Ecotype Genetics

swift ecology

DNA SERVICE GUIDANCE

General guidance

FAQs

General guidance

1 INTRODUCTION

This guidance summarises the DNA analysis service provided by Swift Ecology Ltd (SEL) and the Ecotype Genetics Limited laboratory (EG) at Sussex University. Please also see the FAQs (pages 7-11).

2 SAMPLE TYPES

Species may be identified from field samples including faecal samples, fur samples, tissue samples (e.g. wing punches) and hair samples. 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 a pipistrelle, long-eared or *Myotis* species, or no suspected species has been given, and where the sample has not been identified as potentially mixed species:

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 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 potentially containing 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 multiple species 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
- UK and Ireland small mammal species
- Pine marten
- Fox
- Red and grey squirrel
- European otter

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

C. If a qPCR assay isn't available for the species (or the sample has failed the qPCR assays) standard DNA sequencing will be performed. We will target a region from the mitochondrial genome (cytB, nd1, COI, and 16s genes or 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). If you need more information or if you are interested in identification of a species that is not currently on our full list (see below), please contact us and we can work with you to develop a bespoke assay for your species.

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 success rate for the analysis is very high 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) from receipt at the lab[1] to identification
- Express[2] turnaround (when available) 3 working days **from receipt at the lab** to identification (extra charge)

6 COSTS AND PAYMENT

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

- **Standard: £55 + VAT per sample**
- **Express[3] (when available): £75 + VAT per sample**
- **Multiple species (bats/small mammals only) standard: £65 + VAT per sample**
- **Multiple species (bats/small mammals only) express: £85 + VAT per sample**

Discounted rates for large numbers of samples or non-profit work are available. Payment is required in advance.

NOTE: The Express service is NOT available for species where qPCR is not available (i.e. for species not listed in Groups A-D – see pages 5-6 below)

7 SAMPLE SUBMISSION AND POSTAGE

Follow the link on our DNA web page (https://www.swiftecology.co.uk/dna.php) to the online DNA analysis form and enter contact and sample details[4]. As sample details are added the form will automatically update payment details and assign each sample with a code.

On completion the form will prompt for online payment, managed securely by Stripe online payment processing. The form cannot be submitted without payment. You will receive an automatic email with attached pdf document containing your details, detail of the sample(s) you require to be analysed, instructions for posting and VAT receipt.

Please note that you must comply with packing instructions provided. You may post using standard post, special/recorded delivery or your preferred courier. **Please make sure to include a printout of the *Samples Submitted* page(s) in the package.**

Dispatch samples direct to the lab address below (**Do not send samples direct to Swift Ecology)**:
Ecotype Genetics Limited
Sussex Innovation Centre
Science Park Square
Falmer
Brighton
BN1 9SB

[1] Please take account of postage times.
[2] 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.
[3] The charge for the express service will not be made if the deadline is not met unless delay was the result of sample failure.
[4] All fields with an asterisk are mandatory

8 RESULTS

Once DNA analysis is complete you will automatically receive an email and attached pdf report with your results. The report will contain the species identified, analytical method and the qPCR threshold cycle (Ct) value[5] (or primers/% match/bases if traditional sequencing was used).

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 form the basis of important decisions then replicate samples should be tested. No responsibility can be taken by either SEL of EG 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 Guidance. Any additional tests over and above this will be chargeable, but this will be agreed before proceeding.

5 Online payment is required in advance in order for samples to be analysed.

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 failure to abide by instructions in relation to packaging/postage.

8 Loss in transit is not insured. You may wish to take out your own insurance.

10 LIST OF SPECIES

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.

Red fox	*Vulpes vulpes*
Otter	*Lutra lutra*

[5] The Ct value is the PCR cycle at which the sequence-specific fluorescence generated crosses the fluorescence (background) threshold. The Ct value is inversely correlated to the amount of target nucleic acid, and thus lower values indicated higher amounts of target nucleic acid. Ct values are typically between 15-20.

Pine marten	*Martes martes*

Group B. Small mammals.

Water shrew	*Neomys fodiens*
Common shrew	*Sorex araneus*
Pygmy shrew	*Sorex minutus*
Greater white toothed shrew	*Crocidura russula*
European water vole	*Arvicola amphibius*
Bank vole	*Myodes glareolus*
Field vole	*Microtus agrestis*
Yellow-necked mouse	*Apodemus flavicollis*
Wood mouse	*Apodemus sylvaticus*
House mouse	*Mus musculus*
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' 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*

Note: If your favourite species is not present in this list, contact us! We can undertake standard DNA sequencing or for multiple analysis will try our very best to work with you and design a qPCR assay for the species in question. The design of such a test will normally be free of charge but standard turnaround times will not apply and the Express service will not be available for these species.

Frequently Asked Questions

What does it cost and how long does it take?

No. of samples per shipment	Cost PER SAMPLE excl. VAT	Additional costs PER SAMPLE excl. VAT for:	
		Multiple species sample (bats and small mammals[6] only)*	Express service
1-5	£55	£10	£20
6-14	£50		
15+	£40		
Non-commercial samples†			
Flat rate	£35		

*Please read "Can you identify multiple bat species present in one sample"

†Non-commercial is strictly for University research, bat groups, etc. If you have not sent us non-commercial samples before, you must contact us before choosing this option.

Service	Turnaround time
Standard	10 working days from receipt at the lab
Express*	3 working days from receipt at the lab
Mixed species sample	As above (can be standard or express)

*The Express service is occasionally temporarily unavailable. Please check availability before requesting express analysis. **The Express service is NOT available for species not in groups A-D (see pages 5-6).**

Important notes:

1. SEL aim to reply to emails within 6 working hours; however, all enquiries are dealt with directly by our ecologists, so there may be delays if we are out on site. Please contact us again only if you have not received a reply within one working day.
2. You may send both express and standard service samples in one consignment.
3. One package can contain multiple samples.
4. Turnaround time relates to the period elapsed after the sample has arrived at the lab. Shipments may get to the lab the day after they arrive at the University, depending on time of arrival and staffing at reception.
5. The timings given above assume that there is sufficient DNA of adequate quality to allow the tests to work first time. If they do not, and further testing is required, the target dates may not be met.
6. Costs will periodically be reviewed and may be changed at any time.

Are there any discounts for multiple species samples?

Yes. Please see "What does it cost and how long does it take"

Is there a discount for non-commercial tests?

Yes. Please see "What does it cost and how long does it take"

Is there an express service?

Yes. Please see "What does it cost and how long does it take"

[6] Small mammals included in this analysis are those listed under Group B in Section 10 above. If you require other small mammal species to be included, please contact us first.

Can you identify different bat species present in a sample?

Yes, but the testing procedure is different depending on the species likely to be present (see also "What tests do you use").

1. If you think the species present is one or more of common pipistrelle, soprano pipistrelle, brown long-eared or any of the *Myotis* species, this will be picked up as a matter of course by the initial test performed by the lab. In this case there is no need to ask for a multiple species analysis.

2. If you have specified on the form that you think it is a different species to those above i.e. one of *Rhinolophus* sp., barbastelle, Nathusius' pipistrelle, grey long-eared, *Nyctalus* sp. or serotine, the species you have specified will be tested for first. If this test fails, other species will then be tested for.

3. If, however, you think that the sample may contain a mix of species that might include species from both the above groups, or multiple species from the second group, and you want to establish all the species present, then you can ask for a multiple species test. There is an additional charge for this.

See "What does it cost and how long does it take" and also "What tests do you use"

Why do I have to pay in advance?

Online payment is required before samples will be analysed. This ensures there are no delays to the analysis of samples once they arrive at the lab, and the subsequent issuing of results.

I need a VAT receipt/invoice

This will be issued on completion of the online form and associated payment.

What can you test?

- Faecal samples from mammals (droppings, scat)
- Fur (plucked fur yields more DNA than cut material)
- Tissue samples (do not send entire carcasses. See "How should samples be preserved and packaged")
- Swabs (from bite wounds etc.) – **PLEASE CONTACT US BEFORE SENDING SWABS**.

What tests do you use?

We are the only lab currently offering targeted qPCR analysis for individual bat species. For bats, the process is given in Section 3 in the General Guidance above.

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

Can you identify the sex of an animal or individual animals?

DNA tests for species ID use mitochondrial DNA, of which there are thousands of copies in each cell. Generally speaking we cannot identify individual animals, because to do this requires nuclear DNA, of which there are only two copies in each cell. There are limited circumstances in which the sex of an animal can be established e.g. otter/pine marten – please contact us for more information. If you are interested in being

able to distinguish the sex of your favourite species from samples, please don't hesitate to ask us and we may be able to provide a bespoke service.

Why do I have to provide all this information?

Certain information on the online form does not have to be completed e.g. precise locations/grid references/site description, for example if the information is commercially sensitive. All fields with an asterisk are mandatory.

We have designed the form so the amount of data entry is kept to a minimum.

Can I send samples from outside GB?

Yes, but please make sure all courier costs are covered.

What happens if the sample is lost in transit?

The postal service is not perfect and losses may occasionally occur; neither SEL nor EG will accept responsibility for this. You may wish to use a special delivery service or arrange insurance, e.g. to cover costs associated with obtaining a replacement sample. Please see also "How many bat droppings should I send per sample".

How many bat droppings should I send per sample?

Although it is usually possible to obtain a result from a single dropping, we advise that wherever possible you collect at least 5-10 droppings, submit at least 3 for testing and if possible, keep at least a couple back in the unlikely event of loss in transit. Such losses are very rare but if you have kept a few droppings in reserve this avoids the need to make an additional trip to collect more. Sending several droppings means that the lab has more than one dropping to work on, in case the first test fails.

How should samples be preserved?

- **Do not** store samples in alcohol or other preservative; keep in a cool, dry place. You can refrigerate (or freeze if needed for longer term storage), but please see notes about moisture content below.

- If collecting faecal samples from live bats, or any faecal samples that are for some other reason not completely dry, they should be naturally air-dried for a few days before sending – i.e. do not put damp samples straight into containers. Droppings from live bats have often proved difficult to extract DNA from; possibly due to a bacterial reaction due to the moisture content, which then affects the DNA content of the mucus covering of the dropping, which contains the epithelial cells from the digestive tract of the bat.

- **Do not** send entire bodies of animals; snip off a part of the tissue.

How should samples be packaged?

- **Do not** use glass containers.

- The best containers for bat droppings are small (2 ml) plastic tubes e.g. Eppendorf safe-lock tubes https://online-shop.eppendorf.co.uk/UK-en/Laboratory-Consumables-44512/Tubes-44515/Eppendorf-Safe-Lock-Tubes-PF-8863.html.

- Standard urine sample-type plastic tubes (c. 20 ml) are fine for larger scats but not for bat droppings. Small droppings in a large container move around in transit, which may cause them to break up which can mean it is more difficult to get a result. Also if you are sending multiple samples, you cannot get many of the larger tubes into one package and if there are too many tubes in an envelope it is more likely to come open during transit.

- If you use ziplock-type plastic bags, ensure these are as small as possible; otherwise, the droppings move around and break up in transit and also can be difficult to extract intact. If ziplock bags or tubes are not available, seal bags securely with sticky tape.

- Please retain part of the sample if possible (also see "How many bat droppings should I send per sample), in the unlikely event of loss or damage in transit.

- **Samples must be individually marked with their unique sample number.** Without this they will not be processed. The package must be sent within 2 working days of the sample numbers being allocated.

- Place sample bags/tubes together in a leakproof outer bag along with absorbent material e.g. kitchen towel in case of leakage. This should also be securely sealed. If the sample is likely to leak or smell please add an extra sealable plastic bag. This is in order to comply with UK post office packaging requirements for biological samples. Failure to comply could result in confiscation and destruction of the samples.

- A hard copy of the Transaction details (page 1) and Samples submitted (page 2, etc) from the VAT report must be included in the package.

- We strongly advise the use of padded envelopes as these are more robust and less likely to get damaged in transit than standard envelopes. Several packages have arrived empty recently due to the envelope ripping and the contents falling out. Also, seal outer envelopes securely – tape up the flap thoroughly as well as using the sealing strip.

- Affix correct postage. Sample packages are likely to be classed as large letters, and packages with insufficient postage are held by the PO and will not be delivered to the lab.

How quickly will I get results?

It depends on the service level you select. Please read "What does it cost and how long will it take"

How much confidence should I have in the results?

Unlike DNA sequencing which provides a % match, with qPCR the answer is a binary yes or no. **The technique uses species specific primers; therefore, a positive result is only given if DNA from the matching species is present**. Each species-specific primer is tested against all other species to ensure no cross reactivity. Furthermore, during the reaction, each species test is run in duplicate and a result is only given if both replicates give the same result. The Ct value reflects the amount of DNA present in the sample. The lower the value, the lower the number of polymerase chain reaction cycles that were needed to give a positive result. A Ct value threshold is set by the lab for technical reasons to ensure there is sufficient DNA in the sample to give a result. When a positive result is recorded below this threshold we can be >99% certain that that species is present.

If the sample has been sequenced then a % match is provided. However, there is variation in any individual animal's DNA coding and therefore the match will not always be 100%. We generally consider anything over 95% to be a reliable result.

The test didn't work – what went wrong? Can I have a refund?

The lab's success rate is very high and failures are rare. It is usually not possible to know 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 breaking up in transit so that the mucus covering has been destroyed.

See also "How should samples be preserved and packaged"

The charge for the analysis is not refundable. It covers labour for both SEL and EG, and the primers and other lab materials needed to carry out the analysis.