



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: Northern goshawk (*Accipiter gentilis*) eDNA qPCR Tool: eACGE3 Gene Target: MT-ND4
 Species Code: av-ACGE 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 0.7 95% CI 0.5-1.2 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>)	# Voucher		Sample Sources/Locations
		Detection	Specimens	
av-CAGU	Hermit thrush (<i>Catharus guttatus</i>)	No	1	British Columbia
av-ACGE	Northern goshawk (<i>Accipiter gentilis</i>)	Yes	3	British Columbia
av-COLI	Rock pigeon (<i>Columba livia</i>)	No	1	British Columbia
av-STVU	European starling (<i>Sturnus vulgaris</i>)	No	1	British Columbia
av-MEGA	Turkey (<i>Meleagris gallopavo</i>)	No	1	British Columbia
av-TYPH	Sharp-tailed grouse (<i>Tympanuchus phasianellus</i>)	No	1	Nebraska
av-LALA	Willow ptarmigan (<i>Lagopus lagopus</i>)	No	1	British Columbia
av-CACA	Red knot (<i>Calidris canutus</i>)	No	1	British Columbia
av-POAT	Black-capped chickadee (<i>Poecile atricapillus</i>)	No	1	British Columbia
av-RIRI	Bank swallow (<i>Riparia riparia</i>)	No	1	British Columbia
av-CHMO	Mountain plover (<i>Charadrius montanus</i>)	No	1	California
av-FUAM	American coot (<i>Fulica americana</i>)	No	1	British Columbia
av-BRMA	Marbled murrelet (<i>Brachyramphus marmoratus</i>)	No	1	British Columbia
av-BUIS	Barrow's goldeneye (<i>Bucephala islandica</i>)	No	1	British Columbia
av-FACA	Spruce grouse (<i>Falciennis canadensis</i>)	No	1	British Columbia
av-SPNU	Red-naped sapsucker (<i>Sphyrapicus nuchalis</i>)	No	1	British Columbia
av-AMBA	Baird's sparrow (<i>Ammodramus bairdii</i>)	No	1	British Columbia
av-AMNE	Nelson's sparrow (<i>Ammospiza nelsoni</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1	British Columbia
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia

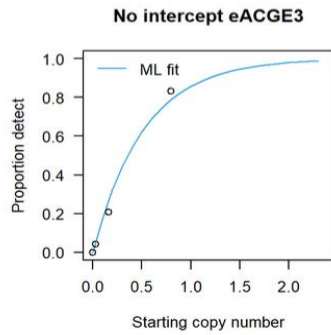
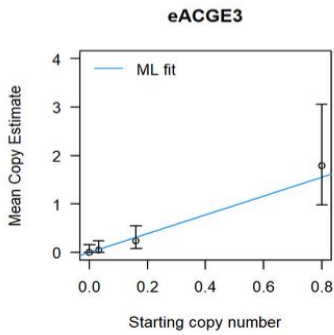
References

- 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/>

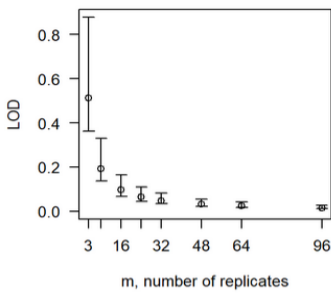


From 8 Technical Replicates

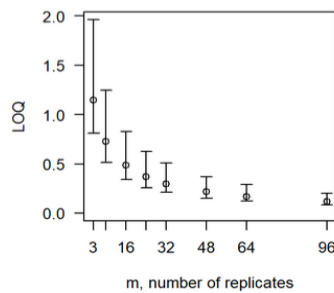
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1	0.069	0.07
2	0.148	0.11
3	0.242	0.15
4	0.357	0.197
5	0.505	0.258
6	0.713	0.349
7	1.07	0.532

Determined using eLowQuant R code⁴.

Limits detect - no intercept eACGE3



Limits quant - no intercept eACGE3

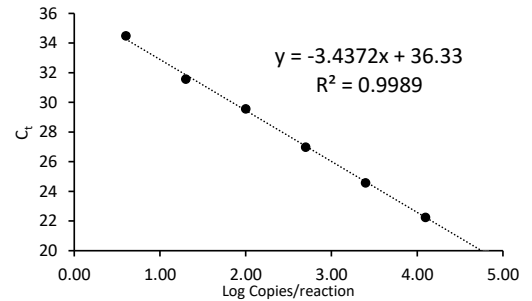


Binomial-Poisson model: No intercept

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



Efficiency 95%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH dehydrogenase subunit 4 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