Helbing Lab
eDNA Inventory

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[1-3].

General eDNA Assay Information

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

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.9 Copies/Rxn
LOQ 2.1 95% CI 1.5-3.4 Copies/Rxn
LOB 0 hits/8
$LOQ_{continuous}$ 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].
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-AMGR	Northwestern Salamander (*Ambystoma gracile*)	No	1	British Columbia
am-AMMC	Long-toed Salamander (*Ambystoma macrodactylum*)	No	1	British Columbia
am-AMMV	Western Tiger Salamander (*Ambystoma mavortium*)	Yes	5	British Columbia
am-AMTI	Tiger salamander *(Ambystoma tigrinum)*	Yes	2	British Columbia
am-ANVA	Wandering Salamander (*Aneides vagrans*)	No	1	British Columbia
am-ENES	Ensatina (*Ensatina eschscholtzii*)	No	1	British Columbia
am-LICA	Bullfrog (*Lithobates (Rana) catesbeiana*)	No	1	British Columbia
am-PLVE	Western Redback Salamander (*Plethodon vehiculum*)	No	1	British Columbia
am-TAGR	Rough-skinned Newt (*Taricha granulosa*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands

References

1. 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

2. 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

3. 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

4. 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

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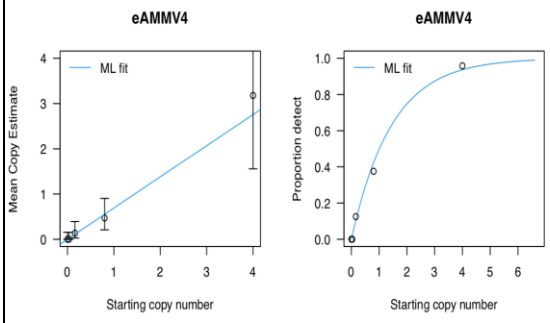
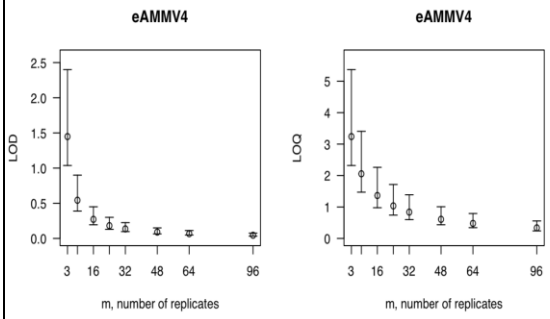
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



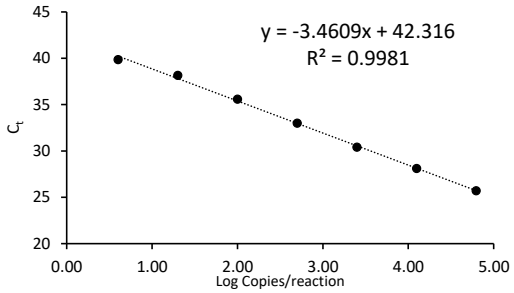
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.19	0.2
2	0.42	0.31
3	0.68	0.42
4	1.01	0.55
5	1.42	0.72
6	2.01	0.98
7	3.02	1.49

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code[4].

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

Efficiency 95%

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 dispalcement 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