



### 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<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Myotis bats eDNA qPCR Tool: eMyotis1 Gene Target: MT-12S  
Species Code: eMyotis 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 0.3 95% CI 0.3-0.6 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 0.8 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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> )	# Voucher		
		Detection	Specimens	Sample Sources/Locations
ma-MYLU	Little brown bat ( <i>Myotis lucifugus</i> )	Yes	4	British Columbia
ma-MYCA	Californian bat ( <i>Myotis californicus</i> )	Yes	1	British Columbia
ma-MYCI	Western Small-footed bat ( <i>Myotis ciliolabrum</i> )	Yes	1	British Columbia
ma-MYEV	Long-eared bat ( <i>Myotis evotis</i> )	Yes	1	British Columbia
ma-MYTH	Fringed bat ( <i>Myotis thysanodes</i> )	Yes	1	British Columbia
ma-MYVO	Long-legged bat ( <i>Myotis volans</i> )	Yes	1	British Columbia
ma-MYYU	Yuma bat ( <i>Myotis yumanensis</i> )	Yes	4	British Columbia
ma-MYSE	Northern myotis bat ( <i>Myotis septentrionalis</i> )	Yes	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	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	Cardillera 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

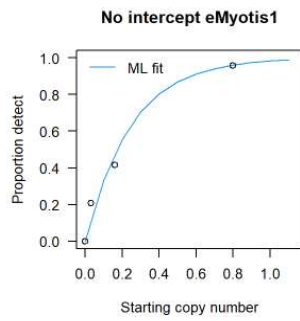
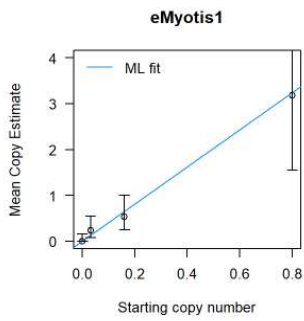
#### References

- 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**

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

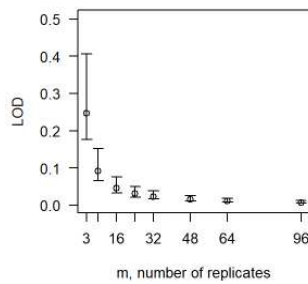
From 8 Technical Replicates



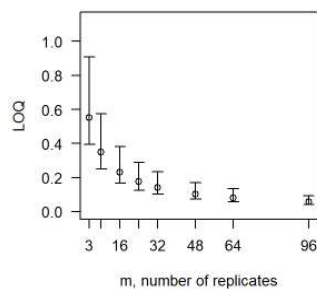
# Detects	# Copies	SE
0	0	0
1	0.03	0.03
2	0.07	0.05
3	0.12	0.07
4	0.17	0.09
5	0.24	0.12
6	0.34	0.17
7	0.51	0.25

Determined using eLowQuant R code<sup>4</sup>.

**Limits detect - no intercept eMyotis**

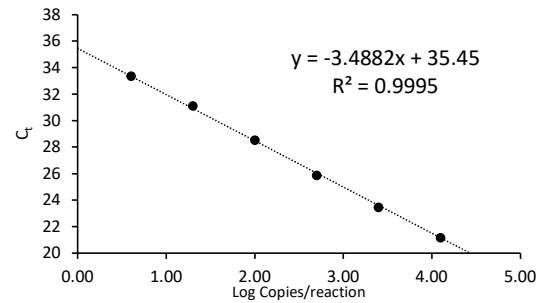


**Limits quant - no intercept eMyotis1**



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

**Applied to reactions with 100% positive hits**



Efficiency 94%

**Field Sample Validation**

Sample Type	Known		Detected	Location
	Presence	# Samples		

**Abbreviations**

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-12S	Mitochondrial 12S ribosomal RNA 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