



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: Spot-tailed earless lizard (*Holbrookia sp*) eDNA qPCR Tool: eHolbrookia1 Gene Target: MT-ND2-trnW-trnA
Species Code: re-Holbrookia eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.8 Copies LOQ 2 95% CI 1.4-3.2 Copies 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: Qiacity

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)	# Voucher		
		Detection	Specimens	Sample Sources/Locations
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
re-ASSE	Six-lined racerunner (<i>Aspidoscelis sexlineatus</i>)	No	1	Texas
re-CHBO	Rubber boa (<i>Charina bottae</i>)	No	1	British Columbia
re-CHPI	Painted turtle (<i>Chrysemys picta</i>)	No	1	British Columbia
re-CHSE	Snapping turtle (<i>Chelydra serpentina</i>)	No	1	British Columbia
re-CLGU	Spotted turtle (<i>Clemmys guttata</i>)	No	1	British Columbia
re-COCO	Western yellow-bellied racer (<i>Coluber constrictor</i>)	No	1	British Columbia
re-COTE	Sharp-tailed snake (<i>Contia tenuis</i>)	No	1	British Columbia
re-ELCO	Alligator lizard (<i>Elgaria coerulea</i>)	No	1	British Columbia
re-HOLA	Plateau spot-tailed earless lizard (<i>Holbrookia lacerata</i>)	Yes	3	Texas
re-HOSU	Tamaulipin spot-tailed earless lizard (<i>Holbrookia subcaudalis</i>)	Yes	4	Texas
re-POMU	Common wall lizard (<i>Podarcis muralis</i>)	No	1	British Columbia
re-SCOL	Texas spiny lizard (<i>Selaporus olivaceus</i>)	No	2	Texas

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

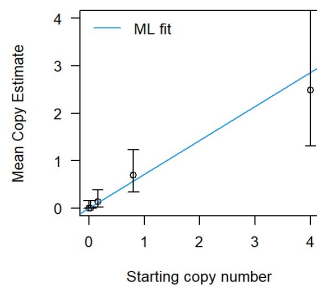
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

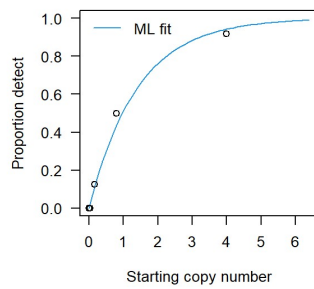
# Detects	# Copies	SE
0	0	0
1	0.19	0.19
2	0.40	0.30
3	0.66	0.40
4	0.97	0.53
5	1.37	0.69
6	1.94	0.94
7	2.91	1.43

Determined using eLowQuant R code⁴.

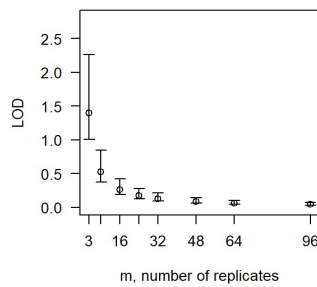
eHolbrookia1



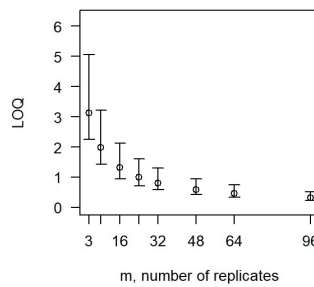
eHolbrookia1



eHolbrookia1



eHolbrookia1

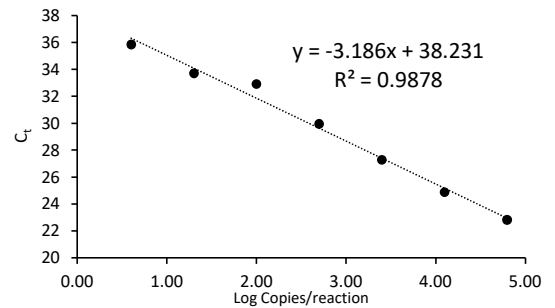


Binomial-Poisson model: No intercept

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 106%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Soil	Y	18	67%	Texas

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2-trnW-trnA	Mitochondrial NADH dehydrogenase 2, tRNA-W and tRNA-A genes
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