

**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: Largescale sucker (*Catostomus macrocheilus*) eDNA qPCR Tool: eCAMA2 Gene Target: MT-ND2  
 Species Code: te-CAMA 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 0.5 95% CI 0.3-0.8 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	
te-CAMA	Largescale sucker ( <i>Catostomus macrocheilus</i> )	Yes	5	British Columbia
te-CACA	Salish sucker [ <i>Catostomus catostomus (chehalis)</i> ]	No	1	Alberta
te-CACO	White sucker ( <i>Catostomus commersonii</i> )	No	1	Ontario
te-COAR	Cisco/Tullibee ( <i>Coregonus artedii</i> )	No	1	Alberta
te-COCL	Lake whitefish( <i>Coregonus clupeaformis</i> )	No	1	Alberta
te-COCO	Slimy sculpin( <i>Cottus cognatus</i> )	No	1	British Columbia
te-ESLU	Northern Pike ( <i>Esox lucius</i> )	No	1	British Columbia
te-ESNI	Chain pickerel ( <i>Esox niger</i> )	No	1	Nova Scotia
te-HIAL	Goldeye ( <i>Hiadon alosoides</i> )	No	1	Alberta
te-LOLO	Burbot ( <i>Lota lota</i> )	No	1	Alberta
te-MIDO	Smallmouth bass ( <i>Micropterus dolomieu</i> )	No	1	British Columbia
te-ONCL	Westslope cutthroat trout ( <i>Oncorhynchus clarkii clarkii</i> )	No	1	Alberta
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 (steelhead) trout ( <i>Oncorhynchus mykiss</i> )	No	1	Alberta
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-PLGR	Flathead chub ( <i>Platygobio gracilis</i> )	No	1	Ontario
te-RHCA	Longnose (nooksack) dace ( <i>Rhinichthys cataractae</i> )	No	1	British Columbia
te-SACO	Bull trout ( <i>Salvelinus confluentus</i> )	No	1	British Columbia
te-SAFO	Brook trout ( <i>Salvelinus fontinalis</i> )	No	1	Alberta
te-SANA	Lake trout ( <i>Salvelinus namaycush</i> )	No	1	British Columbia
te-SASA	Atlantic salmon ( <i>Salmo salar</i> )	No	1	Nova Scotia
te-SAVI	Walleye ( <i>Sander vitreus</i> )	No	1	Washington
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1	British Columbia
ma-FECA	Cat (domestic) ( <i>Felis catus</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands

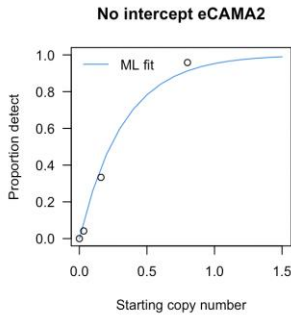
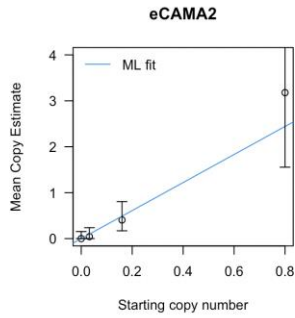
**References**

- 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/>

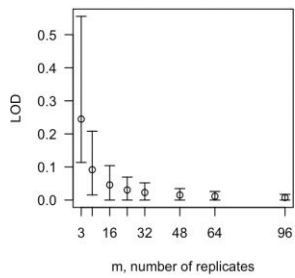


From 8 Technical Replicates

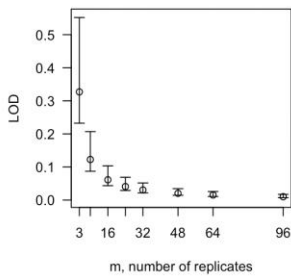
# Detects	# Copies	SE
0	0	0
1	0.033	0.044
2	0.072	0.058
3	0.123	0.079
4	0.175	0.099
5	0.259	0.138
6	0.35	0.178
7	0.512	0.264

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

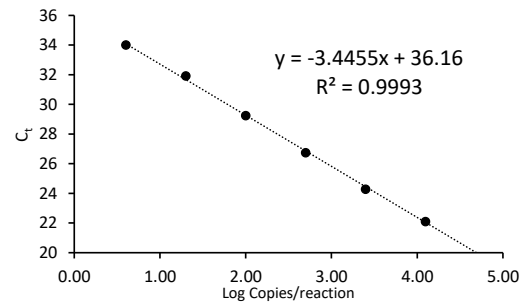
Limits detect - intercept eCAMA2



Limits detect - no intercept eCAMA2



Applied to reactions with 100% positive hits



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

Efficiency 95%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase 2 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