



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: Little brown bat (*Myotis lucifugus*) eDNA qPCR Tool: eMYLU4 Gene Target: MT-TL1
Species Code: ma-MYLU eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.1 95% CI 0.1-0.2 Copies/Rxn LOQ 0.5 95% CI 0.4-0.9 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: 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 (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-MYLU	Little brown bat (<i>Myotis lucifugus</i>)	Yes	4		British Columbia
ma-MYCA	Californian bat (<i>Myotis californicus</i>)	No	1		British Columbia
ma-MYCI	Western Small-footed bat (<i>Myotis ciliolabrum</i>)	No	1		British Columbia
ma-MYEV	Long-eared bat (<i>Myotis evotis</i>)	No	1		British Columbia
ma-MYTH	Fringed bat (<i>Myotis thysanodes</i>)	No	1		British Columbia
ma-MYVO	Long-legged bat (<i>Myotis volans</i>)	No	1		British Columbia
ma-MYYU	Yuma bat (<i>Myotis yumanensis</i>)	No	4		British Columbia
ma-MYSE	Northern myotis bat (<i>Myotis septentrionalis</i>)	No	1		British Columbia
ma-TABR	Brazilian free-tailed bat (<i>Tadarida brasiliensis</i>)	No	1		British Columbia
ma-EPFU	Big brown bat (<i>Eptesicus fuscus</i>)	No	1		Alberta
ma-LABO	Eastern red bat (<i>Lasiurus borealis</i>)	No	1		Alberta
ma-USAR	Grizzly bear (<i>Ursus arctos</i>)	No	1		British Columbia
ma-URAM	American black bear (<i>Ursus americanus</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
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-ALAL	Moose (<i>Alces alces</i>)	No	1		British Columbia
ma-ANPA	Pallid bat (<i>Antrozous pallidus</i>)	No	1		British Columbia
ma-CEEL	Red deer (<i>Cervus elaphus</i>)	No	1		British Columbia
ma-ODHE	Mule deer (<i>Odocoileus hemionus</i>)	No	1		British Columbia
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	No	1		Washington
ma-SOBE	Pacific water/marsh shrew (<i>Sorex bendirii</i>)	No	1		Washington
ma-SONA	Cardilleran water shrew (<i>Sorex navigator</i>)	No	1		Washington
ma-PHVI	Harbour seal (<i>Phoca vitulina</i>)	No	1		British Columbia
ma-PHPH	Harbour porpoise (<i>Phocoena phocoena</i>)	No	1		British Columbia
ma-LOCA	River otter (<i>Lontra canadensis</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, 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



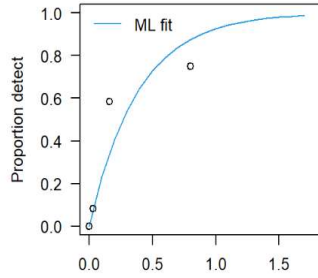
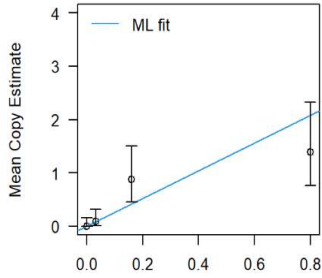
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eMYLU4

No intercept eMYLU4

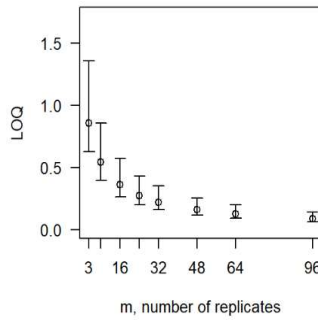
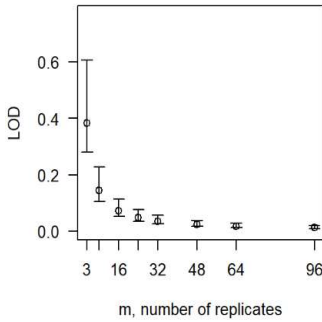
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

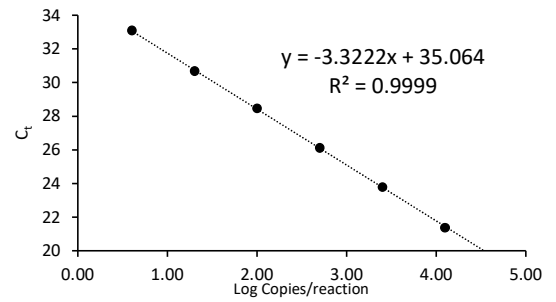


# Detects	# Copies	SE
0	0	0
1	0.05	0.05
2	0.11	0.08
3	0.18	0.11
4	0.27	0.14
5	0.38	0.19
6	0.53	0.26
7	0.80	0.39

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Efficiency 100%

Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-TL1	Mitochondrial tRNA Leucine 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