



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: Brazilian Free-Tailed Bat (*Tadarida brasiliensis*) eDNA qPCR Tool: eTABR1 Gene Target: MT-CYB
Species Code: ma-TABR eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.7 Copies/Rxn LOQ 1.7 95% CI 1.2-2.8 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		
am-LICA	Bullfrog (<i>Lithobates catesbeiana</i>)	No	1		British Columbia
ma-ANPA	Pallid Bat (<i>Antrozous pallidus</i>)	No	1		British Columbia
ma-COTO	Townsend's Big-eared Bat (<i>Corynorhinus townsendii</i>)	No	1		British Columbia
ma-EPFU	Big Brown Bat (<i>Eptesicus fuscus</i>)	No	1		British Columbia
ma-EUMA	Spotted bat (<i>Euderma maculatum</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
ma-LACI	Hoary Bat (<i>Lasiurus cinereus</i>)	No	1		British Columbia
ma-LANO	Silver-haired Bat (<i>Lasionycteris noctivagans</i>)	No	1		British Columbia
ma-MYCA	Californian Myotis (<i>Myotis californicus</i>)	No	1		British Columbia
ma-MYCI	Western Small-footed Myotis (<i>Myotis ciliolabrum</i>)	No	1		British Columbia
ma-MYEV	Long-eared Myotis (<i>Myotis evotis</i>)	No	1		British Columbia
ma-MYLU	Little Brown Myotis (<i>Myotis lucifugus</i>)	No	1		British Columbia
ma-MYTH	Fringed Myotis (<i>Myotis thysanodes</i>)	No	1		British Columbia
ma-MYVO	Long-legged Myotis (<i>Myotis volans</i>)	No	1		British Columbia
ma-MYYU	Yuma Myotis (<i>Myotis yumanensis</i>)	No	1		British Columbia
ma-TABR	Brazilian Free-Tailed Bat (<i>Tadarida brasiliensis</i>)	Yes	5		United States

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. 2020; 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 (2021) 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



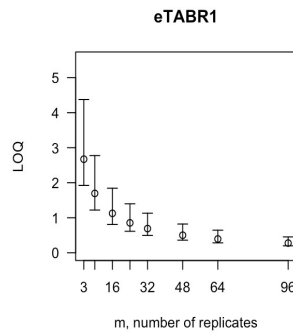
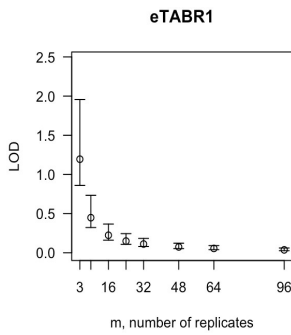
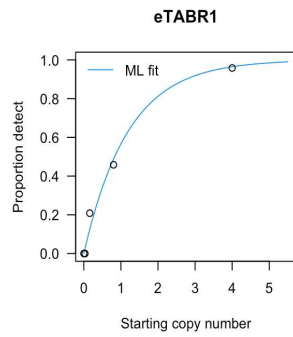
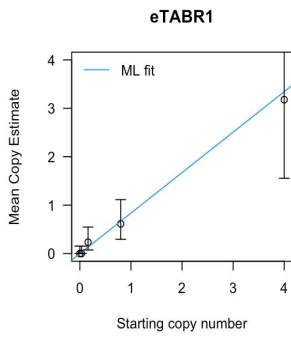
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

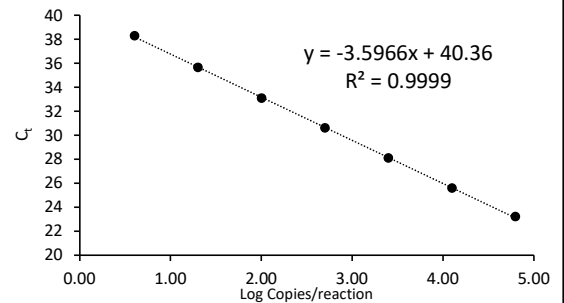
# Detects	# Copies	SE
0	0	0
1	0.16	0.16
2	0.34	0.25
3	0.56	0.35
4	0.83	0.45
5	1.17	0.59
6	1.66	0.8
7	2.49	1.22

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 ≥ 95% positive hits



Efficiency 90%

Field Sample Validation

Known
Sample Type Presence # Samples Detected Location

Abbreviations			
95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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