



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: Chain pickerel (*Esox niger*) eDNA qPCR Tool: eESNI3 Gene Target: MT-ND2
 Species Code: te-ESNI eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.9 95% CI 0.6-1.5 Copies/Rxn LOQ 3.3 95% CI 2.4-5.5 Copies/Rxn LOB 0 hits/8
 LOQ_{continuous} 20 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)

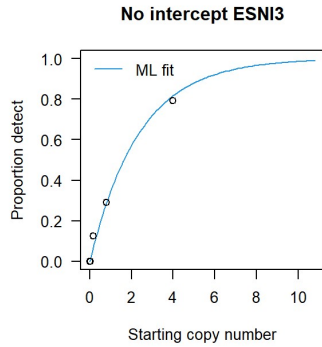
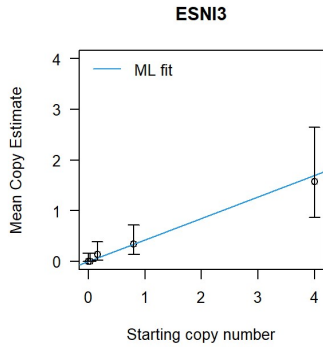
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
te-CACA	Longnose Sucker (<i>Catostomus catostomus</i>)	Yes	1		British Columbia
te-COCO	Slimy Sculpin (<i>Cottus cognatus</i>)	No	1		British Columbia
te-ESLU	Northern Pike (<i>Esox lucius</i>)	No	1		British Columbia
te-ESNI	Chain Pickerel (<i>Esox niger</i>)	Yes	5		Nova Scotia
te-GAAC	Three Spine Stickleback (<i>Gasterosteus aculeatus</i>)	No	1		British Columbia
te-LEGI	Pumpkinseed Sunfish (<i>Lepomis gibbosus</i>)	No	1		British Columbia
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-ONCLle	Westslope Cutthroat Trout (<i>Oncorhynchus clarkii lewisi</i>)	No	1		Alberta
te-ONMY	Rainbow Trout (<i>Oncorhynchus mykiss</i>)	No	1		Alberta
te-PRCY	Round Whitefish (<i>Prosopium cylindraceum</i>)	No	1		Yukon
te-SAFO	Brook Trout (<i>Salvelinus fontinalis</i>)	No	1		Alberta
te-SASA	Atlantic Salmon (<i>Salmo salar</i>)	No	1		Nova Scotia

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

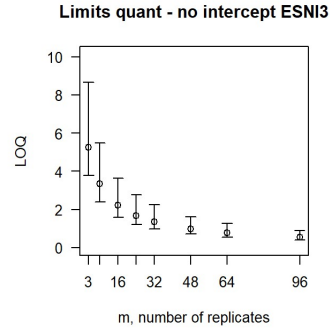
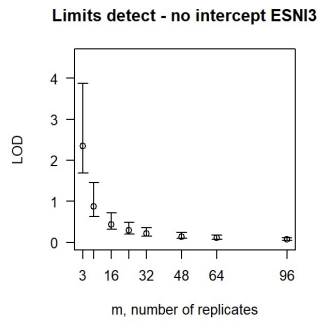
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>



From 8 Technical Replicates

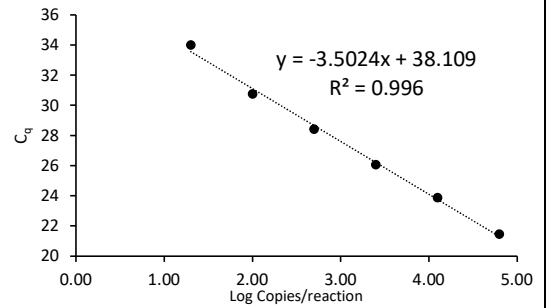
# Detects	# Copies	SE
0	0	NaN
1	0.315	0.321
2	0.678	0.5
3	1.108	0.682
4	1.633	0.895
5	2.311	1.171
6	3.267	1.584
7	4.901	2.414

Determined using eLowQuant R code⁴.



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 91%

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	Mitochondrial NADH dehydrogenase 2 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