

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: Boccaccio rockfish (*Sebastes paucispinus*) eDNA qPCR Tool: eSEPA9 Gene Target: MT-D-Loop
Species Code: te-SEPA eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.9 95% CI 0.6-1.6 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 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		Sample Sources/Locations
			Specimens		
te-SEPA	Boccaccio rockfish (<i>Sebastes paucispinus</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-SEMA	Quillback rockfish (<i>Sebastes malinger</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	Petrable 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-CALufa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Domestic 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, 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

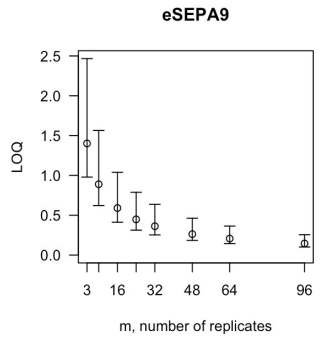
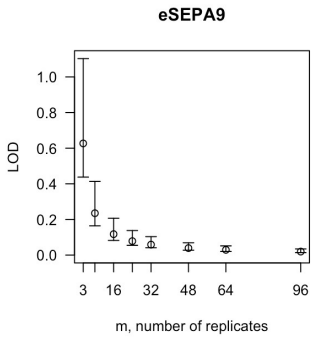
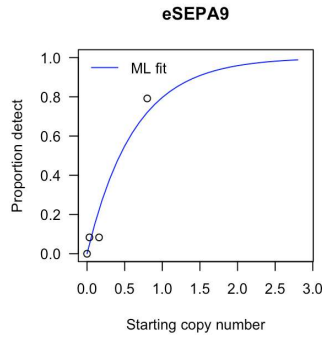
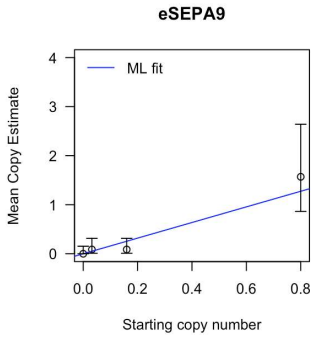
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

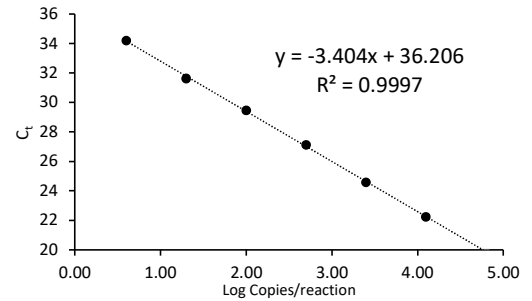
# Detects	# Copies	SE
0	0	0
1	0.08	0.09
2	0.18	0.13
3	0.3	0.18
4	0.44	0.24
5	0.62	0.32
6	0.87	0.43
7	1.31	0.65

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 97%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Yes	6	Yes	Vancouver Aquarium

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-D-Loop	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