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: Northern Leopard Frog (*Lithobates(Rana) pipiens)* | eDNA qPCR Tool: eLIPI1 | Gene Target: MT-RNR1
Species Code: am-LIPI | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.8 Copies/Rxn | LOQ 1.8 95% CI 1.3-2.9 Copies/Rxn | 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: 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 (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
am-ANBO	Western Toad (*Anaxyrus (Bufo) boreas*)	No	1	British Columbia
am-ASTR	Pacific Tailed frog (*Ascaphus truei*)	No	1	British Columbia
am-LICA	Bullfrog (*Lithobates (Rana) catesbeiana*)	No	1	British Columbia
am-LICL	Green Frog (*Lithobates (Rana) clamitans*)	No	1	British Columbia
am-LIPI	Northern Leopard Frog (*Lithobates (Rana) pipiens*)	Yes	1	British Columbia
am-PSRE	Pacific Chorus Frog (P*seudacris (Hyla) regilla*)	No	1	British Columbia
am-RAAU	Northern Red-legged Frog (*Rana aurora*)	No	1	British Columbia
am-TAGR	Rough-skinned Newt (*Taricha granulosa*)	No	1	British Columbia
am-XELA	African Clawed Frog (*Xenopus laevis*)	No	1	South Africa
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

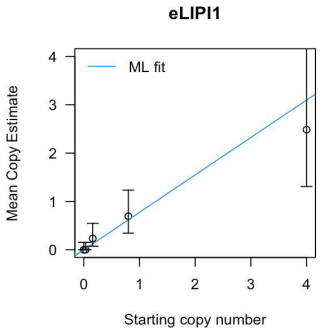
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849

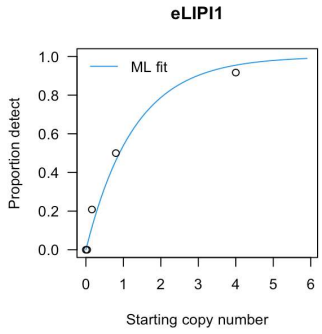
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164

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

Helbing Lab
eDNA Inventory

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



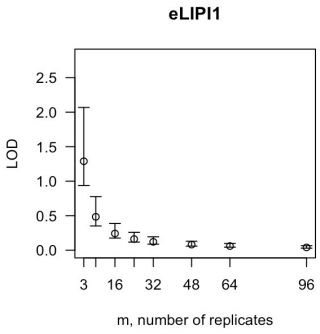


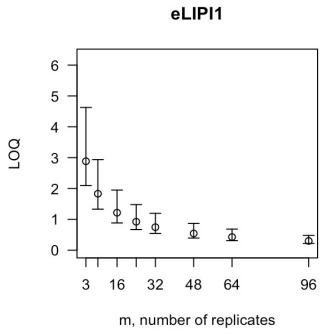
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
https://onlineacademiccommunity.uvic.ca/helbinglab/edna/

From 8 Technical Replicates

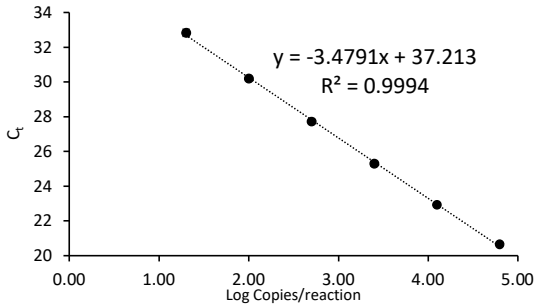
# Detects	# Copies	SE
0	0	0
1	0.17	0.18
2	0.37	0.27
3	0.61	0.37
4	0.9	0.49
5	1.27	0.64
6	1.79	0.86
7	2.69	1.32

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 94%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR1	Mitochondrial 12S ribosomal RNA 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