

### 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 sensitivity<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Salish Sucker (*Catostomus sp. cf. catostomus*) eDNA qPCR Tool: eCACA2 Gene Target: MT-CYB  
 Species Code: te-CACAch eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 2.2 95% CI 1.6-3.6 Copies/Rxn LOQ 8.3 95% CI 6-13.7 Copies/Rxn LOB 0 hits/8  
 LOQ<sub>continuous</sub> 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Immolase

#### eDNA Assay Specificity Test Information

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

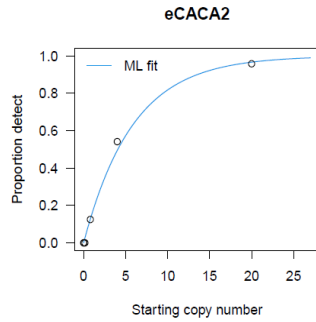
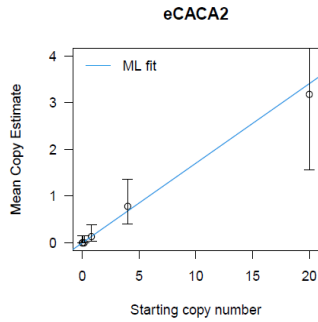
Species	Common Name ( <i>Species</i> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-LICA	American Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	2		British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands
te-CAAU	Goldfish ( <i>Carassius auratus</i> )	No	2		British Columbia
te-CACAch	Salish Sucker ( <i>Catostomus sp. cf. catostomus</i> )	Yes	8		British Columbia
te-COCO	Slimy Sculpin ( <i>Cottus cognatus</i> )	No	2		British Columbia
te-ESLU	Northern Pike ( <i>Esox lucius</i> )	No	1		British Columbia
te-GAAC	Three Spine Stickleback ( <i>Gasterosteus aculeatus</i> )	No	1		British Columbia
te-LEGI	Pumpkinseed Sunfish ( <i>Lepomis gibbosus</i> )	No	2		British Columbia
te-ONCLcl	Coastal Cutthroat Trout ( <i>Oncorhynchus clarkii clarkii</i> )	No	1		British Columbia
te-ONKI	Coho Salmon ( <i>Oncorhynchus kisutch</i> )	No	1		British Columbia
te-ONMY	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	No	1		British Columbia
te-SAMA	Dolly Varden ( <i>Salvelinus malma</i> )	No	1		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. 2020; 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



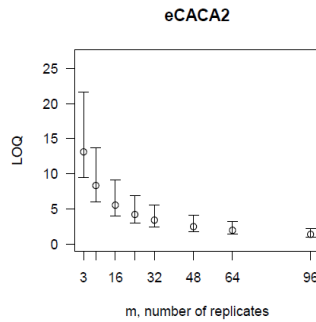
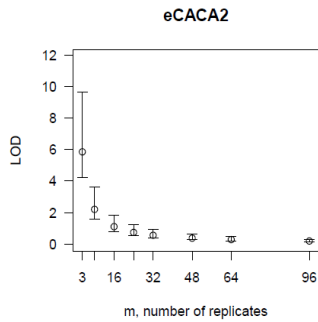
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

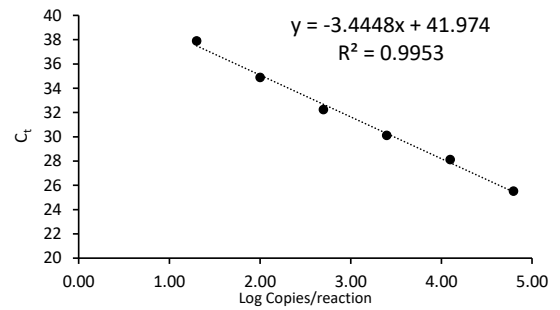
# Detects	# Copies	SE
0	0	0
1	0.78	0.8
2	1.69	1.25
3	2.76	1.7
4	4.07	2.23
5	5.75	2.91
6	8.14	3.95
7	12.19	6

Determined using eLowQuant R code<sup>4</sup>.



Binomial-Poisson model: No intercept  
Determined using eLowQuant R code<sup>4</sup>.  
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Y	3	Y	Manual Pond, British Columbia
Water	Y	1	Y	Agassiz Slough, British Columbia

Abbreviations

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