

**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: Long toed salamander (*Ambystoma macrodactylum*)
Species Code: am-AMMAeDNA qPCR Tool: eAMMA3
eDNA qPCR Format: TaqManGene Target: MT-ND2
Published in:**eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA**LOD 0.5 95% CI 0.3-1 Copies/Rxn LOQ 1.8 95% CI 1.2-3.7 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 Copies/RxnBinomial-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**

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

Species	Common Name (<i>Species</i>)	# Voucher		
		Detection	Specimens	Sample Sources/Locations
am-AMGR	Northwestern salamander (<i>Ambystoma gracile</i>)	No	1	British Columbia
am-AMMA	Long toed salamander (<i>Ambystoma macrodactylum</i>)	Yes	5	British Columbia
am-AMMV	Eastern tiger salamander (<i>Ambystoma mavortium</i>)	No	1	British Columbia
am-AMME	Axlotl (<i>Ambystoma mexicanum</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	Prince Edward Island
am-ENES	Monterey ensatina (<i>Ensatina eschscholtzii</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-TAGR	Rough-skinned newt (<i>Taricha granulosa</i>)	No	1	British Columbia
am-ANAM	American toad (<i>Anaxyrus americanus</i>)	No	1	Ontario
am-RAAU	Northern red-legged frog (<i>Rana aurora</i>)	No	1	British Columbia
am-LICA	North American bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	British Columbia
am-XELA	African clawed frog (<i>Xenopus laevis</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Domestic cat (<i>Felis catus</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*, 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*, 3: 970-981. doi: 10.1002/edn3.220



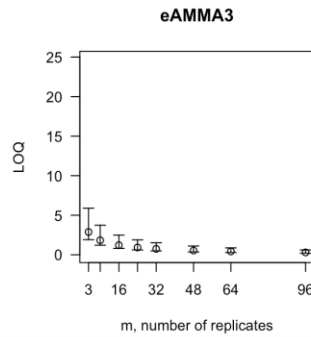
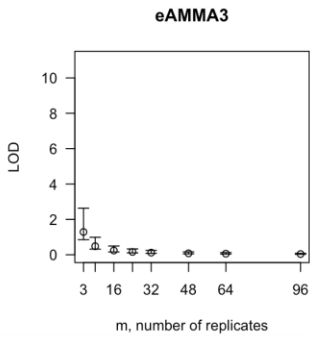
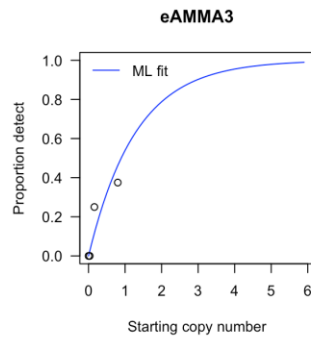
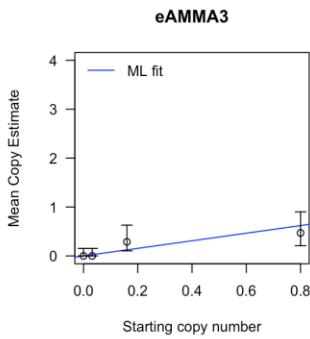
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

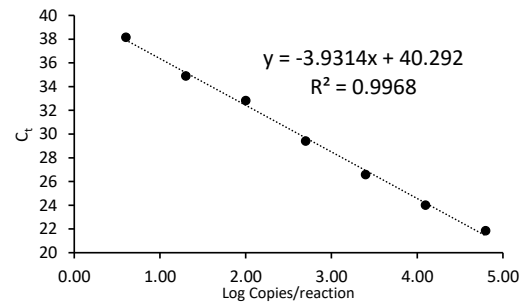
# Detects	# Copies	SE
0	0	0
1	0.17	0.18
2	0.37	0.28
3	0.61	0.39
4	0.89	0.51
5	1.27	0.68
6	1.79	0.92
7	2.68	1.4

Determined using eLowQuant R code⁴.



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

Applied to reactions with 100% positive hits



Efficiency 80%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase subunit 2 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