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*)	eDNA qPCR Tool:	eTHSA2	Gene Target:	MT-CYB
Species Code:	re-THSA	eDNA qPCR Format:	TaqMan	Published in:	

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD	0.3	95% CI	0.2-0.5	Copies/Rxn	LOQ	1.0	95% CI	0.5-1.7 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

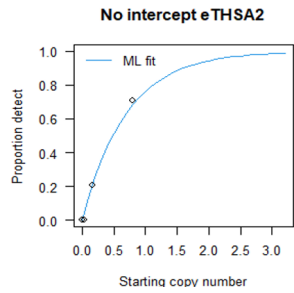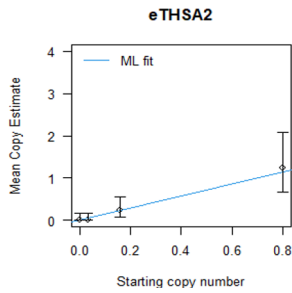### eDNA Assay Specificity Test Information

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

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

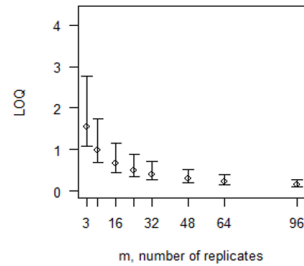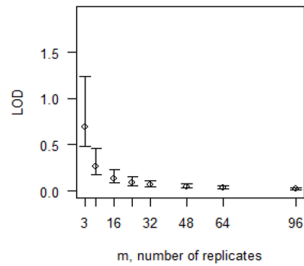## eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eTHSA2

No intercept eTHSA2

Limits detect - no intercept eTHSA2

Limits quant - no intercept eTHSA2

From　　8　　Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.083	0.119
2	0.184	0.148
3	0.297	0.187
4	0.437	0.244
5	0.626	0.33
6	0.88	0.455
7	1.319	0.709

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits

$y = 2.2247x + 18.657$
$R^2 = 0.9985$

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　　106%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

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