



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: Beaver (*Castor canadensis*) eDNA qPCR Tool: emaCACAA3 Gene Target: MT-COI
 Species Code: ma-CACA eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1-0.4 Copies/Rxn LOQ 0.8 95% CI 0.6-1.4 Copies/Rxn LOB 0 hits/8
 LOQ_{continuous} 0 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: Qiacity

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>)	# Voucher		Sample Sources/Locations
		Detection	Specimens	
ma-CACA	Beaver (<i>Castor canadensis</i>)	Yes	4	Ontario
ma-CALU	Wolf (<i>Canis lupus</i>)	No	1	British Columbia
ma-URAM	American black bear (<i>Ursus americanus</i>)	No	1	British Columbia
ma-FECA	Cat (domestic) (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-CALufa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-LOCA	River otter (<i>Lontra canadensis</i>)	No	1	British Columbia
ma-SCCA	Eastern grey squirrel (<i>Sciurus carolinensis</i>)	No	1	British Columbia
ma-ORCU	European rabbit (<i>Oryctolagus cuniculus</i>)	No	1	British Columbia
ma-PEPE	Fisher (<i>Pekania pennanti</i>)	No	1	British Columbia
ma-MAAM	American marten (<i>Martes americana</i>)	No	1	British Columbia
ma-NEVI	American mink (<i>Neovision vision</i>)	No	1	British Columbia
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	No	1	British Columbia
ma-MYLU	Little Brown myotis (bat) (<i>Myotis lucifugus</i>)	No	1	British Columbia
ma-CALA	Coyote (<i>Canis latrans</i>)	No	1	British Columbia
ma-GUGU	Wolverine (<i>Gulo gulo</i>)	No	1	British Columbia
ma-ORAM	Mountain goat (<i>Oreamnos americanus</i>)	No	1	British Columbia
ma-MUMU	House mouse (<i>Mus musculus</i>)	No	1	British Columbia
ma-VUVU	Fox (<i>Vulpes vulpes</i>)	No	1	British Columbia

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

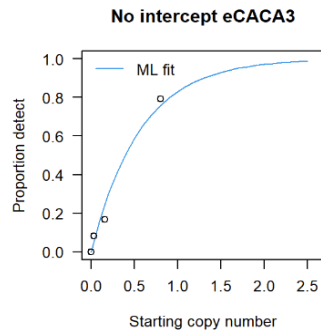
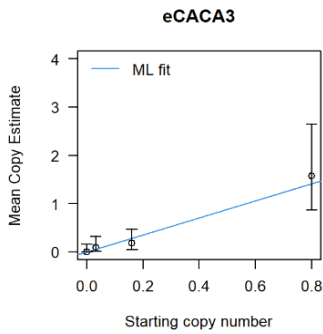


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

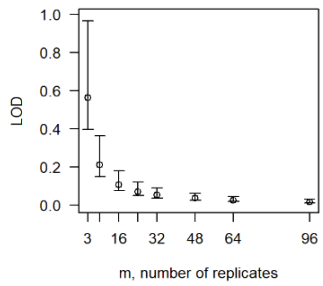
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

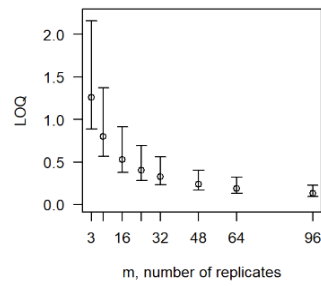
# Detects	# Copies	SE
0	0	0
1	0.065	0.068
2	0.145	0.108
3	0.236	0.146
4	0.348	0.193
5	0.492	0.254
6	0.699	0.349
7	1.048	0.532



Limits detect - no intercept eCACA3

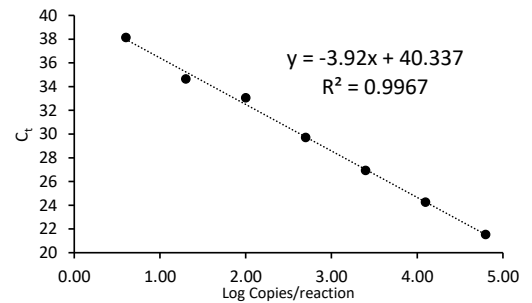


Limits quant - no intercept eCACA3



Determined using eLowQuant R code⁴.

Applied to reactions with ≥ 95% positive hits



Efficiency 80%

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			Location
	Presence	# Samples	Detected	

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COI	Mitochondrial cytochrome oxidase subunit 1 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