

Mole®

DNA extraction from bird blood stored in EtOH

“ GeneMole® is a benchtop instrument for automated nucleic acid purification. Automation reduces the risk of injuries caused by repetitive pipetting, limits exposure to chemicals and infectious agents, and gives you more time to focus on other operations. GeneMole® can process 1-16 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™. ”



Figure 1:
Little egret
(*Egretta garzetta*)

Introduction

Bird blood is nucleated and contains a lot of DNA when compared to un-nucleated mammalian blood. This allows us to obtain sufficiently high quality and quantity of bird DNA from even small blood samples. Reliable field methods for the storage of samples to be used for DNA extraction and amplification are critical to many studies employing molecular techniques. Storage of bird blood in ethanol is one of the classic ways of sample preservation. Such samples can be stored at room temperature for a long time. Here we have used the GeneMole® instrument to extract DNA from blood samples collected on ethanol. The blood samples are from 35 different bird species.

Methods

Blood collected in ethanol coagulated as pellet. A proportion of each pellet was removed from the ethanol and placed in an eppendorf tube. After the ethanol had evaporated, 100 or 200 µl PBS was added and the pellet was ground with a micropestle. DNA extraction was performed on the GeneMole® instrument using the MoleStrips™ DNA Blood kit. Sample and elution volumes were set to either 100 or 200 µl. No proteinase treatment was used.

Results

There are some variations in yields due to differences in blood pellet size and bird species. Generally, small pellets gave lower yield and ratio than bigger pellets. Also some pellets were harder to dissolve than others.

Table 1: Yield and OD values obtained for DNA extracted from bird blood in ethanol. The input volumes and the elution volumes were specified as 200 µl for all samples except for the Trumpeter finches and the Pied flycatchers, where the input and elution volumes were specified as 100 µl.

| N | Common name | Scientific name | 260/ 280 | DNA Yield [µg] |
|---|---------------------------|----------------------------------|-------------|----------------------|
| 1 | Levant sparrowhawk | <i>Accipiter brevipes</i> | 1.76 | 2.90 |
| 1 | Tawny pipit | <i>Anthus campestris</i> | 1.65 | 1.62 |
| 1 | Richards pipit | <i>Anthus richardi</i> | 1.66 | 2.10 |
| 2 | Water pipit | <i>Anthus spinoletta</i> | 1.64 | 1.83 |
| 1 | Squacco heron | <i>Ardeola ralloides</i> | 1.55 | 1.20 |
| 2 | Trumpeter finch | <i>Bucanetes githagineus</i> | 1.83 | 3.95 |
| 1 | Greater short-toed lark | <i>Calandrella brachydactyla</i> | 1.50 | 1.20 |
| 1 | Rufous-tailed scrub robin | <i>Cercotrichas galactotes</i> | 1.75 | 3.13 |

Table 1 continued

| N | Common name | Scientific name | 260/ 280 | DNA Yield [µg] |
|---|---------------------------|----------------------------------|-------------|----------------------|
| 1 | House crow | <i>Corvus splendens</i> | 1.68 | 3.52 |
| 1 | Little egret | <i>Egretta garzetta</i> | 1.46 | 0.83 |
| 1 | Common moorhen | <i>Gallinula chloropus</i> | 1.48 | 1.11 |
| 2 | White throated kingfisher | <i>Halcyon smyrnensis</i> | 1.66 | 1.38 |
| 1 | Booted eagle | <i>Aquila pennata</i> | 1.48 | 1.40 |
| 1 | Olivaceous warbler | <i>Hippolais pallida</i> | 1.42 | 1.02 |
| 2 | Mashed shrike | <i>Lanius nubicus</i> | 1.84 | 5.40 |
| 1 | Broad-billed sandpiper | <i>Limicola falcinellus</i> | 1.75 | 3.91 |
| 1 | White-throated munia | <i>Lonchura malabarica</i> | 1.60 | 1.70 |
| 1 | Little green bee-eater | <i>Merops orientalis</i> | 1.51 | 1.45 |
| 1 | Palestine sunbird | <i>Nectarinia osea</i> | 1.57 | 2.20 |
| 2 | Namaqua dove | <i>Oena capensis</i> | 1.66 | 2.90 |
| 1 | Hooded wheatear | <i>Oenanthe monacha</i> | 1.79 | 6.50 |
| 1 | Spanish sparrow | <i>Passer hispaniolensis</i> | 1.72 | 2.11 |
| 1 | Common redstart | <i>Phoenicurus phoenicuru</i> | 1.76 | 2.92 |
| 1 | Little crane | <i>Porzana parva</i> | 1.68 | 2.05 |
| 1 | Graceful prinia | <i>Prinia gracilis</i> | 1.81 | 4.19 |
| 2 | White-spectacled balbul | <i>Pycnonotus xanthopygos</i> | 1.86 | 7.69 |
| 2 | European stonechat | <i>Saxicola torquatus</i> | 1.69 | 1.64 |
| 2 | Eurasian collared dove | <i>Streptopelia decaocto</i> | 1.70 | 3.20 |
| 2 | Laughing dove | <i>Streptopelia senegalensis</i> | 1.83 | 8.50 |
| 1 | Sardinian warbler | <i>Sylvia melanocephala</i> | 1.55 | 1.10 |
| 1 | Desert lark | <i>Ammomanes deserti</i> | 1.54 | 1.00 |
| 1 | Southern grey shrike | <i>Lanius meridionalis</i> | 1.75 | 4.21 |
| 1 | Mourning wheatear | <i>Oenanthe lugens</i> | 1.53 | 1.10 |
| 1 | Common scops-owl | <i>Otus scops</i> | 1.46 | 1.08 |
| 7 | Pied flycatcher | <i>Ficedula hypoleuca</i> | 1.4 | 3.00 |

DNA extracted from a family of pied flycatchers (parents and 5 nestlings) were amplified with the primers Fhu3F and Fhu3R (Primmer *et al* 1996) that is part of a panel of microsatellite markers used for paternity testing. They were genotyped on an ABI377. Figure 2 shows the screening of the Fhu3 microsatellite in the family of flycatchers.

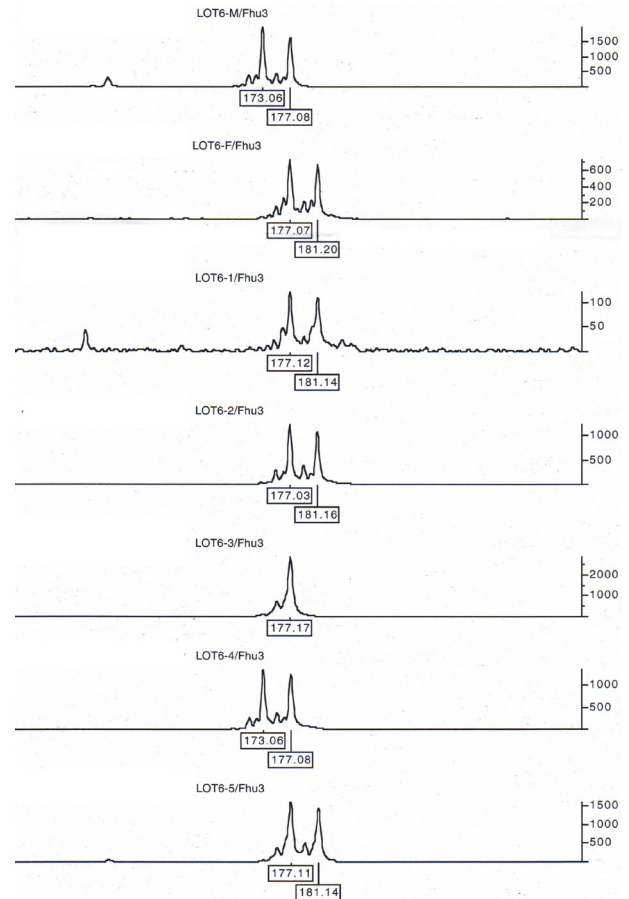


Figure 2: Screening of the Fhu3 microsatellite in a family of Pied flycatchers. Top trace: The expected father; second trace: the mother; lower traces: the 5 nestlings. In this case all the nestlings share alleles with their expected father.

Conclusion

The GeneMole® instrument strongly reduces the hands-on time needed to extract DNA from blood. To reduce the hands-on time even more please see our application note on bird blood stored in buffer.

Acknowledgement

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“Simplify When Possible”

Ordering Information

| Product | Content | Product Number |
|--|-------------------|----------------|
| GeneMole® | Instrument | MG10-000-000 |
| MoleStrips™ DNA Blood | 64 Reagent Strips | MGK20-100-102 |
| MoleStrips™ DNA Blood Convenience Kit ¹ | 32 Reagent Strips | MGK20-100-101 |

¹ The convenience kit contains 32 Reagent Strips and all the required tips, tubes and caps.