



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: Chum salmon (*Oncorhynchus keta*) eDNA qPCR Tool: eONKE9 Gene Target: MT-ND4
Species Code: eONKE eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.6 95% CI 0.4-0.9 Copies/Rxn LOQ 2.1 95% CI 1.5-3.4 Copies/Rxn 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: 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 (<i>Species</i>)	# Voucher		
		Detection	Specimens	Sample Sources/Locations
te-CACAch	Salish sucker (<i>Catostomus catostomus (chehalis)</i>)	No	1	Alberta
te-CACO	White sucker (<i>Catostomus commersonii</i>)	No	1	Ontario
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1	Alberta
te-ESLU	Northern pike (<i>Esox lucius</i>)	No	1	British Columbia
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	1	Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	1	British Columbia
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	Yes	1	British Columbia
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	No	1	British Columbia
te-ONMY	Rainbow (steelhead) trout (<i>Oncorhynchus mykiss</i>)	No	1	Alberta
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	1	British Columbia
te-ONTS	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	No	1	British Columbia
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	No	1	British Columbia
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	1	Alberta
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	1	Alberta
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	No	1	Nova Scotia
te-SAVI	Walleye (<i>Sander vitreus</i>)	No	1	Washington
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
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands

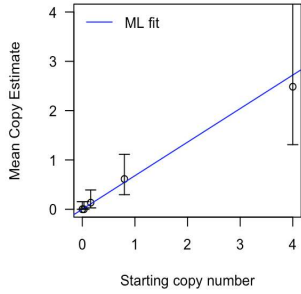
References

- 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

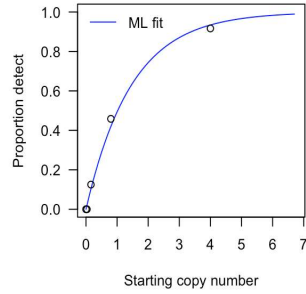


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eONKE9



No intercept eONKE9



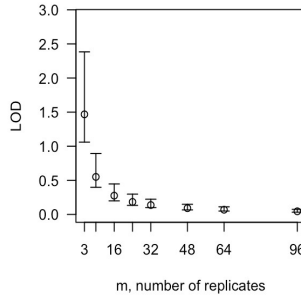
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

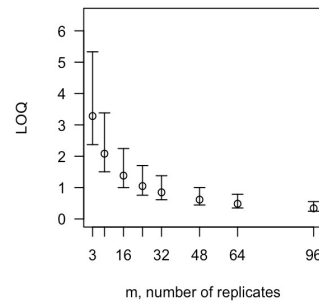
# Detects	# Copies	SE
0	0	0
1	0.2	0.2
2	0.42	0.31
3	0.69	0.43
4	1.02	0.56
5	1.44	0.73
6	2.04	1
7	3.06	1.5

Determined using eLowQuant R code⁴.

Limits detect - no intercept eONKE9



Limits quant - no intercept eONKE9

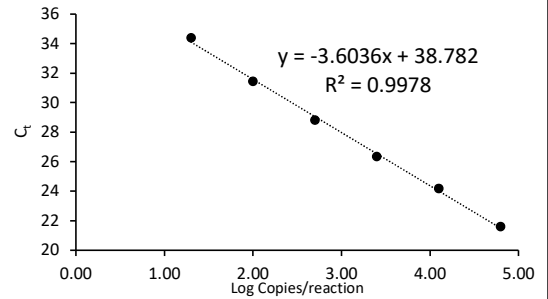


Binomial-Poisson model: No-intercept

Determined using eLowQuant R code⁴.

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

Applied to reactions with 100% positive hits



Efficiency 89%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH dehydrogenase subunit 4 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