



### Helbing/Langlois 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: Sauger (*Sander canadensis*) eDNA qPCR Tool: eSACA1 Gene Target: MT-ND2  
Species Code: te-SACA eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0 95% CI 0-0 Copies/Rxn LOQ 0.1 95% CI 0.1-0.2 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: QIACuity

#### eDNA Assay Specificity Test Information

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

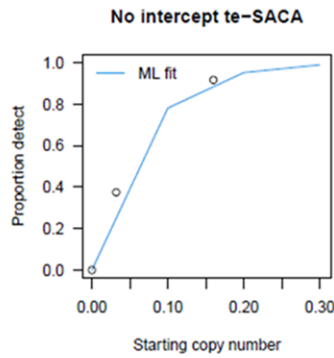
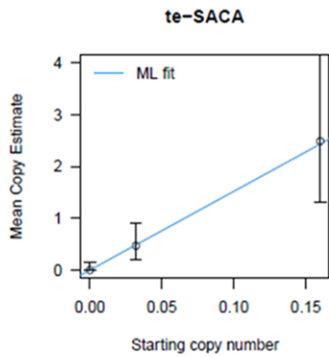
Species	Common Name ( <i>Species</i> )	Detection	# Voucher	
			Specimens	Sample Sources/Locations
te-SACA	Sauger ( <i>Sander canadensis</i> )	Yes	2	Canada: Quebec
te-SAVI	Walleye ( <i>Sander vitreus</i> )	No	2	Canada: Quebec
ma-CALUfa	Canine ( <i>Canis lupus familiaris</i> )	No	1	Canada: Quebec
ma-FECA	Cat ( <i>Felis catus</i> )	No	2	Canada: Quebec
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Canada: Quebec

#### 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

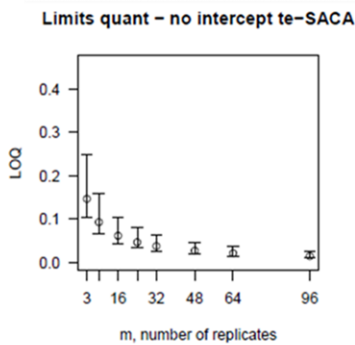
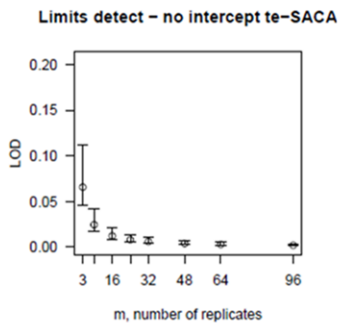


To generate tables for different numbers of replicates, use raw csv data files.

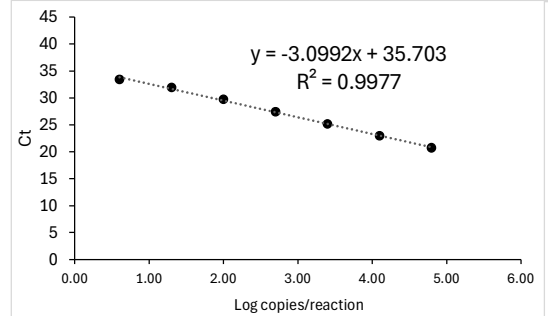
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.008	0.018
2	0.018	0.018
3	0.03	0.021
4	0.044	0.025
5	0.063	0.032
6	0.089	0.044
7	0.134	0.069

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



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



Efficiency 110%

Binomial-Poisson model: No intercept

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

Based on a 2  $\mu$ L DNA input in a total 15  $\mu$ L reaction

Field Sample Validation

Known  
Sample Type Presence # Samples Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial 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