Amphibia-Reptilia

Detectability vs. time and costs in pooled DNA extraction of cutaneous swabs: a study on the amphibian chytrid fungi

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Supplementary material

Supplementary methods

Data-base probing

Peer reviewed articles on which more than 50 samples were processed were selected from Web of Science by combining "*Batrachochytrium*" and "occurrence". Search outcome was initially filtered to articles from 2007 on, to exclude projects anterior to the original publication testing pooled extraction (Hyatt et al., 2007). In addition results were filtered to usage of skin swabs, qPCR and a minimum sample size of 50 samples. From the selected publications, information on whether any kind of pooling was used was extracted.

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Table S1. List of reagents and consumables required for chytrid DNA extraction with Prepman according to the protocol described in methods.

	Price	Volume total	Volume/ sample	n feasible	Price per sample
Prepman	126	10,000	50	200	0.63
Tips 200	84.20	960	4	240	0.35
				TOTAL	0.98

Table S2. List of reagents and consumables required for chytrid DNA extraction with Qiagen according to the protocol described in methods. Items in grey are main components; items in white are reagents necessary to prepare main components.

	Price	Volume total	Volume/ sample	n feasible	Price per sample
Extraction kit	752.00			250	3.01
Lysozyme	149.00	10,000	3.75	2,667	0.06
Buffer	0.79	1,000	0.19	5,319	0.01
Tris-Cl	12.87	870	3.15	276	0.05
Tris	45.90	500	121.10	4	11.12
HCL	12.50	500	70.00	7	1.75
EDTA	21.50	1,000	0.74	1,344	0.02
Triton-x-100	15.20	250	12.00	21	0.73
Ethanol	83.50	1,000	200.00	5	16.70
Tips 200	84.20	960	5	192	0.44
Tips 1000	88.80	960	3	320	0.28
Eppis	14.50			500	0.03
				TOTAL	20.52

Table S3. List of reagents and consumables required for chytrid qPCR according to the protocol described in methods. 2x: in duplicate.

	Cost	Volume total	Volume/ sample	n/ plate	n feasible (2x)	Price per sample (2x)	Price per full plate
KlearKall	1247.80	25000	7.50		1667	0.75	35.94
Bs probe	255.00	1250	0.15		4167	0.06	2.94
Bs primerF	4.09	1250	0.45		1389	0.00	0.14
Bs primerR	4.09	1250	0.45		1389	0.00	0.14
plates	228.00			1	4800	0.05	2.28
seals	168.00			1	9600	0.02	0.84
Tips 20	82.60	960	2	97	9		9.18
Tips 200	84.20	960		3	320		0.26
Tips 1000	88.80	960		1	960		0.09
						TOTAL	51.81

Table S4. Summary of the list of hits from the data-base probing by chronological and alphabetical order. Studies were identified for target chytrid species, *Batrachochytrium dendrobatidis* (*Bd*) or *B. salamandrivorans* (*Bsal*), whether pooling was performed (Pool) and the type of semi-quantitative PCR used (qPCR). y: yes, n: no, PCR: pooling at the PCR level.

Publication	***Bd***	***Bsal***	**Pool**	**qPCR**
Bacigalupe *et al.* 2017	y	n	n	TaqMan
Carvalho *et al.* 2017	y	n	n	TaqMan
Gervasi *et al.* 2017	y	n	n	TaqMan
Horner *et al.* 2017	y	n	n	TaqMan
Lambertini *et al.* 2017	y	n	n	TaqMan
Liew *et al.* 2017	y	n	n	SYBR Green
Rosa *et al.* 2017	y	n	n	TaqMan
Seimon *et al.* 2017	y	n	n	TaqMan
Battaglin *et al.* 2016	y	n	n	TaqMan
Clare *et al.* 2016a	y	n	n	TaqMan
Clare *et al.* 2016b	y	n	n	TaqMan
Grogan *et al.* 2016	y	n	n	TaqMan
Love *et al.* 2016	y	n	n	TaqMan
Rothermel *et al.* 2016	y	n	n	TaqMan
Bielby *et al.* 2015	y	n	n	TaqMan
Bletz *et al.* 2015b	y	n	PCR	TaqMan
Blooi *et al.* 2015	n	y	n	TaqMan
Bosch *et al.* 2015	y	n	n	TaqMan
Bresciano *et al.* 2015	y	n	n	TaqMan
Courtois *et al.* 2015	y	n	n	TaqMan
Crespi *et al.* 2015	y	n	n	TaqMan
D'Aoust-Messier *et al.* 2015	y	n	n	TaqMan
Gabor *et al.* 2015	y	n	n	TaqMan
Hardy *et al.* 2015	y	n	n	TaqMan
Kolby *et al.* 2015	y	n	n	TaqMan
Molur *et al.* 2015	y	n	n	SYBR Green
Piovia-Scott *et al.* 2015	y	n	n	TaqMan
Savage *et al.* 2015	y	n	n	TaqMan
Stockwell *et al.* 2015	y	n	n	TaqMan
Tinsley *et al.* 2015	y	n	n	TaqMan
Valencia-Aguilar *et al.* 2015	y	n	n	TaqMan
Baláž *et al.* 2014	y	n	n	TaqMan
Catenazzi *et al.* 2014	y	n	n	TaqMan
Erismis *et al.* 2014	y	n	n	TaqMan
Galindo-Bustos *et al.* 2014	y	n	n	TaqMan
Heard *et al.* 2014	y	n	n	TaqMan

Table S4. (Cont.)

Publication	***Bd***	***Bsal***	**Pool**	**qPCR**
Jani & Briggs 2014	y	n	n	TaqMan
Langhammer *et al.* 2014	y	n	n	TaqMan
Lenker *et al.* 2014	y	n	n	TaqMan
Martel *et al.* 2014	n	y	n	TaqMan
Rebollar *et al.* 2014	y	n	n	TaqMan
Spitzen-van der Sluijs *et al.* 2014	y	n	n	TaqMan
Huss *et al.* 2013	y	n	n	TaqMan
Ohst *et al.* 2013	y	n	n	TaqMan
Sapsford *et al.* 2013	y	n	n	TaqMan
Villarroel *et al.* 2013	y	n	n	TaqMan
Zampiglia *et al.* 2013	y	n	n	TaqMan