



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: Wood turtle (*Glyptemys insculpta*)
Species Code: re-GLIN

eDNA qPCR Tool: eGLIN1
eDNA qPCR Format: TaqMan

Gene Target: MT-CYB
Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.7 Copies/Rxn LOQ 1.7 95% CI 1.2-2.7 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 Copies/Rxn

Binomial-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>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-LICA	North American Bullfrog (<i>Lithobates (Rana) catesbeianus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
re-CHPI	Painted turtle (<i>Chrysemys picta</i>)	No	1		Ontario
re-CHSE	Snapping turtle (<i>Chelydra serpentina</i>)	No	1		Nova Scotia
re-COTE	Sharp-Tailed Snake (<i>Contia tenuis</i>)	No	1		British Columbia
re-ELCO	Common Wall Lizard (<i>Podarcis muralis</i>)	No	1		British Columbia
re-EMBL	Blandings turtle (<i>Emydoidea blandingii</i>)	No	1		Nova Scotia
re-GLIN	Wood turtle (<i>Glyptemys insculpta</i>)	Yes	2		Nova Scotia
re-POMU	Common Wall Lizard (<i>Podarcis muralis</i>)	No	1		British Columbia
re-THEL	Western Terrestrial Garter Snake (<i>Thamnophis elegans</i>)	No	1		British Columbia
re-THOR	Northwestern Garter Snake (<i>Thamnophis ordinoides</i>)	No	1		British Columbia
re-THSI	Common Garter Snake (<i>Thamnophis sirtalis</i>)	No	1		British Columbia

References

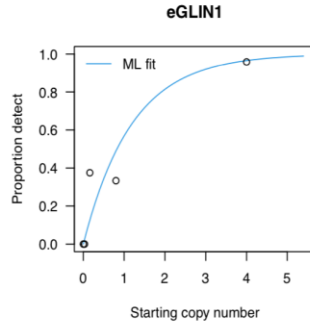
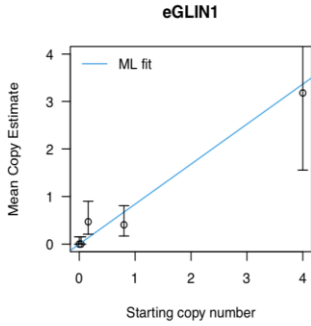
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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, 00: 1-12. doi: 10.1002/edn3.220



eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

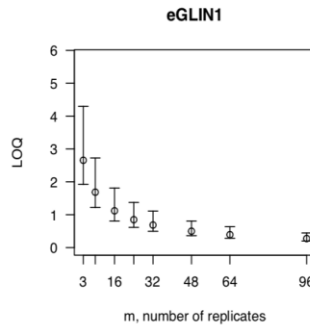
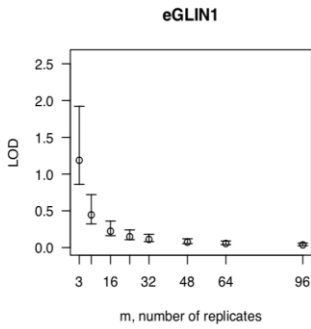
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.16	0.16
2	0.34	0.25
3	0.56	0.34
4	0.82	0.45
5	1.16	0.59
6	1.65	0.8
7	2.47	1.21

Determined using eLowQuant R code⁴.

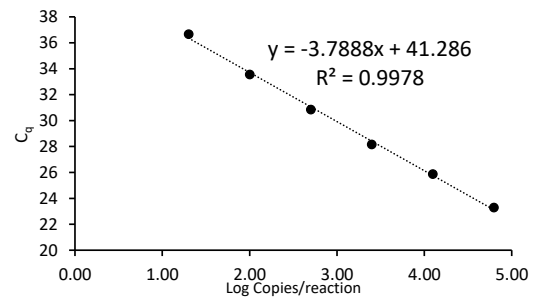


Binomial-Poisson model: No intercept

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 83%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	1	Y	Nova Scotia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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