



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: Goldfish (*Carassius auratus*) eDNA qPCR Tool: eCAAUS Gene Target: MT-CYB
Species Code: te-CAAU eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.8 95% CI 0.4-1.4 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 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: 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>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-CAAU	Goldfish (<i>Carassius auratus</i>)	Yes	7	British Columbia
te-ESLU	Northern Pike (<i>Esox lucius</i>)	No	1	British Columbia
te-GAAC	Three-spined Stickleback (<i>Gasterosteus aculeatus</i>)	No	1	British Columbia
te-MIDO	Smallmouth Bass (<i>Micropterus dolomieu</i>)	No	2	British Columbia
te-MISA	Largemouth Bass (<i>Micropterus salmoides</i>)	No	1	British Columbia
te-ONCL	Cutthroat Trout (<i>Oncorhynchus clarkii</i>)	No	1	Alberta
te-ONGO	Pink Salmon (<i>Oncorhynchus gorbuscha</i>)	No	2	British Columbia
te-ONKE	Chum Salmon (<i>Oncorhynchus keta</i>)	No	2	British Columbia
te-ONKI	Coho Salmon (<i>Oncorhynchus kisutch</i>)	No	1	British Columbia
te-ONMY	Rainbow Trout (<i>Oncorhynchus mykiss</i>)	No	1	Alberta
te-ONNE	Sockeye Salmon (<i>Oncorhynchus nerka</i>)	No	2	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-SACO	Bull Trout (<i>Salvelinus confluentus</i>)	No	2	Alberta
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 Grayling (<i>Thymallus arcticus</i>)	No	1	Alberta
te-THPA	Eulachon (<i>Thaleichthys pacificus</i>)	No	1	British Columbia

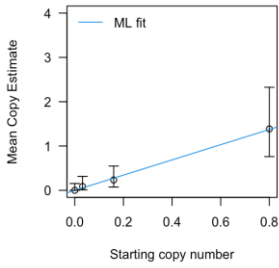
References

- 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, 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, 3: 970-981. doi: 10.1002/edn3.220

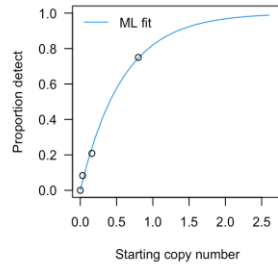


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eCAAU5



eCAAU5

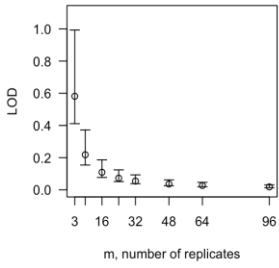


From 8 Technical Replicates

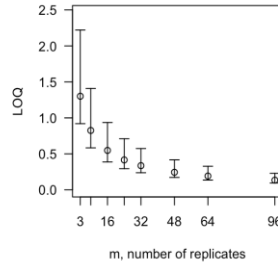
# Detects	# Copies	SE
0	0	0
1	0.078	0.08
2	0.17	0.12
3	0.27	0.17
4	0.4	0.22
5	0.57	0.29
6	0.81	0.4
7	1.21	0.6

Determined using eLowQuant R code⁴.

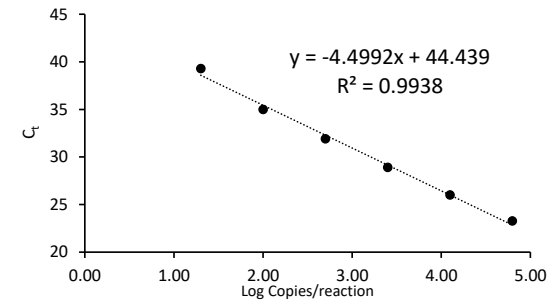
eCAAU5



eCAAU5



Applied to reactions with 100% positive hits



Efficiency 67%

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

Known
Sample Type Presence # Samples Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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