



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 anchovy (*Engraulis mordax*) eDNA qPCR Tool: eENMO2 Gene Target: MT-COX2
 Species Code: te-ENMO 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 0.5 95% CI 0.4-0.8 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)

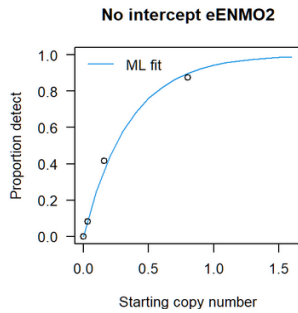
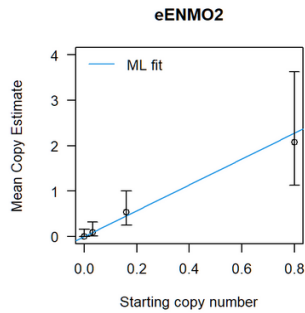
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-CAFA	Dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	2		Netherlands
te-AMPE	Pacific sandlance (<i>Ammodytes personatus</i>)	No	2		British Columbia
te-CLPA	Pacific herring (<i>Clupea pallasii</i>)	No	2		British Columbia
te-ENMO	Northern anchovy (<i>Engraulis mordax</i>)	Yes	7		British Columbia
te-GAMA	Pacific cod (<i>Gadus macrocephalus</i>)	No	2		British Columbia
te-HYPR	Surf smelt (<i>Hypomesus pretiosus</i>)	No	2		British Columbia
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	2		British Columbia
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	No	2		British Columbia
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	No	2		British Columbia
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	2		British Columbia
te-OSMO	Rainbow smelt (<i>Osmerus mordax</i>)	No	2		Alaska
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	No	2		Nova Scotia
te-SPTH	Longfin smelt (<i>Spirinchus thaleichthys</i>)	No	2		Washington
te-THPA	Eulachon (<i>Thaleichthys pacificus</i>)	No	2		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

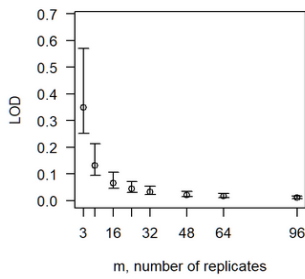
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>



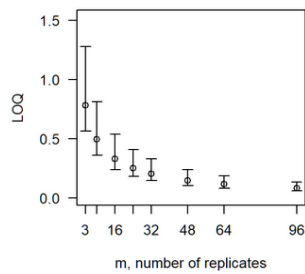
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.047	0.048
2	0.101	0.074
3	0.165	0.101
4	0.243	0.133
5	0.344	0.174
6	0.486	0.235
7	0.729	0.358

Limits detect - no intercept eENMO2

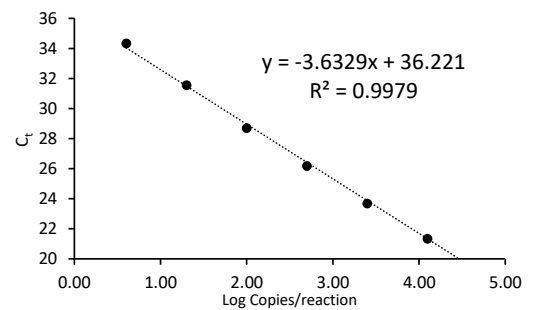


Limits quant - no intercept eENMO2



Determined using eLowQuant R code⁴.

Applied to reactions with 100% positive hits



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

Efficiency 88%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COX2	Mitochondrial cytochrome oxidase 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