

**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: Snowshoe hare (*Lepus americanus*)  
Species Code: ma-LEAMeDNA qPCR Tool: eLEAM1  
eDNA qPCR Format: TaqManGene Target: MT-ND5  
Published in: \_\_\_\_\_**eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA**LOD 0.2 95% CI 0.3-0.4 Copies/Rxn LOQ 0.6 95% CI 0.4-1 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 4 Copies/RxnBinomial-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> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-ALAL	Moose ( <i>Alces alces</i> )	No	1		British Columbia
ma-ANPA	Pallid bat ( <i>Antrozous pallidus</i> )	No	1		British Columbia
ma-CALU	Wolf ( <i>Canis lupus</i> )	No	1		British Columbia
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1		British Columbia
ma-CEEL	Red deer ( <i>Cervus elaphus</i> )	No	1		Washington
ma-FECA	Cat (domestic) ( <i>Felis catus</i> )	No	1		British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands
ma-LEAM	Snowshoe hare ( <i>Lepus americanus</i> )	Yes	2		British Columbia
ma-LOCA	River otter ( <i>Lontra canadensis</i> )	No	1		British Columbia
ma-NEVI	American mink ( <i>Neovision vision</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-ORCU	European rabbit ( <i>Oryctolagus cuniculus</i> )	No	1		British Columbia
ma-PEPE	Fisher ( <i>Pekania pennanti</i> )	No	1		British Columbia
ma-PHPH	Harbour porpoise ( <i>Phocoena phocoena</i> )	No	1		British Columbia
ma-PHVI	Harbour seal ( <i>Phoca vitulina</i> )	No	1		British Columbia
ma-SCCA	Eastern grey squirrel ( <i>Sciurus carolinensis</i> )	No	1		British Columbia
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-URAR	Grizzly bear ( <i>Ursus arctos</i> )	No	1		British Columbia
ma-VUVU	Fox ( <i>Vulpes vulpes</i> )	No	1		British Columbia

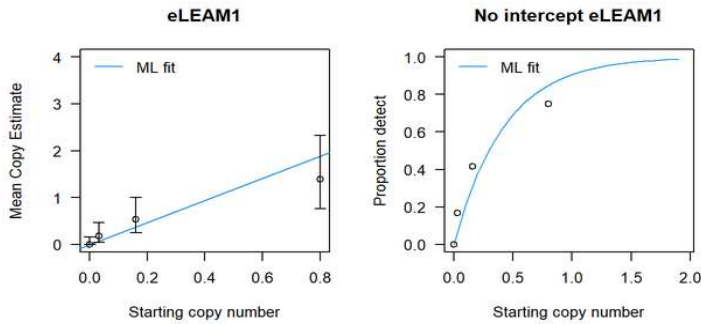
**References**

- 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/>

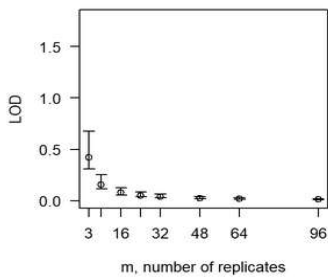


From 8 Technical Replicates

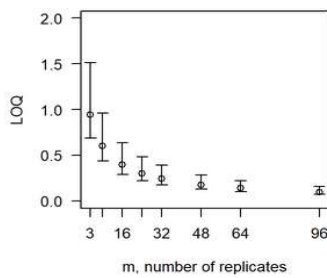
# Detects	# Copies	SE
0	0	0
1	0.057	0.058
2	0.122	0.09
3	0.199	0.122
4	0.294	0.16
5	0.415	0.209
6	0.587	0.283
7	0.881	0.43

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

Limits detect - no intercept eLEAM1



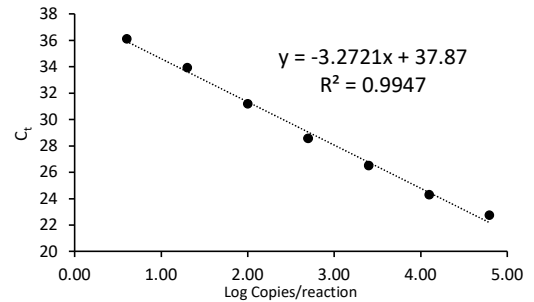
Limits quant - no intercept eLEAM1



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 102%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND5	Mitochondrial NADH dehydrogenase subunit 5 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