



### 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: Bald eagle (*Haliaeetus leucocephalus*) eDNA qPCR Tool: eHALE3 Gene Target: MT-ND2  
Species Code: av-HALE 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 0.5-1.1 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		
am-AMTI	Barred tiger salamander ( <i>Ambystoma tigrinum</i> )	No	2		British Columbia
av-ACGE	Northern goshawk ( <i>Accipiter gentilis</i> )	No	1		British Columbia
av-AQCH	Golden eagle ( <i>Aquila chrysaetos</i> )	No	1		British Columbia
av-ARHE	Great Blue heron ( <i>Ardea herodias</i> )	No	1		British Columbia
av-ASFL	Short-eared owl ( <i>Asio flammeus</i> )	No	1		British Columbia
av-BRCA	Canada goose ( <i>Branta canadensis</i> )	No	1		British Columbia
av-BUIS	Barrow's goldeneye ( <i>Bucephala islandica</i> )	No	1		British Columbia
av-BUVI	Great horned owl ( <i>Bubo virginianus</i> )	No	1		Yukon
av-CACA	Red knot ( <i>Calidris canutus</i> )	No	1		British Columbia
av-CHMI	Common nighthawk ( <i>Chordeiles minor</i> )	No	1		British Columbia
av-EUCA	Rusty blackbird ( <i>Euphagus carolinus</i> )	No	1		Alaska
av-FACA	Spruce grouse ( <i>Falcapennis canadensis</i> )	No	1		British Columbia
av-FARU	Gyr Falcon ( <i>Falco rusticolus</i> )	No	1		British Columbia
av-HALE	Bald eagle ( <i>Haliaeetus leucocephalus</i> )	Yes	1		British Columbia
av-HIHI	Harlequin duck ( <i>Histrionicus histrionicus</i> )	No	1		British Columbia
av-HIRU	Barn swallow ( <i>Hirundo rustica</i> )	No	1		British Columbia
av-JUHY	Dark-eyed junco ( <i>Junco hyemalis</i> )	No	1		British Columbia
av-LALA	Willow ptarmigan ( <i>Lagopus lagopus</i> )	No	1		British Columbia
av-PTAL	Cassin's auklet ( <i>Ptychoramphus aleuticus</i> )	No	1		British Columbia
av-RIRI	Bank swallow ( <i>Riparia riparia</i> )	No	1		British Columbia
av-SICA	White-breasted nuthatch ( <i>Sitta carolinensis</i> )	No	1		British Columbia
av-STVU	European starling ( <i>Sturnus vulgaris</i> )	No	1		British Columbia
av-SYAN	Ancient murrelet ( <i>Synthliboramphus antiquus</i> )	No	1		British Columbia
av-TYAL	Barn owl ( <i>Tyto alba</i> )	No	1		British Columbia
ma-CALUfa	Domestic dog ( <i>Canis lupus familiaris</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-MUMU	House mouse ( <i>Mus musculus</i> )	No	1		British Columbia
te-CLPA	Pacific herring ( <i>Clupea pallasii</i> )	No	1		British Columbia
te-ONCle	Westslope cutthroat trout ( <i>Oncorhynchus clarkii lewisi</i> )	No	1		Alberta

#### 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

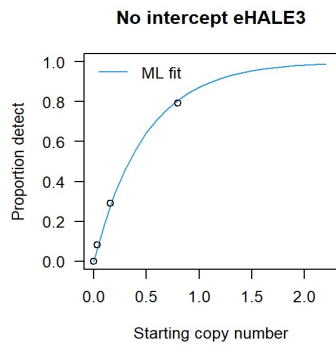
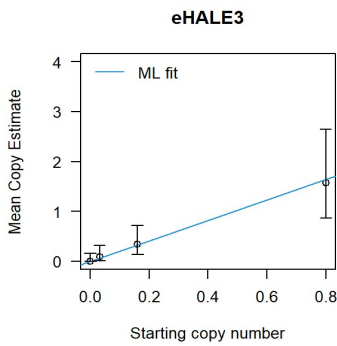
**eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA**

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

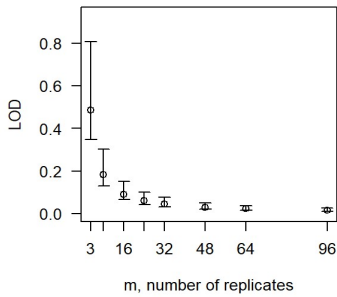
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.065	0.066
2	0.14	0.103
3	0.228	0.141
4	0.336	0.185
5	0.476	0.242
6	0.673	0.327
7	1.009	0.498

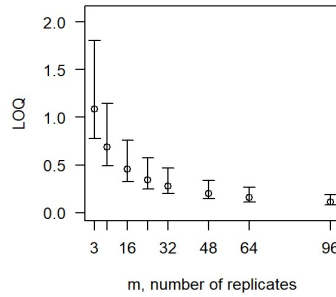
Determined using eLowQuant R code<sup>4</sup>.



**Limits detect - no intercept eHALE3**

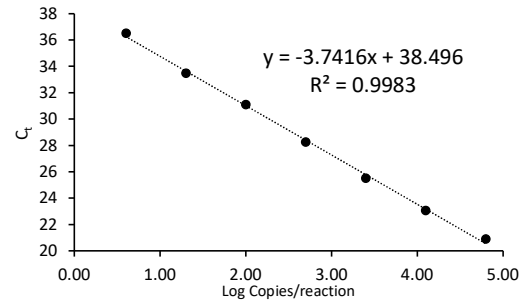


**Limits quant - no intercept eHALE3**



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

**Applied to reactions with ≥ 95% positive hits**



Efficiency 85%

**Field Sample Validation**

Sample Type	Known		Detected	Location
	Presence	# Samples		

**Abbreviations**

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase subunit 2 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