

### 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<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Pink Salmon (*Oncorhynchus gorbuscha*) eDNA qPCR Tool: eONGO5 Gene Target: MT-ND1  
 Species Code: te-ONGO eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 2.5 95% CI 1.8-4 Copies/Rxn LOQ 9.4 95% CI 6.8-15.2 Copies/Rxn LOB 0 hits/8  
 LOQ<sub>continuous</sub> 100 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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
ma-HOSA	Human ( <i>Homo Sapiens</i> )	No	1	Netherlands
te-CAAU	Goldfish ( <i>Carassius auratus</i> )	No	1	British Columbia
te-ESLU	Northern Pike ( <i>Esox lucius</i> )	No	1	British Columbia
te-MIDO	Smallmouth Bass ( <i>Micropterus dolomieu</i> )	No	1	British Columbia
te-MISA	Largemouth Bass ( <i>Micropterus salmoides</i> )	No	1	British Columbia
te-ONCL	Cutthroat Trout ( <i>Oncorhynchus clarkii</i> )	No	1	British Columbia
te-ONGO	Pink Salmon ( <i>Oncorhynchus gorbuscha</i> )	Yes	1	British Columbia
te-ONKE	Chum Salmon ( <i>Oncorhynchus keta</i> )	No	1	British Columbia
te-ONKI	Coho Salmon ( <i>Oncorhynchus kisutch</i> )	No	1	British Columbia
te-ONMY	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	No	1	British Columbia
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-PRCY	Round Whitefish ( <i>Prosopium cylindraceum</i> )	No	1	Yukon
te-SACO	Bull Trout ( <i>Salvelinus confluentus</i> )	No	1	Alberta
te-SAMA	Dolly Varden ( <i>Salvelinus malma</i> )	No	1	British Columbia
te-SASA	Atlantic Salmon ( <i>Salmo salar</i> )	No	1	Nova Scotia
te-THAR	Arctic Greyling ( <i>Thymallus arcticus</i> )	No	1	Alberta
te-THPA	Eaulachon ( <i>Thaleichthys pacificus</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

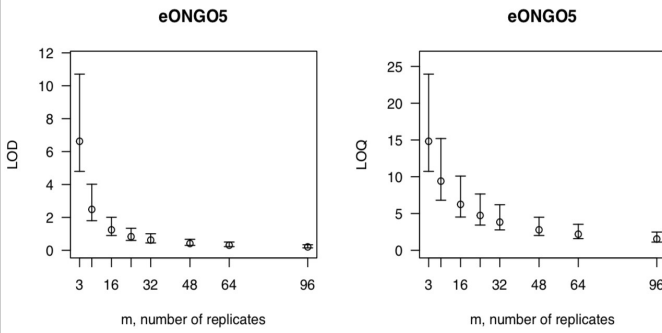
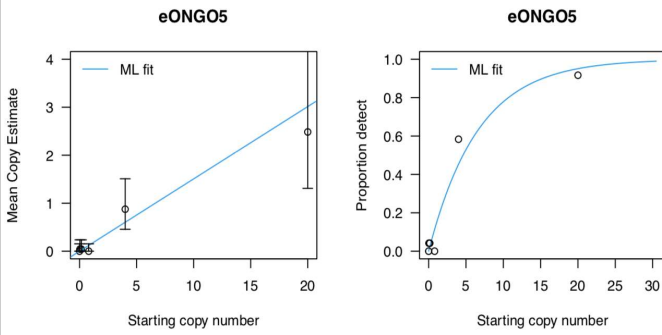
**eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA**

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

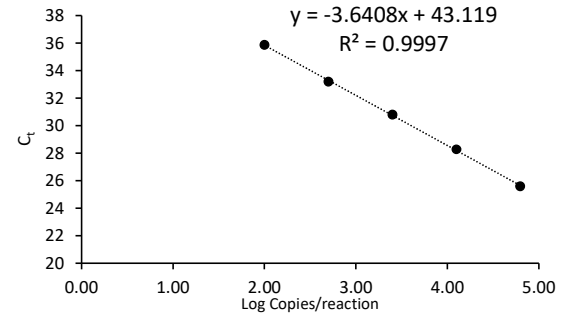
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.89	0.9
2	1.91	1.41
3	3.12	1.92
4	4.6	2.51
5	6.5	3.28
6	7.59	3.51
7	13.8	6.76

Determined using eLowQuant R code<sup>4</sup>.



Applied to reactions with 100% positive hits



Efficiency 88%

Binomial-Poisson model: No intercept  
 Determined using eLowQuant R code<sup>4</sup>.  
 Based on a 2  $\mu$ L DNA input in a total 15  $\mu$ L reaction

**Field Sample Validation**

Sample Type	Known Presence	# Samples	Detected	Location
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**Abbreviations**

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase subunit 1 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