

### 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<sup>1-3</sup>.

#### General eDNA Assay Information

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

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.6 95% CI 0.4-1.0 Copies/Rxn LOQ 2.3 95% CI 1.7-4.0 Copies/Rxn LOB 0 hits/8  
 LOQ<sub>continuous</sub> 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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)

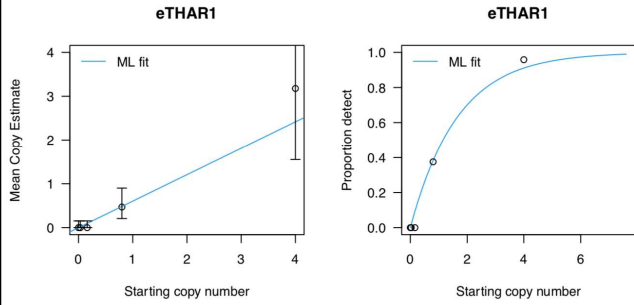
Species	Common Name ( <i>Species</i> )	Detection	# Voucher	
			Specimens	Sample Sources/Locations
am-LICA	American Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands
te-CACAch	Salish sucker ( <i>Catostomus catostomus chehalis</i> )	No	1	British Columbia
te-CACO	White sucker ( <i>Catostomus commersonii</i> )	No	1	Alberta
te-COAR	Cisco/Tullibee ( <i>Coregonus artedii</i> )	No	1	British Columbia
te-COCL	Lake whitefish ( <i>Coregonus clupeaformis</i> )	No	1	Alberta
te-HIAL	Goldeye ( <i>Hiodon alosoides</i> )	No	1	Alberta
te-LOLO	Burbot ( <i>Lota lota</i> )	No	1	Yukon
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> )	No	1	British Columbia
te-ONMY	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	No	1	Alberta and 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-PRWI	Mountain whitefish ( <i>Prosopium williamsoni</i> )	No	1	Alberta
te-SANA	Lake trout ( <i>Salvelinus namaycush</i> )	No	1	Alberta
te-SAVI	Walleye ( <i>Sander vitreus</i> )	No	1	Alberta
te-THAR	Arctic Grayling ( <i>Thymallus arcticus</i> )	Yes	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



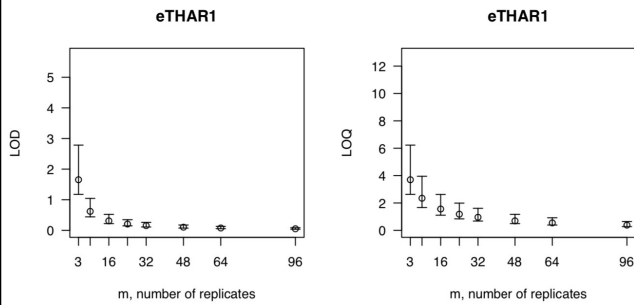
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



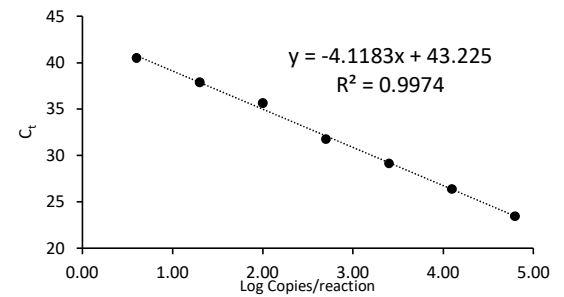
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.22	0.23
2	0.48	0.35
3	0.78	0.48
4	1.15	0.63
5	1.62	0.83
6	2.3	1.12
7	3.44	1.71

Determined using eLowQuant R code<sup>4</sup>.



Applied to reactions with 100% positive hits



Efficiency 75%

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

Field Sample Validation

Sample Type	Known			Location
	Presence	# Samples	Detected	
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