



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: Smoky shrew (*Sorex fumeus*) eDNA qPCR Tool: eSOFU1 Gene Target: MT-ND1
Species Code: ma-SOFU eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-0.7 Copies/Rxn LOQ 1.8 95% CI 1.3-2.7 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 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-SOFU	Smoky shrew (<i>Sorex fumeus</i>)	Yes	3		Quebec
ma-SOCI	Masked shrew (<i>Sorex cinereus</i>)	No	5		Quebec
ma-SOHO	Pygmy shrew (<i>Sorex hoyi</i>)	No	3		Quebec
ma-BLBR	Northern short-tailed shrew (<i>Blarina brevicauda</i>)	No	3		Quebec
ma-SOGA	Gaspé shrew (<i>Sorex gaspensis</i>)	No	1		Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Cell line ATCC
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1		Quebec
ma-FECA	Cat (<i>Felis catus</i>)	No	2		Quebec

References

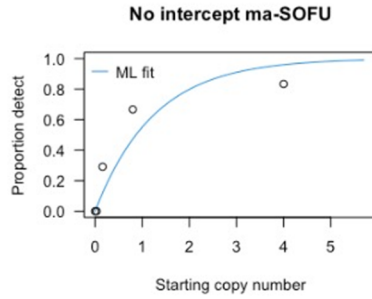
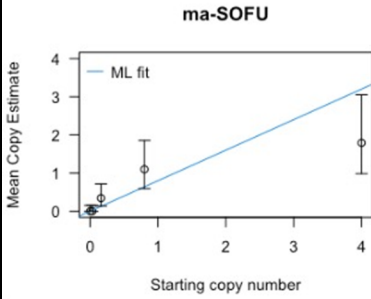
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>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



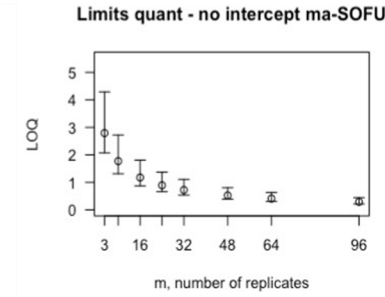
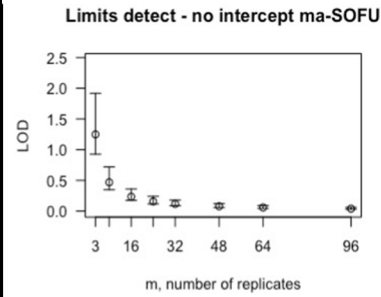
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

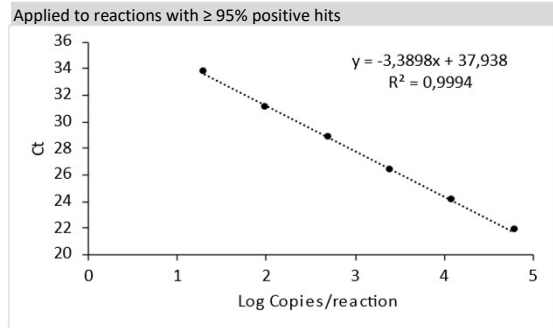
From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.167	0.17
2	0.36	0.263
3	0.588	0.358
4	0.867	0.468
5	1.226	0.611
6	1.728	0.822
7	2.602	1.259



Determined using eLowQuant R code⁴.



Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Efficiency 97%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Soil	Y	2	Y	Parc national de la Jacques-Cartier, Quebec

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 1
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