Helbing Lab
eDNA Inventory

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: Arctic Grayling *(Thymallus arcticus)*
Species Code: te-THAR
eDNA qPCR Tool: eTHAR1
eDNA qPCR Format: TaqMan
Gene Target: MT-RNR1
Published in:

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 1.1-2.6 Copies/Rxn
$LOQ_{continuous}$ 4 Copies/Rxn
LOB 0 hits/8

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-CACAch	Salish sucker (*Catostomus catostomus chehalis*)	No	1	British Columbia
te-CACO	White sucker (*Catostomus commersonii*)	No	1	Alberta
te-COAR	Cisco/Tullibee (*Coregonus artedi*)	No	1	British Columbia
te-COCL	Lake whitefish (*Coregonus clupeaformis*)	No	1	Alberta
te-HIAL	Goldeye (*Hiodon alosoides*)	No	1	Alberta
te-LOLO	Burbot (*Lota lota*)	No	1	Yukon
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)*	No	1	British Columbia
te-ONMY	Rainbow Trout *(Oncorhynchus mykiss)*	No	1	Alberta and British Columbia
te-ONNE	Sockeye Salmon *(Oncorhynchus nerka)*	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PRWI	Mountain whitefish (*Prosopium williamsoni*)	No	1	Alberta
te-SANA	Lake trout (*Salvelinus namaycush)*	No	1	Alberta
te-SAVI	Walleye (*Sander vitreus*)	No	1	Alberta
te-THAR	Arctic Grayling *(Thymallus arcticus)*	Yes	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, 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

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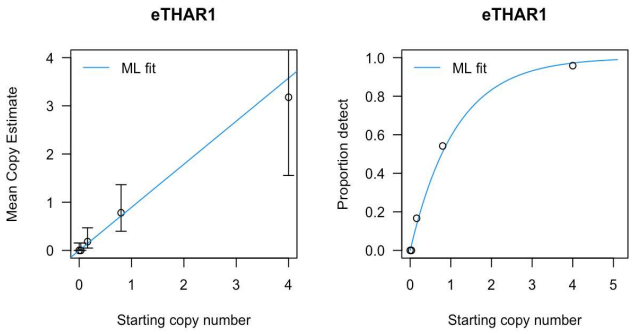
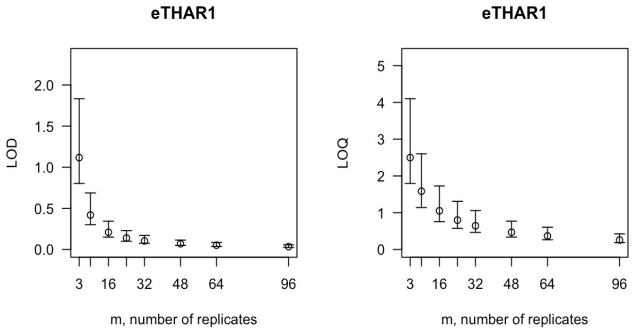
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



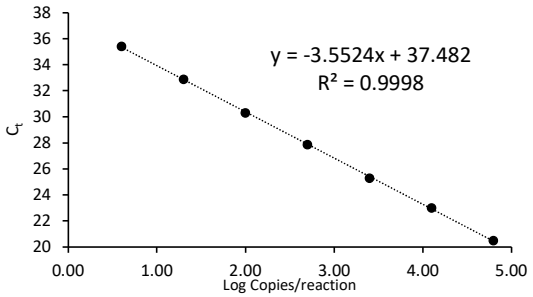
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.15	0.15
2	0.32	0.24
3	0.53	0.32
4	0.78	0.43
5	1.1	0.56
6	1.55	0.75
7	2.33	1.14

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency 91%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	9	Y	Alberta
Water	N	12	Y	Alberta
Water	Y	25	Y	Yukon

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR1	Mitochondrial 12S ribosomal RNA 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