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: Cutthroat Trout *(Oncorhynchus clarkii)*	eDNA qPCR Tool: eONCL4	Gene Target: MT-ND1
Species Code: te-ONCL	eDNA qPCR Format: TaqMan	Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2	95% CI 0.1-0.4	Copies/Rxn	LOQ 0.8	95% CI 0.6-1.4	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)

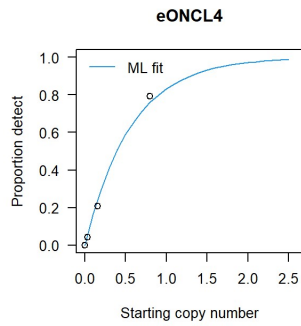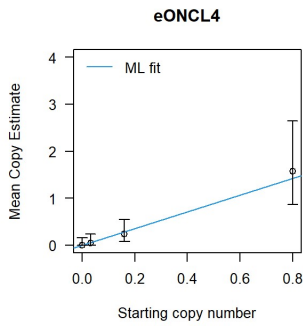| Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo Sapiens*)	No	1	Netherlands
te-ONCLcl	Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*)	Yes	5	British Columbia
te-ONCLle	Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*)	Yes	9	Alberta
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*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	6	Alberta and British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tshawytscha*)	No	1	British Columbia
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	4	Alberta
te-SAFO	Brook Trout (*Salvelinus fontinalis*)	No	4	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	Alberta
te-SASA	Atlantic Salmon (*Salmo salar*)	No	1	Nova Scotia

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, 00: 1-12. doi: 10.1002/edn3.220

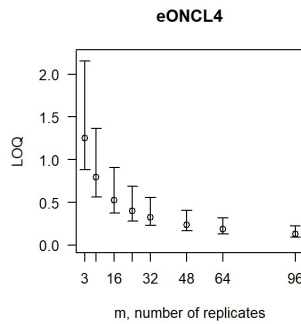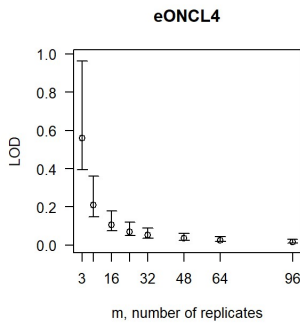## eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eONCL4

Mean Copy Estimate vs Starting copy number — ML fit

eONCL4

Proportion detect vs Starting copy number — ML fit

eONCL4

LOD vs m, number of replicates

eONCL4

LOQ vs m, number of replicates

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	0.00	0.00
1	0.08	0.08
2	0.16	0.12
3	0.26	0.16
4	0.39	0.22
5	0.55	0.28
6	0.78	0.38
7	1.17	0.58

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits

$y = -3.403x + 36.333$
$R^2 = 0.9993$

C_t vs Log Copies/reaction
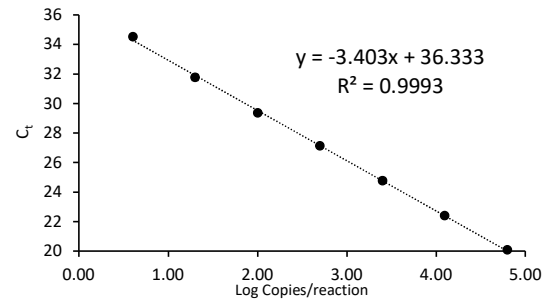
Efficiency   97%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

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