



### 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 sensitivity<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Bighorn sheep (*Ovis canadensis*) eDNA qPCR Tool: eOVCA1 Gene Target: MT-TL1  
Species Code: ma-OVCA eDNA qPCR Format: TaqMan Published in:

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-0.9 Copies/Rxn LOQ 1.7 95% CI 1.1-3.5 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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-BIBI	American bison ( <i>Bison bison</i> )	No	1		British Columbia
ma-CACA	Beaver ( <i>Castor canadensis</i> )	No	1		British Columbia
ma-CALU	Wolf ( <i>Canis lupus</i> )	No	1		British Columbia
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1		British Columbia
ma-DADA	Fallow deer ( <i>Dama dama</i> )	No	1		British Columbia
ma-FECA	Cat ( <i>Felis catus</i> )	No	1		British Columbia
ma-GUGU	Wolverine ( <i>Gulo gulo</i> )	No	1		Ontario
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands
ma-LOCA	River otter ( <i>Lontra canadensis</i> )	No	1		British Columbia
ma-LYRUfa	Western bobcat ( <i>Lynx rufus fasciatus</i> )	No	1		British Columbia
ma-MUMU	Mouse ( <i>Mus musculus</i> )	No	1		British Columbia
ma-MUNI	Least weasel ( <i>Mustela nivalis</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		Washington
ma-ODVI	White-tailed deer ( <i>Odocoileus virginianus</i> )	No	1		British Columbia
ma-ORAM	Mountain goat ( <i>Oreamnos americanus</i> )	No	1		British Columbia
ma-OVCA	Bighorn sheep ( <i>Ovis canadensis</i> )	Yes	1		British Columbia
ma-OVDA	Thinhorn sheep ( <i>Ovis dalli</i> )	No	1		British Columbia
ma-PEPE	Fisher ( <i>Pekania pennanti</i> )	No	1		British Columbia
ma-PUCO	Cougar ( <i>Puma concolor</i> )	No	1		British Columbia
ma-SOBE	Pacific water/marsh shrew ( <i>Sorex bendirii</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

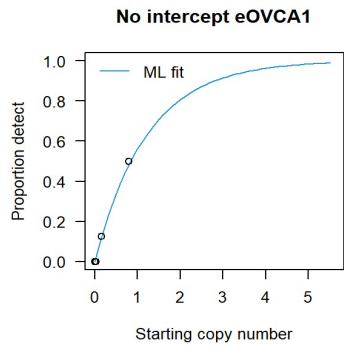
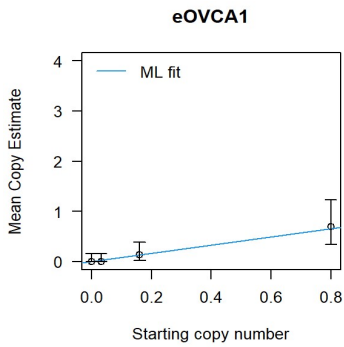
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

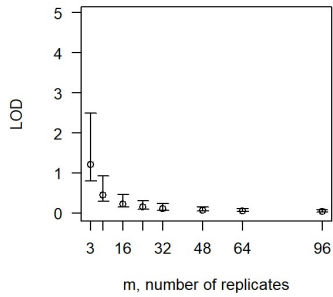
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.162	0.168
2	0.35	0.265
3	0.572	0.365
4	0.843	0.483
5	1.193	0.637
6	1.686	0.866
7	2.529	1.316

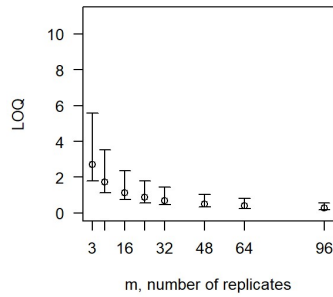
Determined using eLowQuant R code<sup>4</sup>.



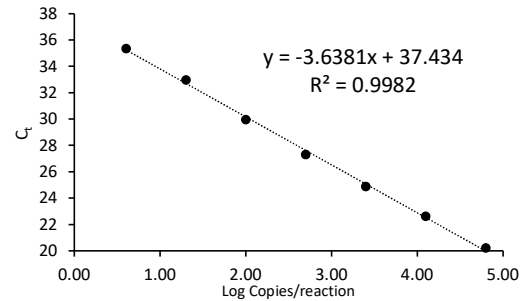
Limits detect - no intercept eOVCA1



Limits quant - no intercept eOVCA1



Applied to reactions with ≥ 95% positive hits



Efficiency 88%

Binomial-Poisson model: No intercept  
Determined using eLowQuant R code<sup>4</sup>.  
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-TL1	Mitochondrially encoded tRNA Leucine 1
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