



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: Wolf (*Canis lupus*) eDNA qPCR Tool: eCALU1 Gene Target: MT-CYTB
 Species Code: ma-CALU eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1 - 0.3 Copies/Rxn LOQ 0.8 95% CI 0.6 - 1.3 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
ma-CALU	Wolf (<i>Canis lupus</i>)	Yes	6	British Columbia
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	Yes	1	British Columbia
ma-CALA	Coyote (<i>Canis latra</i>)	No	1	Ontario
ma-FECA	Cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-ALAL	Moose (<i>Alces alces</i>)	No	1	Ontario
ma-ANPA	Pallid bat (<i>Antrozous pallidus</i>)	No	1	British Columbia
ma-CEEL	Red deer (<i>Cervus elaphus</i>)	No	1	Ontario
ma-ESRO	Gray whale (<i>Eschrichtius robustus</i>)	No	1	British Columbia
ma-LOCA	River otter (<i>Lontra canadensis</i>)	No	1	British Columbia
ma-MUMU	House mouse (<i>Mus musculus</i>)	No	1	British Columbia
ma-ODHE	Mule deer (<i>Odocoileus hemionus</i>)	No	1	Ontario
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	No	1	Ontario
ma-ORCU	European rabbit (<i>Oryctolagus cuniculus</i>)	No	1	British Columbia
ma-PEPE	Flsher (<i>Pekania pennanti</i>)	No	1	British Columbia
ma-PHPH	Harbour porpoise (<i>Phocoena phocoena</i>)	No	1	British Columbia
ma-PHSO	Southern elephant seal (<i>Phoca leonina</i>)	No	1	British Columbia
ma-PHVI	Harbour seal (<i>Phoca vitulina</i>)	No	1	British Columbia
ma-SCCA	Eastern grey squirrel (<i>Sciurus carolinensis</i>)	No	1	British Columbia
ma-SOBE	Pacific water/marsh shrew (<i>Sorex bendirii</i>)	No	1	British Columbia
ma-SONA	Cardillieran water shrew (<i>Sorex navigator</i>)	No	1	British Columbia
ma-USAR	Grizzly bear (<i>Ursus arctos</i>)	No	1	British Columbia
ma-ZACA	California sea lion (<i>Zalophus californianus</i>)	No	1	British Columbia
ma-URAM	American black bear (<i>Ursus americanus</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

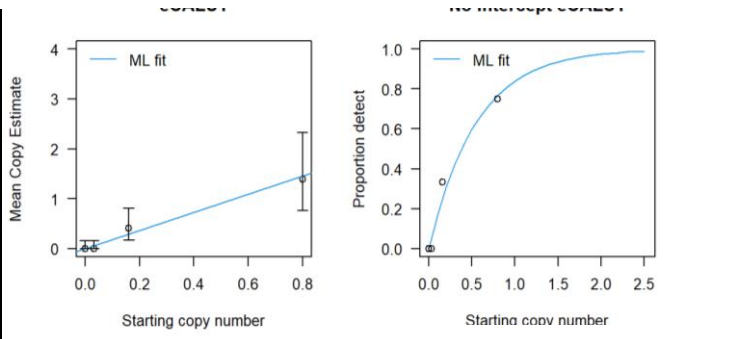


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eCALU1

No intercept eCALU1

To calculate tables for different numbers of replicates, raw csv data files can be accessed here:

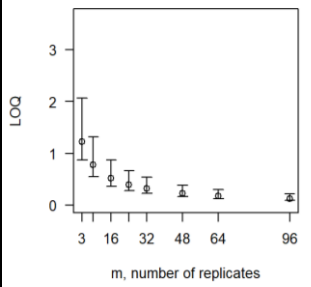


From 8 Technical Replicates

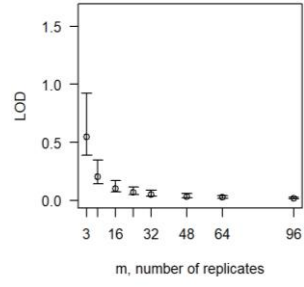
# Detects	# Copies	SE
0	0.00	0.00
1	0.073	0.075
2	0.16	0.12
3	0.26	0.16
4	0.38	0.21
5	0.54	0.27
6	0.76	0.37
7	1.14	0.57

Determined using eLowQuant R code⁴.

Limits quant - no intercept eCALU1

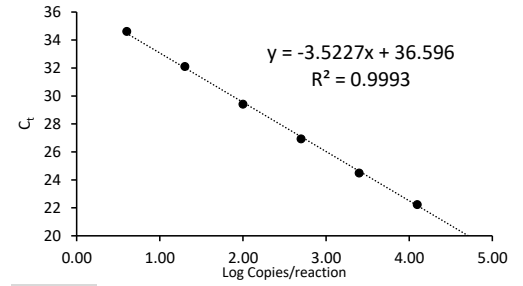


Limits detect - no intercept eCALU1



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 100% positive hits



Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
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Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYTB	Mitochondrially encoded cytochrome b
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