Helbing Lab
eDNA Inventory

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: Eulachon (*Thaleichthys pacificus)* | eDNA qPCR Tool: eTHPA6 | Gene Target: MT-ND1
Species Code: te-THPA | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.6 95% CI 0.4-0.9 Copies/Rxn LOQ 2.2 95% CI 1.6-3.5 Copies/Rxn LOB 0 hits/8
$LOQ_{continuous}$ 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4]. 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 (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
te-AMPE	Pacific sandlance (*Ammodytes personatus*)	No	1	British Columbia
te-ANFI	Sablefish, black cod (*Anoplopoma fimbriatum*)	No	1	British Columbia
te-CLPA	Pacific herring (*Clupea pallasii*)	No	2	British Columbia
te-GAMA	Pacific cod (*Gadus macrocephalus*)	No	1	British Columbia
te-GAAC	Three-spined stickleback (*Gasterosteus aculeatus*)	No	1	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-OPEL	Ling cod (*Ophiodon elongatus*)	No	1	British Columbia
te-OSMO	Rainbow smelt (*Osmerus mordax*)	No	2	Alaska
te-SASA	Atlantic salmon (*Salmo salar*)	No	1	Nova Scotia
te-SPTH	Longfin smelt (*Spirinchus thaleichthys*)	No	2	Washington
ma-CALUfa	Dog (*Canis lupus familiaris*)	No	1	British Columbia
ma-FECA	Cat (*Felis catus*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-THPA	Eulachon (*Thaleichthys pacificus)*	Yes	6	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, 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

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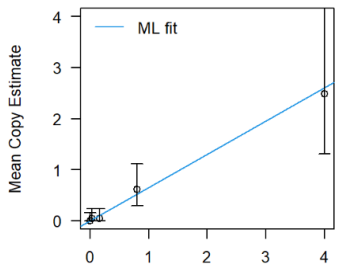
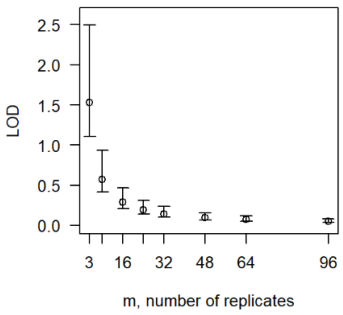
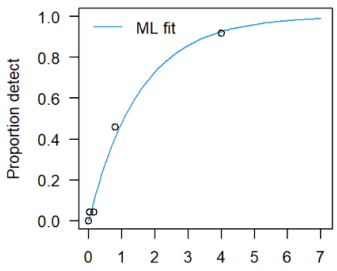
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

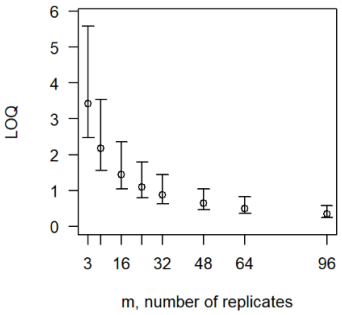
eTHPA6





No intercept eTHPA6



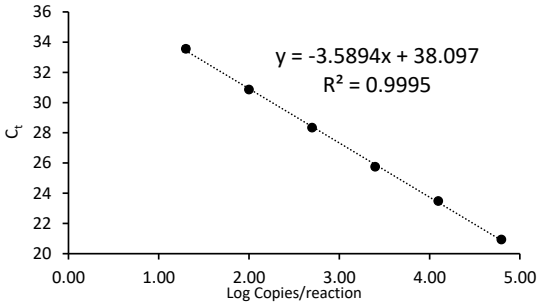


From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.21	0.21
2	0.44	0.33
3	0.72	0.44
4	1.06	0.58
5	1.5	0.76
6	2.13	1.03
7	3.19	1.57

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits



Efficiency 90%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase subunit 1 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