



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: *Coregonus zenithicus* (shortjaw cisco) eDNA qPCR Tool: eCOZE1 Gene Target: MT-COI
Species Code: te-COZE eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1-0.3 Copies/Rxn LOQ 0.7 95% CI 0.5-1.1 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 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: QIAcuity

eDNA Assay Specificity Test Information

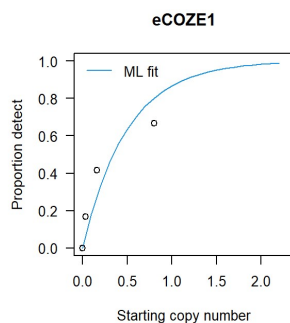
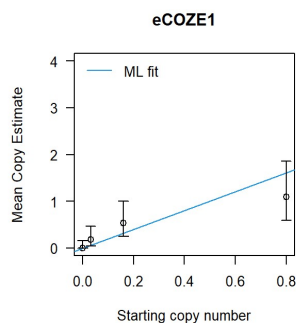
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	# Voucher		
		Detection	Specimens	Sample Sources/Locations
te-COZE	Shortjaw Cisco (<i>Coregonus zenithicus</i>)	Yes	5	Alberta
te-COCL	Lake Whitefish (<i>Coregonus clupeaformis</i>)	No	1	Québec
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	British Columbia
te-CACA	Salish Sucker (<i>Catostomus catostomus</i>)	No	1	British Columbia
te-ESLU	Northern Pike (<i>Esox lucius</i>)	No	1	British Columbia
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-LOLO	Burbot (<i>Lota lota</i>)	No	1	Yukon
te-ONCLie	Cutthroat Trout (<i>Oncorhynchus clarkii</i>)	No	1	Alberta
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 (Steelhead) Trout (<i>Oncorhynchus mykiss</i>)	No	1	Alberta
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-SACO	Bull Trout (<i>Salvelinus confluentus</i>)	No	1	British Columbia
te-SAFO	Brook Trout (<i>Salvelinus fontinalis</i>)	No	1	Alberta
te-SANA	Lake Trout (<i>Salvelinus namaycush</i>)	No	1	British Columbia
te-SASA	Atlantic Salmon (<i>Salmo salar</i>)	No	1	Nova Scotia
te-SAVI	Walleye (<i>Sander vitreus</i>)	No	1	Alberta (Winefred Lake)
te-COAR	Cisco/Tullibee (<i>Coregonus artedii</i>)	No	3	British Columbia (Christina Lake)
te-CACO	White Sucker (<i>Catostomus commersonii</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, 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

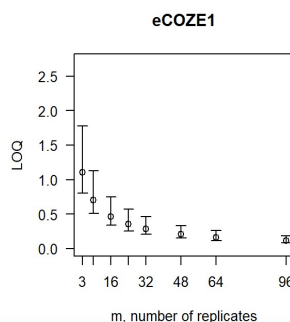
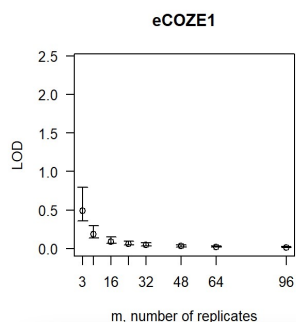
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

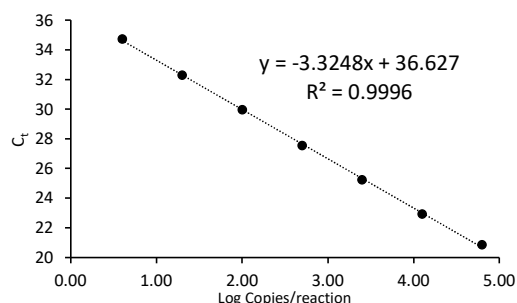
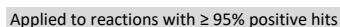
# Detects	# Copies	SE
0	0	0
1	0.066	0.067
2	0.142	0.105
3	0.233	0.143
4	0.343	0.187
5	0.485	0.245
6	0.686	0.331
7	1.029	0.504

Determined using eLowQuant R code⁴.

Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction



Efficiency	100%
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Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COI	Mitochondrial cytochrome oxidase 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