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: Sharp-Tailed Snake (*Contia tenuis*)   eDNA qPCR Tool: eCOTE3   Gene Target: MT-ND4
Species Code: re-COTE   eDNA qPCR Format: TaqMan   Published in: 5

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD __0.4__   95% CI __0.3-0.6__ Copies/Rxn   LOQ __1.3__   95% CI __1-2.2__ 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-LICA	Bullfrog (*Lithobates (Rana) catesbeiana*)	No	2	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
re-COTE	Sharp-Tailed Snake (*Contia tenuis*)	Yes	5	British Columbia
re-ELCO	Alligator Lizard (*Elgaria coerulea*)	No	1	British Columbia
re-POMU	Common Wall Lizard (*Podarcis muralis*)	No	1	British Columbia
re-THEL	Western Terrestrial Garter Snake (*Thamnophis elegans*)	No	1	British Columbia
re-THOR	Northwestern Garter Snake (*Thamnophis ordinoides*)	No	1	British Columbia
re-THSI	Common Garter Snake (*Thamnophis sirtalis*)	No	1	British Columbia

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. 2020; 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

5. Matthias, L, Allison, MJ, Maslovat, CY, Hobbs, J, and Helbing, CC (2021) Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. Ecological Indicators, 131. https://doi.org/10.1016/j.ecolind.2021.108187
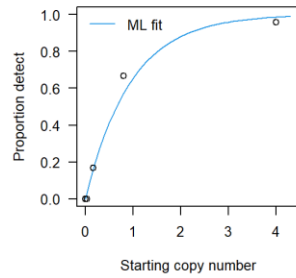
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eCOTE3
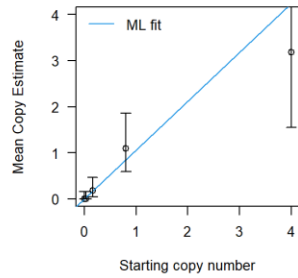
eCOTE3

From   8   Technical Replicates

# Detects	# Copies	SE
0	0.00	0.00
1	0.13	0.13
2	0.27	0.20
3	0.44	0.27
4	0.65	0.36
5	0.92	0.47
6	1.31	0.63
7	1.96	0.96

Determined using eLowQuant R code[4].
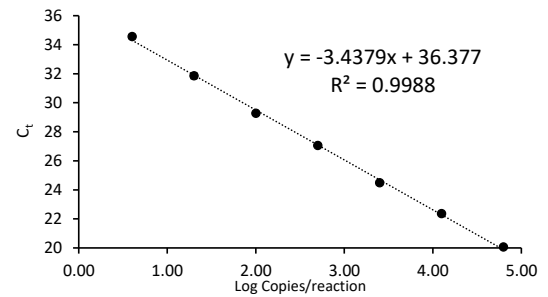
eCOTE3
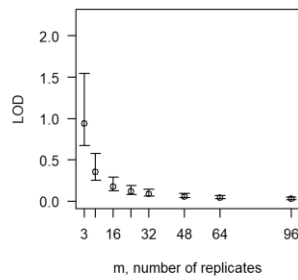
eCOTE3
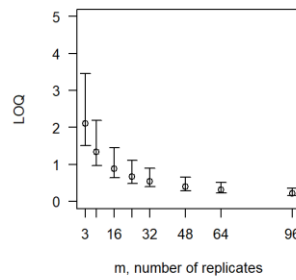
Binomial-Poisson model: ___No intercept___
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits

$y = -3.4379x + 36.377$
$R^2 = 0.9988$

Efficency		95%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH subunit 4 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