel™ system and GelGreen® Agarose Tab™ can be found at:

https://www.minipcr.com/wp-content/uploads/Bluegel_UsersGuide.pdf

and

https://www.minipcr.com/wp-content/uploads/Data-sheet-GelGreen-Tabs-with-TBE-and-GelGreen_vF.pdf.

Mailing Instructions (for amplicons, DNA and ants):

Return several ants of each species for taxonomic identification. Use a separate tube of ethanol for each different ant species. Place the labeled screw-cap tubes containing ethanol and your ant specimens and the labeled Parafilmed tubes containing Chelex/tissue/DNA into the microcentrifuge tube storage box for mailing. Label the box with your last name, the date, and "US Ants." Place the foil-wrapped filter paper discs, pestles and cap locks into the Ziploc bag for mailing. Label the bag with your last name, the date, and "US Ants." Ensure that your PCR tubes are clearly labeled with the sample identification numbers. Tape the PCR tubes inside of the petri dish and tape the dish closed. Label the dish with your last name, the date, and "US Ants." Place the materials into an appropriate package and mail to:

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