



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: Arctic char (*Salvelinus alpinus*) eDNA qPCR Tool: eSAAL1 Gene Target: MT-COX2
Species Code: te-SAAL eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-0.8 Copies/Rxn LOQ 1.7 95% CI 1.3-2.9 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-SAAL	Arctic char (<i>Salvelinus alpinus</i>)	Yes	2		Canada: Quebec (INRS facilities)
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	1		Canada: Quebec
te-LEAP	American brook lamprey (<i>Lethenteron appendix</i>)	No	1		Canada: Quebec
te-PEMA	Sea lamprey (<i>Petromyzon marinus</i>)	No	1		Canada: Quebec
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	1		Canada: Quebec
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1		Canada: Quebec
te-ONMY	Rainbow trout (<i>Oncorhynchus mykiss</i>)	No	1		Canada: Quebec
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	1		Canada: Quebec
te-SATR	Brown trout (<i>Salmo trutta</i>)	No	1		Canada: Quebec
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	No	1		Canada: Quebec
te-LEGI	Pumpkinseed (<i>Lepomis gibbosus</i>)	No	1		Canada: Quebec
te-PIPR	Fathead minnow (<i>Pimephales promelas</i>)	No	1		Canada: Quebec (INRS facilities)
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	1		Canada: Quebec
ma-FECA	Cat (<i>Felis catus</i>)	No	2		Canada: Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Canada: Quebec

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



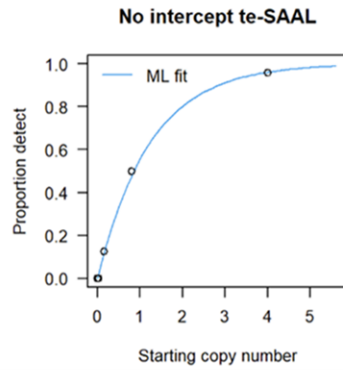
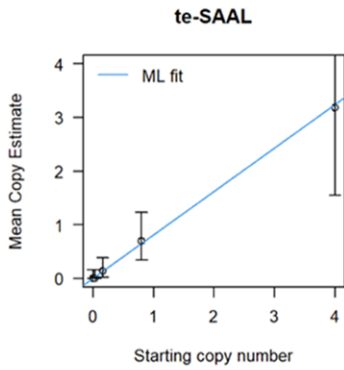
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

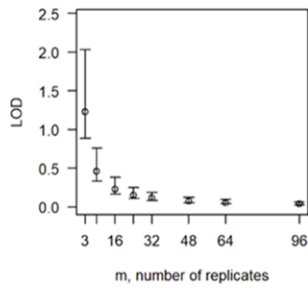
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.163	0.186
2	0.362	0.272
3	0.583	0.36
4	0.855	0.469
5	1.213	0.619
6	1.72	0.85
7	2.602	1.335

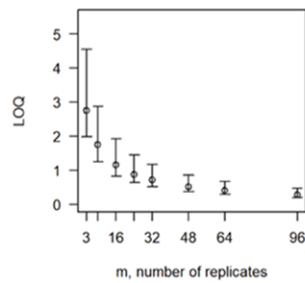
Determined using eLowQuant R code⁴.



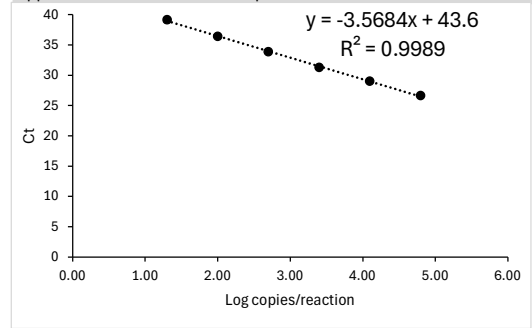
Limits detect - no intercept te-SAAL



Limits quant - no intercept te-SAAL



Applied to reactions with ≥ 95% positive hits



Efficiency 91%

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		
Water	Yes	6	Yes	Rivière Koksoak, Québec, Canada

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COX2	Mitochondrial cytochrome c oxidase subunit II
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