Helbing Lab
eDNA Inventory

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: Brook Trout (*Salvelinus fontinalis*) | eDNA qPCR Tool: BRKT2 | Gene Target: MT-CYB
Species Code: te-SAFO | eDNA qPCR Format: TaqMan | Published in: 5

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-1.2 Copies/Rxn | LOQ 2.1 95% CI 1.3-4.6 Copies/Rxn | LOB 0 hits/8
Binomial-Poisson model for 8 technical replicates
Determined using eLowQuant R code[4]. 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 (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-CALUfa	Dog (*Canis lupus familiaris*)	N	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	N	1	Netherlands
te-CAAU	Goldfish (*Carassius auratus*)	N	2	Alberta
te-ONCLle	Westslope cutthroat trout (*Oncorhynchus clarki lewisi*)	N	2	Alberta
te-ONGO	Pink salmon (*Oncorhynchus gorbuscha*)	N	1	British Columbia
te-ONKE	Chum salmon (*Oncorhynchus keta*)	N	1	British Columbia
te-ONKI	Coho salmon (*Oncorhynchus kisutch*)	N	1	British Columbia
te-ONMY	Rainbow trout (*Oncorhynchus mykiss*)	N	2	Alberta
te-ONNE	Sockeye salmon (*Oncorhynchus nerka*)	N	1	British Columbia
te-ONTS	Chinook salmon (*Oncorhynchus tschawytscha*)	N	1	British Columbia
te-PRCY	Round whitefish (*Prosopium cylindraceum*)	N	1	Yukon
te-SACO	Bull trout (*Salvelinus confluentus*)	N	2	Alberta
te-SAFO	Brook trout (*Salvelinus fontinalis*)	Y	5	Alberta
te-SAMA	Dolly varden (*Salvelinus malma*)	N	1	British Columbia
te-SANA	Lake trout (*Salvelinus namaycush*)	N	2	Alberta

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, 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 (2021) 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

5. Wilcox, T. M., et al. (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. PLoS One 8(3): e59520.

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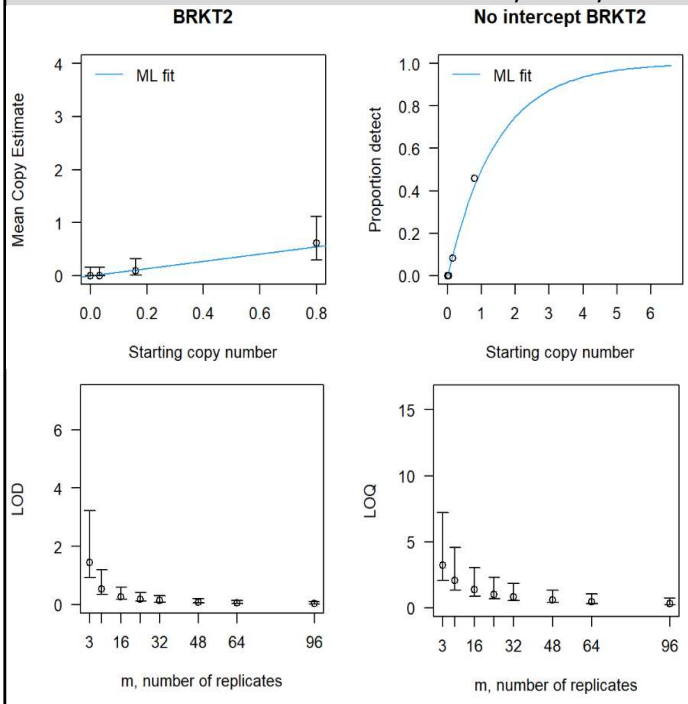
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

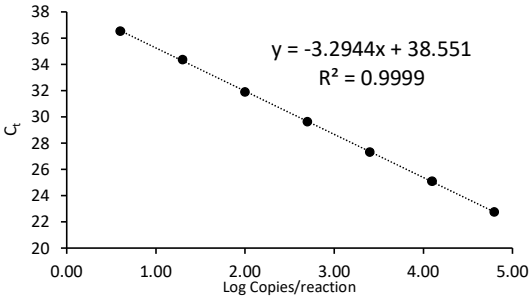


From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.19	0.2
2	0.42	0.32
3	0.68	0.44
4	1.01	0.59
5	1.42	0.77
6	2.01	1.05
7	3.01	1.6

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits



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

Efficiency 101%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome b
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