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: All Salmonid Species
Species Code: te-Salmonid
eDNA qPCR Tool: eSalmo2
eDNA qPCR Format: TaqMan
Gene Target: tRNA-Ile-Gln-Met
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 code[4]. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Qiacuity

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

Species	Common Name (*Species*)	Detection	Detection rate	# Voucher Specimens	Sample Sources/Locations
am-LICA	North American bullfrog (*Lithobates (Rana) catesbeiana*)	No	0%	1	British Columbia
ma-CALUfa	Canine (*Canis lupus familiaris*)	No	0%	1	British Columbia
ma-FECA	Cat (*Felis catus*)	No	0%	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	0%	1	Netherlands
te-AMPE	Pacific sandlance (*Ammodytes personatus*)	No	0%	1	British Columbia
te-ANRO	American eel (*Anguilla rostrata*)	No	0%	1	Prince Edward Island
te-CAAU	Goldfish (*Carassius auratus*)	No	0%	1	Alberta
te-COAR	Cisco/Tullibee (*Coregonus artedi*)	Yes	56%	4	Alberta
te-COCL	Lake whitefish (*Coregonus clupeaformis*)	Yes	100%	1	Alberta
te-COCO	Slimy sculpin (*Cottus cognatus*)	No	0%	1	British Columbia
te-ENMO	Californian anchovy (*Engraulis mordax*)	No	0%	1	British Columbia
te-ESLU	Northern pike (*Esox lucius*)	No	0%	1	British Columbia
te-GAAC	Three-spined stickleback (*Gasterosteus aculeatus*)	No	0%	1	British Columbia
te-GAMA	Pacific cod (*Gadus macrocephalus*)	No	0%	1	British Columbia
te-HIAL	Goldeye (*Hiodon alosoides*)	No	0%	1	Alberta
te-HYPR	Surf smelt (*Hypomesus pretiosus*)	No	0%	2	British Columbia
te-LEGI	Pumpkinseed/Sunfish/Pond perch (*Lepomis gibbosus*)	No	0%	1	British Columbia
te-LOLO	Burbot (*Lota lota*)	No	0%	1	Yukon
te-MIDO	Smallmouth bass (*Micropterus dolomieu*)	No	0%	2	British Columbia
te-MISA	Largemouth bass (*Micropterus salmoides*)	No	0%	1	British Columbia
te-MOAM	White perch (*Morone americana*)	No	0%	1	Quebec
te-MOSA	Striped bass (*Morone saxatilis*)	No	0%	1	Ontario
te-ONCLle	Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	Yes	100%	2	Alberta
te-ONGO	Pink salmon (*Oncorhynchus gorbuscha*)	Yes	100%	2	British Columbia
te-ONKE	Chum salmon (*Oncorhynchus keta*)	Yes	100%	1	British Columbia
te-ONKI	Coho salmon (*Oncorhynchus kisutch*)	Yes	100%	1	British Columbia
te-ONMY	Rainbow (steelhead) trout (*Oncorhynchus mykiss*)	Yes	100%	1	British Columbia and Alberta
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	Yes	100%	1	British Columbia
te-ONTS	Chinook salmon (Oncorhynchus tschawytscha)	Yes	100%	1	British Columbia
te-OPEL	Ling cod (*Ophiodon elongatus*)	No	0%	1	British Columbia
te-PEFL	Yellow perch (*Perca flavescens*)	No	0%	1	Quebec
te-PRCY	Round whitefish (*Prosopium cylindraceum*)	Yes	100%	1	Yukon
te-PRWI	Mountain whitefish (*Prosopium williamsoni*)	Yes	88%	1	Alberta
te-RHCA	Longnose (nooksack) dace (*Rhinichthys cataractae*)	No	0%	1	British Columbia
te-SACA	Sauger (*Sander canadensis*)	No	0%	1	Quebec
te-SACO	Bull trout (*Salvelinus confluentus*)	Yes	92%	2	Alberta
te-SAFO	Brook trout (*Salvelinus fontinalis*)	Yes	96%	2	Alberta
te-SAMA	Dolly varden (*Salvelinus malma*)	Yes	92%	1	British Columbia
te-SANA	Lake trout (*Salvelinus namaycush*)	Yes	100%	2	Alberta
te-SASA	Atlantic salmon (*Salmo salar*)	Yes	100%	1	Newfoundland
te-SATR	Brown trout (*Salmo trutta*)	Yes	100%	1	Quebec
te-seaLE	Rougheye rockfish (Sebastes aleutianus)	No	0%	1	British Columbia
te-SERU	Yelloweye rockfish (*Sebastes ruberrimu* s)	No	0%	1	British Columbia
te-SPTH	Longfin smelt (*Spirinchus thaleichthys*)	No	0%	1	Washington
te-THAR	Arctic grayling (*Thymallus arcticus*)	No	0%	2	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	0%	1	British Columbia

Helbing Lab
eDNA Inventory

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 (*Species*)	Detection	Detection rate	# Voucher Specimens	Sample Sources/Locations
te-ACFU	Lake sturgeon/yellow sturgeon (Acipenser fulvescens)	No	0%	1	Quebec
te-ANFI	Sablefish, black cod (Anoplopoma fimbriatum)	No	0%	1	Alaska
te-CACA	Longnose sucker (Catostomus catostomus)	No	0%	1	Alaska
te-CACO	White sucker (Catostomus commersonii)	No	0%	1	Ontario
te-CAMA	Largescale sucker (Catostomus macrocheilus)	No	0%	1	British Columbia
te-CLPA	Pacific herring (Clupea pallasii)	No	0%	1	British Columbia
am-AMMV	Tiger salamander (Ambystoma mavortium)	No	0%	1	British Columbia
am-ANAM	American toad (Anaxyrus americanus)	No	0%	1	Ontario
am-ANBO	Western toad (Anaxyrus (Bufo) boreas)	No	0%	1	North West Territories
am-LIPI	Northern leopard frog (Lithobates (Rana) pipiens)	No	0%	1	British Columbia
am-PSRE	Pacific chorus frog (Pseudacris (Hyla) regilla)	No	0%	1	Ontario
am-RAAU	Northern red-legged frog (Rana aurora)	No	0%	1	British Columbia
ma-CACA	Beaver (Castor canadensis)	No	0%	1	British Columbia
ma-CALUfa	Dog (Canis lupus familiaris)	No	0%	1	British Columbia
ma-ENLUke	Sea otter (Enhydra lutris kenyoni)	No	0%	1	British Columbia
ma-ESRO	Gray whale (Eschrichtius robustus)	No	0%	1	British Columbia
ma-EUJU	Steller sea lion (Eumetopias jubatus)	No	0%	1	British Columbia
ma-LOCA	River otter (Lontra canadensis)	No	0%	1	British Columbia
ma-ODVI	White-tailed deer (Odocoileus virginianus)	No	0%	1	British Columbia
mo-COFL	Asian clam (Corbicula fluminea)	No	0%	1	Ontario
mo-CRGI	Pacific oyster (Crassostrea gigas)	No	0%	1	British Columbia
mo-DRBU	Quagga mussel (Dreissena bugensis)	No	0%	1	Ontario
mo-DRPO	Zebra mussels (Dreissena polymorpha)	No	0%	1	Ontario
mo-Myspp	Mussel (Mytilus spp.)	No	0%	1	British Columbia
mo-OBOL	Freshwater mussel (Obovaria olivaria)	No	0%	1	Quebec
mo-OSLU	Olympia oyster (Ostrea lurida)	No	0%	1	British Columbia
te-PAVE	English sole (Parophrys vetulus)	No	0%	1	British Columbia

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

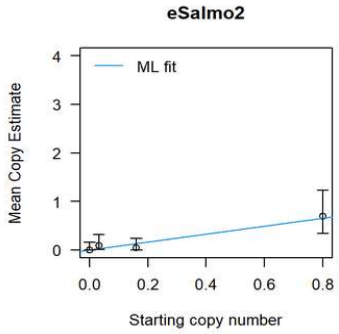
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849

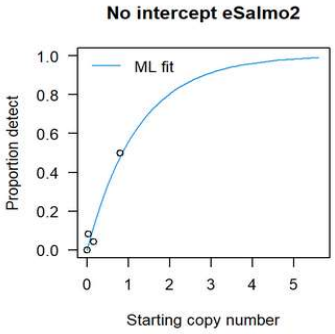
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164

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

Helbing Lab
eDNA Inventory

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



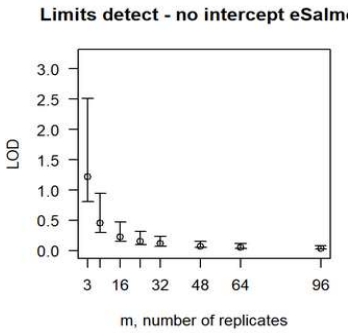


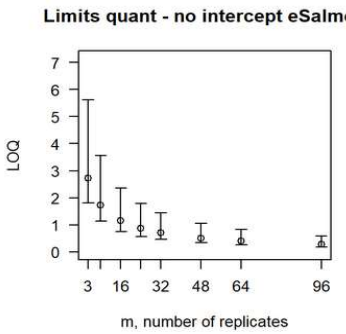
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
https://onlineacademiccommunity.uvic.ca/helbinglab/edna/

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.164	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

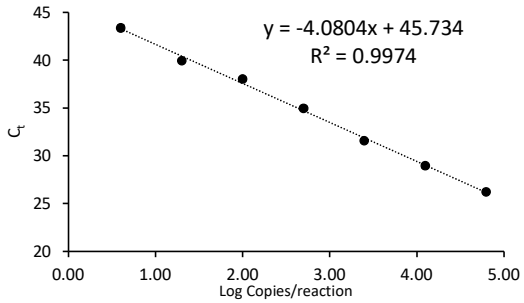
Determined using eLowQuant R code[4].





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

Applied to reactions with ≥ 95% positive hits



Efficiency 76%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

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