

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: Walleye (*Sander vitreus*)
Species Code: te-SAVI
eDNA qPCR Tool: eSAVI2
eDNA qPCR Format: TaqMan
Gene Target: MT-ND1
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.5 Copies/Rxn LOQ 1 95% CI 0.7-1.8 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 (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
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>)	No	2	British Columbia
te-CACO	White sucker (<i>Catostomus commersonii</i>)	No	2	Alberta
te-CAMA	Largescale sucker (<i>Catostomus macrocheilus</i>)	No	2	British Columbia
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1	Alberta
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	2	British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	2	British Columbia
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	2	Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	2	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	British Columbia
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	2	British Columbia
te-PRCY	Round whitefish (<i>Prosopium cylindraceum</i>)	No	1	British Columbia
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	No	2	British Columbia
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	2	British Columbia
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	2	British Columbia
te-SAVI	Walleye (<i>Sander vitreus</i>)	Yes	1	Alberta
te-THAR	Arctic grayling (<i>Thymallus arcticus</i>)	No	2	British Columbia

References

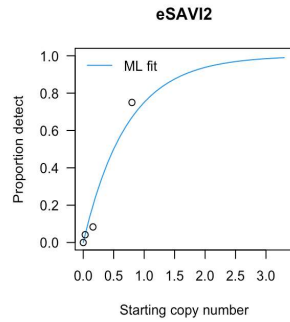
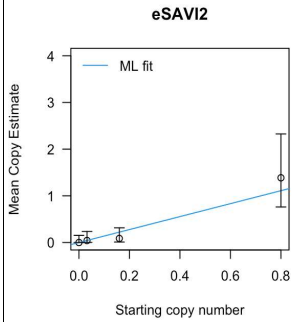
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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 (2021) 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

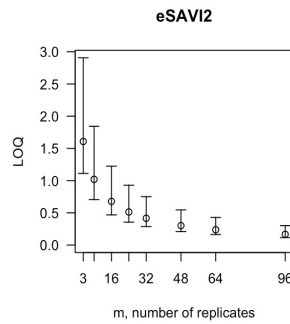
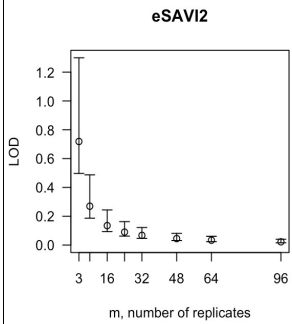
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

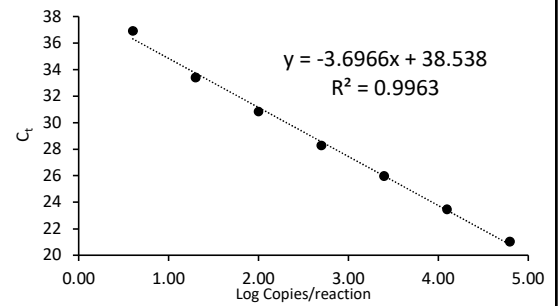


# Detects	# Copies	SE
0	0	0
1	0.096	0.099
2	0.21	0.15
3	0.34	0.21
4	0.5	0.28
5	0.71	0.37
6	1	0.5
7	1.5	0.76

Determined using eLowQuant R code⁴.



Applied to reactions with ≥ 95% positive hits



Efficiency 86%

Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
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Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 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