

Appendix G.12

Environmental DNA – Certificate of Analysis, Bureau Veritas Laboratories



Attention: Melanie MacDonald

McCallum Environmental Ltd 2 Bluewater Road, Suite 115 Halifax, NS Canada B4B 1G7 Client Project #: 17-191 Site Location: FMS C.O.C. #: 20200529 Quote #: N/A PO#: N/A

> Report Date: 2020/06/05 Report #: ME20200605 Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20200529 Received: 2020/05/29, 9:05 AM

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica # Samples Received: 22

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE- DNA [™]	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA [™]	22	22	2020/06/01 2020/06/02	2020/06/02 2020/06/03 2020/06/04	N/A	GUE SOP-00056	ePlant5
General Fish assay (eFish)	22	21	N/A	N/A	2020/06/03 2020/06/04	GUE SOP-00056	eFish1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA[™] assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.



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Methodology for Sample Analysis

Samples received to the laboratory are entered into the Laboratory Information Management System (LIMS) upon receipt. Samples were inspected and assessed for amount of silica beads, silica bead saturation level, coin envelope condition and number of coin envelopes in each bag. Samples were frozen at -20°C until processing in the laboratory. Sample analysis is completed within 10 or 15 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit[™] (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation from the filter, the IntegritE-DNATM assay¹ is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNATM assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNATM assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNATM assay is 30. If the IntegritE-DNATM assay produces a positive detection frequency of ⁻ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNATM assay produces a positive detection frequency of ⁻ 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNATM assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNATM assay for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNATM assay, then the target species or genera assay is performed. Eight technical replicates or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay may be ineffective. Once a assay is positive control (Ultrapure water), and two technical replicates of genera. The cut-off Ct value for target species assay is 50.

¹ 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.



BECKY HENDERSON Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services Email: Becky.Henderson@bvlabs.com Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.

For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2



Client Name: McCallum Environmental Ltd Client Project #: 17-191 Site Location: FMS Sampler Initials: MMD

RESULTS - General Fish assay (eFish)

Client Sample ID	BV Case ID	Sampling Date	Preservation Type	COC Number	IntegritE- DNA [™] Positive detection (Ct≤30) ¹	QC Batch	Cleanup required	IntegritE-DNA [™] Positive detection (Ct≤30) after cleanup	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50) ²	QC Batch
1-A	ME20200020	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1⁵	8/8	200603Q1
1-B	ME20200021	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	8/8	200603Q1
1-C	ME20200022	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	8/8	200603Q1
2-A	ME20200023	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
2-B	ME20200024	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
2-C	ME20200025	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-A	ME20200026	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-B	ME20200027	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q1
3-C	ME20200028	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
4-A	ME20200029	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	0/8	200603Q1
4-B	ME20200030	2020/05/25	Silica	20200529	4/4	200602Q1	No	N/A	N/A	eFish1	1/8	200603Q3
4-C	ME20200031	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-A	ME20200032	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-B	ME20200033	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
5-C	ME20200034	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	2/8	200604Q2
6-A	ME20200035	2020/05/25	Silica	20200529	0/4 ³	200603Q2	Yes ³	4/4	200604Q1	eFish1	8/8	200604Q2
6-B	ME20200036	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	8/8	200604Q2
6-C	ME20200037	2020/05/25	Silica	20200529	0/4 ³	200603Q2	Yes ³	4/4	200604Q1	eFish1	8/8	200604Q2
7-A	ME20200038	2020/05/25	Silica	20200529	0/4 ³	200603Q2	Yes ³	4/4	200604Q1	eFish1	0/8	200604Q2
7-B	ME20200039	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	0/8	200604Q2
7-C	ME20200040	2020/05/25	Silica	20200529	4/4	200603Q2	No	N/A	N/A	eFish1	1/8	200604Q2
Field Blank	ME20200041	2020/05/25	Silica	20200529	0/4 ³	200603Q2	Yes ³	$0/4^4$	200604Q1	eFish1	N/A	N/A

¹ IntegritE-DNATM Assay: Four technical replicates were assayed for each eDNA sample. The cut-off Ct value for IntegritE-DNATM assay was 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

² Target Species Assay: Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

³ The IntegritE-DNA[™] assay failed and cleanup is required.

⁴ Quality of the samples are insufficient for the qPCR assay.

⁵ eFISH1: qPCR primer/probe assay to assess the presence of Fish species eDNA (confirmed to detect several fish including 19 species; Sockeye Salmon (Oncorhynchus nerka), Pink Salmon (Oncorhynchus gorbuscha), Chum Salmon (Oncorhynchus keta), Arctic Grayling (Thymallus arcticus), Cutthroat Trout (Oncorhynchus clarkii), Rainbow Trout (Oncorhynchus mykis), Chinook Salmon (Oncorhynchus tshawytscha), Coho Salmon (Oncorhynchus keta), Arctic Grayling (Thymallus arcticus), Cutthroat Trout (Oncorhynchus clarkii), Rainbow Trout (Oncorhynchus mykis), Chinook Salmon (Oncorhynchus tshawytscha), Coho Salmon (Oncorhynchus kisutch), Atlantic Salmon (Salmo salar), Dolly Varden (Salvelinus condition), Buiny Sculpin (Cottus cognatus), American Eel (Anguilla rostrata), Northern Pike (Esox lucius), Smallmouth Bass (Micropterus dolomieu), Largemouth Bass (Micropterus salmoides), Bull Trout (Salvelinus confluentus), Eulachon (Thaleichthys pacificus)). This assay is designed to be non-specific. It may detect eDNA from other fish species in addition or instead of the specific species listed here, which the assay has been validated for.

GENERAL COMMENTS

The IntegritE-DNA results for Blank sample (BV case ID, ME20200041) were negative before and after clean-up as expected.

Results relate only to the items tested.



Client Name: McCallum Environmental Ltd Client Project #: 17-191 Site Location: FMS Sampler Initials: MMD

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative	Control ¹	qPCR Positive Co	ntrols ²	qPCR Negative Contr	rols ³
QC Batch	Parameter	Date	Detection at:	Pass/Fail	Detection at:	Pass/Fail	Detection at:	Pass/Fail
200602Q1	IntegritE-DNA	2020/06/02	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200603Q2	IntegritE-DNA	2020/06/03	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200604Q1	IntegritE-DNA	2020/06/04		N/A	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
200603Q1	eFish1	2020/06/03	eDNA Isolation Negative Control is assessed using	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass
200603Q3	eFish1	2020/06/03	IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass
200604Q2	eFish1	2020/06/04		N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

¹eDNA Isolation Negative Control: Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNATM only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

²qPCR Positive Controls: Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNATM. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

³qPCR Negative Controls (Ultrapure water): Four technical replicates for IntegritE-DNATM and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNATM, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Ujvabzaele1

Reporter: ALI MIRABZADEH, M.Sc. Senior Analyst, Bureau Veritas Laboratories, DNA Services

Reviewer: HEATHER ALLEN, M.Sc. Supervisor, Bureau Veritas Laboratories, DNA Services



Client Name: McCallum Environmental Ltd Client Project #: 17-191 Site Location: FMS Sampler Initials: MMD

Fish Species Assay Validation Information

et Speci cies Abbi	VA Assay Informations es reviation / Specificity Tests	Various F Fish	ish Spec	ies		_		eDNA qPC eDNA qPC	R Primer/Probe set R Format	eFish1 TaqMan		_		_	_
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Client Name: McCallum Environmental Ltd Client Project #: 17-191 Site Location: FMS Sampler Initials: MMD

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* AMW assay also detects Ambystoma tigninum (ANT) Tiger Salamander. ³ a Fish assay can detect DNA from 12 fish species (Sockeys salmon, Pink salmon, Churn salmon, Arclic grayling, Cuthroat trout, Rainbow trout, Chinook salmon, Coho salmon, Atlantic Salmon, Dolly Varden, Round Whitefish and Silmy Sculpin). This assay is designed to be non-specific. It may detect DNA from other fish species in addition or instead of the specific species listed here, which the assay has been validated for.

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Client Name: McCallum Environmental Ltd Client Project #: 17-191 Site Location: FMS Sampler Initials: MMD

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