Technical summary

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DNA Extraction from Otter Spraint and Tissue Samples

R&D Technical Summary W1-025/TS

Background

A feasibility study[1] into the use of DNA fingerprinting to study the European otter (*Lutra lutra*) was completed in 1998. One of the recommendations of the resulting R&D Technical Report was to develop the technique and extend the programme for a further three years. Whilst funding was not available to undertake all the recommendations made within the feasibility report there was sufficient available to continue sample collection and analysis on a reduced scale. In order to achieve this it was necessary to identify a suitable commercial laboratory to undertake the necessary analysis.

The reader is directed to the Feasibility Study Report for detailed descriptions of all the methods and terminology referenced below.

Project Objective

For the feasibility study all analyses were completed by Aberdeen University as part of a research and development contract. However, the University, as a research organisation, does not have the staff to undertake DNA extraction and analysis on a routine long-term basis. Because of the high level of interest in the technique by conservation organisations it was considered prudent to identify a laboratory that could undertake the work on a regular, commercial basis. The laboratory was initially required to extract and analyse DNA from spraint and tissue samples collected between August 1998 and July 2000.

[1] The Use of DNA Fingerprinting to Study the Population Dynamics of Otters (*Lutra lutra*) in Southern Britain: A Feasibility Study. R&D Technical Report W202. 1999. Environment Agency.

Project Sequence

A detailed search of the internet combined with recommendations from DNA contacts identified several potential organisations. Each was sent a basic description of the project together with detailed DNA extraction and typing methods (Dallas and Piertney, 1998; Coxon *et. al*., 1999). Whilst all organisations expressed initial interest in the project only one laboratory, Tepnel Life Sciences, indicated that they had the technical facilities and staff resources to take on the project.

Tepnel proposed that the extraction would be completed by themselves and that they would subcontract the second stage of the process, the PCR and typing, to a specialist company. The second company was not identified due to commercial confidentiality.

Although Tepnel had extensive experience in DNA extraction and purification, extraction of DNA from otter spraint and tissue samples was new to them. Tepnel was, therefore, awarded a limited contract to confirm their competence in the required methodology. As tissue samples provide pure otter DNA in good concentrations the results of DNA extraction and typing are generally more reliable. Therefore, Tepnel was provided with 24 tissue samples for typing using 9 primer sites. In addition to the 9 primers the SRY site was run as a quality assurance test, as the sex was known of all the animals from which tissue samples were taken. Only 1 primer site for one tissue sample failed to produce a PCR product.

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Two of the full profiles matched animals identified previously from spraint collected near or at the same locations that the
otter carcasses were found.

In addition, 41 spraints were provided to allow further optimisation of the extraction process at Tepnel and to trial their full DNA profiling service with spraint rather than tissue samples. Of the 41 samples, three were subject to full PCR and typing at nine loci plus the SRY locus. Four out of 27 primer sites did not produce a product. The full results of the tissue and spraint sample analyses are presented in *Nucleic Acid Purification and DNA Profile Typing*. Tepnel Document No. NS005, (May 2000).

As part of this pilot Tepnel was then contracted to run a more rigorous test using 20 spraint samples. This also coincided with a change in subcontractor so that further laboratory optimisation was achieved using these spraint samples. Initially only 13 out of the 20 samples yielded sufficient DNA for PCR and typing. Following repetitions, excluding the SRY reactions, 157 out of 180 PCR reactions (using 9 primer sites) succeeded. All samples had at least five primer sites develop, the minimum required for an individual fingerprint to be considered reliable. This compared to an average of 20% of spraints yielding reliable fingerprints previously achieved by Aberdeen University. Full details of the optimisation process and the test run of spraint are presented within *Microsatellite analysis of DNA extracted from Otter spraint samples. Final Report.* Tepnel Life Sciences Report Note 002, (October 2000).

The interpretation of the data provided raised some concerns. All the spraints supplied were collected from less than 500 metres of the same watercourse over a six month period. Comparison of the individual profiles from the first 13 samples found little repetition with only one DNA profile repeated twice. This would indicate a surprisingly high number of otters (9 males and two females) were present on the same stretch of watercourse. Interestingly there was no repetition of individual profiles identified from samples collected during the previous year from the same area and analysed by Aberdeen University. Subsequent repeats of the analyses improved the number of loci that developed but did not reduce the number of different profiles/individual animals identified. If correct, these results would indicate a very mobile, male dominated population.

Subsequently, Tepnel was contracted to extract and analyse spraint DNA from 456 samples collected from the Rivers Tone and Brue in Somerset and the River Itchen in Hampshire. An additional 17 tissue samples from these catchments were also submitted for analysis.

Sufficient DNA was extracted from 328 of the spraint samples. Sixteen of the tissue samples gave positive amplification of DNA. However, the number of primer sites to develop was greatly reduced compared to the previous test runs. Assuming a minimum of five primers need to be fully developed (Coxon et al 1999) for a fingerprint to be considered reliable, then the number of reliable profiles was down to 4.7% for the Tone samples, 5.8% for the Brue and 15.3% for the Itchen compared to an average of 20% achieved by Aberdeen University.

Again a very high number of individual animals were indicated by the different profiles, 64 from the Somerset River Tone samples, three for the Brue and 31 for the River Itchen. Detailed discussions with Tepnel and their subcontractor identified that for this batch of samples only one PCR run was completed for each primer for each sample. The minimum recommended in the methodology is four. The necessary repeat analyses are planned but the results were not available for comment to be included within this report. Once completed it is likely that the number of reliable fingerprints will increase and that the high number of different DNA profiles identified may reduce. Work is currently underway at Oxford University (Laura Bonesi, pers comm) which may help to determine the number of animals identified by the data set. A programme has been developed that identifies individual animals by interpreting a range of PCR gel plate band sizes related to specific bin number assignments for individual alleles. Bin number assignment is currently completed manually.

Detailed analysis of any viable data will be presented in a separate report. The full results to date are presented in Tepnel Final Report no. NS0021, (June 2002) [2].

This work will be relevant to those interested in otter DNA Fingerprinting. This study has examined the feasibility of using DNA extracted from otter spraints to identify individual animals, and to provide population estimates for certain rivers. The technique shows promise but requires further refinement before it can be considered as a viable method to survey otter populations in the field. Further research work is being carried out to refine the methodology with a view to making it accessible to those working on otters in the UK.

This R&D Technical Summary relates to information from R&D Project W1-025 reported in detail in the aforementioned outputs.

Internal Status: Released to Agency Regions
External Status: Public Domain

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[2] Nucleic Acid Purification and DNA Profile Typing of 473 Eurasian Otter Spraint and Tissue Samples. Final Report NS0021. Tepnel Life Sciences. June 2002.

Copies of the Report W202 and this Summary will be available internally from your Regional Libraries or the National Information Centre in Bristol. Externally, this and other R&D outputs are available from the Environment Agency's R&D Dissemination Centre, c/o WRc Information Resources, Frankland Road, Blagrove, Swindon, Wiltshire SN5 8YF, Tel: 01793 865138, Fax: 01793 514562. Website URL: www.eareports.com

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