

### 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<sup>1-3</sup>.

#### 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<sub>continuous</sub> 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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)

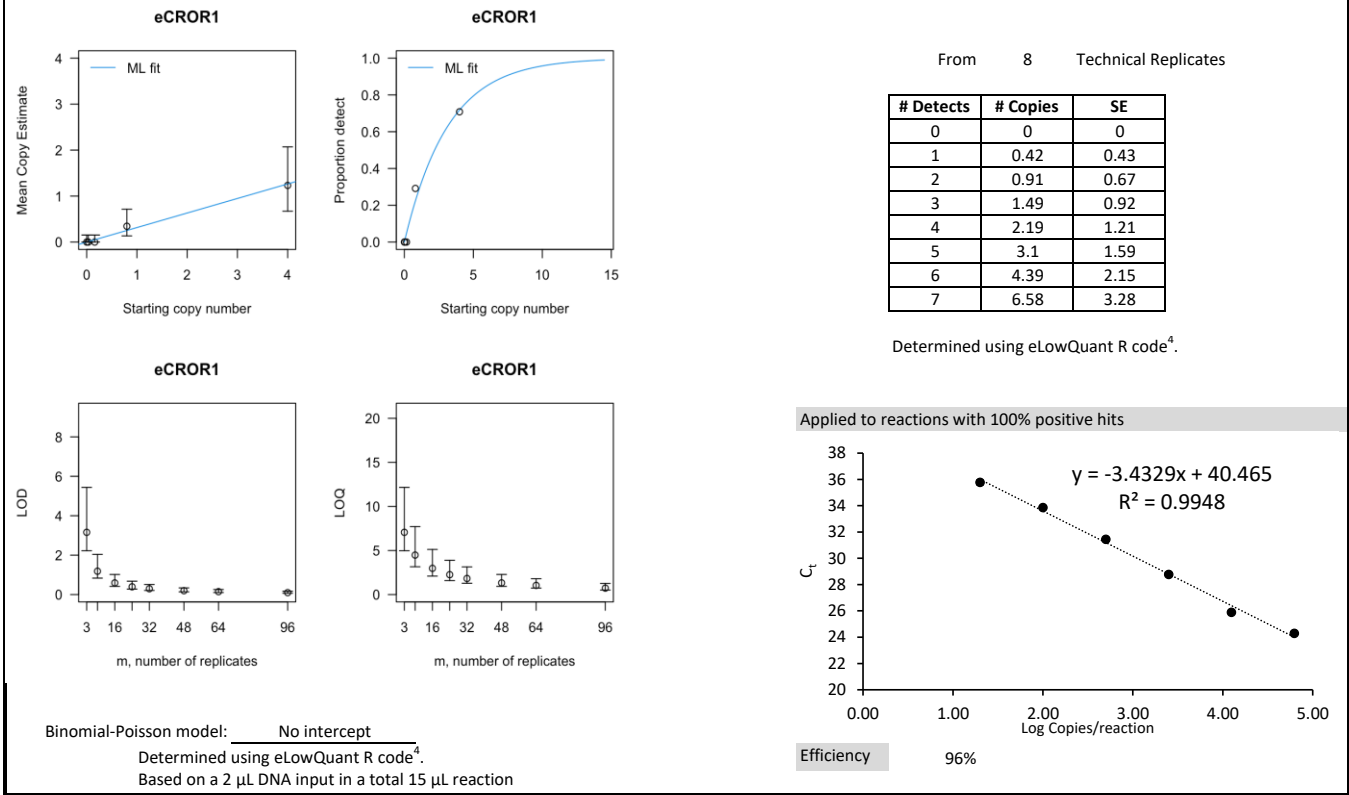
Species	Common Name ( <i>Species</i> )	Detection	# Voucher Specimens	Sample Sources/Locations
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands
re-CHBO	Rubber Boa ( <i>Charina bottae</i> )	No	1	British Columbia
re-CHSE	Snapping Turtle ( <i>Chelydra serpentina</i> )	No	1	Ontario
re-COCN	Eastern Racer ( <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



Field Sample Validation

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

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