

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: Sablefish (*Anoplopoma fimbriatum*) eDNA qPCR Tool: eANFI6 Gene Target: MT-ND2
Species Code: te-ANFI6 eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.7 Copies LOQ 1.3 95% CI 0.9-2.5 Copies 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: 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	
			Specimens	Sample Sources/Locations
ANFI	Sablefish (<i>Anoplopoma fimbriatum</i>)	Yes	6	British Columbia
HIST	Pacific halibut (<i>Hippoglossus stenolepis</i>)	No	6	British Columbia
OPEL	Ling cod (<i>Ophiodon elongatus</i>)	No	1	British Columbia
SECA	Copper rockfish (<i>Sebastes caurinus</i>)	No	1	British Columbia
GAMA	Pacific cod (<i>Gadus macrocephalus</i>)	No	1	British Columbia
SEPN	Canary rockfish (<i>Sebastes pinnigers</i>)	No	1	British Columbia
SEMA	Quillback rockfish (<i>Sebastes malinger</i>)	No	1	British Columbia
SEPR	Red stripe rockfish (<i>Sebastes proriger</i>)	No	1	British Columbia
SEEN	Widow rockfish (<i>Sebastes entomelas</i>)	No	1	British Columbia
FECA	Cat (<i>Felis catus</i>)	No	1	British Columbia
HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
CAFA	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia

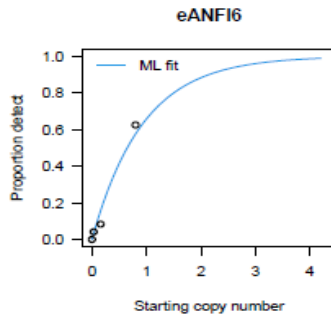
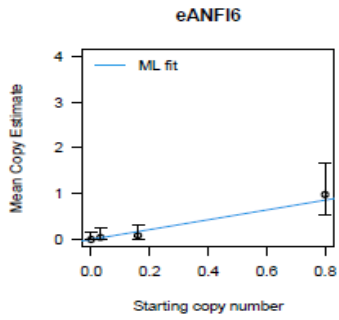
References

- 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





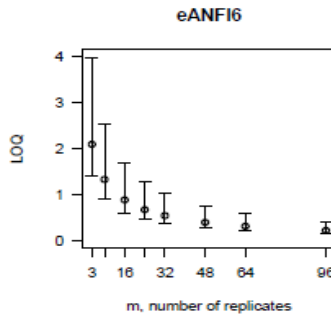
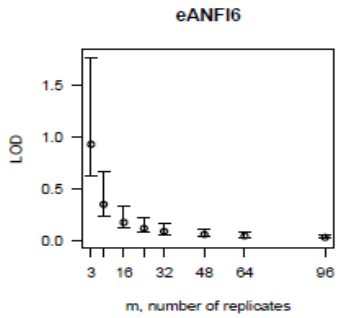
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



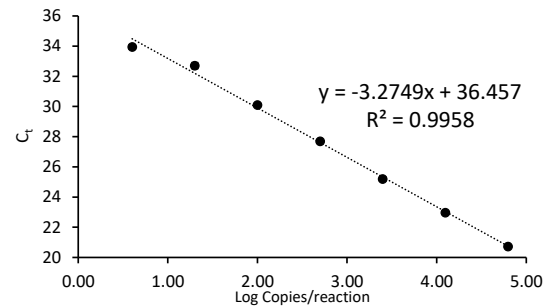
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.124	0.128
2	0.268	0.201
3	0.438	0.276
4	0.646	0.365
5	0.914	0.479
6	1.292	0.651
7	1.94	0.991

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Efficiency 102%

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
Water	Y	6	Y	Vancouver Aquarium

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase 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