

**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: American pickerels (*Esox americanus*) eDNA qPCR Tool: eESAM2 Gene Target: MT-Dloop
 Species Code: te-ESAM eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.9 Copies/Rxn LOQ 2 95% CI 1.5-3.4 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		Sample Sources/Locations
			Specimens		
ma-CALufa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
te-CACAch	Salish sucker (<i>Catostomus catostomus</i>)	No	1		Alberta
te-CACO	White sucker (<i>Catostomus commersonii</i>)	No	1		Ontario
te-CAMA	Largescale sucker (<i>Catostomus macrocheilus</i>)	No	1		British Columbia
te-COAR	Cisco/Tullibee (<i>Coregonus artedii</i>)	No	1		Alberta
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1		Alberta
te-COCO	Slimy sculpin (<i>Cottus cognatus</i>)	No	1		British Columbia
te-ESAMam	Redfin pickerel (<i>Esox americanus americanus</i>)	Yes	4		Ontario and Quebec
te-ESAMve	Grass pickerel (<i>Esox americanus vermiculatus</i>)	Yes	4		Ontario
te-ESLU	Northern pike (<i>Esox lucius</i>)	No	1		British Columbia
te-ESNI	Chain pickerel (<i>Esox niger</i>)	No	1		British Columbia
te-LOLO	Burbot (<i>Lota lota</i>)	No	2		Alberta
te-MYCA	Peamouth chub (<i>Mylocheilus caurinus</i>)	No	1		Ontario
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</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-PLGR	Flathead chub (<i>Platygobio gracilis</i>)	Yes	1		British Columbia
te-PTOR	Mountain whitefish (<i>Prosopium williamsoni</i>)	No	1		British Columbia
te-RHCA	Longnose (nooksack) dace (<i>Rhinichthys cataractae</i>)	No	5		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

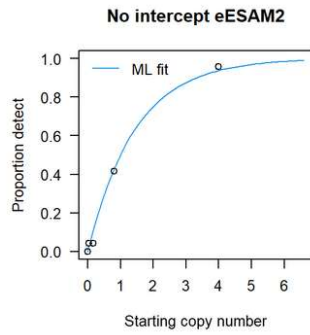
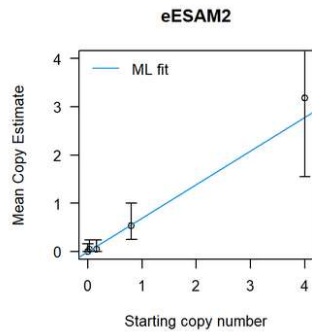
References

- 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/>

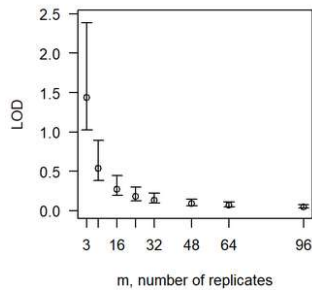


From 8 Technical Replicates

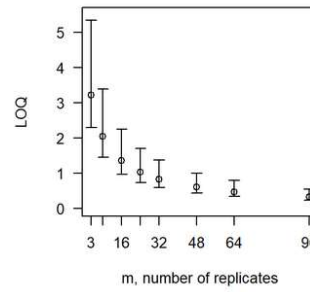
# Detects	# Copies	SE
0	0	0
1	0.192	0.196
2	0.414	0.305
3	0.676	0.417
4	0.997	0.547
5	1.411	0.716
6	1.994	0.969
7	2.991	1.476

Determined using eLowQuant R code⁴.

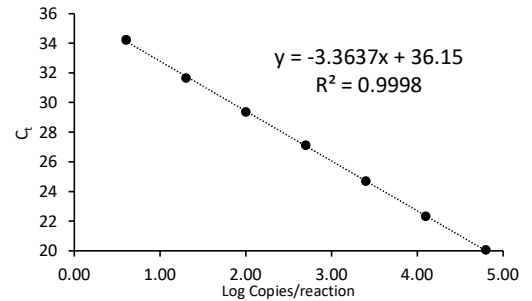
Limits detect - no intercept eESAM2



Limits quant - no intercept eESAM2



Applied to reactions with $\geq 95\%$ positive hits



Efficiency 98%

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

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-Dloop	Mitochondrial displacement loop (D-loop) 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