



**Helbing Laboratory eDNA Technical Bulletin**

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity<sup>1-3</sup>.

**General eDNA Assay Information**

Target Species: Various Fish Species  
Species Code: te-Fish  
eDNA qPCR Tool: eFish1  
eDNA qPCR Format: TaqMan  
Gene Target: MT-RNR1  
Published in: 5

**eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA**

LOD 0.9 95% CI 0.7-1.5 Copies/Rxn LOQ 3.4 95% CI 2.5-5.6 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Immolase

**eDNA Assay Specificity Test Information**

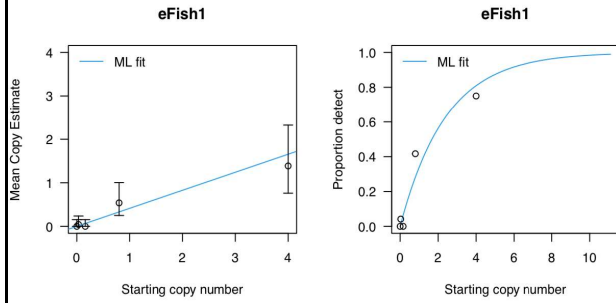
n=25 technical replicates. Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA

Species	Common Name ( <i>Species</i> )	Detection	# Voucher	
			Specimens	Sample Sources/Locations
am-AMMA	Long toed salamander ( <i>Ambystoma macrodactylum</i> )	Yes	1	British Columbia
am-AMMV	Eastern tiger salamander ( <i>Ambystoma mavortium</i> )	No	1	British Columbia
am-AMTI	Barred tiger salamander ( <i>Ambystoma tigrinum</i> )	No	1	British Columbia
am-ANBO	Western toad ( <i>Anaxyrus (Bufo) boreas</i> )	No	1	British Columbia
am-ANVA	Wandering salamander ( <i>Aneides vagrans</i> )	No	1	British Columbia
am-ASMO	Rocky Mountain tailed frog ( <i>Ascaphus montanus</i> )	Yes	1	British Columbia
am-ASTR	Pacific (coastal) tailed frog ( <i>Ascaphus truei</i> )	Yes	1	British Columbia
am-ENES	Monterey ensatina ( <i>Ensatina eschscholtzii</i> )	No	1	British Columbia
am-LICA	North American bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	1	British Columbia
am-LICL	Green frog ( <i>Lithobates (Rana) clamitans</i> )	No	1	British Columbia
am-LIPI	Northern leopard frog ( <i>Lithobates (Rana) pipiens</i> )	No	1	Alberta
am-LISY	Wood frog ( <i>Lithobates (Rana) sylvaticus</i> )	No	1	British Columbia
am-PSMA	Boreal chorus frog ( <i>Pseudacris maculata</i> )	No	1	British Columbia
am-RAAU	Northern red-legged frog ( <i>Rana aurora</i> )	No	1	British Columbia
am-RACA	Cascades frog ( <i>Rana cascadae</i> )	No	1	British Columbia
am-RALU	Columbia spotted frog ( <i>Rana luteiventris</i> )	No	1	British Columbia
am-RAPR	Oregon spotted frog ( <i>Rana pretiosa</i> )	No	1	British Columbia
am-SPIN	Great basin spadefoot toad ( <i>Spea (Scaphiopus) intermontana</i> )	Yes	1	British Columbia
am-TAGR	Rough-skinned newt ( <i>Taricha granulosa</i> )	No	1	British Columbia
am-XELA	African clawed frog ( <i>Xenopus laevis</i> )	No	1	South Africa
ma-HOSA	Human ( <i>Homo Sapiens</i> )	No	1	Netherlands
ma-SOBE	Pacific water/marsh shrew ( <i>Sorex bendirii</i> )	No	1	British Columbia
ma-SOTR	Trowbridge's shrew ( <i>Sorex trowbridgii</i> )	No	1	British Columbia
mo-COFL	Asian clam ( <i>Corbicula fluminea</i> )	No	1	Ontario
mo-DRBU	Quagga mussels ( <i>Dreissena bugensis</i> )	No	1	Ontario
mo-DRPO	Zebra mussels ( <i>Dreissena polymorpha</i> )	No	1	Ontario
te-ANRO	American Eel ( <i>Anguilla rostrata</i> )	Yes	1	Prince Edward Island
te-COCO	Slimy Sculpin ( <i>Cottus cognatus</i> )	Yes	1	Yukon
te-ESLU	Northern Pike ( <i>Esox lucius</i> )	Yes	1	British Columbia
te-GAAC	Three-spined stickleback ( <i>Gasterosteus aculeatus</i> )	Yes	2	British Columbia
te-MIDO	Smallmouth Bass ( <i>Micropterus dolomieu</i> )	Yes	1	British Columbia
te-MISA	Largemouth Bass ( <i>Micropterus salmoides</i> )	Yes	1	British Columbia
te-ONCL	Cutthroat Trout ( <i>Oncorhynchus clarkii</i> )	Yes	2	Alberta and British Columbia
te-ONGO	Pink Salmon ( <i>Oncorhynchus gorbuscha</i> )	Yes	1	British Columbia
te-ONKE	Chum Salmon ( <i>Oncorhynchus keta</i> )	Yes	1	British Columbia
te-ONKI	Coho Salmon ( <i>Oncorhynchus kisutch</i> )	Yes	1	British Columbia
te-ONMY	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	Yes	2	Alberta and British Columbia
te-ONNE	Bull Trout ( <i>Salvelinus confluentus</i> )	Yes	1	British Columbia
te-ONTS	Chinook Salmon ( <i>Oncorhynchus tshawytscha</i> )	Yes	1	British Columbia
te-PRCY	Round Whitefish ( <i>Prosopium cylindraceum</i> )	Yes	1	Alberta
te-SACO	Bull Trout ( <i>Salvelinus confluentus</i> )	Yes	1	British Columbia
te-SAMA	Dolly Varden ( <i>Salvelinus malma</i> )	Yes	1	British Columbia
te-SASA	Atlantic Salmon ( <i>Salmo salar</i> )	Yes	1	Nova Scotia
te-THAR	Arctic grayling ( <i>Thymallus arcticus</i> )	Yes	1	Alberta
te-THPA	Eulachon ( <i>Thaleichthys pacificus</i> )	Yes	1	British Columbia

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

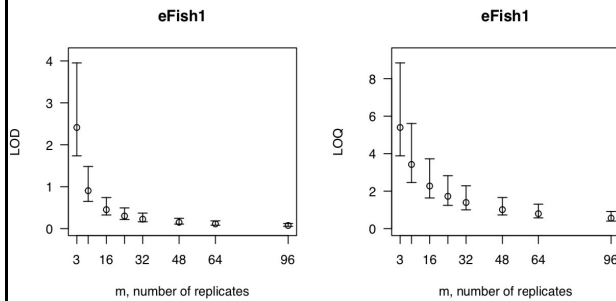
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.32	0.33
2	0.67	0.51
3	1.14	0.7
4	1.68	0.92
5	2.37	1.2
6	3.35	1.62
7	5.02	2.47

Determined using eLowQuant R code<sup>4</sup>.

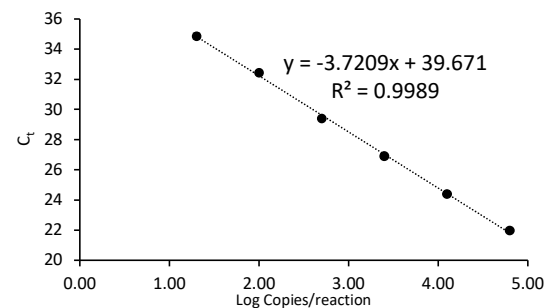


Binomial-Poisson model: No intercept

Determined using eLowQuant R code<sup>4</sup>.

Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 86%

Field Sample Validation

Sample Type	Known			Detected	Location
	Presence	# Samples			
Water	Y	3	Y	Nebraska, United States of America	
Water	Y	80	Y	British Columbia	
Water	Y	36	Y	Alberta	

References

- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. <https://doi.org/10.1371/journal.pone.0213849>
- Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
- Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220
- Klymus, KE, Merkes, CM, Allison, MJ, Goldberg, CS, Helbing, CC, Hunter, ME, Jackson, CA, Lance, RF, Mangan, AM, Monroe, EM, Piaggio, AJ, Stokdyk, JP, Wilson, CC, Richter, CA (2019) Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA. 2020; 2: 271-282. <https://doi.org/10.1002/edn3.29>

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR1	Mitochondrially Encoded 12S RNA gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error