Helbing Lab

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: Northern myotis *(Myotis septentrionalis)*
Species Code: ma-MYSE
eDNA qPCR Tool: eMYSE3
eDNA qPCR Format: TaqMan
Gene Target: MT-COII
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1-0.3 Copies/Rxn
LOQ 0.6 95% CI 0.4-1.1 Copies/Rxn
LOB 0 hits/8
$LOQ_{continuous}$ 4 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)

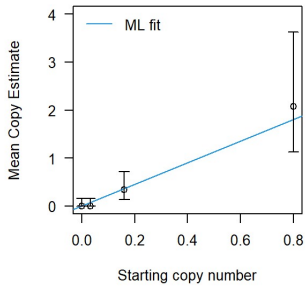
Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-ALAL	Moose (Alces alces)	No	1	British Columbia
ma-ANPA	Pallid bat (Antrozous pallidus)	No	1	British Columbia
ma-CALUfa	Dog (Canis lupus familiaris)	No	1	British Columbia
ma-CEEL	Red deer (Cervus elaphus)	No	1	British Columbia
ma-EPFU	Big brown bat (Eptesicus fuscus)	No	1	British Columbia
ma-FECA	Cat (domestic) (Felis catus)	No	1	British Columbia
ma-HOSA	Human (Homo sapiens)	No	1	Netherlands
ma-LABO	Eastern red bat (Lasiurus borealis)	No	1	Alberta
ma-LOCA	River otter (Lontra canadensis)	No	1	British Columbia
ma-MYCA	Californian myotis (Myotis californicus)	No	1	British Columbia
ma-MYCI	Western Small-footed myotis (Myotis ciliolabrum)	No	1	British Columbia
ma-MYEV	Long-eared myotis (Myotis evotis)	No	1	British Columbia
ma-MYLU	Little Brown myotis (Myotis lucifugus)	No	2	British Columbia
ma-MYSE	Northern myotis (Myotis septentrionalis)	Yes	5	British Columbia
ma-MYTH	Fringed myotis (Myotis thysanodes)	No	2	British Columbia
ma-MYVO	Long-legged myotis (Myotis volans)	No	2	British Columbia
ma-MYYU	Yuma myotis (Myotis yumanensis)	No	2	British Columbia
ma-ODHE	Mule deer (Odocoileus hemionus)	No	1	British Columbia
ma-ODVI	White-tailed deer (Odocoileus virginianus)	No	1	British Columbia
ma-SOBE	Pacific water/marsh shrew (Sorex bendirii)	No	2	Washington
ma-SONA	Cardilleran water shrew (Sorex navigator)	No	2	Washington
ma-TABR	Brazilian free-tailed bat (Tadarida brasiliensis)	No	2	British Columbia and Saint Kitts & Nevis
ma-URAM	American black bear (Ursus americanus)	No	1	British Columbia
ma-URAR	Grizzly bear (Ursus arctos)	No	1	British Columbia

References

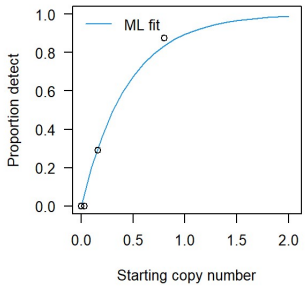
1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. https://doi.org/10.1002/edn3.82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

eMYSE3



No intercept eMYSE3



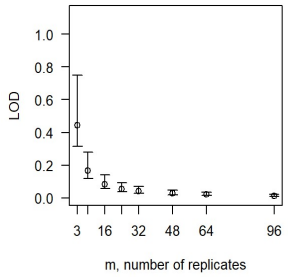
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
https://onlineacademiccommunity.uvic.ca/helbinglab/edna/

From 8 Technical Replicates

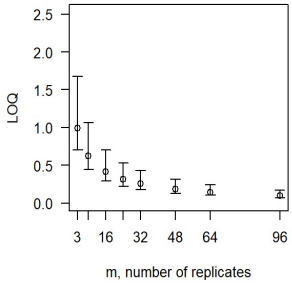
# Detects	# Copies	SE
0	0.00	0.00
1	0.059	0.061
2	0.13	0.094
3	0.21	0.13
4	0.31	0.17
5	0.44	0.22
6	0.62	0.30
7	0.92	0.46

Determined using eLowQuant R code[4].

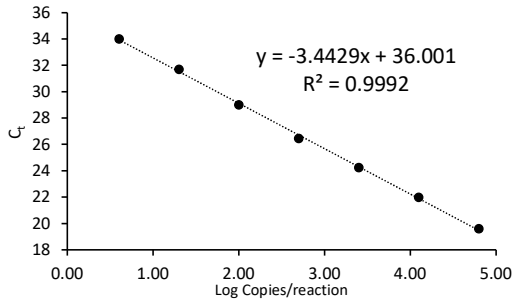
Limits detect - no intercept eMYSE3



Limits quant - no intercept eMYSE3



Applied to reactions with 100% positive hits



Efficiency 95%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COII	Mitochondrial cytochrome oxidase subunit 2 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