

## Sampling for determination of rat genetics for eradication and reinvasion work

The following information has been kindly prepared for PII by Dr Rachel Fewster, Senior Lecturer, Faculty of Science, The University of Auckland, New Zealand.

### Sample Size

For eradication planning, it is recommended that samples from 20 rats are collected from the eradication island **before the eradication** takes place. They need to be stored in >70% ethanol (preferably refrigerated, but not essential) in case a reinvasion occurs. The samples can be tested against any reinvaders.

The suggestion of 20 from the original population is because there is only one chance to sample that population: there is no possibility of increasing the samples after the eradication has taken place. 10 *might* be good enough but with no guarantees, 30 would be great, 50 would be fantastic. With 20, there is probably a good chance of getting a result, without requiring ludicrous amounts of fieldwork effort. (It depends on how difficult rats are to catch in these places - sometimes 20 samples is asking a lot!)

If a reinvasion occurs, you need to **catch as many of the early reinvaders** as you can: one is enough, but the important thing is that the reinvaders should be caught before they start breeding and creating a new population with its own genetic signature.

Then you need to collect samples from the possible invading source populations to investigate the invasion pathway. 10 rats from each source is recommended, which should be sent to the lab and tested against the reinvader: if there's no clear result, then you can always go back to the potential source populations and get more samples. It's a balance between fieldwork time and lab costs, and a law of diminishing returns if you've got populations with enough genetic discrimination.

### Sample Collection

For each rat, snip off a 3-4cm length of tail and put it in the collecting vial. Do not worry if the ethanol doesn't completely cover the sample, but try and immerse as much of the sample as possible. Do NOT stuff too much tissue into a single bottle: it is vital that the ethanol is able to penetrate all the tissue, and only a tiny amount of tissue is actually needed for DNA processing.

If the tail has been eaten, ANY piece of rat flesh can be used instead. Please make a note on the vial if this is the case, and what part of the rat the sample is from (if possible)

NEVER mix samples from two different rats in the same collecting vial: one rat per vial only, please. Make sure blood or tissue from one rat never contaminates the sample from a different rat, e.g. due to a dirty knife.

The closer the sample is taken to the rat's death, the better, but up to 2-3 days should not make a difference to the quality of the sample. This might differ in a hotter climate.

Collecting vials contain 70% - 95% ethanol. *The higher the ethanol content, the better.* After collection, it is best to keep the sample refrigerated. In New Zealand conditions, samples have been stored at room temperature in 70% ethanol for several months without negative consequences.

### **Vial Labelling**

Vials should have plain labels to record the information required for the study. Usually, sticky labels on the outside of the vial can be held on with extra plain tape, but an alternative is to write in pencil and insert the label into the ethanol tube itself so that it can be clearly read from the outside (preferably without opening the bottle again).

The advantage of using labels inside the bottles is that it avoids ink running due to leakage of ethanol, or by getting wet, and it avoids sticky labels falling off. The disadvantage is that it can be difficult to read the labels inside the bottles and can require unnecessary handling of the specimen inside the bottle (to read the label) with the associated risk of contamination. Always write in pencil that will not fade, or (less likely) ink that will not run. Marker pens can also be used that write directly onto the bottles themselves: check for risk of smudging or fading.

### **The details to record with each sample are:**

**Date** – in a standard format

**Species** – e.g. kiore or ship rat; if there is doubt, indicate a best guess with a question mark (it should be possible to tell for sure using genetics, but it is useful to record a field guess to help decide lab processing order and to cross-check genetic results with field records).

**Sex** – male or female; only record this if you are confident, otherwise leave this field blank. (Samples are genetically sexed, but the procedure is not always reliable.)

**Broad Location** – the area where the rat was caught, e.g. West landing.

**Trap number** – if the sample was caught on an established trapping line, record line and number to give exact spatial position. Separately create a record of GPS locations of every trap, so that all samples are spatially referenced. Even if exact spatial location is not required for the study, it is very helpful to record trap number in case there is a query about the sample (e.g. genetic results) and you need to check against the field notes: *this happens a lot!*

Please ***write the notes on the label in pencil*** so that the labels are less likely to be harmed if any ethanol leaks from the vials.

### **Field notes**

It is advisable to keep a separate field notebook replicating the information on the vial label (broad location, date, trap number, species, sex) and adding any extra field notes. Notes such as whether the sample was fresh will help to explain why genetic processing might have failed; if the specimen had been cannibalised it might explain a species mis-identification; etc. We also record strange colourations, e.g. ship rats occur in three different colour morphs, and some have abnormalities such as white tail tips.

*It is good scientific practice to also record morphological characteristics in the field notebook: Head and body length; Tail length; Mass. (These characteristics can also clarify species ID.)*

(NB - if sample collection is to be undertaken by volunteers (members of the public), do not ask for field notes; keep the collection process as straightforward as possible.)

### **Health and safety**

Keep ethanol well away from flames or other source of ignition.

Rats carry leptospirosis which is transmitted particularly through urine. Scratches and cuts must be covered. Gloves should be used. Hands must be cleaned thoroughly with disinfectant before handling food.

Normal procedures for field safety apply.