



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: Pacific Halibut (*Hippoglossus stenolepis*) eDNA qPCR Tool: eHIST2 Gene Target: MT-16S
 Species Code: te-HIST eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 2.1 95% CI 1.5-3.4 Copies/Rxn LOQ 7.8 95% CI 5.6-12.8 Copies/Rxn LOB 0 hits/8
 LOQ_{continuous} 20 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: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

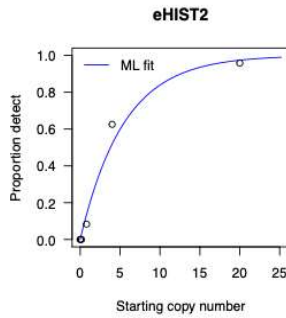
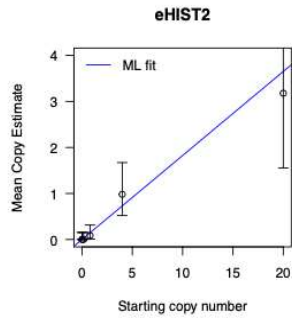
Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
te-ANFI	Sablefish (<i>Anoplopoma fimbria</i>)	No	6	British Columbia
te-GAMA	Pacific cod (<i>Gadus macrocephalus</i>)	No	2	British Columbia
te-HIST	Pacific Halibut (<i>Hippoglossus stenolepis</i>)	Yes	6	British Columbia
te-OPEL	Ling Cod (<i>Ophiodon elongatus</i>)	No	2	British Columbia
te-SEAA	Shortspine thornyhead (<i>Sebastolobus alascanus</i>)	No	1	British Columbia
te-SEBR	Silvergrey Rockfish (<i>Sebastes brevispinis</i>)	No	1	British Columbia
te-SECA	Copper Rockfish (<i>Sebastes caurinus</i>)	No	1	British Columbia
te-SEEN	Widow rockfish (<i>Sebastes entomelas</i>)	No	1	British Columbia
te-SEMA	Quillback Rockfish (<i>Sebastes maliger</i>)	No	1	British Columbia
te-SEPA	Bocaccio rockfish (<i>Sebastes paucispinis</i>)	No	1	British Columbia
te-SEPN	Canary Rockfish (<i>Sebastes pinniger</i>)	No	1	British Columbia
te-SEPR	Redstripe rockfish (<i>Sebastes proriger</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

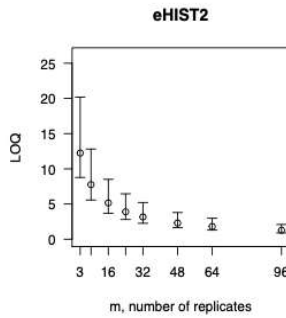
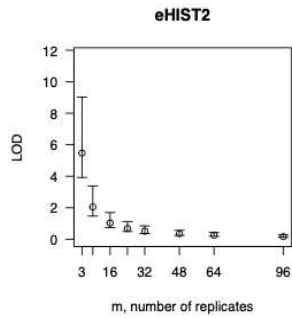
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>



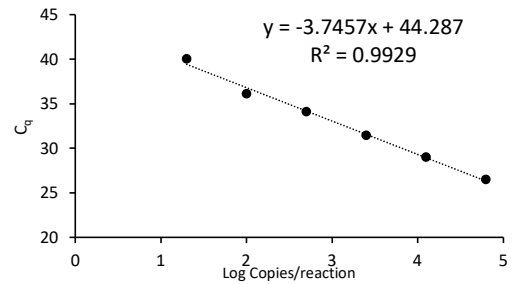
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.731	0.747
2	1.579	1.165
3	2.575	1.587
4	3.797	2.082
5	5.366	2.72
6	7.594	3.686
7	11.387	5.61

Determined using eLowQuant R code⁴.



Applied to reactions with ≥ 95% positive hits



Efficiency 85%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Not Done				

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-16S	Mitochondrial 16S ribosomal RNA 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