



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: Oregon Fairy Shrimp (*Eubranchipus oregonus*) eDNA qPCR Tool: eEUOR1 Gene Target: MT-COI
Species Code: ar-EUOR eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.1 95% CI 0.1-0.1 Copies/Rxn LOQ 0.3 95% CI 0.2-0.4 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 0.8 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: Immolase

eDNA Assay Specificity Test Information

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

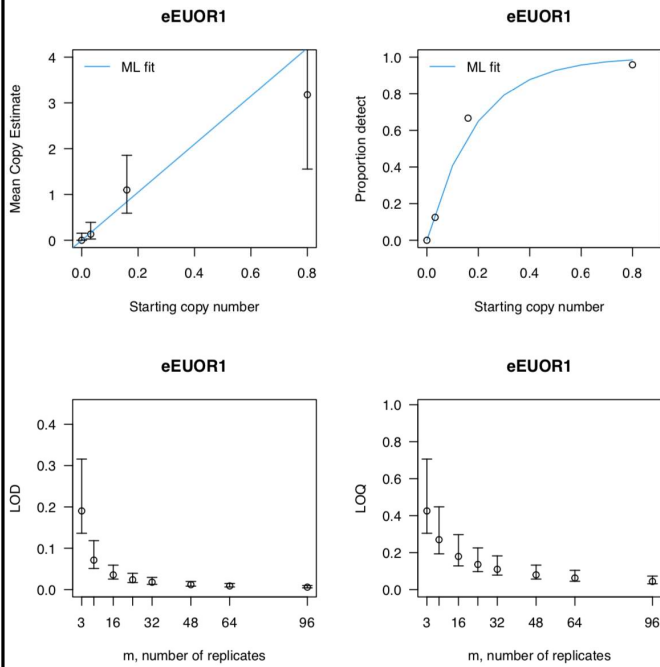
Species	Common Name (Species)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
am-AMMA	Western Tiger Salamander (<i>Ambystoma mavortium</i>)	No	2	British Columbia
am-ANBO	Western Toad (<i>Anaxyrus (Bufo) boreas</i>)	No	2	British Columbia
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	British Columbia
am-PSMA	Boreal chorus frog (<i>Pseudacris maculata</i>)	No	1	British Columbia
ar-EUOR	Oregon Fairy Shrimp (<i>Eubranchipus oregonus</i>)	Yes	5	British Columbia
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-CAAU	Goldfish (<i>Carassius auratus</i>)	No	2	Alberta
te-COCO	Slimy Sculpin (<i>Cottus cognatus</i>)	No	1	British Columbia

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



eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

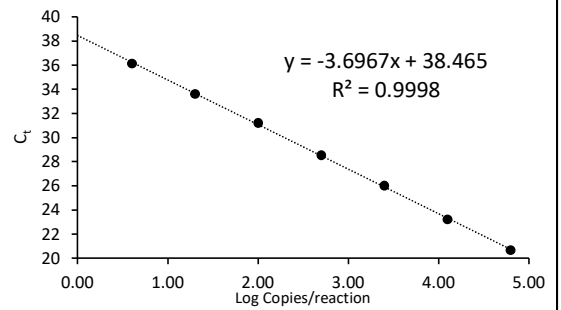


From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.025	0.026
2	0.055	0.04
3	0.09	0.055
4	0.13	0.072
5	0.19	0.095
6	0.26	0.13
7	0.4	0.2

Determined using eLowQuant R code⁴.

Applied to reactions with 100% positive hits



Efficiency 86%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 μ L DNA input in a total 15 μ L reaction

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Y	2	Y	Sugarloaf Mountain, British Columbia
Water	Y	4	Y	University of British Columbia Campus

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COI	Mitochondrial cytochrome oxidase subunit 1 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