10.1071/RD18142_AC

Supplementary Material: *Reproduction, Fertility and Development*, 2019, 31(7), 1289–1295.

Supplementary Material

Non-invasive genetic sexing technique for analysis of short-beaked echidna (*Tachyglossus aculeatus*) populations

Tahlia Perry[A], *Deborah Toledo-Flores*[A], *Wan X. Kang*[A], *Arthur Ferguson*[B], *Belinda Laming*[B], *Enkhjargal Tsend-Ayush*[A], *Shu L. Lim*[A] *and Frank Grützner*[A,C]

[A]The Environment Institute, School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia.

[B]Perth Zoo, 20 Labouchere Road, South Perth, WA 6151, Australia.

[C]Corresponding author. Email: frank.grutzner@adelaide.edu.au

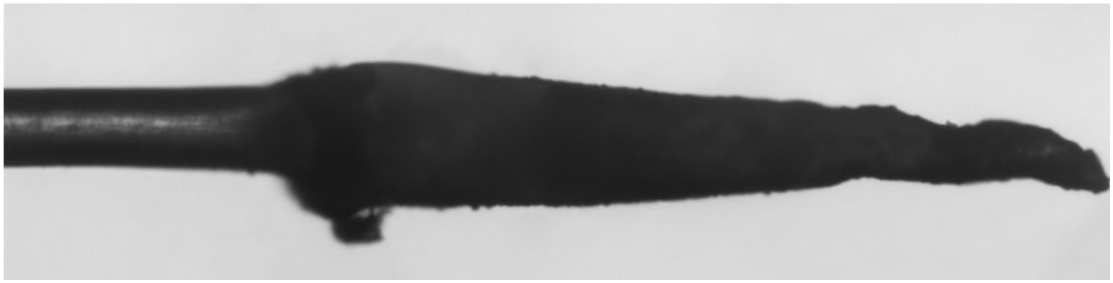

Fig. S1. Image of echidna hair follicle. Echidnas have a distinct conical shaped hair follicle in comparison to the characteristic bulb shaped follicle in humans. For this protocol, the hair shaft was removed from the follicle so that only the follicle was used for DNA extraction.

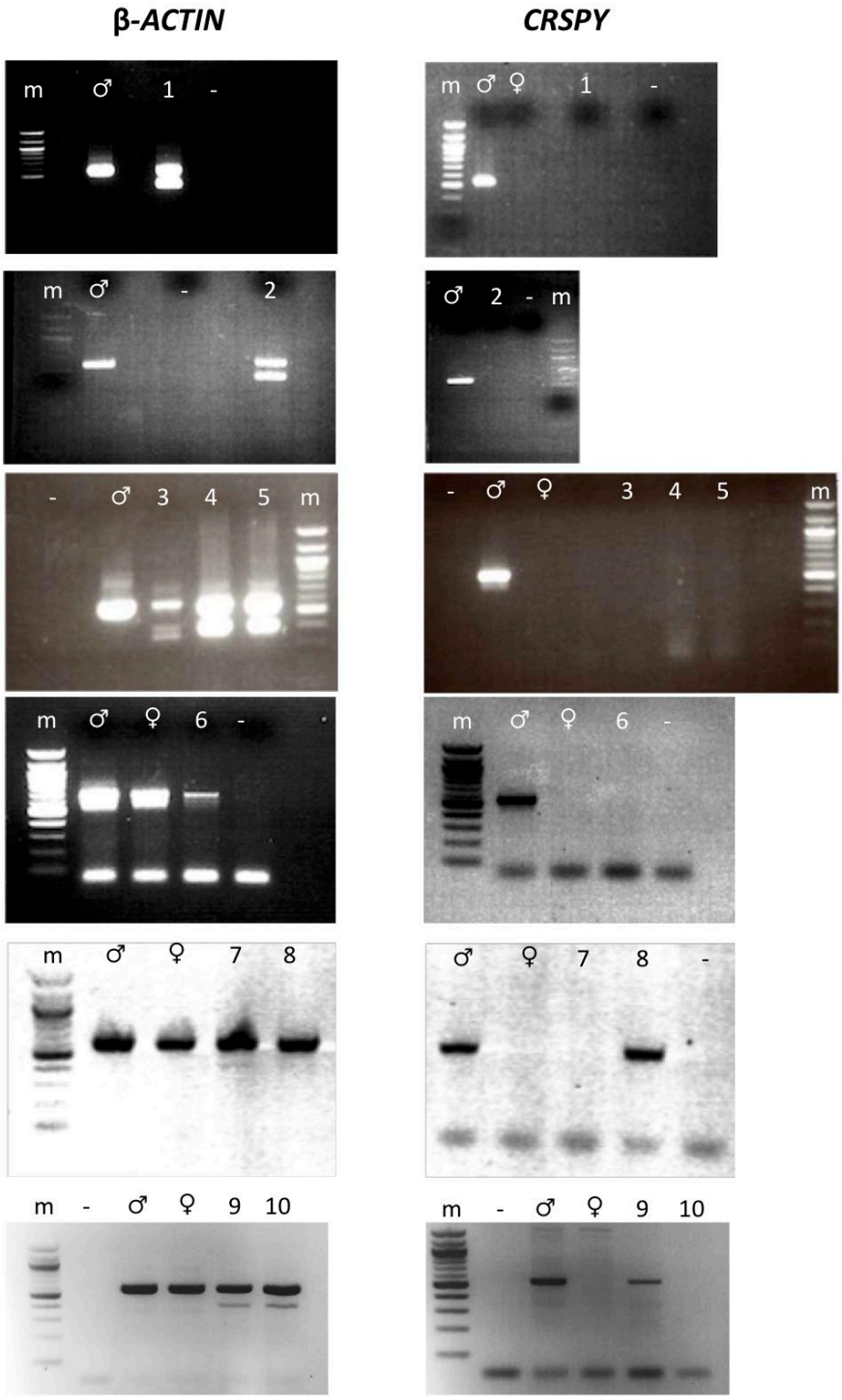

Fig. S2. Original gel images relating to Fig. 3. For Fig. 3, gel images were cropped to appear in an easier to read figure describing the sex of echidnas via PCR. The original gel images are shown here for the amplification of β-*ACTIN* and *CRSPY* for all samples analysed in this paper (ID 1-10). Again, showing that echidnas ID 8 and 9 are male and all remaining are female. ♂ = known male, ♀ = known female, - = negative control. β-*ACTIN* is positive control indicating that genomic extraction was successful for all individuals. Number denotes the ID for echidna DNA used in PCR (see Table 1). 100 bp ladder is used as size marker (m). Both *CRSPY* and β-*ACTIN* are approximately 600 bp in size.

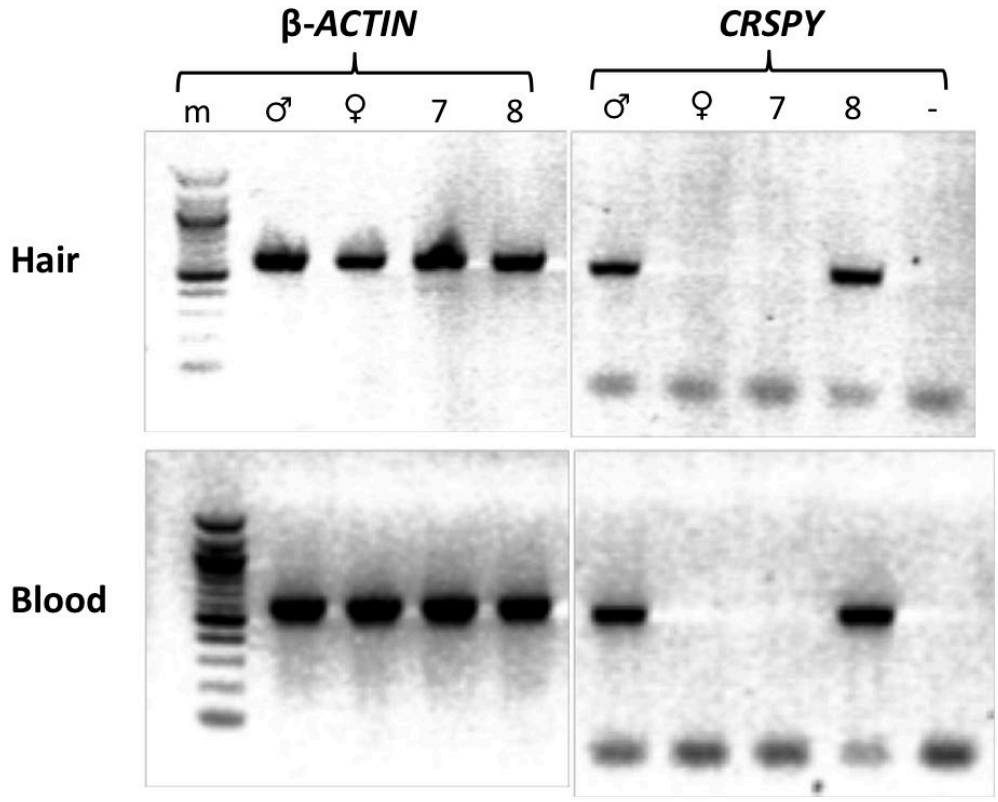

Fig. S3. Confirming sex of two echidnas with PCR of *CRSPY* on gDNA extracted from blood. Amplification of *CRSPY* gives a single band in males only. Single band amplified for echidna ID 8 shows it is male; no amplification in echidna ID 7 indicates it is a female. This is consistent with the amplification pattern from gDNA extracted from hair follicles. ♂ = known male, ♀ = known female, - = negative control. β-*ACTIN* is positive control indicating that genomic extraction was successful for all individuals. 100 bp ladder is used as size marker (m). Both *CRSPY* and β-*ACTIN* are approximately 600 bp in size.