

### 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: Goldeye (*Hiodon alosoides*) eDNA qPCR Tool: eHIAL6 Gene Target: MT-ND2  
Species Code: te-HIAL eDNA qPCR Format: TaqMan Published in:

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

LOD 0.8 95% CI 0.6-1.3 Copies/Rxn LOQ 3 95% CI 2.2-4.9 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 20 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: Qiacity

#### 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-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
te-CACA	Longnose sucker ( <i>Catostomus catostomus</i> )	No	1		British Columbia
te-CACO	White sucker ( <i>Catostomus commersonii</i> )	No	1		Alberta
te-COAR	Cisco/Tullibee ( <i>Coregonus artedii</i> )	No	1		Alberta
te-COCL	Lake whitefish ( <i>Coregonus clupeaformis</i> )	No	1		Alberta
te-COCO	Slimy sculpin ( <i>Cottus cognatus</i> )	No	1		British Columbia
te-ESLU	Northern pike ( <i>Esox lucius</i> )	No	1		British Columbia
te-ESNI	Chain pickerel ( <i>Esox niger</i> )	No	1		Nova Scotia
te-HIAL	Goldeye ( <i>Hiodon alosoides</i> )	Yes	2		Alberta
te-LOLO	Burbot ( <i>Lota lota</i> )	No	1		Alberta
te-ONCLle	Westslope cutthroat trout ( <i>Oncorhynchus clarkii lewisi</i> )	No	1		Alberta
te-ONGO	Pink salmon ( <i>Oncorhynchus gorbuscha</i> )	No	1		British Columbia
te-ONKE	Chum salmon ( <i>Oncorhynchus keta</i> )	No	1		British Columbia
te-ONKI	Coho salmon ( <i>Oncorhynchus kisutch</i> )	No	1		British Columbia
te-ONMY	Rainbow (steelhead) trout ( <i>Oncorhynchus mykiss</i> )	No	1		Alberta
te-ONNE	Sockeye salmon ( <i>Oncorhynchus nerka</i> )	No	1		British Columbia
te-ONTS	Chinook salmon ( <i>Oncorhynchus tshawytscha</i> )	No	1		British Columbia
te-SACO	Bull trout ( <i>Salvelinus confluentus</i> )	No	1		Alberta
te-SAFO	Brook trout ( <i>Salvelinus fontinalis</i> )	No	1		Alberta
te-SANA	Lake trout ( <i>Salvelinus namaycush</i> )	No	1		Alberta
te-SASA	Atlantic salmon ( <i>Salmo salar</i> )	No	1		Nova Scotia
te-SAVI	Walleye ( <i>Sander vitreus</i> )	No	1		Washington

#### 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

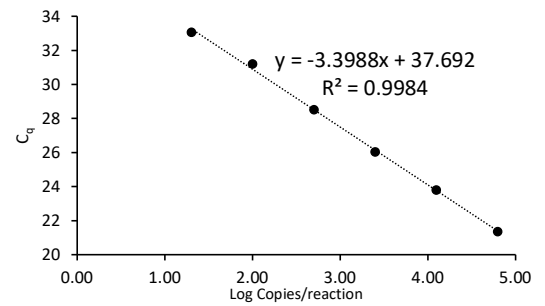
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

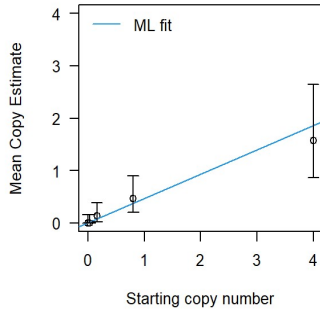
# Detects	# Copies	SE
0	0.00	0.00
1	0.29	0.29
2	0.62	0.46
3	1.01	0.62
4	1.49	0.81
5	2.11	1.06
6	2.98	1.44
7	4.47	2.19

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

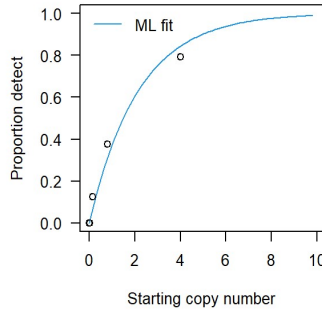
Applied to reactions with 100% positive hits



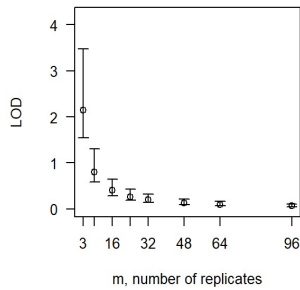
eHIAL6



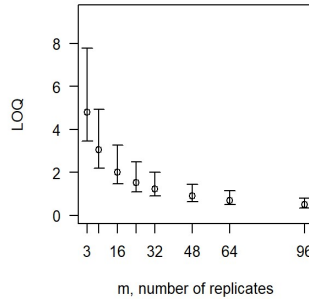
No intercept eHIAL6



Limits detect - no intercept eHIAL6



Limits quant - no intercept eHIAL6



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

Known  
Sample Type Presence # Samples Detected Location

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