Molecular techniques: Extracting DNA from dried dots, PCR and sequencing

Every molecular laboratory has its preferred techniques. Here are the methods that have worked very well for us over the years, but we are always tinkering with protocols.

1. Extracting DNA from filter paper disks. A piece of a dried blood dot is cut from a disk with a razor blade (single sharp edge). Each blade is used twice, once with each corner, then discarded. The size of the piece depends on the density of the drop that had been made, but is typically 3-4 mm square. The piece is then put into a 1.5 mL centrifuge tube, and DNA extracted using the DNeasy kit produced by Qiagen (the 250 extraction kit is #69506). We have tried similar kits offered by other sources, but found the DNeasy kit works the best. This kit uses the standard membrane technology and so is almost fool-proof. We have also tried using various salt extractions, but again we get better results from the DNeasy kit. We avoid phenol-chloroform methods because of the toxicity of the chemicals used. The kit protocol is followed except 50 µL of elution buffer or water is used at the end. If water is used to elute the DNA from the membrane, care is taken that the water is about pH 8. We use Sigma water (# W3500 in the 100 mL size bottle) rather than the FAR more expensive "PCR/molecular grade" water sold, and have never had any problems with the quality of DNA or with PCR results.

2. Extracting DNA from vectors. For several projects we have extracted parasite DNA from vectors. Vectors are preserved in 100% ethanol and kept at –20 C. A single vector is removed, placed into a 1.5 mL vial with its top left open and allowed to air dry for several minutes. The lysis buffer from the DNeasy kit is placed into the vial and the vector ground using a plastic pestle from Sigma. The pestle is discarded after a single use. We originally sought to reuse the pestles by washing them, autoclaving, then exposing them to a UV light source 15 cm away. However, despite all of these efforts, the plastic retained DNA from the earlier sample and contamination presented a problem. This taught the lesson: DNA is amazingly tough unless a laboratory wishes to retrieve old DNA, and then the molecule become very fragile. That is, DNA is tough only when you don't want it to be.

3. PCR. The PuReTaq Ready-to-Go beads produced by Amersham Biosciences (#27-9558-01) are standard in our lab. These are expensive, and the price continues to rise rapidly. However, after trying many other kits using various kinds of polymerase, we again return to the beads. The beads always give the best result with a very low failure rate. We generally use a 25 μL reaction volume and 0.5 mL PCR vials provided in with the beads. However, if there is a high throughput project, we may switch to a smaller reaction volume and 96 well plates. No special PCR Helper is added to the reaction despite using a variety of primers for various genes and for DNA templates from a variety of lizard species and well over 150 bird species.

4. Primers. A description of primers used for various purposes is provided in the Research Results portion of the website and will be updated as we design new primers for specific parasite genera and species.

5. Sequencing. We use the ABI BigDye chemistry and ABI Prism machines for sequencing.

6. Microsatellite genotyping. We use the ABI labeled primers. They are expensive, but the quality is very high and about 1000 samples can be genotyped for $85 worth of labeled primers. Thus, we have not gone to third party sources for the primers. Genotyping is done on the ABI Prism instrument and analyzed with the GeneMapper 3.7 software.