



**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: Asian grass carp (Ctenopharyngodon idella) eDNA qPCR Tool: eCTID1 Gene Target: MT-ND4  
 Species Code: te-CTID eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0.3 95% CI 0.2-0.6 Copies/Rxn LOQ 1.3 95% CI 0.9-2.3 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 (Species)	# Voucher		Sample Sources/Locations
		Detection	Specimens	
te-CTID	Asian grass carp ( <i>Ctenopharyngodon idella</i> )	Yes	4	Ontario
te-COCL	Lake whitefish ( <i>Coregonus clupeaformis</i> )	No	1	Alberta
te-COCO	Slimy sculpin ( <i>Cottus cognatus</i> )	No	1	British Columbia
te-CACO	White sucker ( <i>Catostomus commersonii</i> )	No	1	Alberta
te-CACA	Longnose sucker ( <i>Catostomus catostomus</i> )	No	1	Alberta
te-CAMA	Largescale sucker ( <i>Catostomus macrocheilus</i> )	No	1	Ontario
te-COAR	Cisco/Tullibee ( <i>Coregonus artedii</i> )	No	1	Alberta
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>Hiodon alosoides</i> )	No	1	Alberta
te-LOLO	Burbot ( <i>Lota lota</i> )	No	1	Yukon
te-MYCA	Peamouth chub ( <i>Mylocheilus caurinus</i> )	No	1	British Columbia
te-ONCLie	Westslope cutthroat trout ( <i>Oncorhynchus clarkii lewisii</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	Ontario
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	Alberta
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands
ma-FECA	Cat (domestic) ( <i>Felis catus</i> )	No	1	British Columbia

**References**

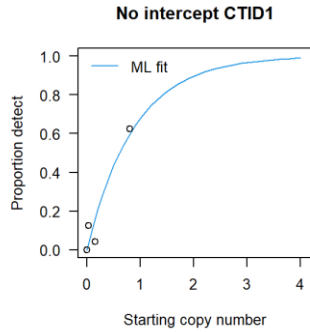
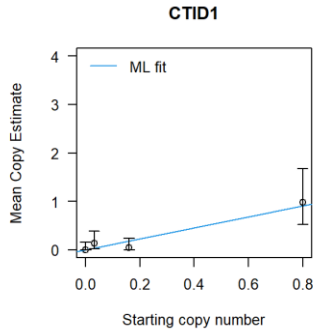
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>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

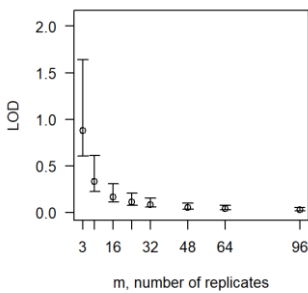
From 8 Technical Replicates



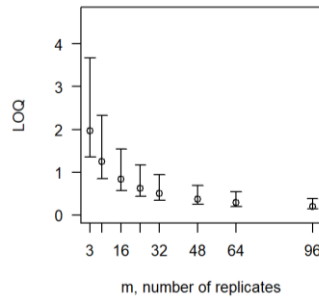
# Detects	# Copies	SE
0	0.00	0.00
1	0.12	0.12
2	0.25	0.19
3	0.42	0.26
4	0.61	0.34
5	0.87	0.45
6	1.23	0.61
7	1.84	0.93

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

Limits detect - no intercept CTID1

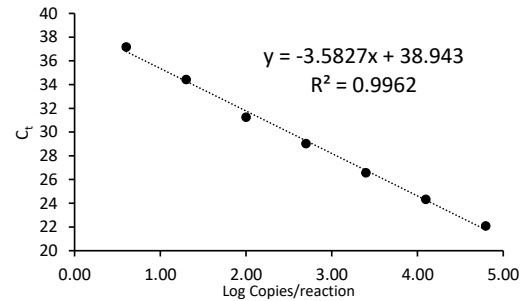


Limits quant - no intercept CTID1



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 90%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

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