

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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Day 1 – Sample Set Up and Protein Digestion (1-1.5 hours)

1. ☐ Unwrap samples, record Blood # and Band # in notebook.
2. ☐ Add 100-200 μL of cell lysis buffer to bring samples up to 600-700 μL , stir samples with pipette tip to break up clumps; vortex.
3. ☐ Transfer ~ 300 μL of each sample into clean tube labeled with assay # (1-24).

Recipe for ProK solution: In bottle, mix ☐ 50 mg dehydrated powder, ☐ 1250 μL glycerol, ☐ 50 μL 1M Tris HCl, ☐ 1200 μL dH₂O. Vortex.

4. ☐ Turn on the H₂O bath or hot block. Add 20 μL of Pro-K to samples, using tip to break up clumps, stir/vortex. (Keep ProK on ice while working.)
5. ☐ Incubate at 55°C in hot block or water bath for at least 2 hours (preferably overnight).

Day 2 – Phenol Extraction (2-3 hours)

1. ☐ Take the phenol:chloroform out of the fridge; allow to come to near-room temperature (10-15 min).
2. ☐ Add 200 μL of ddH₂O to bring digested samples back up to 500 μL . Stir or vortex briefly.
3. ☐ ☐ ☐ Add 500 μL of phenol:chloroform (pH 7.8-8.0) to DNA sample.
4. ☐ ☐ ☐ Shake for approximately 2 minutes until an emulsion forms (or vortex $\sim 30\text{s}$ each.)
5. ☐ ☐ ☐ Centrifuge at 12,000g at room temperature for 8 minutes. Label fresh tubes with assay #.
6. ☐ ☐ ☐ Use a disposable transfer pipette to transfer the upper (water) layer to a fresh tube, being cautious not to transfer any of the proteins (found between the layers) or other waste materials (found in the bottom phenol layer).

Repeat steps 3-6 until no protein is visible at the interface of the water and phenol layers (1-2 repetitions is typical – 2-3 extractions total).

7. ☐ Add 500 μL of chloroform to the sample.
8. ☐ Shake the samples for 1 minute and centrifuge for 6 minutes. Label clean tubes with the original sample number.
9. ☐ Remove the upper layer and place in the clean labeled tube.

Day 2 con't – Ethanol precipitation

1. ☐ Add 50 μL of 3M sodium acetate and 1000 μL of ice cold 100% EtOH. Put in freezer for at least 2 hours, preferably overnight.

Day 3 – Ethanol Pour-offs (total 1.5 hours, but < 1 hr. of active time; a lot of time waiting for long spins to finish)

2. ☐ Spin 25 minutes at room temperature. Carefully pour off EtOH.
3. ☐ ☐ Rinse pellet in 500 μ L 70% EtOH (make sure to get pellet off the bottom of the tube) and centrifuge 10 minutes.
4. ☐ ☐ Pour off EtOH.

Repeat 70% EtOH wash 2x.

5. ☐ Cover rack loosely with plastic wrap, leaving tubes open. Let dry for a few hours or overnight.

Day 4 – Rehydration (<10 min.)

1. ☐ Re-hydrate the samples the following morning with 200 μ l of 1 x TE.
2. Allow to rehydrate for ~2 weeks