# A PCR TEST TO DETECT BANDICOOT DNA FROM PREDATOR SCATS

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*Perameles gunnii* at Austin's Ferry, Tasmania
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**BACKGROUND: Several Australian bandicoot species have become extinct since European settlement, leaving seven species extant in Australia. Bandicoots are vulnerable to predation by introduced foxes, dogs and cats.**

**Two bandicoot species occur in Tasmania: the southern brown bandicoot (*Isoodon obesulus*) and the eastern barred bandicoot (*Perameles gunnii*). The latter has almost completely disappeared from the rest of its range in Victoria. The establishment of a fox population in Tasmania would pose a serious threat to these species.**

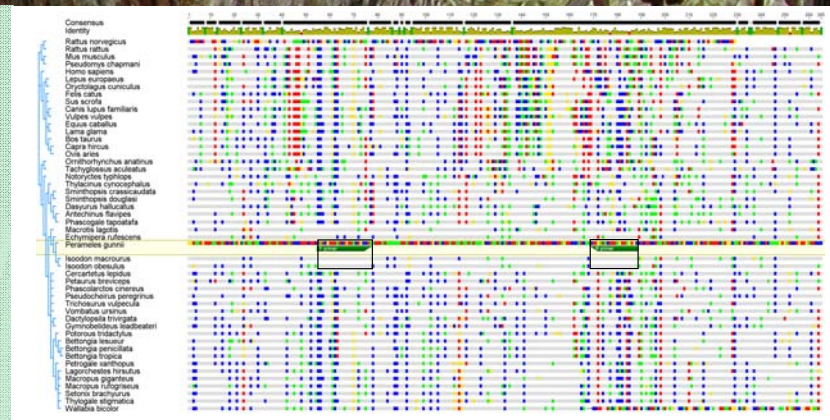
**DNA from trace samples can provide valuable information for wildlife managers about species distributions and predator-prey interactions. Detection of introduced foxes in Tasmania relies upon the use of fox-specific primers to amplify faecal DNA[1]. New genetic tests to detect predation of native wildlife, such as bandicoots, would be useful for monitoring the impacts of introduced predators.**

## DESIGNING DIAGNOSTIC BANDICOOT PRIMERS:

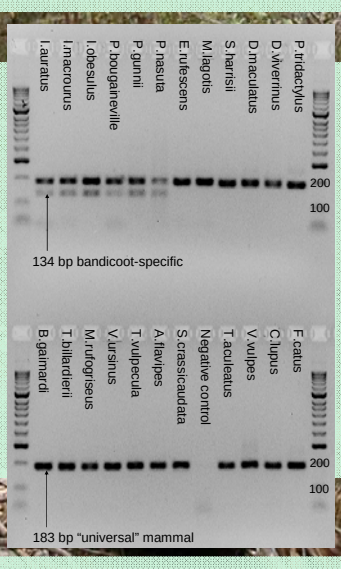
**Mammalian mitochondrial *ND2* sequences were aligned using Geneious software[2]. Sequences were obtained from GenBank[3] (March 2011) and from unpublished data (Linzi Wilson-Wilde). We included sequences from:**

i) all available mammals native to Tasmania;
ii) all available mammals introduced to Tasmania;
iii) representatives of other groups of mammals.

**Primers were designed that were 100% conserved among all *Isoodon* and *Perameles* sequences then available. The primers had multiple mismatched nucleotides relative to all other sequences screened. These new bandicoot-specific primers amplify a 134 bp fragment of the *ND2* gene.**




**Some of the sequences used for primer design. *Perameles gunnii* is the reference sequence. For all other sequences, grey nucleotides show agreement with the reference and coloured nucleotides show mismatch with the reference. The black boxes denote the conserved primer sequences in *Isoodon* and *Perameles*.**




## NEW PRIMERS SPECIFICALLY AMPLIFY BANDICOOT DNA:

**Bandicoot primers were tested for their specificity to bandicoot DNA. A set of "universal" mammal primers, amplifying a 183 bp fragment[1], was also used as a control for DNA quality.**

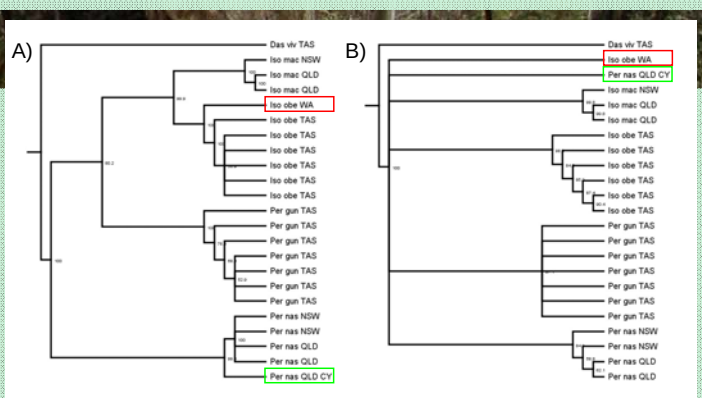
**Amplification was successful from all individuals tested from six bandicoot species: *Isoodon auratus* (n=2), *Isoodon macrourus* (n=4), *Isoodon obesulus* (n=8) *Perameles bougainville* (n=1), *Perameles gunnii* (n=9), *Perameles nasuta* (n=8)**

**No amplification was detected from DNA from 40 other non-target marsupial, monotreme and eutherian mammal species tested. These PCR tests included all large mammalian predators in Tasmania (fox, dog, cat, eastern quoll, spotted-tailed quoll and devil).**

## DNA SEQUENCING TO DISTINGUISH BANDICOOT SPECIES:

**We will use DNA sequencing to determine which bandicoot species is represented in an unknown sample, by comparison with a reference sequence database. We are currently sequencing the *ND2* region from multiple individuals from each bandicoot species.**

**Preliminary results indicate that the 134 bp fragment amplified by the bandicoot-specific primers will provide sufficient resolution to discriminate among bandicoot species. Sequencing additional individuals may enable us to determine the provenance of samples, for example by distinguishing Tasmanian from mainland individuals.**




**Neighbour-joining trees based on preliminary sequencing data from four bandicoot species:**
**A) 882bp of the *ND2* gene amplified with universal mammalian primers and**
**B) 134bp of the *ND2* gene amplified with our new bandicoot-specific primers.**

**References:**

[1]Berry *et al* (2007) Wildlife Research 34: 1-7
[2] Drummond *et al* (2011) Geneious v5.4
Available from http://www.geneious.com/
[3] Benson *et al* (2011) Nucleic Acids Research 39 (Database issue): D32-7

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