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[1-3].

General eDNA Assay Information

Target Species: *Wild boar (Sus scrofa)*	eDNA qPCR Tool: eSUSC1 Gene Target: MT-CYB
Species Code: ma-SUSC	eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD   0.1   95% CI   0.1-0.2   Copies/Rxn   LOQ   0.6   95% CI   0.4-0.9   Copies/Rxn   LOB   0   hits/8

$LOQ_{continuous}$ 0.8   Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].   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 (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-ALAL	Moose (*Alces alces*)	No	2	British Columbia
ma-CALU	Wolf (*Canis lupus*)	No	1	British Columbia
ma-CALUfa	Domestic dog (*Canis lupus familiaris*)	No	3	British Columbia
ma-CEEL	Red deer (*Cervus elaphus*)	No	2	Washington
ma-EPFU	Big brown bat (*Eptesicus fuscus*)	No	2	British Columbia
ma-FECA	Domestic cat (*Felis catus*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
ma-LOCA	River otter (*Lontra canadensis*)	No	2	British Columbia
ma-MUMU	House mouse (*Mus musculus*)	No	1	British Columbia
ma-MYCI	Western Small-footed myotis (bat) (*Myotis ciliolabrum*)	No	2	British Columbia
ma-NEVI	American mink (*Neovision vision*)	No	3	Ontario
ma-ODHE	Mule deer (*Odocoileus hemionus*)	No	2	British Columbia
ma-ORCU	European rabbit (*Oryctolagus cuniculus*)	No	1	British Columbia
ma-PEPE	Fisher (*Pekania pennanti*)	No	2	British Columbia
ma-SCCA	Eastern grey squirrel (*Sciurus carolinensis*)	No	1	British Columbia
ma-SOBE	Pacific water/marsh shrew (*Sorex bendirii*)	No	2	Washington
ma-SUSC	Wild boar (*Sus scrofa*)	Yes	4	Ontario
ma-TABR	Brazilian free-tailed bat (*Tadarida brasiliensis*)	No	3	Tennessee
ma-URAM	American black bear (*Ursus americanus*)	No	2	British Columbia
ma-URAR	Grizzly bear (*Ursus arctos*)	No	3	British Columbia

References

1. 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

2. 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

3. 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

4. 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
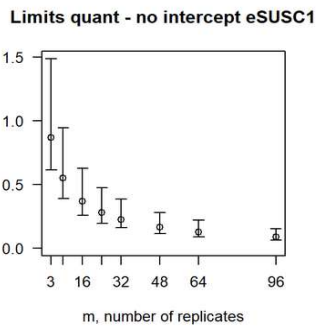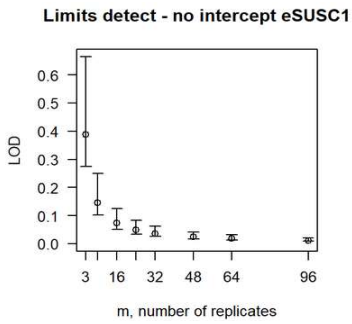
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eSUSC1

No intercept eSUSC1

To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
https://onlineacademiccommunity.uvic.ca/helbinglab/edna/
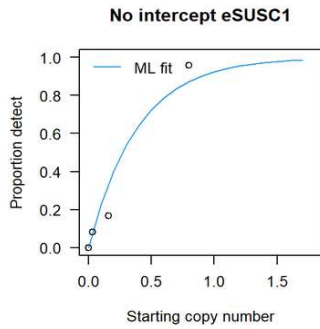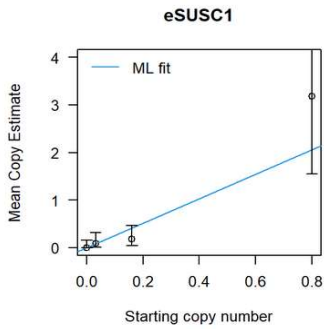
From ____8____ Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.052	0.053
2	0.112	0.083
3	0.183	0.113
4	0.27	0.149
5	0.382	0.195
6	0.539	0.264
7	0.809	0.402

Determined using eLowQuant R code[4].

Limits detect - no intercept eSUSC1

Limits quant - no intercept eSUSC1

Applied to reactions with ≥ 95% positive hits

$y = -3.4682x + 36.627$
$R^2 = 0.9991$
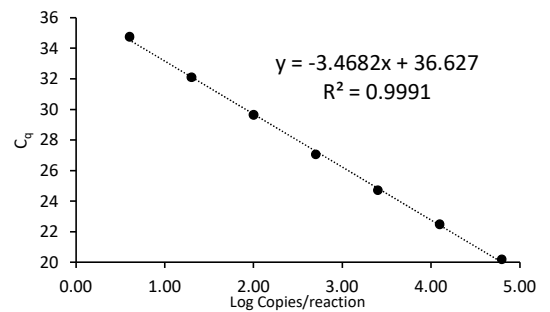
Binomial-Poisson model: ____No intercept____
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency   94%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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