



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: Western Tiger Salamander (*Ambystoma mavortium*) eDNA qPCR Tool: eAMMV4 Gene Target: MT-Dloop
Species Code: am-AMMV eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.9 Copies/Rxn LOQ 2 95% CI 1.4-3.2 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴.

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>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-AMGR	Northwestern Salamander (<i>Ambystoma gracile</i>)	No	1		British Columbia
am-AMMC	Long-toed Salamander (<i>Ambystoma macrodactylum</i>)	No	1		British Columbia
am-AMMV	Western Tiger Salamander (<i>Ambystoma mavortium</i>)	Yes	5		British Columbia
am-AMTI	Tiger salamander (<i>Ambystoma tigrinum</i>)	Yes	2		British Columbia
am-ANVA	Wandering Salamander (<i>Aneides vagrans</i>)	No	1		British Columbia
am-ENES	Ensatina (<i>Ensatina eschscholtzii</i>)	No	1		British Columbia
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1		British Columbia
am-PLVE	Western Redback Salamander (<i>Plethodon vehiculum</i>)	No	1		British Columbia
am-TAGR	Rough-skinned Newt (<i>Taricha granulosa</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands

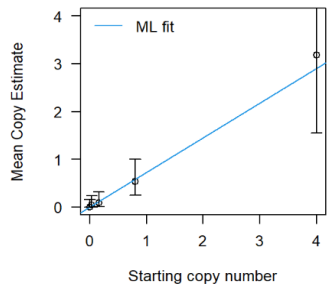
References

- 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, 3: 970-981. doi: 10.1002/edn3.220

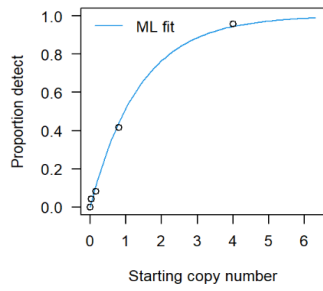


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eAMMV4



No intercept eAMMV4

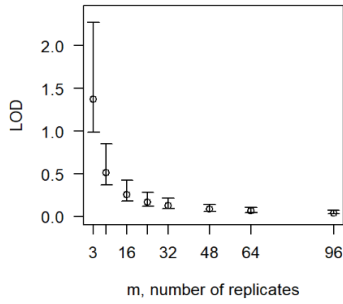


From 8 Technical Replicates

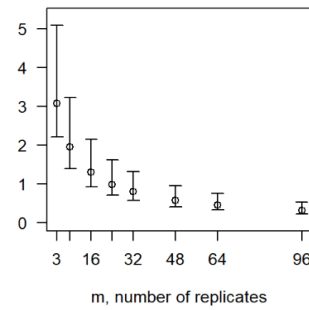
# Detects	# Copies	SE
0	0	0
1	0.18	0.19
2	0.4	0.29
3	0.65	0.4
4	0.96	0.52
5	1.35	0.69
6	1.91	0.93
7	2.87	1.41

Determined using eLowQuant R code⁴.

Limits detect - no intercept eAMMV4

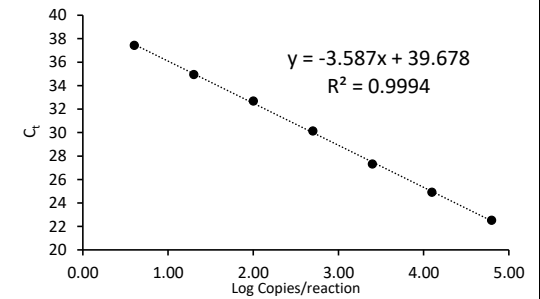


Limits quant - no intercept eAMMV4



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 90%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	13	Y	Southwestern British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-DLOOP	Mitochondrial displacement loop (Dloop) 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