



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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Striped bass (*Morone saxatilis*) eDNA qPCR Tool: eMOSA2 Gene Target: MT-ATP8
Species Code: te-MOSA eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.6 95% CI 0.5-1 Copies/Rxn LOQ 2.4 95% CI 1.7-3.9 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
te-MOSA	Striped bass	Yes	2	Canada: Quebec: Fleuve St-Laurent, Lac St-François, Lac St-Pierre, Lac St-Louis
te-MOAM	White perch (<i>Morone americana</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

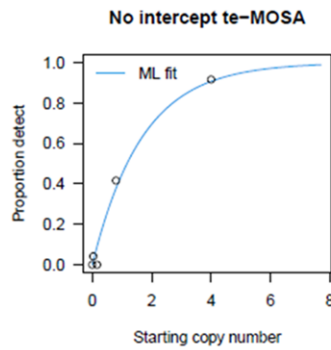
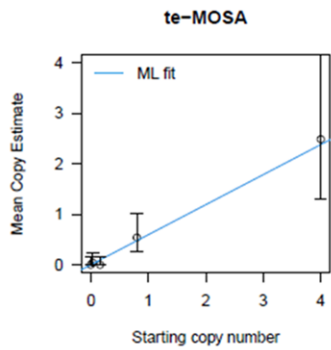
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>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

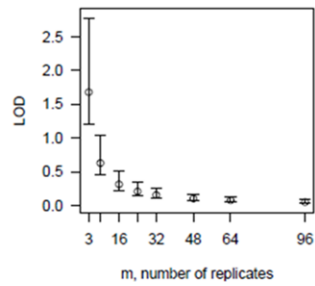
From 8 Technical Replicates



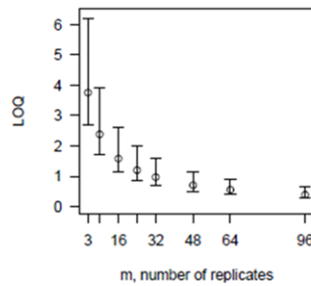
# Detects	# Copies	SE
0	0	0
1	0.209	0.214
2	0.532	0.395
3	0.797	0.492
4	1.132	0.616
5	1.595	0.8
6	2.24	1.069
7	3.325	1.617

Determined using eLowQuant R code⁴.

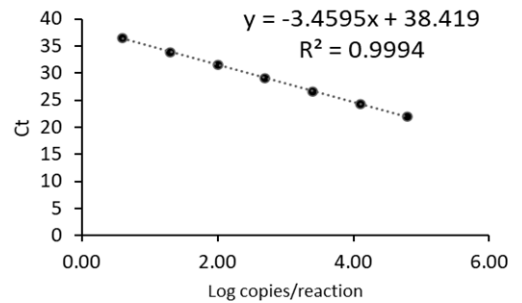
Limits detect – no intercept te-MOSA



Limits quant – no intercept te-MOSA



Applied to reactions with ≥ 95% positive hits



Efficiency 95%

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		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ATP8	Mitochondrial ATP synthase membrane subunit 8
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