



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: Quillback Rockfish (*Sebastes maliger*) eDNA qPCR Tool: eSEMA3 Gene Target: MT-Dloop
Species Code: te-SEMA eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1 95% CI 0.7-1.7 Copies LOQ 3.9 95% CI 2.8-6.5 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
te-SEMA	Quillback rockfish (<i>Sebastes maliger</i>)	Yes	5	British Columbia
te-seaLA	Shortspine thornyhead (<i>Sebastolobus alascanus</i>)	No	2	British Columbia
te-SEAU	Pacific Ocean perch (<i>Sebastes alutus</i>)	No	2	British Columbia
te-SEBA	Redbanded rockfish (<i>Sebastes babcocki</i>)	No	2	British Columbia
te-SEBR	Silvergrey rockfish (<i>Sebastes brevispinus</i>)	No	2	British Columbia
te-SECA	Copper rockfish (<i>Sebastes caurinus</i>)	No	2	British Columbia
te-SECR	Darkblotched rockfish (<i>Sebastes crameri</i>)	No	2	British Columbia
te-SEDI	Splitnose rockfish (<i>Sebastes diploproa</i>)	No	2	British Columbia
te-SEEL	Greenstriped rockfish (<i>Sebastes elongatus</i>)	No	2	British Columbia
te-SEEN	Widow rockfish (<i>Sebastes entomelas</i>)	No	2	British Columbia
te-SEFL	Yellowtail rockfish (<i>Sebastes flavidus</i>)	No	2	British Columbia
te-SEGO	Chilipepper rockfish (<i>Sebastes goodei</i>)	No	2	British Columbia
te-SEHE	Rosethorn rockfish (<i>Sebastes helvomaculatus</i>)	No	2	British Columbia
te-SEJO	Shortbelly rockfish (<i>Sebastes jordani</i>)	No	2	British Columbia
te-SEPA	Bocaccio rockfish (<i>Sebastes paucispinus</i>)	No	2	British Columbia
te-SEPI	Canary rockfish (<i>Sebastes pinnigers</i>)	No	2	British Columbia
te-SEPR	Red stripe rockfish (<i>Sebastes proriger</i>)	No	2	British Columbia
te-SERE	Yellowmouth rockfish (<i>Sebastes reedi</i>)	No	2	British Columbia
te-SERU	Yelloweye rockfish (<i>Sebastes ruberrimus</i>)	No	2	British Columbia
te-SESA	Stripetail rockfish (<i>Sebastes saxicola</i>)	No	2	British Columbia
te-SEVA	Harlequin rockfish (<i>Sebastes variegatus</i>)	No	2	British Columbia
te-SEZA	Sharpchin rockfish (<i>Sebastes zacentrus</i>)	No	2	British Columbia
te-ANFI	Sablefish, black cod (<i>Anoplopoma fimbriatum</i>)	No	1	British Columbia
te-GAMA	Pacific cod (<i>Gadus macrocephalus</i>)	No	1	British Columbia
te-ATST	Arrowtooth flounder (<i>Atheresthes stomias</i>)	No	1	British Columbia
te-EOJO	Petrale sole (<i>Eopsetta jordani</i>)	No	1	British Columbia
te-GACH	Walleye pollock (<i>Gadus chalcogrammus</i>)	No	1	British Columbia
te-GLZA	Rex sole (<i>Glyptocephalus zachirus</i>)	No	1	British Columbia
te-HIEL	Flathead sole (<i>Hippoglossoides elassodon</i>)	No	1	British Columbia
te-LEBI	Rock sole (<i>Lepidopsetta bilineata</i>)	No	1	British Columbia
te-MIPA	Dover sole (<i>Microstomus pacificus</i>)	No	1	British Columbia
te-OPEL	Ling cod (<i>Ophiodon elongatus</i>)	No	1	British Columbia
te-PAVE	English sole (<i>Parophrys vetulus</i>)	No	1	British Columbia
te-HIST	Pacific halibut (<i>Hippoglossus stenolepis</i>)	No	1	British Columbia
te-AMPE	Pacific sandlance (<i>Ammodytes personatus</i>)	No	1	British Columbia
te-THPA	Eulachon (<i>Thaleichthys pacificus</i>)	No	1	British Columbia
te-HYPR	Surf smelt (<i>Hypomesus pretiosus</i>)	No	1	British Columbia
te-CLPA	Pacific herring (<i>Clupea pallasii</i>)	No	1	British Columbia
te-ONMY	Rainbow (steelhead) trout (<i>Oncorhynchus mykiss</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-CAFA	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1	British Columbia

References

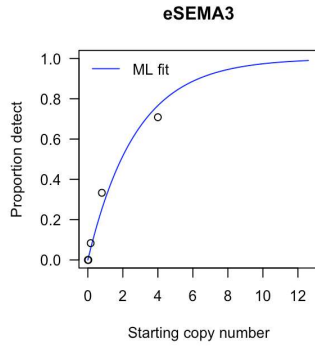
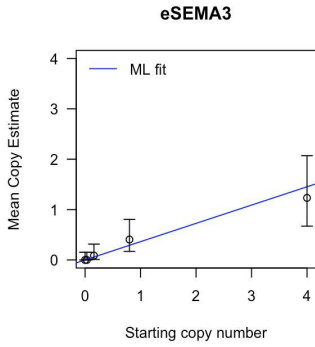
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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

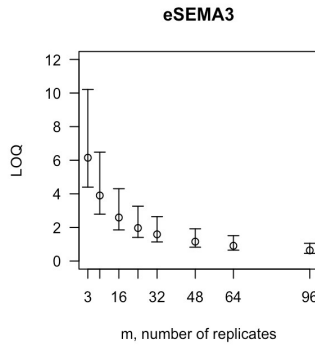
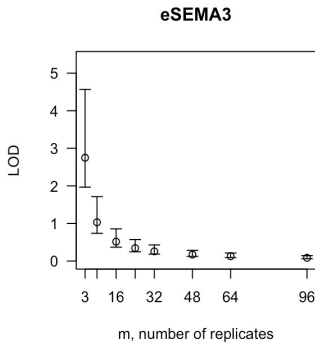
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.37	0.38
2	0.79	0.59
3	1.29	0.8
4	1.91	1.05
5	2.7	1.37
6	3.82	1.86
7	5.72	2.82

Determined using eLowQuant R code⁴.

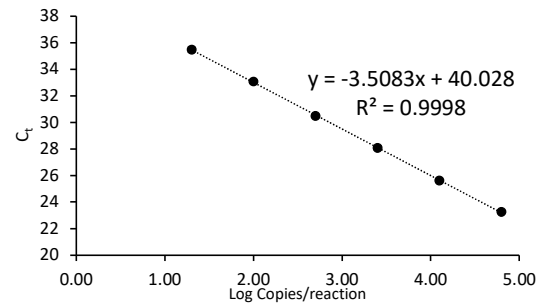


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Applied to reactions with ≥ 95% positive hits



Efficiency 93%

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-Dloop	Mitochondrial D-Loop region
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