



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: Ribbonsnake (*Thamnophis sauritus*)
Species Code: re-THSA

eDNA qPCR Tool: eTHSA2
eDNA qPCR Format: TaqMan

Gene Target: MT-CYB
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.7 95% CI 0.5-1.1 Copies/Rxn LOQ 2.7 95% CI 1.9-4.3 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 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: Immolase

eDNA Assay Specificity Test Information

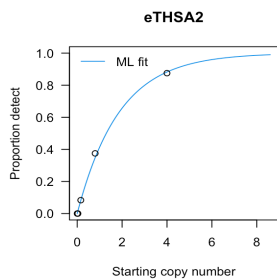
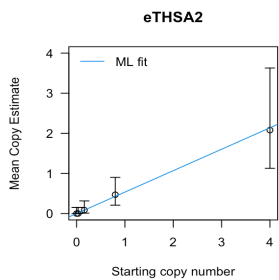
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (<i>Species</i>)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-CALUfa	Canine (<i>Canis lupis familiaris</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo Sapiens</i>)	No	1	Netherlands
re-CHPI	Painted turtle (<i>Chrysemys picta</i>)	No	2	Ontario
re-CHSE	Snapping turtle (<i>Chelydra serpentina</i>)	No	2	Nova Scotia
re-CLGU	Spotted Turtle (<i>Clemmys guttata</i>)	No	2	Ontario
re-COCOMO	Western yellow-bellied racer (<i>Coluber constrictor mormon</i>)	No	2	British Columbia
re-DIPU	Ring-necked Snake (<i>Diadophis punctatus</i>)	No	2	Nova Scotia
re-EMBL	Blandings turtle (<i>Emydoidea blandingii</i>)	No	2	Nova Scotia
re-GLIN	Wood turtle (<i>Glyptemys insculpta</i>)	No	2	Nova Scotia
re-GRGE	Northern map turtle (<i>Graptemys geographica</i>)	No	2	Ontario
re-HEPL	Eastern hog-nosed snake (<i>Heterodon platirhinos</i>)	No	2	Ontario
re-NESI	Northern Watersnake (<i>Nerodia sipedon sipedon</i>)	No	2	Ontario
re-PASP	Gray ratsnake (<i>Pantherophis spiloides</i>)	No	2	Ontario
re-PAVU	Eastern Foxsnake (<i>Pantherophis vulpinus (P. gloydi)</i>)	No	2	Ontario
re-PICA	Gopher snake (<i>Pituophis catenifer</i>)	No	2	British Columbia
re-SICA	Massasauga (<i>Sistrurus catenatus</i>)	No	2	Ontario
re-STOD	Common musk turtle (<i>Sternotherus odoratus</i>)	No	2	Ontario
re-THSA	Ribbonsnake (<i>Thamnophis sauritus</i>)	Yes	8	Nova Scotia
re-THSI	Common Garter Snake (<i>Thamnophis sirtalis</i>)	No	2	Nova Scotia

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, 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

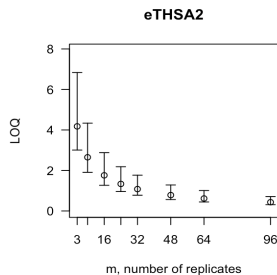
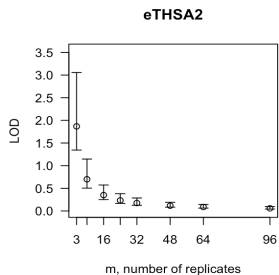
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



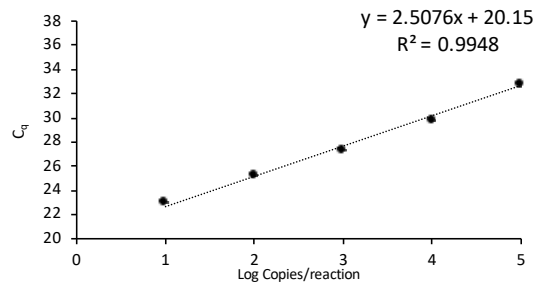
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.25	0.255
2	0.538	0.396
3	0.879	0.541
4	1.3	0.71
5	1.83	0.93
6	2.59	1.26
7	3.89	1.91

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Efficiency 90%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

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