DNA Extractions using Qiagen DNeasy Kits with Extraction Beads

Corrie Moreau – Field Museum (September 2009)

These are the instructions I use for DNA extractions of individual or parts of ants, but are useful for almost any insect or tissue. They are rather comprehensive as they were written so that someone with any level of laboratory experience can follow the protocol.

Instructions for extracting DNA from 24 or less ant specimens (increase accordingly if necessary, but I suggest not doing more than 24 extractions at a time in case something goes wrong).

First create a list of the ant specimens you will be extracting DNA from (use an extraction worksheet or write in your lab notebook). You must be very diligent to never mix up a number or tube as this will lead to confusing the DNA of one ant specimen for another ant specimen. If you ever realize you have done this, it may be necessary to throw out the entire DNA extraction (but check with your supervisor first). Be sure to note this on your extraction worksheet or lab notebook.

Before you begin the DNA extractions it is very important to make sure everything you will be using is DNA free.

- Get six 96 tube racks, forceps, small beaker, Petri dish, and anything else you use for the protocol and put under the UV light bench for 15 minutes or longer (the UV will glow bluish when turned on). The UV light destroys DNA that could contaminate your new extractions.
- While UVing the above materials, thoroughly clean your lab bench with 10% bleach and ethanol. Be sure it is entirely dry before putting your extraction materials on the bench.
- When above materials are done being UVed, bring to lab bench with new, gloved hands.
- Check that the "rotisserie" oven is turn on and set at 55-56˚C.

Next make sure you are set up to begin the extractions.

- I find that if you stagger the tubes in the racks by putting eight tubes in the first row of the rack in positions 1, 3, 5, 7, 9, 11, 13, 15 and then skip a row and then add the next eight tubes in positions 2, 4, 6, etc. and then the last eight tubes in the last row in positions 1, 3, 5, etc. this will allow room for opening all the tubes without the lids getting too much in the way.
- As you open the 1.5mL tubes (now and in all future steps) be careful not to put your thumb or fingers on the inside of the lid (you can introduce contamination this way).
- Put the vials currently holding the specimens you intend to extract in one of the newly UVed 96 tube racks in the order in which you have entered them on your extraction sheet.
- In the next UVed rack put one clean, lid-closed 1.5mL tube for every specimen you will be extracting in the 96 tube rack. Number these tubes on the top using a lab pen (Sharpie, VW pen, etc.) with the same numbering system as the worksheet (does not have to be the collection code; can be a number 1-24, etc.)

- Next put the same number of Qiagen extraction filter tubes in the next 96 tube rack (you will have to peel each tube from its individually sealed holder). Number these with the same system you numbered the 1.5mL tubes above (i.e. 1-24, etc.)
- In the next two 96 tube racks set up the same number of empty Qiagen collection chambers/tubes as the number of extractions you are doing.
- In the last rack place closed 1.5mL tubes (again the same number as you are extracting). These you must label with the "Tough-Spots" stickers writing the collection code from your extraction sheet (not #1-24, etc., but the actual specimen collection codes). These numbers should be the same collection codes as found on the vials/tubes you are taking the whole ant specimens from.

You are now ready to begin the extractions.

- Be sure to have a box of KimWipes ready.
- Make sure all your tubes are always in the same order as your extraction worksheet/lab notebook! This will help you to minimize errors.
- Open each of the 1.5mL tubes you labeled #1-24 with the lids opened away from you again being careful not to let your thumb or fingers touch the inside of the lid (these are the vials you will be placing the specimen into and adding the Qiagen chemicals to leave three hours to overnight).
- Fill your Petri dish with PCR water.
- Fill your small beaker with 95% ethanol and place forceps in this beaker (you will use these to take the individual ant specimens from the tubes).
- Begin with the first vials of specimens. Take one single ant specimen out of the vial using the forceps and place in the Petri dish of water (be sure that the ant you have taken out does not have another ant or larvae in it's mandibles or jaws).
- Leave in the water for 10 seconds. No need to shake around.
- Remove ant specimen from the PCR water using the forceps and place gently on a clean KimWipe.
- Leave on KimWipe for a few seconds to get most to all of the water off.
- Place the ant specimen in a labeled, open 1.5mL tube corresponding to the correct extraction number. Always check everything twice!
- Once you have placed an ant specimen in the open 1.5mL tube, leave the tube open to allow to the ant specimen to dry, but turn cap towards you to indicate that this vial now has an ant specimen inside (this will help you to not accidentally put two ants in the same vial).
- Clean forceps in the beaker of ethanol and wipe with a clean KimWipe.
- You are now ready to begin with the next specimen.
- Continue until all ant specimens are in an individual 1.5mL tube.

With one single ant specimen in each tube, you are now ready to move to the next step.

- Again clean your forceps in the beaker of ethanol and wipe clean with a KimWipe.
- Get a vial of clean Qiagen tungsten carbide beads (or a clean pestle if you do not have Qiagen tungsten carbide beads).
- Place one bead in each tube with the ant specimen and close the tubes.

- Load the vials with the ant specimen and tungsten carbide bead into the Qiagen extraction machine (be sure to load both racks equally – they must be balanced) (or grind each specimen with a clean pestle).
- You will turn on machine for 20 seconds (no more than 20 seconds) at 30.0 1/s frequency!
- Take the tubes with the now shattered ant with the metal bead inside and place back in the rack in numerical order (again staggering the tubes in the rack).

You will now begin to follow the Qiagen DNA extraction instructions (be sure to always use barrier/filtered tips for pipetting).

- Open all the tubes (again with the lids open away from you).
- As you are opening each tube, check the lid to see if the metal bead cracked the lid (this happens rather frequently). If a tube has a cracked lid, carefully cut off the lid at the plastic hinge making sure not to lose the material inside after you have added the buffer and ethanol (outlined below) and vortexed, etc. Then cut the lid off a new tube and place on this tube. Be sure to label with the same exact number that was on the tube before you cut the lid off!
- Add 180uL of Buffer ATL to each tube.
- Add 20uL ProK to each tube (pipetting the mixture up and down), closing each tube as you finish this step.
- Once you have added the Buffer ATL and ProK to each tube, place each tube in the "rotisserie" at 55-56˚C. Make sure the tube is pushed down so that the lid/cap is resting on the metal holder (sometimes the tubes slip out of the metal holder if not).
- Turn "rotisserie" on so the tubes are rotating while they are "cooking" at 55-56˚C.
- Leave tubes in "rotisserie" for three hours minimum to overnight (but not more than 30 hours).

After three hours to overnight, you are ready to finish the extractions.

- Remove the tubes from the "rotisserie" and place back in the 96 tube rack.
- Get a small erlenmeyer flask from the glassware cabinet (you will place the "dirty" metal extraction beads in here).
- Now follow the instructions in the Qiagen kit.
- Add the Buffer AL to each tube as per the instructions and vortex. Then add the 100% ethanol as instructed and vortex. After you pipette off this mixture (using a barrier/filtered tip) and add to the Qiagen filter containing extraction tubes, dump the "dirty" tungsten carbide bead in a small erlenmeyer flask (you will clean these when you are finished the DNA extractions).

Notes about the Qiagen extraction kit.

- Always spin your tubes in the centrifuge with the hinges angled down. You must always put your hinges in the same orientation in the centrifuge to insure maximm DNA recovery.
- On the last step, only add 200uL Buffer AE once. Do not do this step a second time as suggested in the instructions.

- Also once you have added the 200uL Buffer AE, wait 10 minutes before spinning in the centrifuge for the final step. This insures that maximum DNA is removed from the column.
- When spinning in centrifuge for final spin with collection barrier tubes placed within labeled final tubes, only put a tube in every other centrifuge hole and makes sure the caps of the final tubes are not overlaid or they will snap off. If this occurs make sure you know which caps belong to which samples BEFORE taking any of the tubes out of the centrifuge.

Cleaning the Qiagen tungsten carbide beads.

- UV the forceps and a container for 15 or more minutes to hold the metal extraction beads in the last steps (the plastic lid of a 96 well plate will work or the lid of an empty tip box).
- After placing all the "dirty" metal extraction beads in the small erlenmeyer flask, add some distilled water and swish around. Get an empty beaker to pour into. As you will want to pour off the water and not lose any of the metal beads, pour this into the beaker so that any that fall out land in the beaker and not down the sink.
- Once you have poured off as much water as possible without pouring the metal beads out, get three Kim Wipes and fold in half and place over mouth of erlenmeyer flask. Then dump over so the KimWipes absorb the excess water (repeat if necessary, but beads do not have to be completely dry).
- Next add 0.4M HCL to the erlenmeyer flask and "dirty" metal beads filling about ¼ full.
- Swish around for about 1-2 minutes to clean metal extraction beads (some may appear tarnished, but this is fine).
- You must pour this 0.4M HCL into the properly labeled waste jar in the fume hood. Again be careful to not lose the beads (you can leave a little of the HCL in the erlenmeyer flask if you are worried you will lose the beads).
- Now add a little distilled water and again swirl around to wash the beads. Carefully pour off water making sure to not lose the metal beads in sink. Again use three folded KimWipes over the mouth of the erlenmeyer flask to absorb last of the water.
- Now line the UVed holder with several KimWipes. Dump the beads in and rub them with another clean KimWipe.
- Now put container and metal extraction beads under the UV light for 15 minutes. Every 5 minutes or so roll the beads around to expose all sides to the light.
- Once done, using forceps place beads in a new, clean 1.5mL tube. Only put 24 metal beads in each tube.

Special notes:

- If you ever are worked that you have touched something that can possibly contaminate your samples, always throw away your gloves and get new ones.
- ALWAYS use barrier/filter tips for DNA extractions (remember always use barrier/filter tips for any lab work up to PCR).

- It is best to do all DNA extractions in clear (non-colored) tubes. This allows for the visualization of contaminants in your tube such as pigments from the ant specimens or other items.
- If you are worried you may have ruined or contaminated an individual tube or sample, make a note of it on the worksheet. This will help narrow down potential problems later.
- Many of the Qiagen buffers have very similar initials/names, so be extra careful you are using the correct buffer in the correct order.
- **Remember there is no such thing as being too careful in the lab.**