



Helbing/Langlois 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: Atlantic salmon (*Salmo salar*) eDNA qPCR Tool: te-SASA2 Gene Target: MT-ND2
Species Code: te-SASA eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.6 Copies/Rxn LOQ 1.3 95% CI 1-2.2 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: 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>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	Yes	6		Canada: Quebec: Fleuve St-Laurent, riviere Metabetchouane Canada: Quebec: Fleuve St-Laurent, riviere Ouasiemsca Malbaie
te-SATR	Brown trout (<i>Salmo trutta</i>)	No	1		Canada: New Brunswick: Mc Quarrie Brook
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	1		Canada: British Columbia: Indian River
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	1		Canada: Quebec: Fleuve St-Laurent, riviere Trinite
te-LAAP	Lethenteron appendix (<i>Lampetra appendix</i>)	No	1		Canada: Quebec: Fleuve St-Laurent, riviere du Sud
te-PEMA	Sea lamprey (<i>Petromyzon marinus</i>)	No	1		Canada: Quebec: Fleuve St-Laurent, riviere Ste-Marguerite
te-COCL	Lake white fish (<i>Coregonus clupeaformis</i>)	No	1		Canada: Quebec: Fleuve St-Laurent, riviere St-Nicolas
te-ONMY	Rainbow trout (<i>Oncorhynchus mykiss</i>)	No	1		Québec, Canada
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	1		Québec, Canada
ma-FECA	Cat (<i>Felis catus</i>)	No	1		Québec, Canada
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Québec, Canada

References

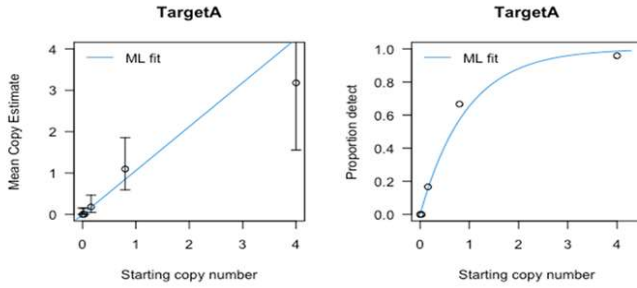
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>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

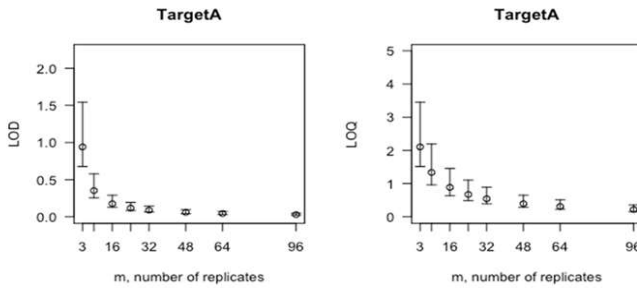
To generate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

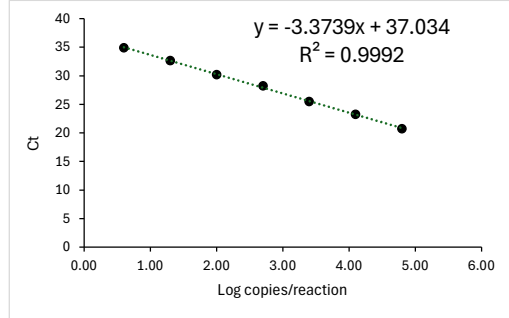


# Detects	# Copies	SE
0	0	0
1	0.078	0.127
2	0.277	0.21
3	0.515	0.313
4	0.739	0.396
5	1.071	0.533
6	1.569	0.76
7	2.445	1.232

Determined using eLowQuant R code⁴.



Applied to reactions with $\geq 95\%$ positive hits



Efficiency 98%

Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	48	Y	Rivière Jacques-Cartier, Québec, Canada

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase subunit 2
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