



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: Eastern Foxsnake (*Pantherophis vulpinus* (*P. gloydi*)) eDNA qPCR Tool: ePAVU5 Gene Target: MT-ND4
Species Code: re-PAVU eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-1.1 Copies/Rxn LOQ 1.9 95% CI 1.2-4.1 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		
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo Sapiens</i>)	No	1		Netherlands
re-CHPI	Painted turtle (<i>Chrysemys picta</i>)	No	2		Ontario
re-CHSE	Snapping turtle (<i>Chelydra serpentina</i>)	No	2		Ontario
re-CLGU	Spotted Turtle (<i>Clemmys guttata</i>)	No	2		Ontario
re-DIPU	Ring-necked Snake (<i>Diadophis punctatus</i>)	No	2		Nova Scotia
re-EMBL	Blandings turtle (<i>Emydoidea blandingii</i>)	No	2		Ontario
re-GLIN	Wood turtle (<i>Glyptemys insculpta</i>)	No	1		Nova Scotia
re-GRGE	Northern map turtle (<i>Graptemys geographica</i>)	No	2		Ontario
re-HEPL	Eastern hog-nosed snake (<i>Heterodon platirhinos</i>)	No	2		Ontario
re-LATR	Milksnake (<i>Lampropeltis triangulum</i>)	No	2		Ontario
re-NESI	Northern Watersnake (<i>Nerodia sipedon sipedon</i>)	No	2		Ontario
re-PASP	Gray ratsnake (<i>Pantherophis spiloides</i>)	No	2		Ontario
re-PAVU	Eastern Foxsnake (<i>Pantherophis vulpinus</i> (<i>P. gloydi</i>))	Yes	6		Ontario
re-SICA	Massasauga (<i>Sistrurus catenatus</i>)	No	2		Ontario
re-STOD	Common musk turtle (<i>Sternotherus odoratus</i>)	No	2		Ontario
re-THSA	Ribbonsnake (<i>Thamnophis sauritus</i>)	No	2		Ontario
re-THSI	Common Garter Snake (<i>Thamnophis sirtalis</i>)	No	2		Ontario and Nova Scotia

References

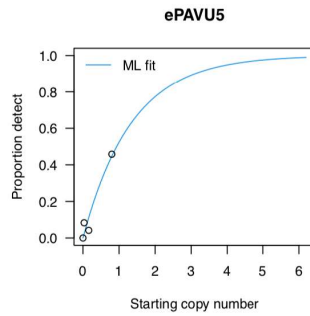
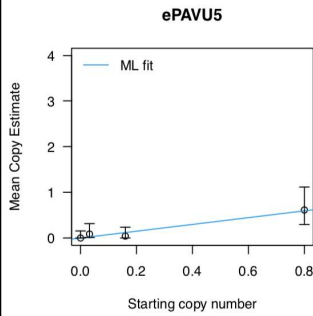
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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

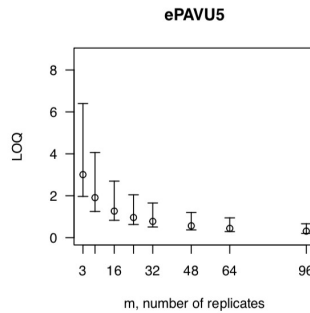
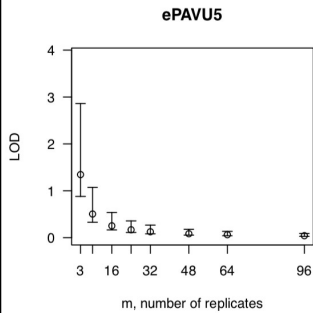
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



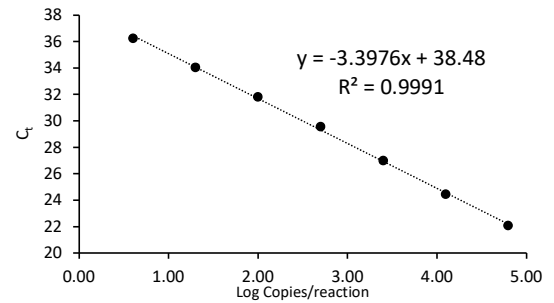
# Detects	# Copies	SE
0	0	0
1	0.18	0.19
2	0.39	0.29
3	0.63	0.41
4	0.93	0.54
5	1.32	0.71
6	1.87	0.97
7	2.8	1.47

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 97%

Field Sample Validation

Known
Sample Type Presence # Samples Detected Location

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