



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: All Salmonid Species eDNA qPCR Tool: eSalmo2 Gene Target: tRNA-Ile-Gln-Met
Species Code: te-Salmonid eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-0.9 Copies/Rxn LOQ 1.7 95% CI 1.1-3.6 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: Qiacyuity

eDNA Assay Specificity Test Information (High n)

n=25 technical replicates. Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA

^aIndicates less consistent hits

Species	Common Name (Species)	Detection	# Voucher Specimens	Sample Sources/Locations
am-LICA	North American bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	Southwestern British Columbia
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	1	Southwestern British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1	Southwestern British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
te-AMPE	Pacific sand lance (<i>Ammodytes personatus</i>)	No	1	Southwestern British Columbia
te-ANRO	American eel (<i>Anguilla rostrata</i>)	No	1	Prince Edward Island
te-CAAU	Goldfish (<i>Carassius auratus</i>)	No	1	Southern Alberta
te-COAR	Cisco/Tullibee (<i>Coregonus artedii</i>)	Inconsistent ^a	5	Northeastern Alberta
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	Yes	1	Northeastern Alberta
te-COCO	Slimy sculpin (<i>Cottus cognatus</i>)	No	1	Southwestern British Columbia
te-ENMO	Californian anchovy (<i>Engraulis mordax</i>)	No	1	Southwestern British Columbia
te-ESLU	Northern pike (<i>Esox lucius</i>)	No	1	Southwestern British Columbia
te-GAAC	Three-spined stickleback (<i>Gasterosteus aculeatus</i>)	No	1	Western British Columbia
te-GAMA	Pacific cod (<i>Gadus macrocephalus</i>)	No	1	Western British Columbia
te-HIAL	Goldeye (<i>Hiodon alosoides</i>)	No	1	Northeastern Alberta
te-HYPR	Surf smelt (<i>Hypomesus pretiosus</i>)	No	2	Southwestern British Columbia
te-LEGI	Pumpkinseed/Sunfish/Pond perch (<i>Lepomis gibbosus</i>)	No	1	Southwestern British Columbia
te-LOLO	Burbot (<i>Lota lota</i>)	No	1	Yukon
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	2	Southwestern British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	1	Southwestern British Columbia
te-MOAM	White perch (<i>Morone americana</i>)	No	1	Southern Quebec
te-MOSA	Striped bass (<i>Morone saxatilis</i>)	No	1	Ontario
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	Yes	2	Southern Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	Yes	2	Western British Columbia
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	Yes	1	Southwestern British Columbia
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	Yes	1	Southwestern British Columbia
te-ONMY	Rainbow (steelhead) trout (<i>Oncorhynchus mykiss</i>)	Yes	1	British Columbia, Alberta
te-ONNE	Sockeye Salmon (<i>Oncorhynchus nerka</i>)	Yes	1	British Columbia
te-ONTS	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	Yes	1	Southwestern British Columbia
te-OPEL	Ling cod (<i>Ophiodon elongatus</i>)	No	1	Southwestern British Columbia
te-PEFL	Yellow perch (<i>Perca flavescens</i>)	No	1	Southern Quebec
te-PRCY	Round whitefish (<i>Prosopium cylindraceum</i>)	Yes	1	Yukon
te-PRWI	Mountain whitefish (<i>Prosopium williamsoni</i>)	Inconsistent ^a	2	Southern Alberta
te-RHCA	Longnose (nooksack) dace (<i>Rhinichthys cataractae</i>)	No	1	Southern British Columbia
te-SACA	Sauger (<i>Sander canadensis</i>)	No	1	Southern Quebec
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	Yes	2	Alberta
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	Yes	2	Alberta
te-SAMA	Dolly varden (<i>Salvelinus malma</i>)	Yes	1	Western British Columbia
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	Yes	2	Southern Alberta
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	Yes	1	Newfoundland
te-SATR	Brown trout (<i>Salmo trutta</i>)	Yes	1	Eastern Quebec
te-seaLE	Rougheye rockfish (<i>Sebastes aleutianus</i>)	No	1	Southwestern British Columbia
te-SERU	Yelloweye rockfish (<i>Sebastes ruberrimus</i>)	No	1	Southwestern British Columbia
te-SPTH	Longfin smelt (<i>Spirinchus thaleichthys</i>)	No	1	Washington State, USA
te-THAR	Arctic grayling (<i>Thymallus arcticus</i>)	Inconsistent ^a	3	Northern Alberta
te-THPA	Eulachon (<i>Thaleichthys pacificus</i>)	No	1	Western British Columbia

eDNA Assay Specificity Test Information (Low n)				
n=2 technical replicates. Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA				
Species	Common Name (<i>Species</i>)	Detection	# Voucher Specimens	Sample Sources/Locations
te-ACFU	Lake sturgeon/yellow sturgeon (<i>Acipenser fulvescens</i>)	No	1	Quebec
te-ANFI	Sablefish, black cod (<i>Anoplopoma fimbriatum</i>)	No	1	Alaska
te-CACA	Longnose sucker (<i>Catostomus catostomus</i>)	No	1	Alaska
te-CACO	White sucker (<i>Catostomus commersonii</i>)	No	1	Ontario
te-CAMA	Largescale sucker (<i>Catostomus macrocheilus</i>)	No	1	British Columbia
te-CLPA	Pacific herring (<i>Clupea pallasii</i>)	No	1	British Columbia
am-AMMV	Tiger salamander (<i>Ambystoma mavortium</i>)	No	1	British Columbia
am-ANAM	American toad (<i>Anaxyrus americanus</i>)	No	1	Ontario
am-ANBO	Western toad (<i>Anaxyrus</i> (<i>Bufo</i>) <i>boreas</i>)	No	1	Northwest Territories
am-LIPI	Northern leopard frog (<i>Lithobates</i> (<i>Rana</i>) <i>pipiens</i>)	No	1	British Columbia
am-PSRE	Pacific chorus frog (<i>Pseudacris</i> (<i>Hyla</i>) <i>regilla</i>)	No	1	Ontario
am-RAAU	Northern red-legged frog (<i>Rana aurora</i>)	No	1	British Columbia
ma-CACA	Beaver (<i>Castor canadensis</i>)	No	1	British Columbia
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-ENLUke	Sea otter (<i>Enhydra lutris kenyoni</i>)	No	1	British Columbia
ma-ESRO	Gray whale (<i>Eschrichtius robustus</i>)	No	1	British Columbia
ma-EUJU	Steller sea lion (<i>Eumetopias jubatus</i>)	No	1	British Columbia
ma-LOCA	River otter (<i>Lontra canadensis</i>)	No	1	British Columbia
ma-ODVI	White-tailed deer (<i>Odocoileus virginianus</i>)	No	1	British Columbia
mo-COFL	Asian clam (<i>Corbicula fluminea</i>)	No	1	Ontario
mo-CRGI	Pacific oyster (<i>Crassostrea gigas</i>)	No	1	British Columbia
mo-DRBU	Quagga mussel (<i>Dreissena bugensis</i>)	No	1	Ontario
mo-DRPO	Zebra mussels (<i>Dreissena polymorpha</i>)	No	1	Ontario
mo-Myspp	Mussel (<i>Mytilus</i> spp.)	No	1	British Columbia
mo-OBOL	Freshwater mussel (<i>Obovaria olivaria</i>)	No	1	Quebec
mo-OSLU	Olympia oyster (<i>Ostrea lurida</i>)	No	1	British Columbia
te-PAVE	English sole (<i>Parophrys vetulus</i>)	No	1	British Columbia

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

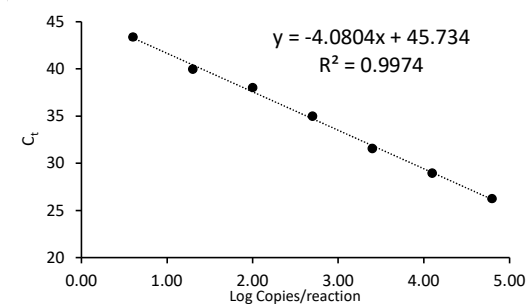
To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

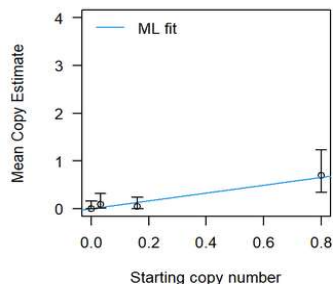
# Detects	# Copies	SE
0	0	0
1	0.164	0.169
2	0.352	0.267
3	0.575	0.368
4	0.849	0.487
5	1.202	0.642
6	1.698	0.872
7	2.548	1.326

Determined using eLowQuant R code⁴.

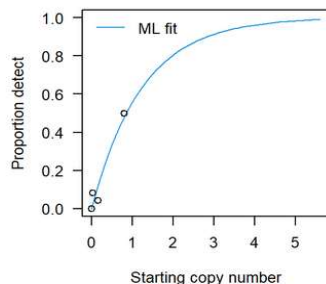
Applied to reactions with ≥ 95% positive hits



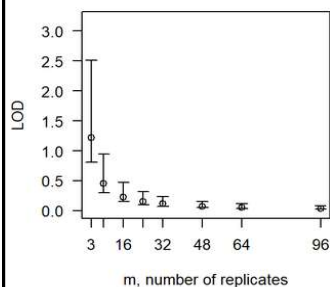
eSalmo2



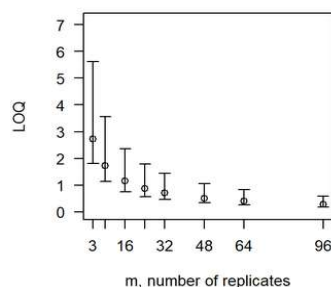
No intercept eSalmo2



Limits detect - no intercept eSalmo2



Limits quant - no intercept eSalmo2



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 Presence	# Samples	Detected	Location
Water	Yes	7	Yes	British Columbia
Sediment	Yes	3	Yes	British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-tRNA-Ile-Gln-Met	Mitochondrial tRNA encoding genes (multiple)
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