



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: Coho Salmon (*Oncorhynchus kisutch*) eDNA qPCR Tool: eONKI4 Gene Target: MT-ND1
Species Code: te-ONKI eDNA qPCR Format: TaqMan Published in: 5

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.7 Copies/Rxn LOQ 1.6 95% CI 1.2-2.5 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
am-LICA	American Bullfrog (<i>Lithobates(Rana) catesbeiana</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-ONCL	Cuttthroat 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>)	Yes	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-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. 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
- Hocking, MD, MacAdams, J, Sneiderman, RB, Allison, MJ, Bergman, LC, Koop, B, Starzomski, B, Lesperance, ML, and Helbing, CC (2022) Establishing the signal above the noise in field applications: Accounting for an environmental background in the detection and quantitation of salmonid eDNA. Fishes, 7: 266. doi: 10.3390/fishes7050266

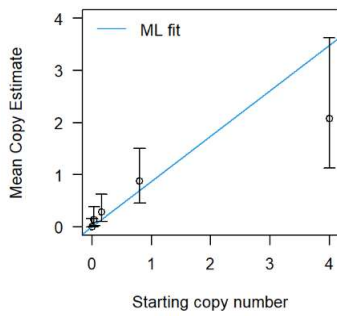


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

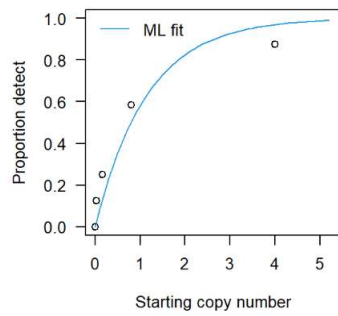
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

eONKI4

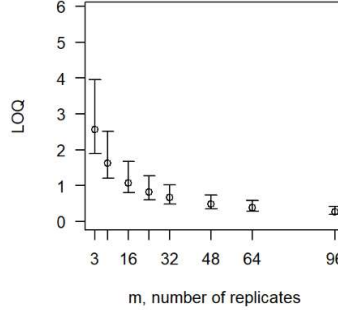
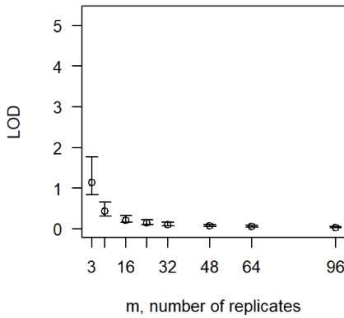


No intercept eONKI4



# Detects	# Copies	SE
0	0	0
1	0.15	0.16
2	0.33	0.24
3	0.54	0.33
4	0.8	0.43
5	1.13	0.56
6	1.59	0.76
7	2.39	1.16

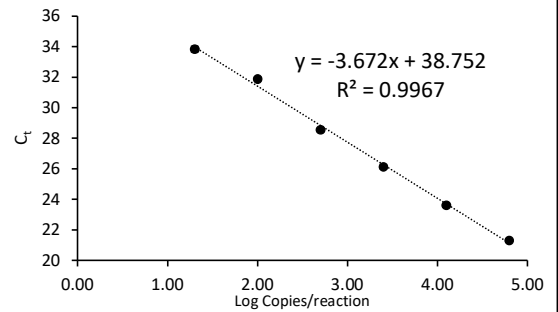
Determined using eLowQuant R code⁴.



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 87%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Yes	6	Yes	Squamish, British Columbia
Water	No	6	Yes	Ts'il'os Provincial Park, British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase subunit 1 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