Indiana University Bloomington
IUScholarWorks

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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For additional information, visit the Ketterson/Nolan Lab community on the IUScholarWorks repository

MICROSATELLITE PCR AND GENOTYPING INSTRUCTIONS
Prepared by Danielle Whittaker & Nicole Gerlach, 2006-2012

Set-up / Primer Preparation
Primers should be mixed up to 100mM stock solutions.

Multiplex Primer Mix: 20 µL ea of F and R primer stock solutions plus enough ddH_2O to bring up to 1 mL (so for a mix with 3 loci: 6 x 20 µL = 120 total µL of primers and 880 µL H_2O.) Aliquot into 9 x 111 µL Eppendorf tubes. Keep frozen and away from light until use.

PCR (takes ~1 hr. to set up; ~4.5 hours in the machine)

1. Sign up for run on 3730 using web-based program. (http://www.bio.indiana.edu/~mobrien/, Acct #_____, 1 hr. per plate)

NOTE: If pipetting DNA from individual tubes rather than a storage plate, do step 5 first (make sure to get the liquid at the very bottom of the plate wells), cover plate, then do steps 2-4 (pipette PCR cocktail against well walls to avoid cross-contamination).

2. Thaw aliquot(s) of primer mix (keep away from light) and the multiplex master mix completely.
3. Prepare PCR cocktail, shake gently. Spin down (pulse) in centrifuge to get liquid from lid. Aliquot 86 µL to each well of a 12-tube strip for multi-channel.
    a. Multiplex: 550 µL Master Mix, 110 µL primer mix, 385 µL RNAse-free H_2O), shake gently. (If you are not doing a full plate, the mix is 5 µL Master Mix, 1 µL primer mix, and 3.5 µL H_2O per well).
    b. Sexing: 200 µL Flexibuffer, 80 µL $MgCl_2$, 100 µL dNTPs, 150 µL 1:10 soln P2 primer, 150 µL 1:10 soln P8 primer, 250 µL RNAse-free H_2O, 20 µL Taq.
4. Add 9.5 µL cocktail mixture to each well of multiple-well flexible PCR plate. Tap on counter to get liquid to bottom.
5. Add 0.5-0.75 µL DNA to wells using multi-channel pipette.
6. Put sticker sealer on plate and make sure all wells are sealed.
7. Run in PCR machine. After run, leave in machine or cover with foil and put in fridge.

Multiplex Protocol:
LID = 105˚ No Wait
1 – T=95˚ 0:15:00
2 – T=94˚ 0:00:30
3 – T=57˚ 0:01:30
4 – T=72˚ 0:01:30
5 – Go to 2, 40x
6 – T=60˚ 0:30:00
7 – Hold 4.0˚

Sexing Protocol:
LID = 105˚ No Wait
1 – T=94˚ 0:01:30
2 – T=48˚ 0:00:45
3 – T=72˚ 0:00:45
4 – T=94˚ 0:00:30
5 – Go to 2, 30x
6 – T=48˚ 0:01:00
7 – T=72˚ 0:05:00
8 – Hold 4.0˚

Dilutions and setting up for 3730 (takes ~1 hr.)

1. Add 19.5 µl dH_20 to each well in a new plate (can use multi-channel and trough).
2. Make ladder mix (990 µl dH_2O plus 10 µl GS500 LIZ-labeled ladder). Aliquot 82 µL to each well of strip for multi-channel. Add 10 µl of mix to each well of walled plate (for running on 3730).
3. Remove PCR plate from machine (or fridge if it's there) and use multi-channel pipette to add 0.5 µl of PCRed DNA to the plate with the dH_2O. Pipette up and down a few times to mix and then transfer 0.5 µl of dilution to plate with ladder.
4. Seal walled plate with foil seal, put in PCR machine; run DENATURE program. (LID = 105˚ Wait, 95˚ for 0:05:00)
5. Seal dilutions plate with sticker seal and cover both that plate and original PCR plate with foil and put in -20˚C freezer.
6. Go get ice.
7. When program is finished (~5 mins), remove plate from machine and immediately place on ice (careful, very hot!).
8. Remove foil seal, replace with septa seal and carry over to sequencing facility. Make sure to pick up Septa seal after run.

Genotyping on the 3730

1. At DNA facility, spin plate in Beckman centrifuge briefly (allow it to come up to 2000 rpm, then stop.)
2. Place plate in black base, snap white cover over top (make sure it snaps on!).
3. Open right drawer on 3730, open door on right, and place plate on stack (notch in top right corner) (plates are taken from the bottom first, when the plate is done, it will be moved to the left stack.)
4. On computer, click on Run Scheduler.
    a. In the "Add Plate" box, enter a plate name, click enter, choose "yes".
    b. In the "New Plate Dialogue" window:
        i. Change application to "Genemapper generic"
        ii. Choose "septa"
        iii. Enter your name for "Owner" and "Operator"
5. In Plate Editor:
    a. In Sample Name, can enter the name of your plate, then fill down
    b. In Results Group, enter CISAB_LAB (fill down)
    c. In Instrument Protocol, enter Fragment Analysis or Genemapper Generic (fill down)
6. Click green arrow on top left of screen to initiate run
7. When run is complete:
    a. Retrieve plate from drawer, or from stack of used plates. Rinse septa seal in ddH_2O and place in drawer to dry. Discard used plate.
    b. Data can be downloaded from the IMBI server.

MICROSATELLITE PRIMER MIXES

Mix 1:
880 µL ddH_2O
20 µL Dpu01 F (green)
20 µL Dpu01 R
20 µL GF05 F (blue)
20 µL GF05 R
20 µL Cumu28 F (red)
20 µL Cumu28 R

Mix 2:
880 µL ddH_2O
20 µL Gf01b F (green)
20 µL Gf01b R
20 µL Dpu16 F (blue)
20 µL Dpu16 R
20 µL GF14 F (red)
20 µL GF14 R

Mix D:
840 µL ddH_2O
20 µL C08 F (green)
20 µL C08 R
20 µL A03 F (blue)
20 µL A03 R
20 µL G08 F (blue)
20 µL G08 R
20 µL Ju05 F (red)
20 µL Ju05 R

Mix E: (don't use, too much overlap between GF05 and Gf01b)
840 µL ddH_2O
20 µL Dpu01 F (green)
20 µL Dpu01 R
20 µL GF05 F (blue)
20 µL GF05 R
20 µL Gf01b F (green)
20 µL Gf01b R
20 µL Dpu16 F (blue)
20 µL Dpu16 R