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TECHNICAL NOTE

Skin swabs with FTA® cards as a dry storage source for amphibian DNA

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Abstract
Amphibians are the most endangered group of vertebrates, and conservation measures increasingly rely on information drawn from genetic markers. The present study explores skin swabs with Whatman FTA® cards as a method to retrieve PCR-amplifiable amphibian DNA. Swabs from ten adult great crested newts (*Triturus cristatus*) were used to compare FTA® card-based protocols with tissue sampling based on toe clips. PCR success rates were measured for seven microsatellite markers and one mtDNA marker (ND4) after 6 months of sample storage. We demonstrate that the merging of eight FTA® card punches from Qiagen-based DNA extraction always led to successful amplifications in at least one replicate, at an overall PCR success rate of 78%. The newly established protocol has the potential for wide application to future DNA-based amphibian studies.

Keywords *Triturus cristatus* · DNA sampling · Non-invasive techniques · Amphibian conservation

Research to address the well-documented global amphibian biodiversity crisis is increasingly involving information drawn from DNA (e.g. McCartney-Melstead and Shaffer 2015). However, due to their lack of dead keratinised tissue, the non-destructive collecting of samples from amphibians usually involves the removal of, for example, toes or tail tips (e.g. Arntzen et al. 1999). Alternative methods based on swabs are increasingly applied, but can be prone to contamination, can yield in insufficient DNA for PCR amplification, and can involve rather torturous procedures such as forcing the mouth to open for buccal sampling (Poschadel and Möller 2004; Broquet et al. 2007; Prunier et al. 2012; Müller et al. 2013; Pichlmüller et al. 2013; Ringler 2018).

Whatman FTA® (Flinders Technology Associates) cards are impregnated with protein denaturants that cause lysis of cells, designed to fix and store DNA directly from tissue while preventing growth of bacteria. However, while FTA® cards are widely used in forensic medicine and epidemiology, their application to conservation-relevant studies is still underexplored (Smith and Burgoyne 2004). They have been successfully used to retrieve DNA from skin swabs in fish (e.g. Kashiwagi et al. 2015), but comprehensive trials for amphibians are as yet lacking (see however e.g. Maddock et al. 2014). In the present paper, we establish a protocol to retrieve amplifiable DNA through FTA® card-based skin swabs of great crested newts (*Triturus cristatus*, for more information on the study species see e.g. Jehle et al. 2011).

Sampling took place on the 2nd of May 2015 at a pond on Gorse Hill Nature Reserve, northwest England. Five male and five female *T. cristatus* (body lengths 126–154 mm) were caught using mesh traps described in Madden and Jehle (2013). Each individual was swabbed with a single FTA® card along the body including the tail (once dorsally, laterally on both sides, and ventrally), and the distal half of the most distal front toe was removed using sterilised scissors; all individuals were immediately released after handling at the place of capture. FTA® cards were stored dry at room temperature in individual envelopes, and corresponding toe tips were stored in 1.5 ml absolute ethanol.

Laboratory work took place approximately 6 months after fieldwork. A Harris wheeler punch was used to remove circles with approximately 2 mm diameter from the FTA® card, cleaned by punching three holes on a filter paper between use (broadly following Ahmed et al. 2011). In Experiment 1, between one and five punches from each card (two replicates each) were washed three times with 200 µl FTA® purification reagent for 3–5 min, before washing them twice with 200 µl 1 M TE buffer. Dried punches were stored at − 20 °C

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Table 1 Average PCR success rates for seven microsatellite loci and mtDNA ND4

Locus	Expt.	Individual PCR success rates										Mean (%)
		1	2	3	4	5	6	7	8	9	10	
*Tcri*13	1	9/10	8/10	8/10	7/10	8/10	8/10	7/10	7/10	8/10	8/10	77
	2	3/3	3/3	2/3	3/3	3/3	2/3	2/3	3/3	3/3	3/3	90
	3	4/4	4/4	4/4	3/4	4/4	4/4	3/4	4/4	4/4	4/4	95
*Tcri*27	1	4/10	7/10	5/10	7/10	5/10	4/10	8/10	8/10	4/10	7/10	59
	2	3/3	3/3	3/3	2/3	2/3	3/3	2/3	2/3	2/3	3/3	83
	3	4/4	4/4	3/4	3/4	4/4	3/4	3/4	3/4	4/4	4/4	88
*Tcri*29	1	7/10	6/10	7/10	5/10	9/10	7/10	6/10	7/10	7/10	5/10	66
	2	2/2	2/2	1/2	1/2	2/2	2/2	1/2	1/2	2/2	2/2	80
	3	4/4	4/4	4/4	4/4	3/4	3/4	3/4	4/4	4/4	4/4	93
*Tcri*35	1	8/10	6/10	5/10	8/10	4/10	8/10	6/10	6/10	5/10	4/10	60
	2	1/2	1/2	1/2	1/2	2/2	2/2	2/2	1/2	1/2	2/2	70
	3	3/4	4/4	4/4	4/4	4/4	3/4	3/4	3/4	4/4	4/4	90
*Tcri*36	1	5/10	6/10	4/10	7/10	7/10	5/10	6/10	6/10	5/10	5/10	56
	2	1/3	2/3	1/3	1/3	1/3	2/3	2/3	2/3	1/3	1/3	53
	3	3/4	4/4	4/4	3/4	4/4	3/4	3/4	3/4	4/4	4/4	88
*Tcri*43	1	8/10	7/10	6/10	8/10	7/10	7/10	8/10	8/10	8/10	6/10	73
	2	3/3	3/3	2/3	2/3	2/3	3/3	3/3	3/3	3/3	2/3	87
	3	4/4	3/4	3/4	3/4	4/4	4/4	4/4	4/4	4/4	3/4	90
*Tcri*46	1	6/10	7/10	8/10	7/10	7/10	6/10	8/10	8/10	6/10	6/10	73
	2	2/2	2/2	2/2	2/2	1/2	1/2	2/2	2/2	2/2	2/2	90
	3	4/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	93
ND4	1	7/10	7/10	7/10	6/10	8/10	8/10	6/10	7/10	6/10	6/10	68
	2	3/3	3/3	2/3	3/3	2/3	2/3	2/3	2/3	3/3	3/3	83
	3	4/4	3/4	4/4	4/4	4/4	3/4	3/4	4/4	4/4	3/4	88
Totals	1	68%	68%	63%	69%	69%	66%	69%	71%	61%	59%	66
	2	86%	90%	67%	71%	71%	81%	76%	76%	81%	86%	78
	3	94%	94%	91%	88%	97%	84%	81%	91%	100%	91%	91

Data are shown across 10 individual *Triturus cristatus* (one FTA® card per individual), as number of successful replicates. For details on the protocols for Experiments 1–3 see text

for up to a week and directly added to the PCR mastermix. In Experiment 2, two or three replicate sets of eight punches from each FTA® card were pooled for extraction using the Qiagen DNeasy Blood & Tissue kit, following the manufacturer's instruction. For Experiment 3 we used the Qiagen kit to extract DNA from the entire toe clips, performing four replicate PCRs for each genetic marker. DNA was quantified using a Jenway 6305 fluorometer and calibrated to 10 ng/µl whenever the measurement was above this value. All PCRs were carried out in 10 µl reaction volumes (1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 U polymerase (GoTaq) and 0.1 mM of each primer in the manufacturer's buffer, using 1 µl DNA extract). We amplified seven nuclear microsatellite markers using procedures described in Krupa et al. (2002), and a mtDNA region (ND4) as described in Wielstra et al. (2013; a subset of PCR products were sequenced to confirm the correct template was amplified, detailed data not shown). PCR success rates were evaluated on 1.5% agarose gels stained with GelRed, using Bioline Hyperladder I as size standard.

For Experiment 1, PCR success rates were 66% (Table 1), and tended to decrease when more than three punches were used per reaction (detailed data not shown). Success rates were markedly higher (78%) for Experiment 2, with at least one replicate resulting in a PCR product for all individuals and markers. PCR success rates based on tissue extracts (Experiment 3) were 91%. There was no consistent pattern of individual (FTA® card)-based PCR success rates across the three experiments (failed PCRs are likely due to punches taken from areas of the card which contain insufficient or no DNA, Cox et al. 2010).

Because FTA® cards enable the storage of DNA without the need for liquid preservatives such as ethanol, they can be particularly useful for field work in difficult locations (Bunting et al. 2014). Although replicates were required to achieve PCR success for each individual, we recommend Experiment 2 as a reliable and generally applicable protocol. We however acknowledge that further experiments are required to test the suitability of our swabbing protocol for

next generation sequencing-based approaches (e.g. Meilink et al. 2015). Handling of amphibians elicits a physiological stress response (Narajan et al. 2012), and potential harmful long-term effects of swabbing, while considered unlikely, were beyond the scope of the present study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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