



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: 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 0.2 95% CI 0.1-0.3 Copies/Rxn LOQ 0.7 95% CI 0.5-1.2 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: 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		Sample Sources/Locations
			Specimens		
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	2		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
re-COTE	Sharp-Tailed Snake (<i>Contia tenuis</i>)	Yes	5		British Columbia
re-ELCO	Alligator Lizard (<i>Elgaria coerulea</i>)	No	1		British Columbia
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

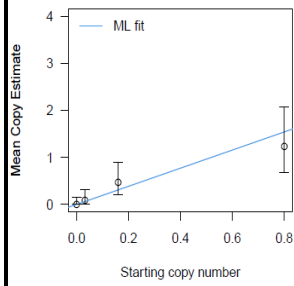
References

- 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, 3: 970-981. doi: 10.1002/edn3.220
- 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>

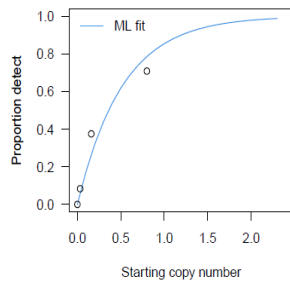


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eCOTE3



eCOTE3

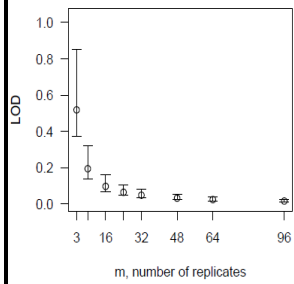


From 8 Technical Replicates

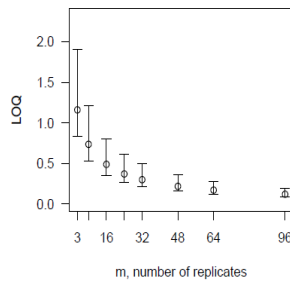
# Detects	# Copies	SE
0	0	0
1	0.07	0.07
2	0.15	0.11
3	0.24	0.15
4	0.36	0.2
5	0.51	0.26
6	0.72	0.35
7	1.08	0.53

Determined using eLowQuant R code⁴.

eCOTE3



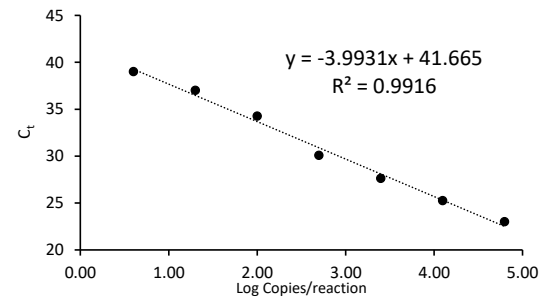
eCOTE3



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 78%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
ACO Swab	Y	22	Y	Salt Spring Island, British Columbia
Soil	Y	14	Y	Salt Spring Island, British Columbia

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