



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: Southern bog lemming (*Synaptomys cooperi*) eDNA qPCR Tool: eSYCO1 Gene Target: MT-ND2
Species Code: ma-SYCO eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.9 95% CI 0.6-1.6 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)

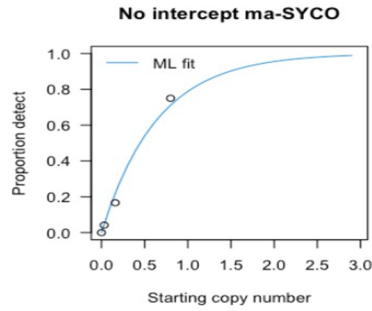
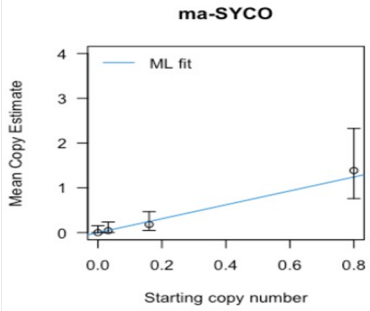
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-SYCO	Southern bog lemming (<i>Synaptomys cooperi</i>)	Yes	2		Quebec
ma-MIPI	Woodland vole (<i>Microtus pinetorum</i>)	No	2		Quebec
ma-ZAHU	Meadow jumping mouse (<i>Zapus hudsonius</i>)	No	3		Quebec
ma-SYBO	Northern bog lemming (<i>Synaptomys borealis</i>)	No	1		Quebec
ma-CLGA	Gapper's red-backed vole (<i>Clethrionomys gapperi</i>)	No	3		Quebec
ma-PEsp	Deer mouse or White-footed mouse (<i>Peromyscus sp.</i>)	No	3		Quebec
ma-NAIN	Woodland jumping mouse (<i>Napaeozapus insignis</i>)	No	3		Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Cell line ATCC
ma-CALufa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		Quebec
ma-FECA	Domestic 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



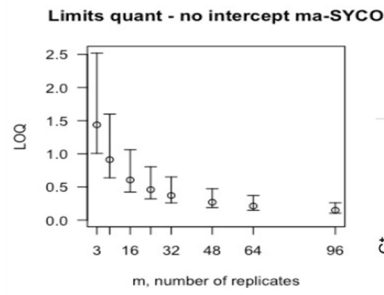
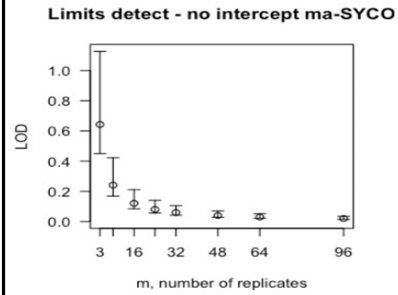
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



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

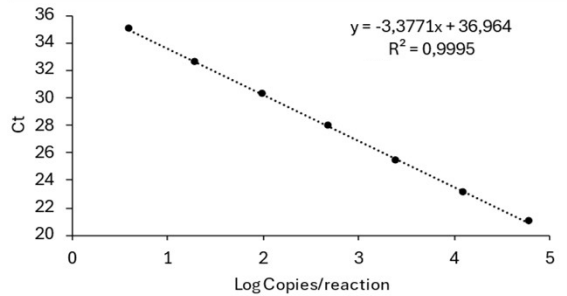
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.086	0.088
2	0.185	0.138
3	0.303	0.188
4	0.446	0.248
5	0.631	0.325
6	0.892	0.44
7	1.338	0.67



Determined using eLowQuant R code⁴.

Applied to reactions with ≥ 95% positive hits



Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 μL DNA input in a total 15 μL reaction

Efficiency 98%

Field Sample Validation

Sample Type	Known			
	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