



**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: Bison (*Bison bison*) eDNA qPCR Tool: eBIBI4 Gene Target: MT-ND4  
 Species Code: ma-BIBI eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

**eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA**

LOD 0.2 95% CI 0.1-0.3 Copies/Rxn LOQ 0.7 95% CI 0.5-1.1 Copies/Rxn LOB 0 hits/8  
 LOQ<sub>continuous</sub> 4 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		Sample Sources/Locations
		Detection	Specimens	
ma-BIBI	Bison ( <i>Bison bison</i> )	Yes	1	British Columbia
ma-MUMU	House mouse ( <i>Mus musculus</i> )	No	1	British Columbia
ma-ORAM	Mountain goat ( <i>Oreamnos americanus</i> )	No	1	British Columbia
ma-ALAL	Moose ( <i>Alces alces</i> )	No	1	British Columbia
ma-CEEL	Red deer ( <i>Cervus elaphus</i> )	No	1	British Columbia
ma-RATA	Boreal woodland caribou ( <i>Rangifer tarandus</i> )	No	1	British Columbia
ma-ODHE	Mule deer ( <i>Odocoileus hemionus</i> )	No	1	Washington
ma-DADA	Fallow deer ( <i>Dama dama</i> )	No	1	British Columbia
ma-SOBE	Pacific water/marsh shrew ( <i>Sorex bendirii</i> )	No	1	British Columbia
ma-EPFU	Big brown bat ( <i>Eptesicus fuscus</i> )	No	1	British Columbia
ma-GUGU	Wolverine ( <i>Gulo gulo</i> )	No	1	British Columbia
ma-URAM	American black bear ( <i>Ursus americanus</i> )	No	1	British Columbia
ma-USAR	Grizzly bear ( <i>Ursus arctos</i> )	No	1	British Columbia
ma-NEVI	American mink ( <i>Neovision vision</i> )	No	1	British Columbia
ma-LOCA	River otter ( <i>Lontra canadensis</i> )	No	1	British Columbia
ma-CALU	Wolf ( <i>Canus lupus</i> )	No	1	British Columbia
ma-SCCA	Eastern grey squirrel ( <i>Sciurus carolinensis</i> )	No	1	British Columbia
ma-ORCU	European rabbit ( <i>Oryctolagus cuniculus</i> )	No	1	British Columbia
ma-PEPE	Fisher ( <i>Pekania pennanti</i> )	No	1	British Columbia
ma-SUSC	Wild boar ( <i>Sus scrofa</i> )	No	1	British Columbia
ma-CALUfa	Dog ( <i>Canis lupus familiaris</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

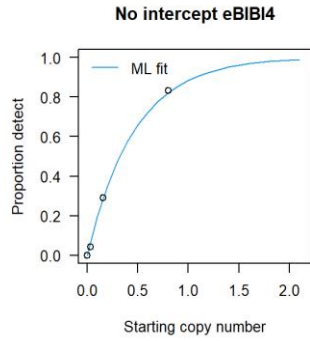
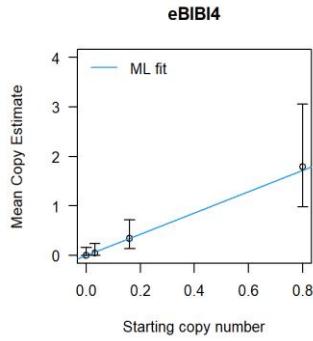
**References**

- 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/>

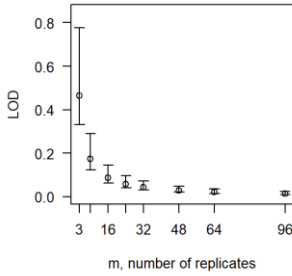


From 8 Technical Replicates

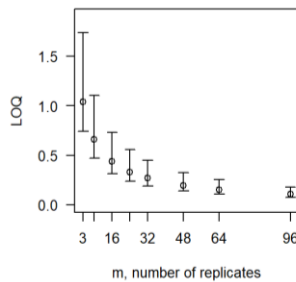
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0	0	0
1	0.062	0.063
2	0.13	0.10
3	0.22	0.14
4	0.32	0.18
5	0.46	0.23
6	0.64	0.31
7	0.97	0.48

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

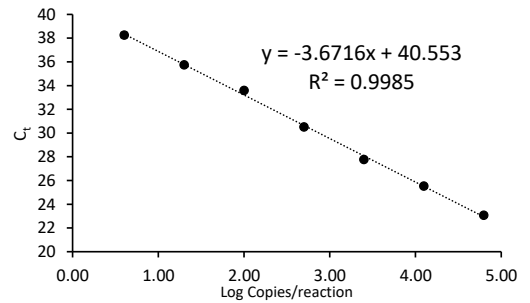
Limits detect - no intercept eBIBI4



Limits quant - no intercept eBIBI4



Applied to reactions with 100% positive hits



Efficiency 87%

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

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
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Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrially encoded NADH dehydrogenase 4
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