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 sensitivity [1-3].

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 0.3   95% CI 0.2-0.5 Copies/Rxn   LOQ 1.1   95% CI 0.8-1.8 Copies/Rxn   LOB 0 hits/8

$LOQ_{continuous}$ 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code [4].   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)

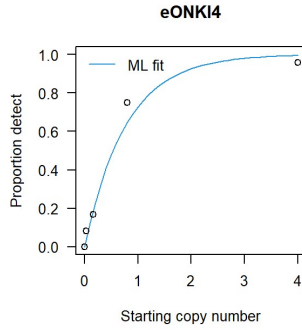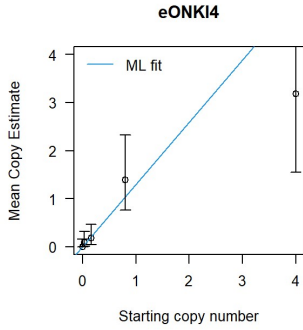| Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
am-LICA	American Bullfrog *(Lithobates(Rana) catesbeiana)*	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-ONCL	Cuttthroat Trout *(Oncorhynchus clarkii)*	No	1	British Columbia
te-ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*	No	1	British Columbia
te-ONKE	Chum Salmon *(Oncorhynchus keta)*	No	1	British Columbia
te-ONKI	Coho Salmon *(Oncorhynchus kisutch)*	Yes	1	British Columbia
te-ONMY	Rainbow Trout *(Oncorhynchus mykiss)*	No	1	British Columbia
te-ONNE	Sockeye Salmon *(Oncorhynchus nerka)*	No	1	British Columbia
te-ONTS	Chinook Salmon *(Oncorhynchus tschawytscha)*	No	1	British Columbia
te-THAR	Arctic Grayling *(Thymallus arcticus)*	No	1	Alberta

References

1. 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

2. 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

3. 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

4. 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

5. 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

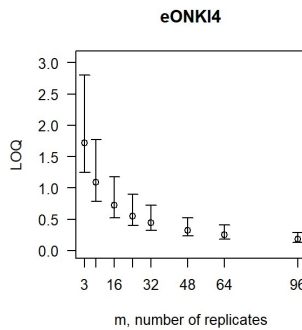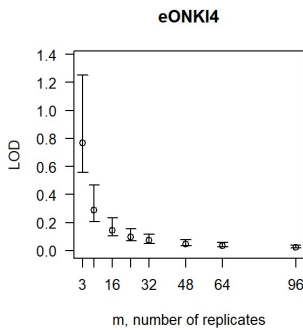## eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eONKI4

eONKI4

eONKI4

eONKI4

Binomial-Poisson model: ___No Intercept___

Determined using eLowQuant R code[4].

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

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	0.10	0.10
2	0.22	0.16
3	0.36	0.22
4	0.53	0.29
5	0.76	0.38
6	1.10	0.52
7	1.60	0.79

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits

$y = -3.5343x + 36.779$
$R^2 = 0.9963$
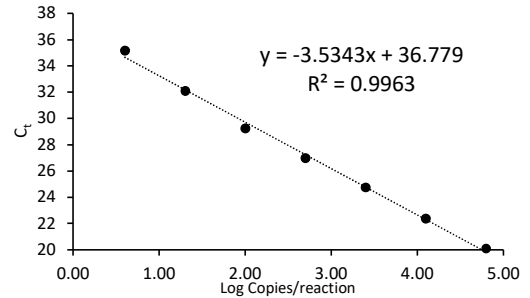
Efficiency ___92%___

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
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