

## Biological Analysis Report

NW-DIST-2023-02-08-01

Florida DEP Laboratory  
2600 Blair Stone Road  
Tallahassee, FL 32399-2400  
DOH Accreditation E31780

Overflow Analyses Performed By:  
University of West Florida  
11000 University Parkway  
Pensacola, FL 32514  
DOH Accreditation E71969

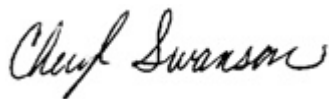
Event Description: **Freeport Run**  
Request ID: **RQ-2023-02-06-37**  
Customer: **NW-DIST**  
Project ID: **SMP**

Send Reports to:  
FL Dept. of Environmental Protection  
160 W Government Street  
Suite 208  
Pensacola, FL 32502-5794  
Attn: Myranda Butler

For additional information please contact  
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Toxicity  
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Certified by: Cheryl A. Swanson, Program Administrator

Date Certified: 29-MAR-2023 09:27



**NON-CONFORMANCE REPORT INCLUDED**

## Case Narrative

Unless otherwise noted, all samples included in this report were received in accordance with protocols referenced in Chapter 62-160, Florida Administrative Code (F.A.C.). Results published in this report pertain only to the samples as submitted to, and received by the laboratory. All times in this report are adjusted to the applicable Eastern Time Zone (EST or EDT).

Results for the following analytical groups are included in this report: Molecular and Overflow.

Scientific notation may be used in reporting very large or small values. Values reported using scientific notation will take the form of the following example: 1.3E+03, which is equivalent to  $1.3 \times 10^3$  or 1300.

Unless otherwise noted, analytical values for soil and sediment samples are reported on a dry weight basis, and analytical values for waste and tissue samples are reported on a wet weight basis.

Results for TNI accredited tests met requirements established by The NELAC Institute. A double asterisk (\*\*) is used to indicate an analyte/matrix/method for which the laboratory is not TNI accredited by the Florida Department of Health Environmental Laboratory Certification Program or where accreditation for that field of testing is not applicable.

Any significant anomalies or deviations from established protocols are documented in Non-Conformance Reports, which, where appropriate, are included within this analytical report. Additional comments related to specific analytical tests may be included as remarks following the analytical results for each sample. Such comments and remarks are for informational purposes only and are not intended to convey judgement about the usability of the reported data.

A quality control report on the performance of the test method for the submitted samples is included. Uncertainty associated with the analytical results contained in this report can be estimated from the reported quality assurance results and from published quality control acceptance limits for each analytical test. Matrix quality control results (matrix spike recoveries and matrix sample precision) pertain only to the matrix sample tested and do not necessarily reflect test method performance for other samples.

Typical matrix quality control (QC) measurements may include matrix spike recovery, matrix spike duplicate recovery, matrix spike precision and matrix sample precision. Not all matrix QC results may be available or reportable; where they are not an explanation is provided. Typical reasons for unavailable QC results include, but are not limited to, a) insufficient matrix sample to perform some or all QC measurements; b) analyte concentration in the sample replicated was too low for a meaningful measurement of precision and c) analyte concentration in the matrix sample spiked was too high (relative to the amount of analyte spiked) for a meaningful measurement of recovery. Where matrix QC results are unavailable, other method performance metrics (e.g., LCS recovery, LCS precision, surrogate recovery) may be used to assess performance of the method. Comments explaining any missing QC measurements are not intended to convey any adverse conclusions about the quality of the reported data.

Precision is reported as relative percent difference unless otherwise noted.

Quality Control codes as defined below may be used in this report to indicate results that are associated with one or more quality control elements which did not fall within established test method criteria. Such results may be qualified as estimates using a J qualifier as required by 62-160 F.A.C. Explanations are included in the report for any results that were reported as estimates for other reasons.

QC Codes used in this report may include:

- LCS – Recovery for the batch Laboratory Control Sample (LCS) was outside existing control limits;
- MS – Recovery for the batch matrix spike (MS) was outside existing control limits;
- CCV – Recovery for a continuing calibration verification (CCV) standard was outside existing control limits;
- SUR – Recovery of a surrogate (SUR) for associated analytes was outside existing control limits;
- RPD – The precision, measured as relative percent difference (RPD), of batch replicate measurements was outside existing control limits;
- RSD – The precision, measured as relative standard deviation (RSD), of batch replicate measurements was outside existing control limits;
- SMP – Sample - used precision derived from replicate analyses of a sample;

The following data qualifiers are used, where applicable, in this report as specified in 62-160 F.A.C.

- A - Value reported is the mean of two or more determinations.
- B - Results based on colony counts outside the acceptable range.
- I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
- J - Estimated value and/or the analysis did not meet established quality control criteria.
- K - Actual value is known to be less than value given.
- L - Actual value is known to be greater than value given.
- N - Presumptive evidence of presence of material.
- O - Sampled, but analysis lost or not performed.
- Q - Sample held beyond normal holding time.
- T - Value reported is less than the criterion of detection.
- U - Material was analyzed for but not detected. The reported value is the method detection limit for the sample analyzed.
- V - Analyte was detected in both sample and method blank.
- X - Too few individuals to calculate SCI value.
- Y - The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate.
- Z - Colonies were too numerous to count (TNTC).

Quality control information from overflow laboratories may not be included in this report. Please refer to the associated report from the overflow laboratory for additional information.

**Sample Location: Seven Runs upstream of Hwy 81**

**Collection Date/Time: 02/06/2023 10:10**

**Field ID: G3NW0116**

**Matrix: W-SURF-FRH**

| Sample ID | Ref. Method           | Component                    | Result | Code | Units      | Batch ID | QC Codes |
|-----------|-----------------------|------------------------------|--------|------|------------|----------|----------|
| 2385899   | SM 9223 B Quanti-Tray | Escherichia Coli-Quanti-Tray | 275    |      | MPN/100 mL |          |          |

**Sample Location: Little Black Creek upstream of Black Creek**

**Collection Date/Time: 02/06/2023 10:35**

**Field ID: G3NW0200**

**Matrix: W-SURF-FRH**

| Sample ID | Ref. Method           | Component                    | Result  | Code | Units      | Batch ID | QC Codes |
|-----------|-----------------------|------------------------------|---------|------|------------|----------|----------|
| 2385898   | SM 9223 B Quanti-Tray | Escherichia Coli-Quanti-Tray | 233     |      | MPN/100 mL |          |          |
| 2385907   | SOP-PCR-1.0           | HF183-qPCR**                 | 1.7E+03 | UQ   | TSC/100 mL | P425465  |          |
|           | SOP-PCR-1.2           | GULL2-qPCR**                 | 1.8E+03 | UQ   | TSC/100 mL | P427500  |          |
|           | SOP-PCR-1.3           | GFD-qPCR**                   | 360     | UQ   | TSC/100 mL | P427499  |          |
|           | SOP-PCR-1.4           | BacR-qPCR**                  | 1.5E+03 | UQ   | TSC/100 mL | P425465  |          |
|           | SOP-PCR-1.5           | DG3-qPCR**                   | 1.3E+03 | UQ   | TSC/100 mL | P425465  |          |

Ref. Method and Comment:

SOP-PCR-1.0: See non-conformance report # 9761.

SOP-PCR-1.2: Precision data unavailable for Gull2-qPCR component. See non-conformance report # 9761.

SOP-PCR-1.3: Precision data unavailable for GFD-qPCR component. See non-conformance report # 9761.

SOP-PCR-1.4: See non-conformance report # 9761.

SOP-PCR-1.5: See non-conformance report # 9761.

**Sample Location: Mouth of Mullet Creek @ Hwy 20**

**Collection Date/Time: 02/06/2023 11:20**

**Field ID: G3NW0137**

**Matrix: W-SURF-FRH**

| Sample ID | Ref. Method           | Component                    | Result | Code | Units      | Batch ID | QC Codes |
|-----------|-----------------------|------------------------------|--------|------|------------|----------|----------|
| 2385900   | SM 9223 B Quanti-Tray | Escherichia Coli-Quanti-Tray | 496    |      | MPN/100 mL |          |          |

## Non-Conformance Report

**NCR ID: 9761**

### Event(s)

NW-DIST-2023-02-08-01

### Job(s)

TLH-2023-02-08-72

### Sample(s)

2385907

### Test(s)

**NCR Type: SHIPPING/RECEIVING**

**NCR Category: Samples arrived late**

Observation: Samples arrived late, samples received within the acceptable temperature range.

Resolution: Samples will be qualified as appropriate.

Authorized by/Date: Kyle Klug 2/22/2023

The Non-Conformance Report details exceptions or problems encountered with the events/jobs/samples/test.

Please address questions to:

Chemistry Colin Wright (850) 245-8085

Biology Cheryl Swanson (850) 245-8177

## Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P425465

| Component  | Result  | Code | Units      |
|------------|---------|------|------------|
| HF183-qPCR | 3.3E+03 | U    | TSC/100 mL |

## Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.2  
Batch ID: P427500

| Component  | Result  | Code | Units      |
|------------|---------|------|------------|
| GULL2-qPCR | 3.6E+03 | U    | TSC/100 mL |

Reference Method: SOP-PCR-1.3  
Batch ID: P427499

| Component | Result | Code | Units      |
|-----------|--------|------|------------|
| GFD-qPCR  | 720    | U    | TSC/100 mL |

Reference Method: SOP-PCR-1.4  
Batch ID: P425465

| Component | Result  | Code | Units      |
|-----------|---------|------|------------|
| BacR-qPCR | 3.0E+03 | U    | TSC/100 mL |

Reference Method: SOP-PCR-1.5  
Batch ID: P425465

| Component | Result  | Code | Units      |
|-----------|---------|------|------------|
| DG3-qPCR  | 2.6E+03 | U    | TSC/100 mL |

## Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0  
Batch ID: P425465

| Component  | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|------------|---------|---------|-----------|----------------|
| HF183-qPCR | 97.0    | 96.4    | P/P       | 50 - 175       |

Reference Method: SOP-PCR-1.2  
Batch ID: P427500

| Component  | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|------------|---------|---------|-----------|----------------|
| GULL2-qPCR | 84.6    | 103     | P/P       | 50 - 300       |

Reference Method: SOP-PCR-1.3  
Batch ID: P427499

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|-----------|---------|---------|-----------|----------------|
| GFD-qPCR  | 91.4    | 100     | P/P       | 50 - 175       |

Reference Method: SOP-PCR-1.4  
Batch ID: P425465

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|-----------|---------|---------|-----------|----------------|
| BacR-qPCR | 105     | 111     | P/P       | 50 - 175       |

Reference Method: SOP-PCR-1.5  
Batch ID: P425465

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|-----------|---------|---------|-----------|----------------|
|-----------|---------|---------|-----------|----------------|

Quality Assurance Report  
Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.5  
Batch ID: P425465

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|-----------|---------|---------|-----------|----------------|
| DG3-qPCR  | 99.0    | 108     | P/P       | 50 - 175       |

Quality Assurance Report  
Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0  
Batch ID: P425465

| Spiked Sample | Component  | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|------------|---------|---------|-----------|----------------|
| 2385071       | HF183-qPCR | 83.0    | 85.7    | P/P       | 50 - 175       |
| 2385072       | HF183-qPCR | 88.6    |         | P         | 50 - 175       |
| 2385073       | HF183-qPCR | 93.8    |         | P         | 50 - 175       |
| 2385804       | HF183-qPCR | 78.9    |         | P         | 50 - 175       |
| 2385851       | HF183-qPCR | 76.8    |         | P         | 50 - 175       |
| 2385907       | HF183-qPCR | 79.0    |         | P         | 50 - 175       |
| 2386142       | HF183-qPCR | 73.6    |         | P         | 50 - 175       |
| 2386201       | HF183-qPCR | 94.3    |         | P         | 50 - 175       |
| 2386202       | HF183-qPCR | 83.9    |         | P         | 50 - 175       |
| 2386211       | HF183-qPCR | 105     |         | P         | 50 - 175       |

Reference Method: SOP-PCR-1.2  
Batch ID: P427500

| Spiked Sample | Component  | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|------------|---------|---------|-----------|----------------|
| 2385907       | GULL2-qPCR | 80.2    |         | P         | 50 - 300       |

Reference Method: SOP-PCR-1.3  
Batch ID: P427499

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|-----------|---------|---------|-----------|----------------|
| 2385907       | GFD-qPCR  | 90.8    |         | P         | 50 - 175       |

Reference Method: SOP-PCR-1.4  
Batch ID: P425465

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|-----------|---------|---------|-----------|----------------|
| 2385071       | BacR-qPCR | 94.3    | 95.2    | P/P       | 50 - 175       |
| 2385072       | BacR-qPCR | 93.6    |         | P         | 50 - 175       |
| 2385073       | BacR-qPCR | 92.9    |         | P         | 50 - 175       |
| 2385804       | BacR-qPCR | 88.0    |         | P         | 50 - 175       |
| 2385851       | BacR-qPCR | 71.4    |         | P         | 50 - 175       |
| 2385907       | BacR-qPCR | 81.5    |         | P         | 50 - 175       |
| 2386142       | BacR-qPCR | 70.2    |         | P         | 50 - 175       |
| 2386201       | BacR-qPCR | 89.3    |         | P         | 50 - 175       |
| 2386202       | BacR-qPCR | 80.3    |         | P         | 50 - 175       |
| 2386211       | BacR-qPCR | 102     |         | P         | 50 - 175       |

Reference Method: SOP-PCR-1.5  
Batch ID: P425465

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|-----------|---------|---------|-----------|----------------|
|---------------|-----------|---------|---------|-----------|----------------|

## Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5  
 Batch ID: P425465

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---------------|-----------|---------|---------|-----------|----------------|
| 2385071       | DG3-qPCR  | 89.9    | 96.2    | P/P       | 50 - 175       |
| 2385072       | DG3-qPCR  | 100     |         | P         | 50 - 175       |
| 2385073       | DG3-qPCR  | 101     |         | P         | 50 - 175       |
| 2385428       | DG3-qPCR  | 103     |         | P         | 50 - 175       |
| 2385429       | DG3-qPCR  | 105     |         | P         | 50 - 175       |
| 2385430       | DG3-qPCR  | 99.3    |         | P         | 50 - 175       |
| 2385804       | DG3-qPCR  | 104     |         | P         | 50 - 175       |
| 2385851       | DG3-qPCR  | 96.8    |         | P         | 50 - 175       |
| 2385907       | DG3-qPCR  | 104     |         | P         | 50 - 175       |
| 2386142       | DG3-qPCR  | 92.1    |         | P         | 50 - 175       |
| 2386201       | DG3-qPCR  | 105     |         | P         | 50 - 175       |
| 2386202       | DG3-qPCR  | 88.9    |         | P         | 50 - 175       |
| 2386211       | DG3-qPCR  | 105     |         | P         | 50 - 175       |

## Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0  
 Batch ID: P425465

| Replicated Lab Sample | Component  | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|-----------------------|------------|-----------|-------------------|-----------|----------------|
| 2385071               | HF183-qPCR | 3.22      | Spike             | P         | 0 - 25         |

Reference Method: SOP-PCR-1.4  
 Batch ID: P425465

| Replicated Lab Sample | Component | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|-----------------------|-----------|-----------|-------------------|-----------|----------------|
| 2385071               | BacR-qPCR | 1.00      | Spike             | P         | 0 - 25         |

Reference Method: SOP-PCR-1.5  
 Batch ID: P425465

| Replicated Lab Sample | Component | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|-----------------------|-----------|-----------|-------------------|-----------|----------------|
| 2385071               | DG3-qPCR  | 6.79      | Spike             | P         | 0 - 25         |

\* Sample, spike and/or laboratory control sample precision (LCS) is reported.

## Quality Assurance Report Surrogates

Lab Sample ID: 2385907  
 Field Sample ID: G3NW0200

| Reference Method | Surrogate  | % Rec. | Pass/Fail | Control Limits |
|------------------|------------|--------|-----------|----------------|
| SOP-PCR-1.0      | SKETA-qPCR | 94.7   | P         | 50 - 300       |
| SOP-PCR-1.2      | SKETA-qPCR | 95.1   | P         | 50 - 300       |
| SOP-PCR-1.3      | SKETA-qPCR | 93.8   | P         | 50 - 300       |

## Quality Assurance Report Surrogates

Lab Sample ID: 2385907  
 Field Sample ID: G3NW0200

| Reference Method | Surrogate  | % Rec. | Pass/Fail | Control Limits |
|------------------|------------|--------|-----------|----------------|
| SOP-PCR-1.4      | SKETA-qPCR | 95.8   | P         | 50 - 300       |
| SOP-PCR-1.5      | SKETA-qPCR | 90.5   | P         | 50 - 300       |

## Quality Assurance Report Summary

| Ref. Method | Analyte    | LCS % Recovery |      | MS % Recovery |      |      | LCS | Precision<br>SMP | MS   |
|-------------|------------|----------------|------|---------------|------|------|-----|------------------|------|
| SOP-PCR-1.0 | HF183-qPCR | 96.4           | 97.0 | 83.0          | 85.7 | 88.6 |     |                  | 3.22 |
| SOP-PCR-1.2 | GULL2-qPCR | 103            | 84.6 | 80.2          |      |      |     |                  |      |
| SOP-PCR-1.3 | GFD-qPCR   | 100            | 91.4 | 90.8          |      |      |     |                  |      |
| SOP-PCR-1.4 | BacR-qPCR  | 111            | 105  | 80.3          | 102  | 94.3 |     |                  | 1.00 |
| SOP-PCR-1.5 | DG3-qPCR   | 108            | 99.0 | 89.9          | 96.2 | 100  |     |                  | 6.79 |
|             |            |                |      | 101           |      |      |     |                  |      |

## Reference Method Descriptions

| Method                | Description                                                                                                                                        | Associated Samples        |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| SM 9223 B Quanti-Tray | E. coli by Colilert-18/QT by UWF in Pensacola                                                                                                      | 2385898, 2385899, 2385900 |
| SOP-PCR-1.0           | Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction                        | 2385907                   |
| SOP-PCR-1.2           | Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction | 2385907                   |
| SOP-PCR-1.3           | Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction                          | 2385907                   |
| SOP-PCR-1.4           | Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction                    | 2385907                   |
| SOP-PCR-1.5           | Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction                         | 2385907                   |

Preparation and Analysis Log

| Ref. Method           | Received Date | Prep Date/Time   | Prepared By    | Analysis Date/Time | Analyzed By   | Associated Samples        |
|-----------------------|---------------|------------------|----------------|--------------------|---------------|---------------------------|
| SM 9223 B Quanti-Tray | 02/06/2023    | 02/06/2023 15:30 |                | 02/07/2023 11:30   | UWF Lab       | 2385898, 2385899, 2385900 |
| SOP-PCR-1.0           | 02/08/2023    | 02/08/2023 16:00 | Claudia Castro | 02/20/2023 23:05   | Roberta Tatti | 2385907                   |
| SOP-PCR-1.2           | 02/08/2023    | 02/08/2023 16:00 | Claudia Castro | 03/15/2023 14:00   | Roberta Tatti | 2385907                   |
| SOP-PCR-1.3           | 02/08/2023    | 02/08/2023 16:00 | Claudia Castro | 03/15/2023 14:00   | Roberta Tatti | 2385907                   |
| SOP-PCR-1.4           | 02/08/2023    | 02/08/2023 16:00 | Claudia Castro | 02/20/2023 12:18   | Roberta Tatti | 2385907                   |
| SOP-PCR-1.5           | 02/08/2023    | 02/08/2023 16:00 | Claudia Castro | 02/20/2023 15:32   | Roberta Tatti | 2385907                   |