



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: Northern Pacific rattlesnake (*Crotalus oreganus*) eDNA qPCR Tool: eCROR1 Gene Target: MT-CYB
Species Code: re-CROR eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1.2 95% CI 0.8-2 Copies/Rxn LOQ 4.5 95% CI 3.2-7.7 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)

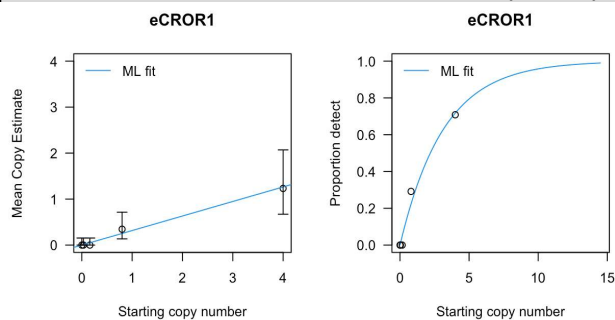
Species	Common Name (Species)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
re-CHBO	Rubber Boa (<i>Charina bottae</i>)	No	1	British Columbia
re-CHSE	Snapping Turtle (<i>Chelydra serpentina</i>)	No	1	Ontario
re-COCN	Eastern Racer (<i>Coluber constrictor</i>)	No	1	British Columbia
re-COTE	Sharp-Tailed Snake (<i>Contia tenuis</i>)	No	1	British Columbia
re-CROR	Northern Pacific rattlesnake (<i>Crotalus oreganus</i>)	Yes	6	British Columbia
re-ELCO	Alligator Lizard (<i>Elgaria coerulea</i>)	No	1	British Columbia
re-GLIN	Wood Turtle (<i>Glyptemys insculpta</i>)	No	1	Nova Scotia
re-PICA	Gopher Snake (<i>Pituophis catenifer</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



eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

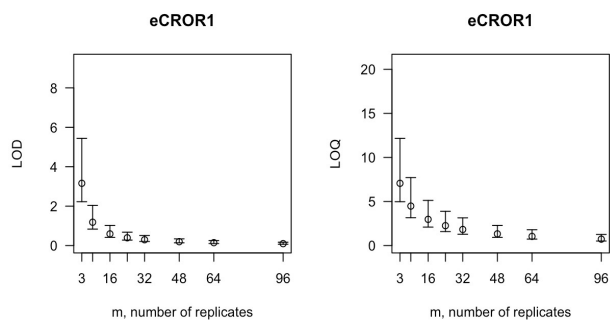


To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.42	0.43
2	0.91	0.67
3	1.49	0.92
4	2.19	1.21
5	3.1	1.59
6	4.39	2.15
7	6.58	3.28

Determined using eLowQuant R code⁴.

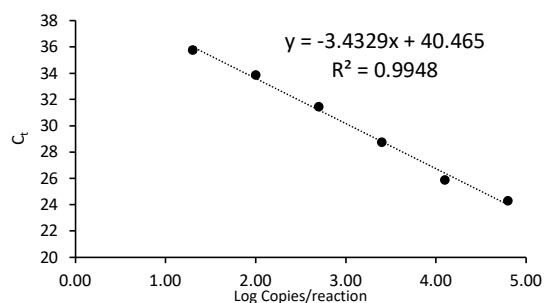


Binomial-Poisson model: No intercept

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 96%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Soil/Sand	Y	10	1	British Columbia
Rock Swab	Y	5	N	British Columbia

Release of DNA into environment appears to be an impediment to reliable detection

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