1 Sex determination of Eastern White-crowned Sparrows (*Zonotrichia leucophrys leucophrys*) using
2 wing chord length
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Extracting DNA from Avian Blood

1. When ready to extract DNA: label an autoclaved 1.5 mL tube for each sample to be extracted. Set aside 24 tubes for each set of extractions (23 individuals, and 1 blank, to ensure you do not contaminate your samples along the way). Add all the chemicals as you would to real samples to the blank (except the blood).

2. Add a small amount (approx. 100 – 200 µL) of the blood/lysis buffer sample (making sure to take a small amount of the aggregate or clot of blood, as that is where the DNA is concentrated) to 400 µL of Cell Lysis Buffer and 3 µL of 20 mg/mL of Proteinase K (stored at -20 $^{o}$C). If you hold the pipette firmly and support it with your other hand, you should be able to add the lysis buffer with one tip without touching the blood, thus using less tips. If you cannot do this, and you do expose the tip to the blood, then use a new pipette tip each time. Use new tips for the Proteinase K in each sample.

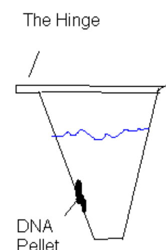
3. Flick each tube and vortex briefly to mix, then spin them down very briefly in the centrifuge (hold “short” on the centrifuge, until it reaches 10 000 RPM, then release the button).

4. Incubate at 60 $^{o}$C for 2 h. Invert the tubes a little every 15 min or so, to speed along the digestion, or use the shaking heat block to do the same thing (set to 300 – 400 rpm). Alternatively, samples can be incubated overnight to ensure full digestion.

5. Once the digestion is finished, spin down the tubes briefly to get the condensed liquid to the bottom. After, add 200 µL of 7.5 M ammonium acetate solution to precipitate the proteins out of the samples. Vortex each sample for 15 sec until well mixed. Centrifuge on max for 7 min. The proteins (reddish-brown) should be collected at the bottom. Carefully pour off the top aqueous phase into a new-labelled tube. Place the old tubes with the blots and proteins into the biohazard waste.

6. Add 900 µL of room temperature 100% isopropanol (2-proponal) and quickly invert the samples a few times. This should bring the DNA out of solution since it is not soluble in alcohol. It will have a white stringy appearance. The more DNA present, the larger the blob. If the blood sample was small or very dilute, you may not see anything, and that is fine.

7. Spin the tubes down on max in the centrifuge for 7 min. Orient the hinge of the tube is oriented at the top when placed into the centrifuge so DNA will pellet on the side where the hinge is.




8. Decant the solution into a beaker by pouring the liquid out slowly so you do not disturb the pellet. Keep an eye on the pellet as you pour to ensure it is still there. Blot the mouth of each tube on a clean piece of paper towel, then invert the samples and let dry for 30 sec.

9. Add 200 µL ice-cold 70% ethanol to each sample. Rock the samples side to side so the ethanol washes the inside of the vial—this will help dissolve any of the remaining ammonium acetate. Try not to let the pellet dislodge.

10. Centrifuge the tubes on max for 10 min.

11. Decant the 70% ethanol, the same way as the isopropanol. Blot the mouth of each tube on a clean piece of paper towel, then invert the samples and let dry for approx. 20 – 30 min.

12. Add 100 – 200 µL 1X TE to each tube. Adjust volume added based on the size of the pellet.

13. Flick the tubes vigorously to dissolve the pellets. Tap the tubes down to get most of the liquid down. Ensure pellets are fully dislodged from the side of the tube and fully dissolved in the TE.

14. Congratulations, your DNA is extracted! You can now Nanodrop the stock solution and calculate what dilution is needed to make a 20 ng/ µL working solution.

Notes

Protocol modified from: Elizabeth A. MacDougall-Shackleton Lab, Western University, London, ON, Canada

Protocol originally modified from: Miller S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16(3), 1215. https://doi.org/10.1093/nar/16.3.1215