



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: Brook stickleback (*Culaea inconstans*)
Species Code: te-CUIN

eDNA qPCR Tool: eCUIN2
eDNA qPCR Format: TaqMan

Gene Target: MT-ND2
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1 - 0.3 Copies/Rxn LOQ 0.8 95% CI 0.5 - 1.3 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)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
te-CUIN	Brook stickleback (<i>Culaea inconstans</i>)	Yes	5		British Columbia
te-COCO	Slimy sculpin (<i>Cottus cognatus</i>)	No	1		British Columbia
te-SAVI	Walleye (<i>Sander vitreus</i>)	No	1		Alberta
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	1		British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	1		British Columbia
te-MOSA	Striped bass (<i>Morone saxatilis</i>)	No	1		Ontario
te-LOLO	Burbot (<i>Lota lota</i>)	No	1		Yukon
te-ESNI	Chain pickerel (<i>Esox niger</i>)	No	1		Nova Scotia
te-ESAMam	Redfin pickerel (<i>Esox americanus americanus</i>)	No	1		British Columbia
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1		Alberta
te-CACO	White sucker (<i>Catostomus commersonii</i>)	No	1		Ontario
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	No	1		British Columbia
te-ONMY	Rainbow (steelhead) trout (<i>Oncorhynchus mykiss</i>)	No	1		British Columbia
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	1		British Columbia
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	1		British Columbia
te-ONCLe	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	1		Alberta
ma-CALUfa	Dog (<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

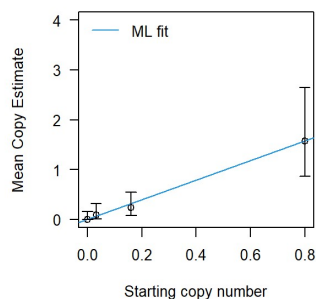
References

- 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

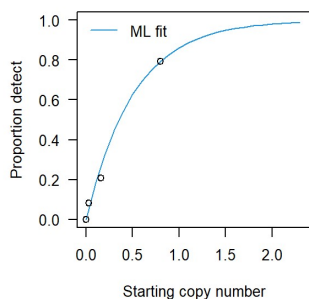
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

eCUIN2



Intercept eCUIN2

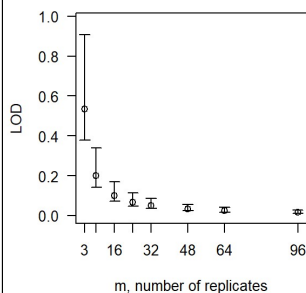


From 8 Technical Replicates

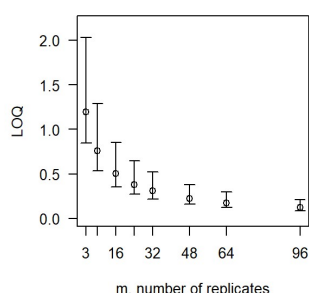
# Detects	# Copies	SE
0	0	0
1	0.72	0.073
2	0.15	0.11
3	0.25	0.16
4	0.37	0.21
5	0.53	0.27
6	0.74	0.36
7	1.11	0.55

Determined using eLowQuant R code⁴.

Limits detect - no intercept eCUIN2



Limits quant - no intercept eCUIN2

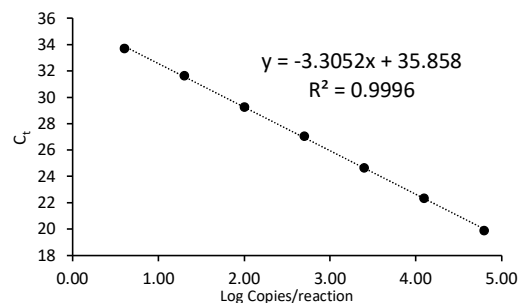


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Applied to reactions with 100% positive hits



Efficiency 101%

Field Sample Validation

Sample Type **Known Presence** **# Samples** **Detected** **Location**

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrially encoded NADH dehydrogenase 2
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