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: North American Bullfrog *(Lithobates [Rana] catesbeiana)*
Species Code: am-LICA
eDNA qPCR Tool: eLICA5
eDNA qPCR Format: TaqMan
Gene Target: MT-ND5
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.7 95% CI 0.5-1.2 Copies LOQ 2.8 95% CI 2-4.5 Copies LOB 0 hits/8

$LOQ_{continuous}$ 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code [4]. 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-LICA	Bullfrog (*Lithobates (Rana) catesbeiana*)	Yes	5	British Columbia
am-ANBO	Western Toad (*Anaxyrus (Bufo) boreas*)	No	2	Northwest Territories
am-LICL	Green frog *(Lithobates [Rana] clamitans)*	No	1	British Columbia
am-LIPI	Northern leopard frog *(Lithobates [Rana] pipiens)*	No	2	Alberta
am-LISY	Wood frog *(Lithobates [Rana] sylvaticus)*	No	1	British Columbia
am-PSRE	Pacific chorus frog *(Pseudacris [Hyla] regilla)*	No	1	British Columbia
am-PSMA	Boreal chorus frog *(Pseudacris maculata)*	No	1	Ontario
am-RAAU	Northern red-legged frog *(Rana aurora)*	No	1	British Columbia
am-RACA	Cascades frog *(Rana cascadae)*	No	1	British Columbia
am-RALU	Columbia spotted frog *(Rana luteiventris)*	No	1	British Columbia
am-RAPR	Oregon spotted frog *(Rana pretiosa)*	No	1	British Columbia
am-SPIN	Great basin spadefoot toad *(Spea [Scaphiopus] intermontana)*	No	1	British Columbia
am-XELA	African clawed frog *(Xenopus laevis)*	No	1	South Africa
am-AMGR	Northwestern salamander *(Ambystoma gracile)*	No	1	British Columbia
am-AMMA	Long toed salamander *(Ambystoma macrodactylum)*	No	1	British Columbia
am-AMMV	Eastern tiger salamander *(Ambystoma mavortium)*	No	1	British Columbia
am-ANBO	Western toad *(Anaxyrus [Bufo] boreas)*	No	1	British Columbia
am-ANAM	American toad *(Anaxyrus americanus)*	No	1	British Columbia
ma-CALUfa	Canine (*Canis lupus familiaris*)	No	1	British Columbia
ma-FECA	Cat (*Felis catus*)	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, 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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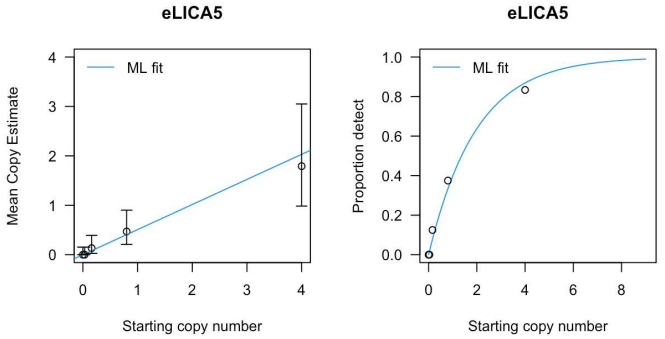
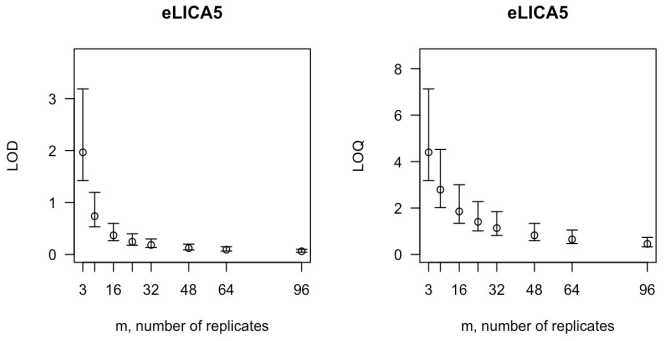
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



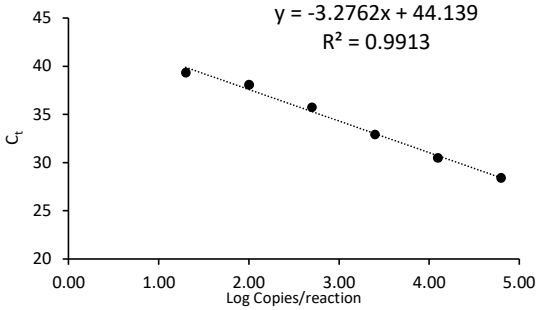
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.26	0.27
2	0.57	0.42
3	0.93	0.57
4	1.37	0.75
5	1.93	0.98
6	2.73	1.32
7	4.10	2.01

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



Efficiency 102%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Freshwater	Y	1	Y	Victoria, BC

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND5	Mitochondrial NADH dehydrogenase subunit 5 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