



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: American Eel (*Anguilla rostrata*)
Species Code: te-ANRO

eDNA qPCR Tool: eANRO1
eDNA qPCR Format: TaqMan

Gene Target: MT-CYB
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.9 Copies/Rxn LOQ 2.1 95% CI 1.5-3.4 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: QIACuity

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

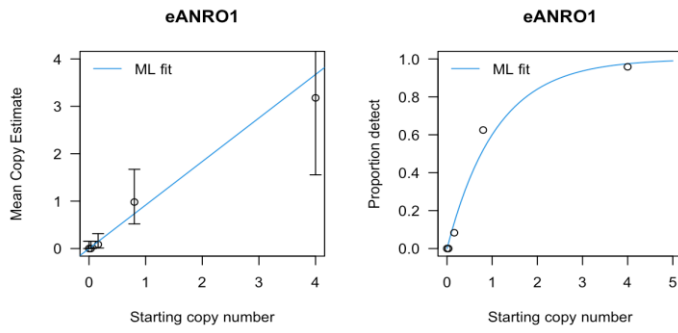
Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-ANRO	American Eel (<i>Anguilla rostrata</i>)	Yes	8	Prince Edward Island
te-COCO	Slimy Sculpin (<i>Cottus cognatus</i>)	No	1	Yukon
te-ESLU	Northern Pike (<i>Esox lucius</i>)	No	1	British Columbia
te-MIDO	Smallmouth Bass (<i>Micropterus dolomieu</i>)	No	1	British Columbia
te-MISA	Largemouth Bass (<i>Micropterus salmoides</i>)	No	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-SACO	Bull Trout (<i>Salvelinus confluentus</i>)	No	1	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 (2021) 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



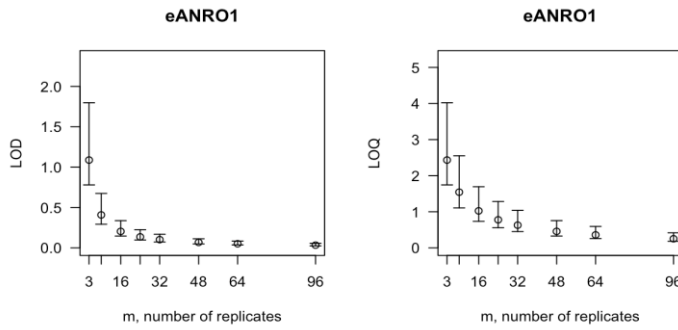
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



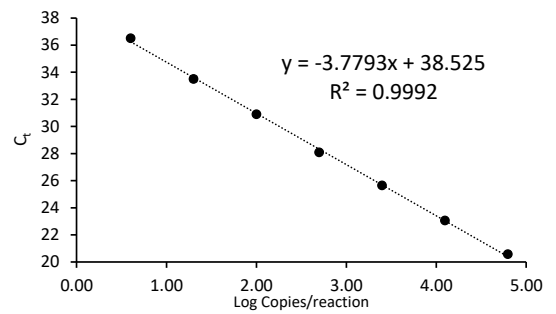
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.19	0.2
2	0.42	0.31
3	0.68	0.42
4	1.01	0.55
5	1.42	0.72
6	2.01	0.98
7	3.02	1.49

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

Efficiency 84%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	30	Y	Prince Edward Island

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