



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 Sandlance (*Ammodytes personatus*)
Species Code: te-AMPE
eDNA qPCR Tool: eAMPE5
eDNA qPCR Format: TaqMan
Gene Target: MT-RNR2
Published in: 5

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.5 Copies/Rxn LOQ 1.1 95% CI 0.8-1.9 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: Immolase

eDNA Assay Specificity Test Information

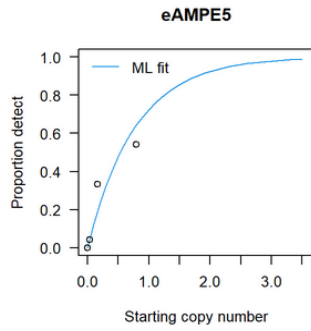
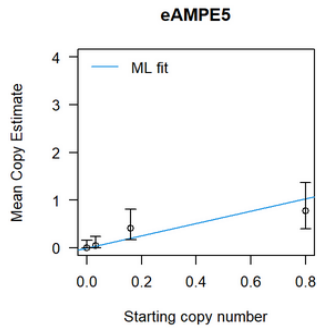
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-AMPE	Pacific Sand Lance (<i>Ammodytes personatus</i>)	Yes	5	British Columbia
te-HYPR	Surf Smelt (<i>Hypomesus pretiosus</i>)	No	2	British Columbia
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-ONNE	Sockeye Salmon (<i>Oncorhynchus nerka</i>)	No	1	British Columbia
te-ONTS	Chinook Salmon (<i>Oncorhynchus tshawytscha</i>)	No	1	British Columbia
te-THPA	Eulachon (<i>Thaleichthys pacificus</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. 2020; 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
- Robinson, CLK, Bergman, LC, Allison, MJ, Huard, J, Sutherst, J, and Helbing, CC (2022) The utility of environmental DNA to detect intertidal habitat use by forage fish. Ecological Indicators, 142: 109306. doi: 10.1016/j.ecolind.2022.109306

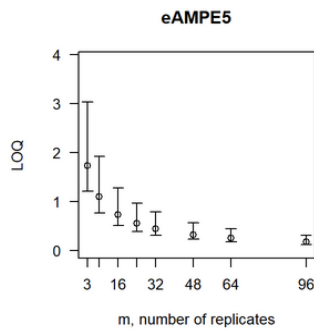
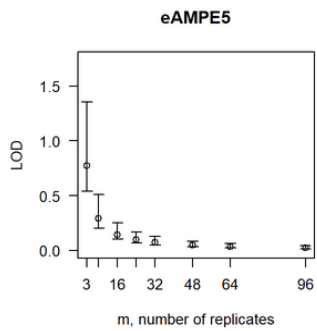
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

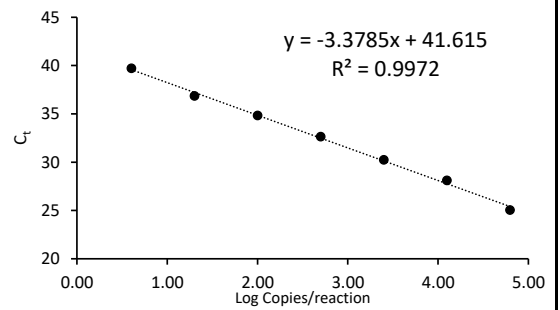
# Detects	# Copies	SE
0	0	0
1	0.1	0.11
2	0.22	0.17
3	0.36	0.23
4	0.54	0.3
5	0.76	0.39
6	1.1	0.53
7	1.6	0.81

Determined using eLowQuant R code⁴.



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 98%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Sand	Y	5	Y	British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR2	Mitochondrial 16S ribosomal RNA
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