Wildlife species identification from field samples by DNA analysis

GENERAL CLIENT GUIDANCE

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1 INTRODUCTION

This document gives a summary of the DNA analysis service provided by Swift Ecology Ltd (SEL) and the Waterford Institute of Technology (WIT). For further details please see our FAQs.

2 SAMPLE TYPES

Species may be identified from field samples including faecal samples, fur samples, tissue samples (e.g. wing punches), hair samples and owl pellets. Other samples might be tested by special arrangement, (e.g. eggshell, feather or bite wound analysis – please consult us first).

3 TESTING PROCESS

We are the only lab currently offering targeted qPCR analysis for individual bat species.

For bats, the process is as follows:

A. Where the suspected species is one or more of a pipistrelle, long-eared or *Myotis* species, or no suspected species has been given:

1. Assuming >5 faecal pellets are present in the sample, a proportion of these will first be tested for common or soprano pipistrelle, brown long-eared, or *Myotis* genus bats. If either common or soprano pipistrelle or BLE is present, a result is provided and no further testing is done. If *Myotis* is present, a further test is run to identify the particular species.

2. If this test fails to identify a species, a further test will be run for all other bat species on the same sample.

3. If this test fails another extract is taken from the original sample and the above process repeated.

4. If this test fails then sequencing is carried out.

B. Where the suspected species is a horseshoe, *Nyctalus* species, barbastelle or serotine, this will be tested for first; other tests will follow if this fails.

C. For samples identified as mixed/multiple species, testing will not stop once one species has been identified, but will continue until all species have been tested for. NB There are additional charges for mixed samples.

Any additional tests over and above that described above will be chargeable, but this will be agreed before proceeding.

The general DNA analysis protocol (all species) is as follows.

A. DNA is extracted from ~0.2g faecal material or 1-5 faecal pellets using a standard protocol as described in Croose *et al* (2016).

B. Where available species identification is carried out using species specific real-time (qPCR) assays. These assays target a variety of mitochondrial genes/regions such as cytB, nd1, d-loop and COI. At the moment qPCR assays are available for:

- All UK and Ireland bat species
- All UK and Ireland small mammal species
- European Hedgehog
- Pine marten
- Fox
- Fallow, Red and Sika deer
- Red and grey squirrel
- European otter

Other species may be tested by sequencing. For a full list of species that can be identified by either qPCR or sequencing, see Appendix 1.

Further information on the use of qPCR in species identification can be found in Moran *et al* (2008).

C. If a qPCR assay is not available for the species or the sample has failed the qPCR assays standard PCR reactions are carried out to obtain a product for DNA sequencing. Depending on the expected species the primers used will target a 300-500 bp product from the mitochondrial genome (cytB, nd1, COI,and 16s genes and d-loop region). The sequence obtained is then compared to sequences in the GenBank® DNA database using **BLAST** (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

References

Croose, E., Birks, J.D., O'Reilly, C., Turner,P., Martin, J. and MacLeod, E.T. (2016) Sample diversity adds value to non-invasive genetic assessment of a pine marten (*Martes martes*) population in Galloway Forest, Southwest Scotland. Mammal Research 61, 131-139

Moran, S, Turner, P., and O'Reilly, C. (2008) Non-invasive genetic identification of small mammal species using real-time PCR. Molecular Ecology Resources 8, 1267–1269.

4 FAILED TESTS

The lab's success rate is 95% and failures are rare. It is usually not possible to know exactly why a particular sample has failed, but the most likely reasons are:

- The sample was not from the target species group, and therefore could not be identified (although the lab will always attempt to identify species to other groups if possible);

- There was insufficient or inadequate quality DNA in the sample to allow amplification. The latter may be caused by:
    - Moisture content of the sample;
    - DNA having been lost or degraded from the sample through exposure; or
    - The sample having become broken up in transit so that the mucus covering has been destroyed.

- Cross contamination prior to submission by handling or contact with other samples may result in failure of sequencing tests.

5 TURNAROUND TIMES

- Normal turnaround 2 weeks (10 working days[1]) from receipt at the lab[2] to identification
- Express* turnaround (when available) 3 working days from receipt at the lab to identification (extra charge)

The express service is normally available; however, there may be occasions, such as at particularly busy periods or over holidays, when it is not possible to run this service. *Please check current availability on our website*

6 COSTS AND PAYMENT

Costs may be reviewed at any time. At the time of writing costs will be:

£50 + VAT per sample

The additional cost for the express service (when available) will be:

£20 + VAT per sample

The charge for the express service will not be made if the deadline is not met (unless any delay was the result of sample failure).

[1] Please note bank holidays in Ireland do not necessarily correspond to UK bank holidays

[2] Please take account of carriage times to Ireland. If sent by UPS most samples arrive within 2-3 working days but some may take longer.

The additional cost for mixed species samples will be:

£10 + VAT per sample

Discounted rates for large numbers of samples or non-profit work can be negotiated.

Payment is required in advance.

Please see our FAQs. for further information.

7 SAMPLE SUBMISSION AND CARRIAGE

Download a sample submission form from our website and follow the instructions provided. You will be provided with sample numbers, import licence, shipping label and VAT receipt.

We have an account with UPS for sample carriage. At the time of writing the cost for this service is £16.50 plus VAT for a package weighing up to 1 kg (this can contain any number of samples up to the weight limit given, within certain size constraints). Please note that sample carriage must comply with carriage and packing instructions provided. For further information please see our FAQs.

Samples must not be sent to the lab using Royal Mail, due to post office restrictions on carriage of biological samples. Do not send samples direct to SEL.

8 REPORTING

Test results will be added to the sample submission form *Analytical Report* sheet at the lab and returned to SEL who will forward to the client. The report will contain the analytical method, the sequence analysed, the species match and any comments.

9 TERMS AND CONDITIONS

By using the service, you agree to our terms and conditions as below.

1 Costs and procedures will be regularly reviewed and may be revised at any time.

2 We take great care to ensure the reliability of the DNA analysis but there is an element of uncertainty in any biological analysis. Undue weight should not be given to single analyses and if the data are to be the basis of important decisions then replicate samples should be tested. No responsibility can be taken for the subsequent use or misuse of data.

3 The cost charged represents the labour and materials required to undertake an analysis, and is incurred whether or not that analysis produces a result. No refunds are given for failed tests where the failure was due to circumstances outside the lab's control.

4 In the case of a failed initial test, further tests will be carried out as detailed in Section 3 of the General Client Guidance or FAQs “What tests do you use”. Any additional tests over and above this will be chargeable, but this will be agreed before proceeding.

5 Payment is required in advance. Shipping labels and sample numbers will not be provided until payment has been received.

6 We accept no responsibility for delays caused by equipment breakdown, industrial action, postal disruption or any other factors outside our control. In the unlikely event of such delays, we will inform the client as soon as possible and will make every effort to process samples at the first opportunity.

7 We accept no responsibility for any action arising from the continued use of Royal Mail to send samples or from failure to abide by restrictions imposed by the courier or by Irish Customs.

8 In the unlikely event of loss in transit the carriage cost will be refunded by the courier. However no other costs resulting from this can be reimbursed.

APPENDIX 1

Species can be identified as below. If the species you require is not listed (e.g. non-UK bat species), please contact us as we can often identify species other than those listed here.

Group A. Carnivores.

Wildcat*	*Felis sylvestris*
Red fox	*Vulpes vulpes*
Otter	*Lutra lutra*
Pine marten	*Martes martes*
Badger	*Meles meles*
Stoat	*Mustela erminea*
Weasel	*Mustela nivalis*
Polecat*	*Mustela putorius*

Group B. Small mammals.

Water shrew	*Neomys fodiens*
Common shrew	*Sorex araneus*
Pygmy shrew	*Sorex minutus*
French shrew	*Sorex coronatus*
Lesser white toothed shrew	*Crocidura suaveolens*
Greater white toothed shrew	*Crocidura russula*
European water vole	*Arvicola amphibius*
Bank vole	*Myodes glareolus*
Field vole	*Microtus agrestis*
Common vole	*Microtus arvalis*
Yellow-necked mouse	*Apodemus flavicollis*
Wood mouse	*Apodemus sylvaticus*
Harvest mouse	*Micromys minutus*
House mouse	*Mus musculus*
Brown rat	*Rattus norvegicus*
Black rat	*Rattus rattus*
Hazel dormouse	*Muscardinus avellanarius*

Group C. Bats.

Greater horseshoe bat	*Rhinolophus ferrumequinum*
Lesser horseshoe bat	*Rhinolophus hipposideros*
Western barbastelle	*Barbastella barbastellus*
Serotine	*Eptesicus serotinus*
Alcathoe bat	*Myotis alcathoe*
Bechstein's bat	*Myotis bechsteinii*
Brandt's bat	*Myotis brandtii*
Daubenton's bat	*Myotis daubentonii*
Mouse-eared bat	*Myotis myotis*

Whiskered bat	*Myotis mystacinus*
Natterer's bat	*Myotis nattereri*
Leisler's bat (Lesser noctule)	*Nyctalus leisleri*
Noctule bat	*Nyctalus noctula*
Nathusius's pipistrelle	*Pipistrellus nathusii*
Common pipistrelle	*Pipistrellus pipistrellus*
Soprano pipistrelle	*Pipistrellus pygmaeus*
Brown long-eared bat	*Plecotus auritus*
Grey long-eared bat	*Plecotus austriacus*

Group D. Other mammals

Red squirrel	*Sciurus vulgaris*
Grey squirrel	*Sciurus carolinensis*
European hedgehog	*Erinaceus europaeus*
European hare	*Lepus europaeus*
Mountain/Irish hare	*Lepus timidus*
Fallow deer	*Dama dama*
Red deer	*Cervus elaphus*
Sika deer	*Cervus nippon*
Roe deer	*Capreolus capreolus*
Muntjac	*Muntiacus reevesi*
Chinese water deer	*Hydropotes inermis*
Edible dormouse	*Glis glis*
Mole	*Talpa europaea*
Wild boar*	*Sus scrofa*
Beaver	*Castor fiber*

Group E. Owls

Barn owl	*Tyto alba*
Snowy owl	*Bubo scandiacus*
Tawny owl	*Strix aluco*
Little owl	*Athene noctua*
Long-eared owl	*Asio otus*
Short-eared owl	*Asio flammeus*

Mitochondrial DNA sequences from these species may be indistinguishable from those of domestic congeners (e.g. cats, ferrets and pigs).