

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: Brook trout (*Salvelinus fontinalis*) eDNA qPCR Tool: eSAFO6 Gene Target: MT-CO3
Species Code: te-SAFO 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/Rxn LOQ 1.1 95% CI 0.8-1.8 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: Qiacity

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		
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
te-COAR	Cisco/Tullibee (<i>Coregonus artedii</i>)	No	2		Alberta
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1		Alberta
te-ESLU	Northern pike (<i>Esox lucius</i>)	No	1		British Columbia
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	2		British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	2		British Columbia
te-ONCLE	Westslope cutthroat trout (<i>Oncorhynchus clarki lewisi</i>)	No	2		Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	2		British Columbia
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	No	2		British Columbia
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	No	2		British Columbia
te-ONMY	Rainbow trout (<i>Oncorhynchus mykiss</i>)	No	2		Alberta
te-ONTS	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	No	1		British Columbia
te-PRCY	Round whitefish (<i>Prosopium cylindraceum</i>)	No	1		Yukon
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	No	2		Alberta
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	Yes	5		Alberta
te-SAMA	Dolly varden (<i>Salvelinus malma</i>)	No	2		British Columbia
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	2		Alberta
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	No	1		Halifax
te-THAR	Arctic grayling (<i>Thymallus arcticus</i>)	No	2		Alberta

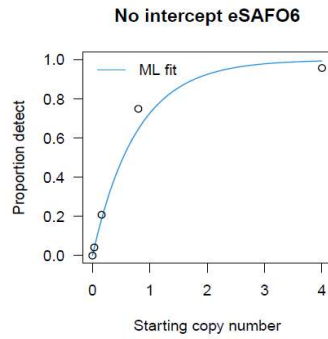
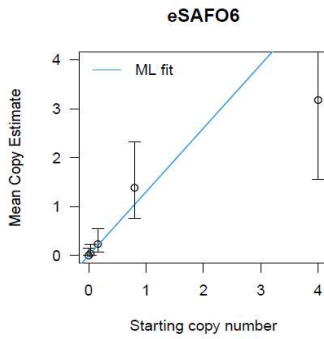
References

- 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

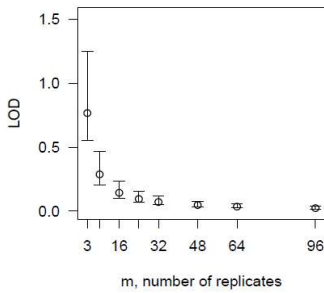
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

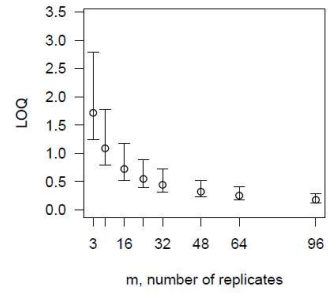


# Detects	# Copies	SE
0	0	0
1	0.103	0.105
2	0.221	0.163
3	0.361	0.222
4	0.533	0.291
5	0.754	0.381
6	1.065	0.515
7	1.598	0.784

Limits detect – no intercept eSAFO6

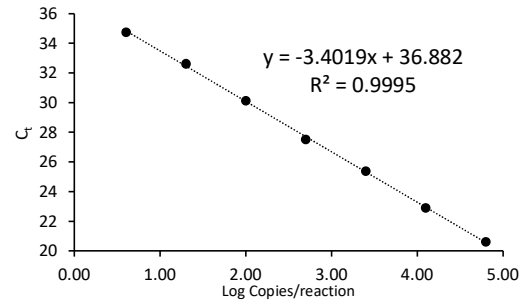


Limits quant – no intercept eSAFO6



Determined using eLowQuant R code⁴.

Applied to reactions with ≥ 95% positive hits



Efficiency 97%

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		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CO3	Mitochondrial cytochrome oxidase subunit 3 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