



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: Rainbow Trout (*Oncorhynchus mykiss*) eDNA qPCR Tool: eONMY5 Gene Target: MT-ND2
Species Code: te-ONMY eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.7 95% CI 0.5-1.1 Copies/Rxn LOQ 2.5 95% CI 1.8-4 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>)	# Voucher		
		Detection	Specimens	Sample Sources/Locations
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-COCO	Slimy sculpin (<i>Cottus cognatus</i>)	No	1	Yukon
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-ONCLcl	Cutthroat Trout - Coastal (<i>Oncorhynchus clarki clarkii</i>)	No	2	British Columbia
te-ONCLle	Cutthroat Trout - Westslope (<i>Oncorhynchus clarki lewisi</i>)	No	3	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>)	Yes	7	British Columbia and 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-SACO	Bull Trout (<i>Salvelinus confluentus</i>)	No	3	Alberta
te-SAFO	Brook Trout (<i>Salvelinus fontinalis</i>)	No	2	Alberta
te-SAMA	Dolly Varden (<i>Salvelinus malma</i>)	No	2	British Columbia
te-THAR	Arctic Grayling (<i>Thymallus arcticus</i>)	No	1	Alberta

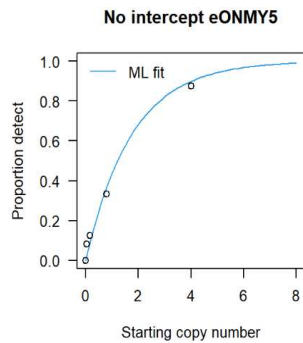
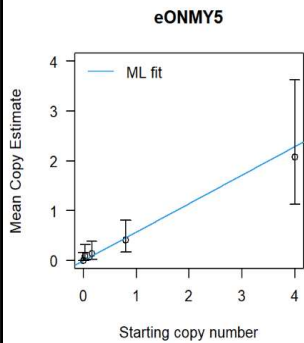
References

- 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, 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

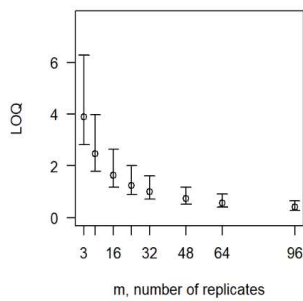
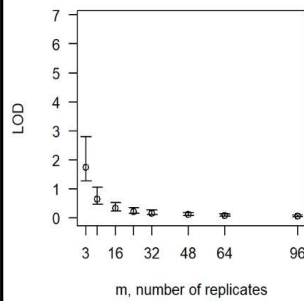
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.23	0.24
2	0.51	0.37
3	0.82	0.5
4	1.21	0.66
5	1.71	0.86
6	2.42	1.17
7	3.63	1.78

Determined using eLowQuant R code⁴.

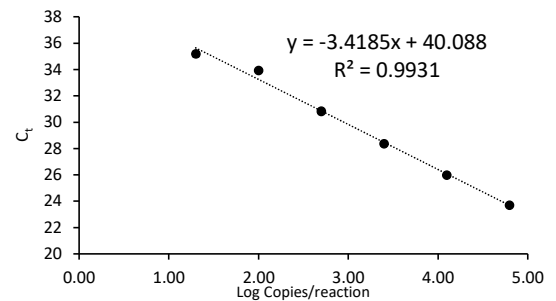


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 96%

Field Sample Validation

Known
Sample Type Presence # Samples Detected Location

Abbreviations

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