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[1-3].

General eDNA Assay Information

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

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD	0.3	95% CI	0.2-0.6	Copies/Rxn	LOQ	1.3	95% CI	0.9-2.4	Copies/Rxn	LOB	0	hits/8

	LOQ$_{continuous}$	4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].

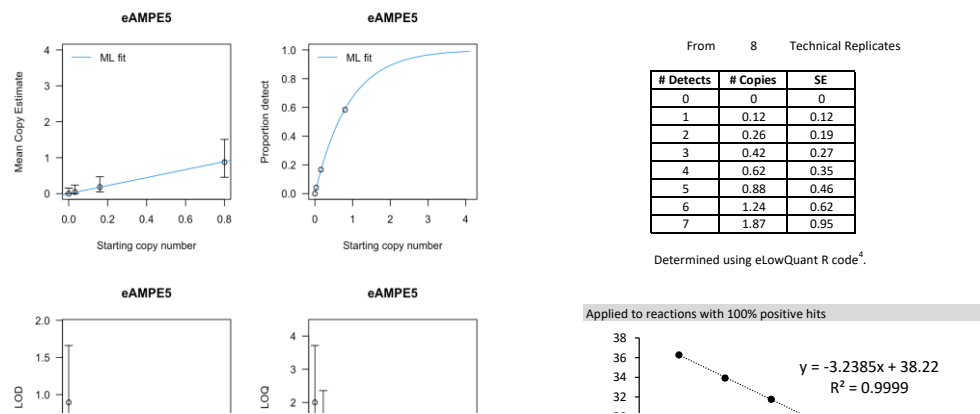Enzyme: QIAcuity

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)
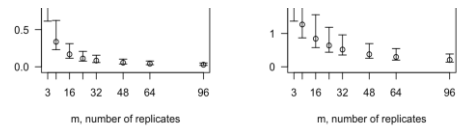
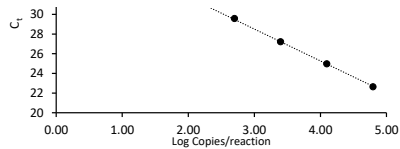| Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-AMPE	Pacific Sand Lance (*Ammodytes personatus*)	Yes	5	British Columbia
te-HYPR	Surf Smelt (*Hypomesus pretiosus*)	No	2	British Columbia
te-ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum Salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

References

1. 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
2. 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
3. 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
4. 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
5. 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

eAMPE5 — Mean Copy Estimate vs Starting copy number (ML fit)

eAMPE5 — Proportion detect vs Starting copy number (ML fit)

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.12	0.12
2	0.26	0.19
3	0.42	0.27
4	0.62	0.35
5	0.88	0.46
6	1.24	0.62
7	1.87	0.95

Determined using eLowQuant R code[4].

eAMPE5 — LOD

eAMPE5 — LOQ

Applied to reactions with 100% positive hits

$y = -3.2385x + 38.22$
$R^2 = 0.9999$

Binomial-Poisson model: ___No intercept___

Determined using eLowQuant R code[4].

Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency	104%

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