



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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Pacific Water Shrew (*Sorex bendirii*) eDNA qPCR Tool: eSOBE7 Gene Target: MT-ND4
Species Code: ma-SOBE eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.5 Copies/Rxn LOQ 1.1 95% CI 0.8-2 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-SOBE	Pacific water/marsh shrew (<i>Sorex bendirii</i>)	Yes	3		Washington
ma-SOCI	Masked shrew (<i>Sorex cinereus</i>)	No	1		Washington
ma-SOMO	Dusky/montane shrew (<i>Sorex monticolus</i>)	No	1		Washington
ma-SONA	Cardilleran water shrew (<i>Sorex navigator</i>)	No	1		Washington
ma-SOPaPa	American water shrew (<i>Sorex palustris palustris</i>)	No	1		Washington
ma-SORO	Olympic water shrew (<i>Sorex rowherii</i>)	No	1		Washington
ma-SOTR	Trowbridge's shrew (<i>Sorex trowbridgii</i>)	No	1		Washington
ma-SOVA	Wandering (vagrant) shrew (<i>Sorex vagrans</i>)	No	1		Washington
ma-ALAL	Moose (<i>Alces alces</i>)	No	1		British Columbia
ma-CEEL	Red deer (<i>Cervus elaphus</i>)	No	1		British Columbia
ma-MUMU	House mouse (<i>Mus musculus</i>)	No	1		British Columbia
ma-MYLU	Little Brown myotis (<i>Myotis lucifugus</i>)	No	1		British Columbia
ma-NEVI	American mink (<i>Neovision vision</i>)	No	1		British Columbia
ma-ODHE	Mule deer (<i>Odocoileus hemionus</i>)	No	1		British Columbia
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	No	1		Washington
ma-URAM	American black bear (<i>Ursus americanus</i>)	No	1		British Columbia
ma-USAR	Grizzly bear (<i>Ursus arctos</i>)	No	1		British Columbia
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands

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

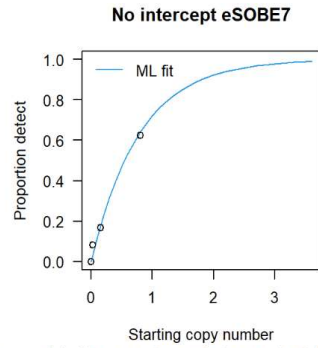
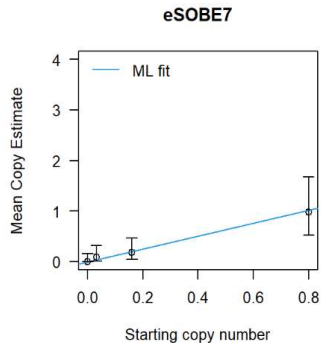
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

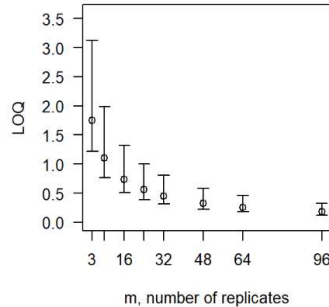
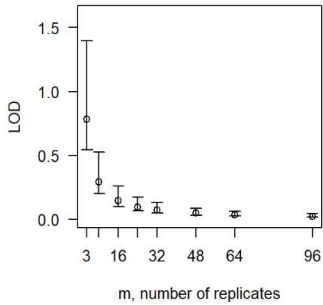
# Detects	# Copies	SE
0	0	0
1	0.11	0.11
2	0.23	0.17
3	0.37	0.23
4	0.54	0.3
5	0.77	0.4
6	1.09	0.54
7	1.63	0.82

Determined using eLowQuant R code⁴.



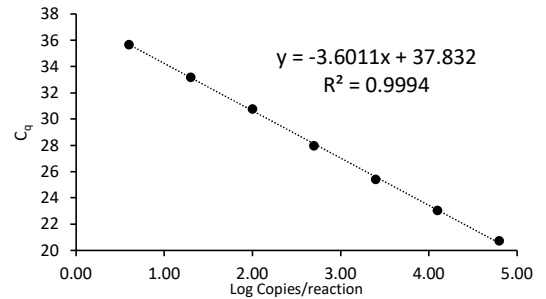
Limits detect - no intercept eSOBE7

Limits quant - no intercept eSOBE7



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

Applied to reactions with ≥ 95% positive hits



Efficiency 90%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH-ubiquinone oxidoreductase chain 4 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