Helbing Lab
eDNA Inventory

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[1-3].

General eDNA Assay Information

Target Species: *Spot-tailed earless lizard (Holbrookia sp)*	eDNA qPCR Tool: eHolbrookia1	Gene Target: MT-ND1
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 code[4].   When the LOQ < LOD, use the LOD for the LOQ.   Enzyme: Qiacuity

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

References

1. 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

2. 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

3. 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

4. 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
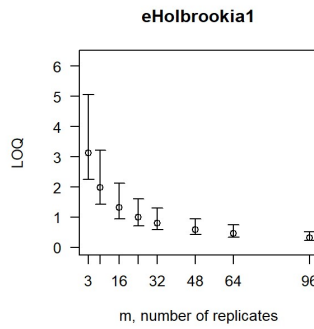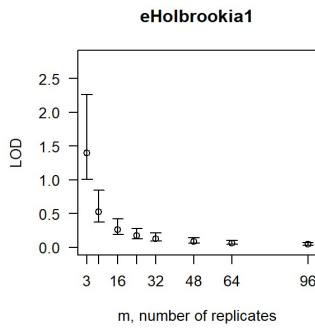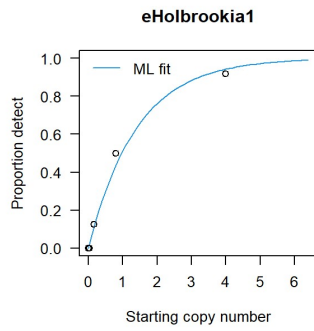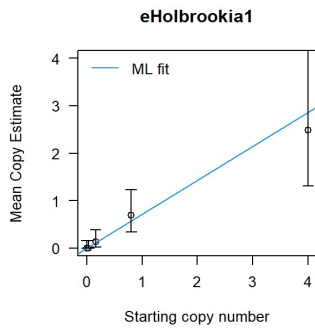
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eHolbrookia1

eHolbrookia1

eHolbrookia1

eHolbrookia1

Binomial-Poisson model: ___No intercept___

Determined using eLowQuant R code[4].

Based on a 2 µL DNA input in a total 15 µL reaction

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 code[4].

Applied to reactions with 100% positive hits

$$y = -3.186x + 38.231$$
$$R^2 = 0.9878$$
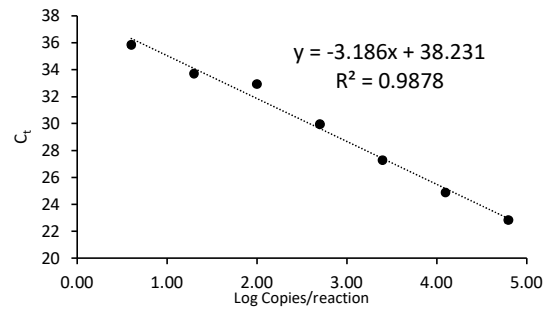
Efficiency	100%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 1 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