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: 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 | 0.2-0.5 | Copies | LOQ | 1.1 | 95% CI | 0.7-1.9 | Copies | LOB | 0 | hits/8 |

$LOQ_{continuous}$ = 4 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: Immolase

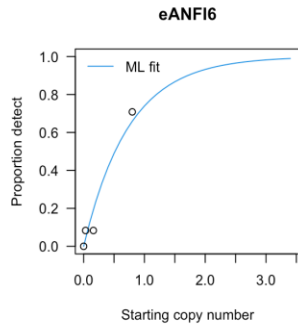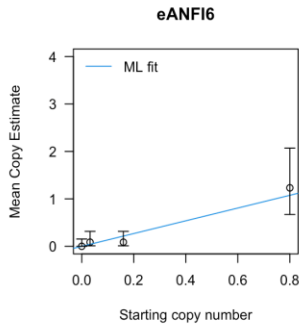### eDNA Assay Specificity Test Information

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

Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ANFI	Sablefish (*Anoplopoma fimbriatum*)	Yes	6	British Columbia
HIST	Pacific halibut (*Hippoglossus stenolepis*)	No	6	British Columbia
OPEL	Ling cod (*Ophiodon elongatus*)	No	1	British Columbia
SECA	Copper rockfish (*Sebastes caurinus*)	No	1	British Columbia
GAMA	Pacific cod (*Gadus macrocephalus*)	No	1	British Columbia
SEPN	Canary rockfish (*Sebastes pinnigers*)	No	1	British Columbia
SEMA	Quillback rockfish (*Sebastes malinger*)	No	1	British Columbia
SEPR	Red stripe rockfish (*Sebastes proriger*)	No	1	British Columbia
SEEN	Widow rockfish (*Sebastes entomelas*)	No	1	British Columbia
FECA	Cat (*Felis catus*)	No	1	British Columbia
HOSA	Human (*Homo sapiens*)	No	1	Netherlands
CAFA	Dog (*Canis lupus familiaris*)	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
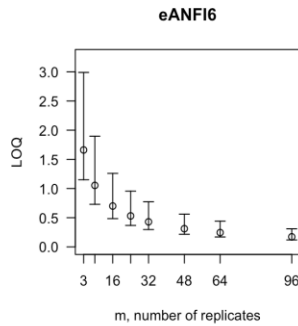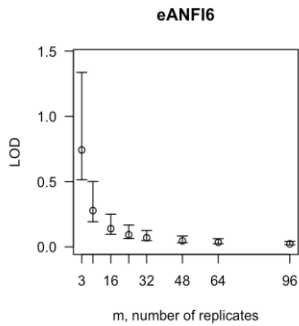
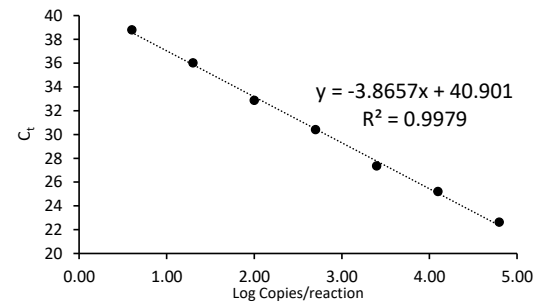## eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eANFI6

eANFI6

From   8   Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.099	0.1
2	0.21	0.16
3	0.35	0.22
4	0.52	0.29
5	0.73	0.38
6	1.03	0.51
7	1.55	0.78

Determined using eLowQuant R code[4].

eANFI6

eANFI6

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

Applied to reactions with 100% positive hits

$y = -3.8657x + 40.901$
$R^2 = 0.9979$

| Efficiency | 81% |

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