



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: Odocoileus spp. eDNA qPCR Tool: eOdocoileus2 Gene Target: MT-COI Annealing Temperature: 62 °C
 Species Code: ma-ODspp eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1 95% CI 0.7-1.7 Copies/Rxn LOQ 3.9 95% CI 2.8-6.5 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)

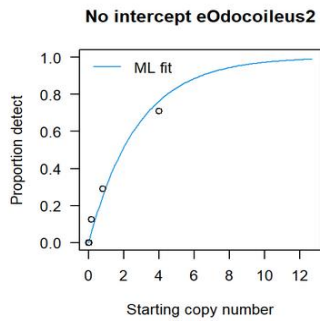
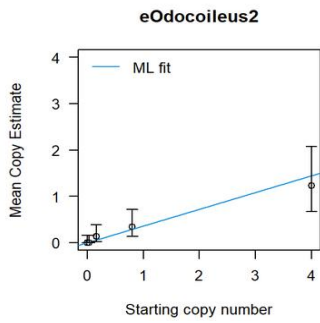
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-ALAL	Moose (<i>Alces alces</i>)	No	1		British Columbia
ma-ALAM	Northern (American) moose (<i>Alces americanus</i>)	No	1		British Columbia
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-CECA	Elk (<i>Cervus canadensis</i>)	Yes	1		British Columbia
ma-CEEL	Red deer (<i>Cervus elaphus</i>)	No	1		British Columbia
ma-CENI	Sika deer (<i>Cervus nippon</i>)	No	1		British Columbia
ma-DADA	Fallow deer (<i>Dama dama</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
ma-LEAM	Snowshoe hare (<i>Lepus americanus</i>)	No	1		British Columbia
ma-LOCA	River otter (<i>Lontra canadensis</i>)	No	1		British Columbia
ma-LYCA	Canada lynx (<i>Lynx canadensis</i>)	No	1		British Columbia
ma-LYRUfa	Western Bobcat (<i>Lynx rufus fasciatus</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>)	Yes	2		British Columbia
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	Yes	2		British Columbia
ma-ORAM	Mountain goat (<i>Oreamnos americanus</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-RATA	Boreal woodland caribou (<i>Rangifer tarandus</i>)	No	1		British Columbia
ma-SUSC	Wild boar (<i>Sus scrofa</i>)	No	1		Ontario
ma-URAM	American black bear (<i>Ursus americanus</i>)	No	1		British Columbia
ma-URAR	Grizzly bear (<i>Ursus arctos</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

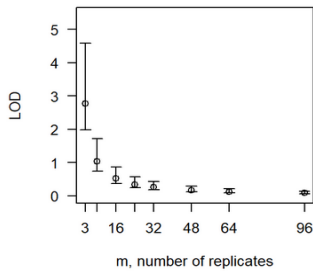
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>



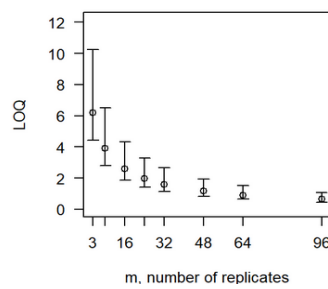
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.369	0.377
2	0.796	0.587
3	1.3	0.802
4	1.918	1.053
5	2.714	1.378
6	3.836	1.865
7	5.748	2.835

Limits detect - no intercept eOdocoileu

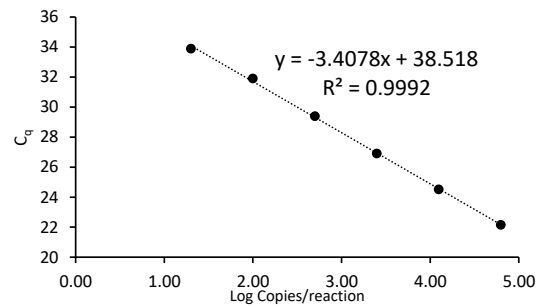


Limits quant - no intercept eOdocoileu



Determined using eLowQuant R code⁴.

Applied to reactions with $\geq 95\%$ positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

Efficiency 97%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

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