



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 sensitivity¹⁻³.

General eDNA Assay Information

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

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1 95% CI 0.7-1.7 Copies/Rxn LOQ 3.9 95% CI 2.8-6.4 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: QIAcuity

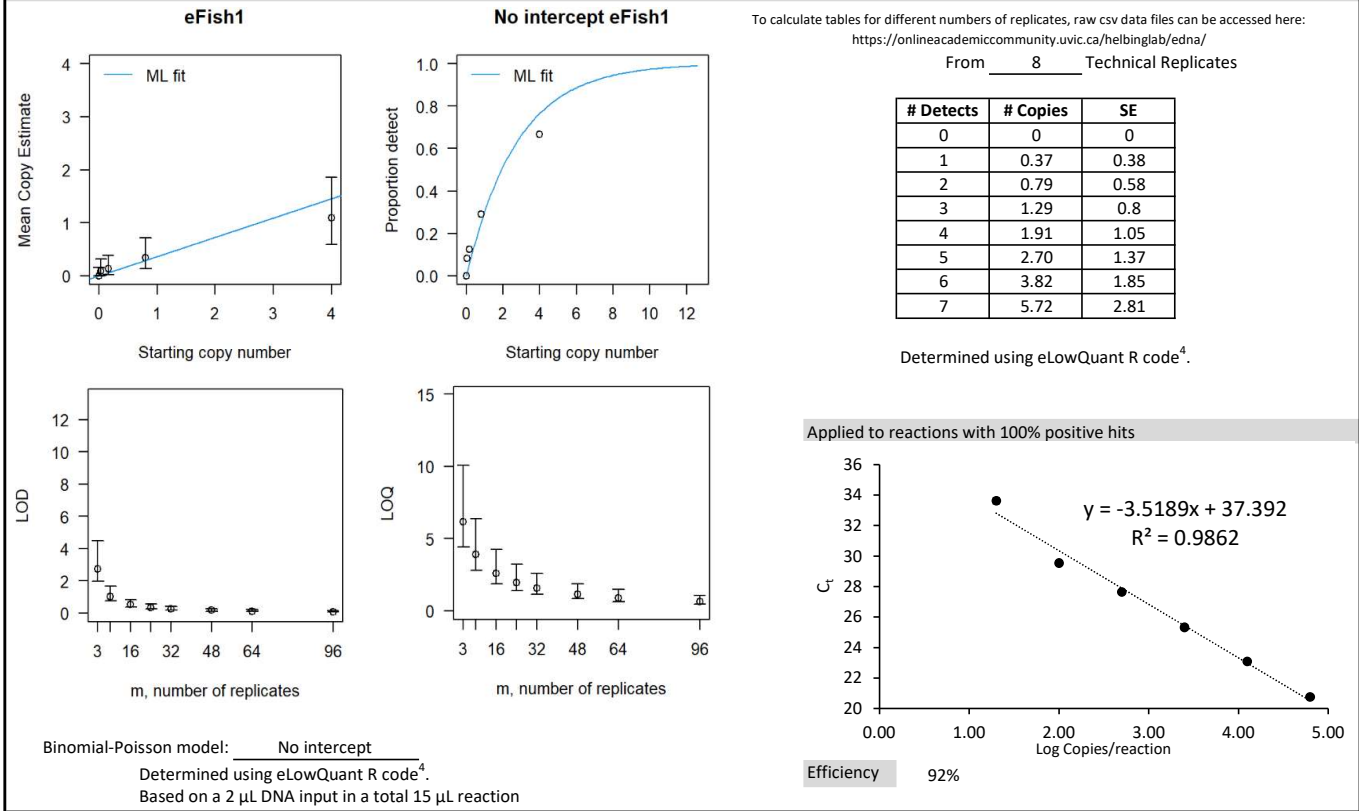
eDNA Assay Specificity Test Information

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



Field Sample Validation

Sample Type	Known			Location
	Presence	# Samples	Detected	
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