

Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement

This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (<https://scholarworks.iu.edu/dspace/handle/2022/7911>).

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DNA Extraction Protocol for Junco Blood Samples

Compiled by Danielle Whittaker, October 10, 2006

You will need:

- Pipets & pipet tips
- 1.5 ml Eppendorf tubes: for each sample, you will need 6 tubes. Label the first five on the top with the sample number, and the sixth tube will need to be labeled with sample number, "DNA", and the date of the extraction. On the sixth tube, write the information on the side as well as the top.
- Proteinase K (in freezer)
- Phenol-chloroform (in deli fridge)
- Chloroform (in deli fridge)
- 100% and 70% ethanol (in freezer)
- 3M Sodium Acetate
- 1X TE Buffer

Day 1: Digestion

1. Prepare the samples. In our case, that means taking approximately half of the original sample (around 200 μ l) and putting it in a new 1.5 ml Eppendorf tube. The blood samples are stored in Longmyer's solution, which is a lysis buffer. This means that the red blood cells are burst open (lysed) and their contents are released outside of the cell membrane. The end result is that the samples are gooey and sticky and difficult to pipet. What I do is first cut the tip off a 1000 μ l pipet tip, and then use it with a 1000 μ l pipet to maneuver about half of the blob into the new tube. I use the snap-top to "cut" the blob in half.
2. Add 1000 μ l Longmyer's solution (lysis buffer) to the sample (in the new tube!).
3. Add 6 μ l Proteinase K to the sample (in the new tube!) and vortex thoroughly.
4. Digest overnight at 55°C in a heating block or water bath.

Day 2: Phenol-Chloroform Extraction

First, prepare an aliquot of the digestion.

1. Transfer 200 microliters from each sample to the new tubes.
2. Store the remainder of the digestions in the old tubes in the refrigerator. You can always extract more DNA from the remainder later if you need to.

You will need to do most of this work in the fume hood.

1. Add 200 μ l phenol-chloroform to each sample.
2. Vortex briefly until an emulsion is formed.
3. Centrifuge for 5 minutes at 12000 rpm.
4. The sample will have separated into an organic phase (at the bottom), an aqueous phase (at the top), and the proteins at the interface (the middle). We want the aqueous phase, which has the DNA in it. Pipet off the top layer and put it in a new tube.

5. During this first step, you will probably have some difficulty, as there will be a huge gooeey blob of protein in the middle. Sometimes you can pull this out and put it in the waste container, and then proceed to pipet off the aqueous layer.
6. Pour out the organic phase (which is the phenol) into the phenol waste bottle. Discard tip and old tube.
7. Repeat steps 1-4 two more times, for a total of 3 phenol-chloroform steps. For these later steps, however, I usually use 100 μ l of phenol-chloroform because the aqueous layer is often small.
8. Now repeat with chloroform to remove the rest of the phenol: Add 100 μ l chloroform, centrifuge 5 minutes, and pipet off top layer into new tube.
9. Precipitating the DNA: Add 10 μ l 3M sodium acetate and 250 μ l 100% ethanol (cold ethanol from freezer!). Shake gently; you should see the DNA coming together as a small white blob.
10. Put in -20°C freezer for at least 1 hour.
11. Spin at 12000 rpm for 30 minutes.
12. DNA pellet will be on bottom of tube. Pipet off supernatant (it's ok if you don't get it all).
13. Add 200 μ l cold 70% ethanol. Spin 5 minutes. Pipet off supernatant.
14. Repeat 13.
15. Now, let the pellet dry for 1 hour to overnight, depending on how much ethanol you left in the tube. You want the pellet to be completely dry.

Day 3: Reconstituting the DNA.

1. Add 200 μ l 1X TE Buffer.
2. Mix gently by shaking.
3. Let sit at room temperature for one week.
4. Store in refrigerator.