

### 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: Largemouth Bass (*Micropterus salmoides*) eDNA qPCR Tool: eMISA2 Gene Target: MT-COI  
 Species Code: te-MISA eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0.6 95% CI 0.5-1 Copies/Rxn LOQ 2.4 95% CI 1.8-3.9 Copies/Rxn LOB 0 hits/8  
 LOQ<sub>continuous</sub> 20 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: 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> )	# Voucher		Sample Sources/Locations
		Detection	Specimens	
am-LICA	Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo Sapiens</i> )	No	1	Netherlands
te-COCO	Slimy Sculpin ( <i>Cottus cognatus</i> )	No	1	Yukon
te-MIDO	Smallmouth Bass ( <i>Micropterus dolomieu</i> )	No	1	British Columbia
te-MISA	Largemouth Bass ( <i>Micropterus salmoides</i> )	Yes	1	British Columbia
te-ONCL	Cutthroat Trout ( <i>Oncorhynchus clarkii</i> )	No	1	British Columbia
te-ONGO	Pink Salmon ( <i>Oncorhynchus gorbuscha</i> )	No	1	British Columbia
te-ONKE	Chum Salmon ( <i>Oncorhynchus keta</i> )	No	1	British Columbia
te-ONKI	Coho Salmon ( <i>Oncorhynchus kisutch</i> )	No	1	British Columbia
te-ONMY	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	No	1	British Columbia
te-ONNE	Sockeye Salmon ( <i>Oncorhynchus nerka</i> )	No	1	British Columbia
te-ONTS	Chinook Salmon ( <i>Oncorhynchus tshawytscha</i> )	No	1	British Columbia
te-PRCY	Round Whitefish ( <i>Prosopium cylindraceum</i> )	No	1	Yukon
te-SAMA	Dolly Varden ( <i>Salvelinus malma</i> )	No	1	British Columbia
te-SASA	Atlantic Salmon ( <i>Salmo salar</i> )	No	1	Nova Scotia
te-THAR	Arctic Greyling ( <i>Thymallus arcticus</i> )	No	1	Alberta
te-THPA	Eaulachon ( <i>Thaleichthys pacificus</i> )	No	1	British Columbia

#### References

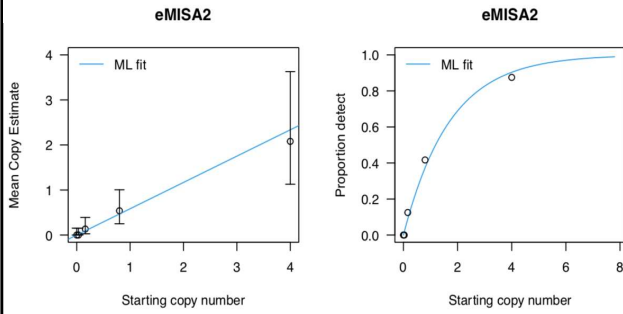
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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

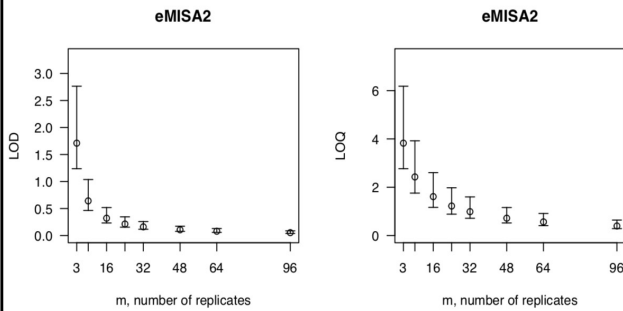
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



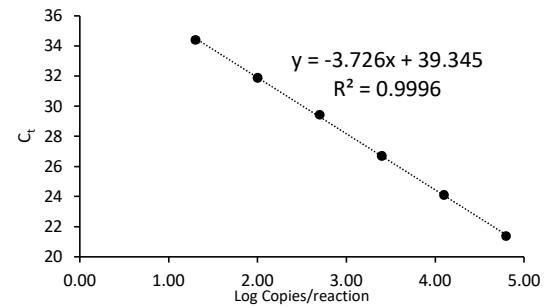
# Detects	# Copies	SE
0	0	0
1	0.23	0.23
2	0.49	0.36
3	0.81	0.49
4	1.19	0.65
5	1.68	0.85
6	2.37	1.15
7	3.56	1.75

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



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 86%

Field Sample Validation

Known  
 Sample Type Presence # Samples Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COI	Mitochondrial cytochrome oxidase subunit 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