*prep*GEM® Blood

Chinstrap penguin, Antarctica. Photograph by Carlo Olavarria

A rapid DNA extraction method for avian blood samples

Selina Patel, Craig Millar.

Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, University of Auckland, Private Bag 92109, New Zealand

Introduction

DNA analysis of avain blood samples from both domestic and wild bird species has increased substantially over the past decade. There are now DNA-based tests for sexing, genotyping and species identification for most avian taxa. The majority of these involve PCR based methods such as RFLP analysis and sequencing. Such analyses rely on the ability to quickly and efficiently extract DNA from a range of tissue types. Blood is one of the most commonly used types of tissue as it is easy to collect and store. Unlike mammalian blood, avian blood has nucleated red blood cells, making it a rich source of DNA. Due to this unique property, very small amounts of avian blood can be used for DNA analysis.

Once collected blood can be stored using a range of methods including Queen's lysis buffer (Seutin et al 1991), FTA® cards, in ethanol or frozen whole. DNA required for downstream applications relies on the recovery of PCR quality DNA for which a quick, inexpensive and reliable method would be beneficial for screening purposes. Here we report an extraction method which can be applied to multiple storage methods.

Methods

1. Sample preparation and extraction

Avian blood samples for this study were stored using four methods:

1) blood on FTA® cards
2) blood in 95% ethanol
3) frozen blood
4) blood in Queen's lysis buffer.

The whole blood in ethanol and the frozen whole blood were diluted 1:10 prior to extractions, this was done in order to standardize the amount of blood per extraction (whole blood in Queen's lysis buffer is already a 1:10 dilution).

DNA was extracted using *prep*GEM® Blood as per the manufacturer's instructions. For whole blood on FTA cards, a 2 mm x 2 mm piece was cut and the DNA extracted using the *prep*GEM Storage Card kit.

	Add blood or FTA card to enzyme & buffer
	Heat to 75°C and incubate for 15 min
	Increase temp. to 95°C and incubate for 15 min (blood) or 5 min (FTA card)
	Spin at max. for 2 min (blood) or 5min (FTA card)

2. PCR

DNA extracts were quantified using a Qubit fluorometer kit (Invitrogen) and the Qubit ssDNA assay.

PCRs were run in 25 µl volume reactions using 1-2 µl of DNA extract. PCR was performed to amplify the 648 bp COI barcoding region as well as a region of the CHDI gene used for molecular sexing. Primers and PCR conditions used for barcoding are described by Patel et al, 2010. P2/P8 primers used for molecular sexing are described in Griffiths et al, 1998. The PCR cycles for molecular sexing were:

94°C for 2 min,
94°C for 30 s
52°C for 30 s
72°C for 30 s
x 5
94°C for 30 s
47°C for 30 s
72°C for 30 s
x 30
Hold at 20°C.

The resulting PCR products were digested with *Hae* III to differentiate between the two sexes. All PCR products were visualized by gel electrophoresis. A sample from each of the storage methods was chosen for DNA sequencing. PCR products were purified using the AMPure® XP PCR purification kit (Beckman Coulter) and cycle sequenced using the Big dye 3.1 chemistry. Samples were then analyzed on an ABI Prism 3130 XL genetic analyzer. Sequencing was carried out bidirectionally and the sequences were analysed using Sequence Scanner V1.0 (Applied Biosystems).

Sequence quality is routinely measured using QV (quality values) or PHRED scores, which are assigned to individual bases. The higher the QV values the better is the sequence qualit. QV values of 20 and above are deemed acceptable. Sequencing scores wererepresented as a percentage of individual bases in each sequence with QV values of greater than 20.

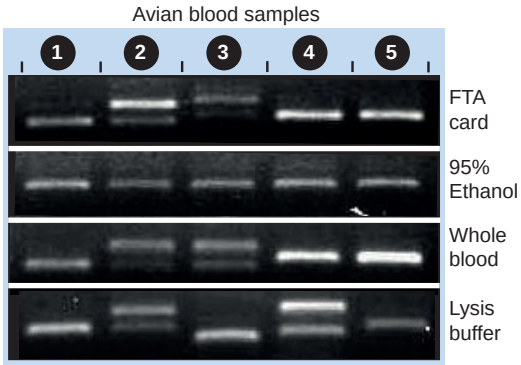

Figure 1 Examples of the amplification of nuclear (CHDI) DNA using *prep*GEM Blood and *prep*GEM Storage Card kits.

Results and discussion

DNA was extracted from the four different blood storage methods. In all samples PCR amplification was achieved with both the CHDI sex specific fragment (from nuclear DNA; Figure 1) and the COI barcoding fragment (from mitochondrial DNA) In addition, sequencing from four of the barcoding products resulted in high quality sequences (Table 1).

Blood storage method	Avian samples	CHDI sexing	COI amplification success / sequencing scores
FTA card	Chicken 1 Chicken 2 Petrel 1 Petrel 2 Petrel 3	Male Female Female Male Male	100% Forward – 90.6%, Reverse – 86.4%
Ethanol	Black stilt 1 Black stilt 2 Black stilt 3 Black stilt 4 Black stilt 5	Male Male Male Male Male	100% Forward – 92%, Reverse – 78.5%
Whole blood	Chicken 3 Chicken 4 Chicken 5 Petrel 4 Petrel 5	Male Female Female Male Male	100% Forward – 90.7%, Reverse – 88.9%
Queen's lysis buffer	Swamp hen 1 Petrel 6 Passerine 1 Passerine 2 Petrel 3	Male Female Male Female Male	100% Forward – 90.1%, Reverse – 89.7%

Table 1 A summary of the results indicating the range of species used, the sex of the individuals and the amplification success and sequencing scores (unedited) for the barcoding region.

Results and discussion

Figure 2 shows the average amount of DNA extracted from the five samples for each storage method. Interestingly the FTA card and Queen's lysis buffer gave a significantly higher DNA yield compared to the ethanol and whole blood. However all storage methods had DNA concentrations higher than the 0.5 ng / µl stated in the *prep*GEM Blood protocol for mammalian blood. This is expected given that avian blood cells are nucleated.

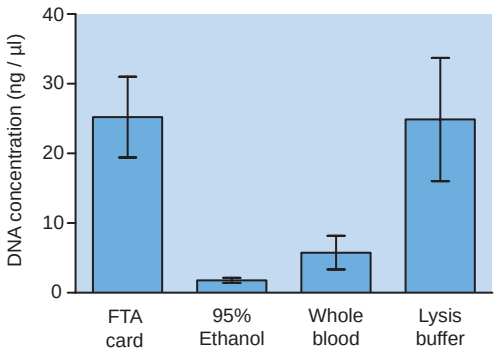

Figure 2 Average DNA yields for the four different storage methods. Error bars are one standard deviation.

Conclusions

Our findings indicate that both *prep*GEM Blood and *prep*GEM Storage Card are versatile and work well with avian blood. Furthermore these kits provide relatively large amounts of DNA from different storage methods. The extraction method is rapid and reproducible and provides DNA that can be reliably used for sexing and generating high quality DNA sequences.

References

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Seutin G., White B. N. and Boag P. T. (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology **69**:82-90.