

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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: White sucker (*Catostomus commersonii*) eDNA qPCR Tool: eCACO4 Gene Target: MT-ND2
Species Code: te-CACO eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.8 Copies/Rxn LOQ 1.6 95% CI 1-3.1 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. 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	
			Specimens	Sample Sources/Locations
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-CACA	Longnose sucker (<i>Catostomus catostomus</i>)	No	2	British Columbia
te-CACO	White sucker (<i>Catostomus commersonii</i>)	Yes	3	Alberta
te-CAMA	Largescale sucker (<i>Catostomus macrocheilus</i>)	No	2	British Columbia
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1	Alberta
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	2	British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	2	British Columbia
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	2	Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	2	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	2	British Columbia
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	1	British Columbia
te-PRCY	Round whitefish (<i>Prosopium cylindraceum</i>)	No	1	British Columbia
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	No	2	British Columbia
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	2	British Columbia
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	2	British Columbia
te-SAVI	Walleye (<i>Sander vitreus</i>)	No	1	Alberta
te-THAR	Arctic grayling (<i>Thymallus arcticus</i>)	No	2	Alberta

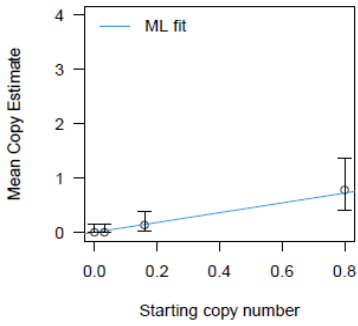
References

- 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. 2020; 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, 00: 1-12. doi: 10.1002/edn3.220

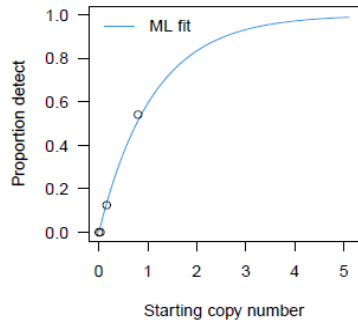


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eCACO4



eCACO4

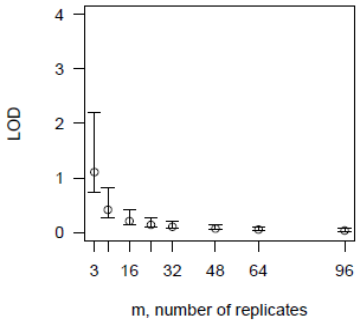


From 8 Technical Replicates

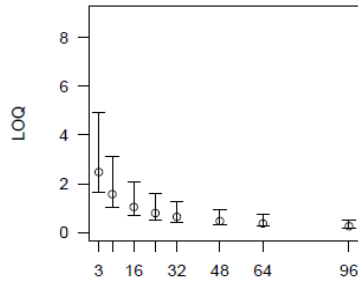
# Detects	# Copies	SE
0	0	0
1	0.15	0.15
2	0.32	0.24
3	0.52	0.33
4	0.77	0.44
5	1.09	0.58
6	1.54	0.78
7	2.31	1.19

Determined using eLowQuant R code⁴.

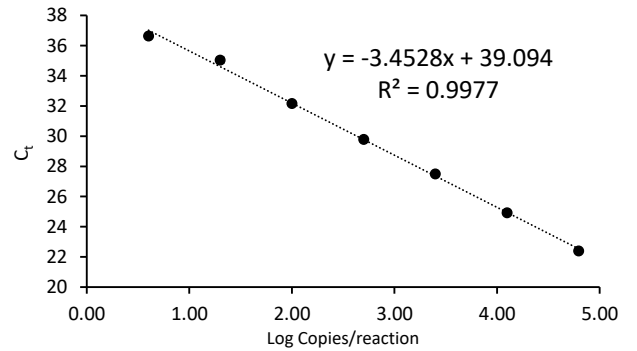
eCACO4



eCACO4



Applied to reactions with 100% positive hits



Efficiency 95%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 µL DNA input in a total 15 µL reaction

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