

**Source Identification of Fecal Coliform Bacteria Impairment in Rapid Creek,  
South Dakota  
DNA Sampling Plan**

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**Prepared For:**



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**Project Location:** The project will be located on the impaired segment of Rapid Creek which has a length of 74 stream miles, beginning at Canyon Lake, running through the center of Rapid City, and ending where Rapid Creek empties in the Cheyenne River.

**Project Abstract:** Rapid Creek is a vital natural resource for wild brown trout, tourism and recreation in Rapid City, SD. Continuous parks and green spaces all along the creek creates and enhances a quality of life unparalleled in our region. Expanding urban development has and continues to impact Rapid Creek's water quality. Regular water testing by the South Dakota Department of Environment and Natural Resources (SDDENR) has resulted in listed total maximum daily load (TMDL) impairments for fecal coliform bacteria since 1998. A study by the SDDENR and the South Dakota School of Mines and Technology in 2010 determined that the proposed study area would require a 95 percent reduction in the fecal coliform loading in high and moist flow zones in order to meet the TMDL standard. The specific sources of fecal coliform bacteria contamination has yet to be identified. Through diverse partnerships with local residents, a small local business (H2E, Incorporated), Source Molecular Corporation, the SDDENR, the United States Geological Survey, Pennington County and the City of Rapid City, the West Dakota Water Development District will collect and analyze deoxyribonucleic acid (DNA) samples from Rapid Creek to identify sources of fecal coliform bacteria. By defining and understanding the source(s) of bacteria in Rapid Creek and taking the lead on corrective action, the West Dakota Water Development District hopes to provide a healthy and accessible urban waterway for future generations to use and enjoy.

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## Background

Lower Rapid Creek has been listed as an impaired water body due to exceedances of fecal coliform bacteria criteria by the South Dakota Department of Environment and Natural Resources (SDDENR) in every listing cycle since 1998. Urban runoff pollution is a widespread and complex issue that was identified as a primary contributor to the overall bacterial load in Rapid Creek by the SDDENR in 2004. Within the proposed study area, Rapid Creek is listed for a variety of beneficial uses including cold water permanent fish life propagation, warm water permanent fish life propagation, immersion recreation, limited contact recreation, fish and wildlife propagation, recreation and stock watering, and irrigation. Rapid Creek is a nationally renowned wild brown trout fishery and a cornerstone of the fishing industry in the Black Hills. The creek also travels through the center of Rapid City and serves as an aesthetic feature for hikers, bicyclers, runners, and walkers. A big question mark has been placed on the fundamental value and the variety of beneficial uses of Rapid Creek due to the bacterial impairment designation. Recreational use of Rapid Creek also had the potential to make people ill, since the water concentrations of *Escherichia coli* exceeded the standard for over 1,000 samples. However, before effective remedial action can be taken, the sources of contamination must be identified. The West Dakota Water Development District (WDWDD) and its partners have developed a two phase project to first characterize the bacterial water quality impairment in Rapid Creek and then to lead a targeted effort to mitigate the impairment.

## Scope and Objectives

The objective of Phase 1 is to identify the source(s) of fecal coliform bacteria in Rapid Creek through microbial source tracking genetic and molecular techniques. The project will be located on the impaired segment of Rapid Creek which has a length of 74 stream miles, beginning at Canyon Lake on the west side of Rapid City, running through the center of Rapid City, and ending where Rapid Creek empties into the Cheyenne River. Sampling locations have been strategically chosen as sites that would provide the most information to identify and isolate possible sources of contamination. Sampling locations also correspond with existing USGS and SDDENR monitoring sites in order to utilize flow, fecal coliform bacteria, TSS, and *E. coli* data that is already being recorded. By sampling for fecal coliform, TSS and *E. coli* in conjunction with DNA testing we hope to identify the major sources of contamination to Rapid Creek. The WDWDD has obligated \$53,014.00 to sample four locations monthly for one year.

The objective of Phase 2 is to take appropriate measures to restore the water quality of Rapid Creek and to develop best management practices for its protection. Direct action will be taken by the WDWDD and its many partners to remediate contaminant sources identified in Phase 1.

## Collaboration

The proposed project will address the ongoing environmental concern in Rapid Creek through the collaborative efforts of community stakeholders and the WDWDD.

The WDWDD has fostered partnerships with local residents (Paul Squillace and Tim Rogers), a small local business (H2E, Incorporated), a private commercial laboratory (Source Molecular Corporation), and the United States Geological Survey (USGS). Rapid City's local government (City of Rapid City) has been notified of the prospective project and it has assigned a staff member to assist with the study. In addition, the WDWDD is in the process of engaging the SDDENR and SDSMT as potential partners and collaborators.

From planning to completion, each partner will play an important role in the success of the proposed project. H2E, Incorporated (H2E) is a small local environmental consulting firm hired by the WDWDD to fill program management roles. H2E has volunteered their time to research and develop a DNA Testing Plan for Rapid Creek. They will be contracted to complete the following tasks:

- Manage project budget.
- Oversee all aspects of the project to insure quality work.
- Establish and maintain working relationships with all project partners and the public.
- Write quarterly progress reports and provide project updates at all WDWDD public meetings.
- Collaborate with the SDDENR and USGS for sample collection.
- Analyze and interpret the DNA testing results provided by Source Molecular Corporation.

Paul Squillace, former United States Geological Survey (USGS) Research Hydrologist and Tim Rogers, the Environmental Manager of Black Hills Power are local residents who have volunteered their time and expertise to assist with this project. Paul Squillace will be available to consult the WDWDD and H2E on the design of the DNA Testing Plan and the collection of samples. Tim Rogers has helped to foster partnerships with the City of Rapid City, the SDDENR and the USGS and reviewed grant applications.

Source Molecular Corporation is private commercial laboratory that has volunteered to provide assistance in crafting an appropriate DNA Testing Plan and will be contracted to complete laboratory tests on all collected samples. Tests will identify and quantify the fecal contamination from four of five of the following host sources based on sampling location: human, dog, deer, bird, and cattle. Site specific host source tests are identified under the Sampling Locations section of this report and were chosen based on upstream land use and possible point and non-point sources. Source Molecular will provide a DNA quantification report that will include all analysis results and a thorough analytical method explanation.

The USGS and the City of Rapid City will be conducting affiliated research that will investigate the primary sources of bacteria in stormwater, which has been a persistent question for stormwater management in Rapid City. The two major objectives of this study will be to quantify

bacterial loads for various infrastructure elements along drainage flow paths and to identify the primary source species for the bacteria in the stormwater discharges.

The SDSMT will also be conducting affiliated research that will investigate the pathogenicity of the bacteria in Rapid Creek. Dr. Lisa Kunza and Dr. Linda DeVeaux are applying a novel pathogenicity metric, which determines presence/absence as well as relative abundance of 30 genes, to water samples collected from Rapid Creek.

The USGS, City of Rapid City, SDSMT and the WDWDD will collaborate their project efforts to obtain as much data and information about Rapid Creek as possible.

## Sampling Locations

Sampling locations have been strategically chosen as sites that would provide the most information to identify and isolate possible sources of contamination. All sampling locations will be tested for human, dog, cattle, deer and bird host sources. Maps of each of the following preliminary sampling locations can be found in Appendix B.

1. DENR Site WQM 69 – Dark Canyon (460669)
  - Selected to coordinate with the DENR WQM 69 sites and to provide base information for Rapid Creek before the urban impacts of Rapid City.
2. USGS Station 6413200 – Below Canyon Lake
  - Selected to determine the effects of the bird population in Canyon Lake and the large concentration of septic systems in the Wonderland Drive drainage basin.
3. USGS Station 641600 and DENR Site WQM 173– St Patrick Street
  - Selected to coordinate with both the USGS and DENR sampling sites and to help determine the effects of Rapid City’s downtown area.
4. DENR Site WQM 92 – Below Wastewater Treatment Plant (460692)
  - Gives a good perspective of the possible impacts of the WWTP and the cow feedlots in the area.

## Sampling Procedure

Through collaboration with the SDDENR, the USGS and Source Molecular, H2E will collect monthly DNA samples for one year, from sites along the impaired segment of Rapid Creek from Canyon Lake to where the creek empties into the Cheyenne River. These samples may be collected at established USGS gauging stations or SDDENR sampling locations and will demonstrate seasonal variations and the effects of urban stormwater runoff on the water quality in Rapid Creek. Shipping kits, including sterile sample bottles will be obtained from Source Molecular to help insure quality assurance and quality control. Specific details regarding sample collection materials, methods, preservation and shipment can be found below.

## List of Required Materials

- 500 ml leakproof sterile bottles
- Ice packs
- Zip lock bags
- Cooler
- Sharpie marker
- Pen
- Disposable gloves
- Paper towels
- Freezer
- Chain of Custody
- Waders

## Sample Collection

The Water Grab Sample technique listed below will be implemented to collect a 500 mL sample from each sampling location on a monthly basis. Real time flow data will be recorded from USGS gaging station 06412810 and photo documentation will be taken at the time that each sample is collected.

1. Label each sample container with the date, time, and site number/name.
2. Record all applicable information on the Chain of Custody Sheet (see Appendix A).
3. Water samples will be collected before any other work is done at a site.
4. Collect water samples before stirring up the stream bottom, or collect samples upstream of agitated water. Samples will be collected by wading into the centroid of flow or mid-channel in the stream and reaching as far upstream as possible to avoid collecting stirred up water. If this is not possible due to high flow or other safety reasons, samples will be collected while standing on the edge of the water or on a rock.
5. Without touching the inside or lip of the sterile sample bottles or caps, hold the uncapped bottle upside down and submerge it about 1 foot below the surface mid-stream until filled. Tip the bottle upright and allow water to fill the bottle. Remove the bottle from the water and screw on the cap.
6. Store and transport samples using the Sample Shipment Directions below.
7. Complete the Chain of Custody Sheet (see Appendix A) and include in sample shipment.

## Sample Collection QA/QC

In addition to Source Molecular's lab analysis QA/QC, sample collection QA/QC will be implemented. The key aspects of quality control associated with sample collection are as follows:

1. Field personnel will be thoroughly trained in proper sample collection protocol.

2. Field personnel will be thoroughly trained to recognize and avoid potential sources of sample contamination.
3. Sample gear or equipment that comes in direct contact with the water sample will be made of non-contaminating materials and will be cleaned between sampling events.
4. Sample containers will be sterile and the recommended type of material.
5. Conditions for sample collection, preservation and holding times will be followed.

Additional QA/QC sampling will be shipped to Source Molecular for analysis. One blank per sampling trip will be analyzed. Duplicate samples will also be sporadically taken and sent to the lab to help confirm accurate results. Human bacteria is most harmful to other humans than other species and it is generally easier to control if it is identified. Therefore, human bacteria identification and validation will be prioritized first and all duplicate samples will test for human host specie.

### Sample Shipment

After collection, samples will be immediately shipped to Source Molecular via an overnight courier using the recommended packing instructions below:

- Wrap leakproof sterile bottles with abundant paper towels and put them individually in zip lock bags.
- Ice packs will be placed in zip lock bags and packed along with sample(s).
- Wrap the zip lock bag with abundant paper towels and insert everything in another zip lock bag.
- Ice packs will not directly touch the samples (adding additional packing material will prevent this).
- As an added precaution, the zip lock bags and packing material will be put in two overlapping garbage bags.
- The garbage bags will be wrapped tightly and everything put in a sturdy cooler.

Ship the sample(s) via overnight courier to:

Source Molecular Corporation  
4985 SW 74<sup>th</sup> Court  
Miami, Florida 33155 USA

### Sample Analysis

Source Molecular Corporation is a private commercial laboratory who aims to fill the void between source identification research and real-world implementation of the technology. Source Molecular will be contracted to provide sample analysis services. Source Molecular maintains quality control for sample analysis with the latest scientific laboratory equipment and highly

trained, degreed personnel. Source Molecular's QA/QC summary can be found in Appendix D. Source Molecular uses scientifically rigorous molecular analytical methods that enable it to identify with high confidence any contamination, to avoid false positives. Their stringent methodologies and expertise in understanding fecal contamination help insure accurate results and the success of this project.

Source Molecular will perform quantification fecal contamination testing from human, deer, bird, and dog or cattle host sources for each sample. The test results will be delivered back to the WDWDD within 5-10 business days. Source Molecular will also provide a DNA quantification report that will include all analysis results and a thorough analytical method explanation for each set of tests.

## Project Budgets

The WDWDD has obligated \$53,014.00 for DNA sample collection, testing and analysis. The detailed DNA testing plan budget can be found in Appendix C.

## Sharing Results

Throughout the duration of the project, WDWDD bi-monthly public meetings will be held to share information between the project partners and with the community. Meeting announcements will be posted in several local newspapers (Rapid City Journal, New Underwood Post, and Hill City Prevailer) to notify and invite the public to attend. The meetings will allow the projects partners to collaborate ideas, track progress, and share results with the public and stakeholders. The collected water quality data and project progress will be recorded in written reports and shared with the public at the meetings. The meeting minutes will be posted at the WDWDDs office and on the web after each meeting. At the conclusion of the project, a final report will be developed that evaluates the data collected and the potential sources of contamination in Rapid City. All reports and the results from the DNA testing will be made available to the public via the WDWDD website and upon request.



## Appendices

### Appendix A: Chain of Custody

# Chain Of Custody Record



**SHIPPING ADDRESS:**  
 4985 SW 74th Court, Miami, FL 33155 USA  
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733  
 Email: info@sourcemolecular.com

Company Name:		Analysis Requested (see pg. 2)			<b>Comments</b>
Contact Name(s):		Mark boxes with "X"			
E-mail Address(es):					
Phone:					
Address:					
City/State/Zip:					
Country:					
Sample ID	Media	Sample Date	Sample Time	# of Containers	Quantification (Yes, No, If Positive)

<b>Completed by Client:</b> SAMPLES DELIVERED BY _____ SAMPLER NAME _____ SAMPLE SITE _____ SIGNATURE _____	<b>Completed by Source Molecular:</b> SAMPLES RECEIVED BY _____ RECEIVED DATE/TIME _____ TEMPERATURE _____ CONDITION _____ SIGNATURE _____
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## Explanation of Terms Used in Chain-of-Custody Record

<b>Sample ID:</b>	The specific sample identification number, unique to each sample set.
<b>Media:</b>	The sample media (e.g. water, soil).
<b>Sample Date:</b>	The date that the sample was taken, including month, day, and year.
<b>Sample Time:</b>	The time the sample was taken.
<b># of Containers:</b>	The number of containers for that particular sample set.
<b>Analysis:</b>	The type(s) of analysis required for that particular sample or sample set.
<b>Quantification:</b>	The type of testing to be performed. Select "Yes" for quantification analysis. Select "No" for only presence or absence analysis. Select "If Positive" for presence or absence with quantification of the host biomarkers that are positive.
<b>Samples Delivered By:</b>	The signature of the individual who is delivering the samples to the laboratory. Under most circumstances, this will be one of the individuals who performed the sampling.
<b>Sampler Name:</b>	The name of the individual(s) performing the sampling.
<b>Sample Site:</b>	The name of the site being sampled.
<b>Samples Received By:</b>	The name of the individual who receives the samples for the laboratory.
<b>Received Date/Time:</b>	The date and time the samples are delivered to the lab, including hour, month, day, and year.
<b>Temperature:</b>	The temperature of the samples upon arrival, in degrees Celsius.
<b>Condition:</b>	The condition of the samples upon arrival.
<b>Signature:</b>	The signature of the individual who receives the samples for the laboratory.

## Recommended Quantities

### Water samples:

500mL of water in leak proof bottles per sample.

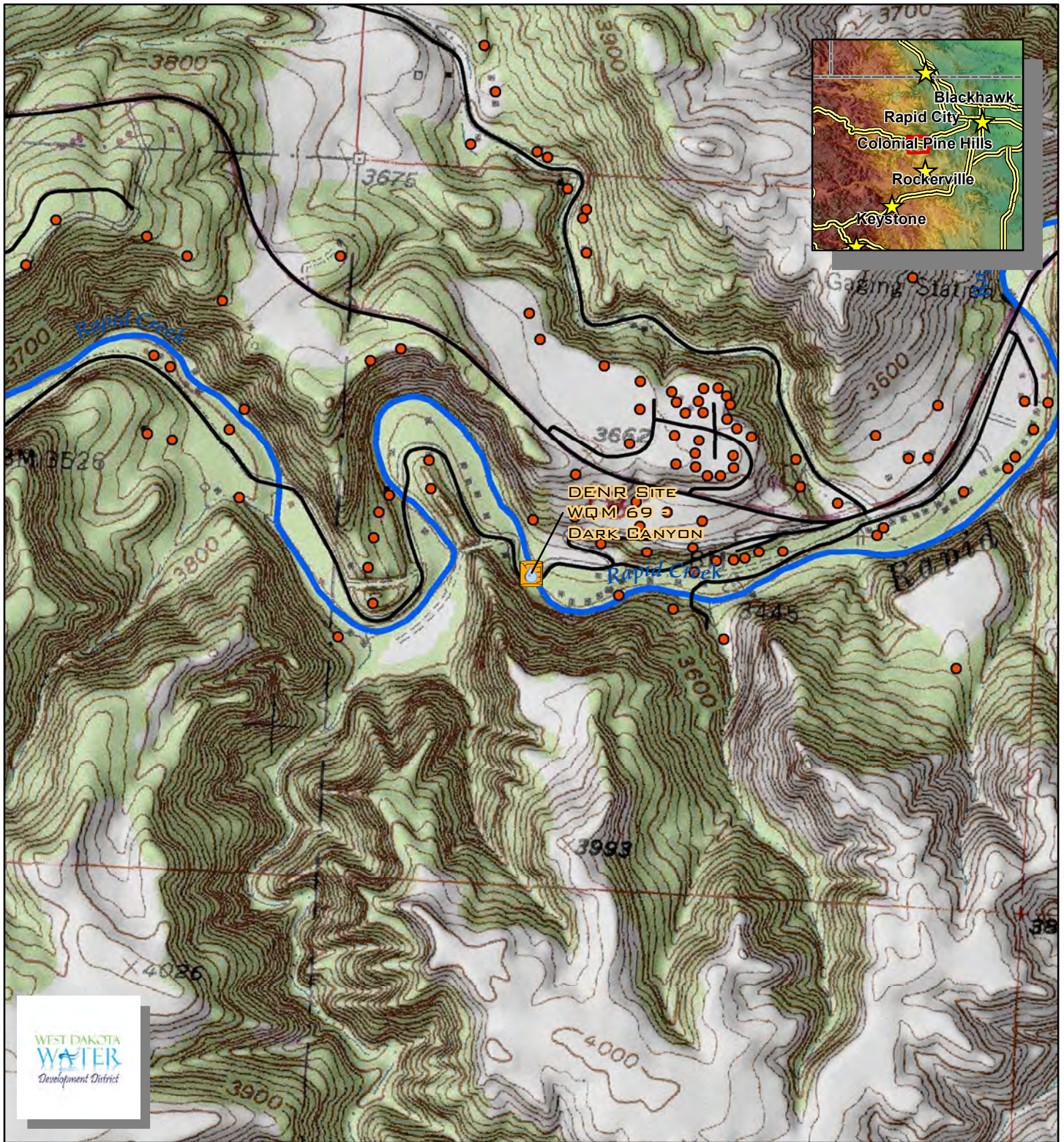
### Filters, sediment, shellfish or other sample types:

Contact us for details.




## Available Analysis

<b>Human</b>	Human Bacteroidetes ID: Dorei ( <i>Default</i> ) Human Bacteroidetes ID: EPA Human Bacteroidetes ID: Steri Human Bacteroidetes ID: Spp. Human Bacteroidetes ID: Fragilis
<b>Cattle</b>	Cow Bacteroidetes ID: EPA 1 ( <i>Default</i> ) Cow Bacteroidetes ID: EPA 2
<b>Pig</b>	Pig Bacteroidetes ID
<b>Bird</b>	Bird Fecal ID
<b>Gull</b>	Gull Fecal ID
<b>Goose</b>	Goose Bacteroidetes ID
<b>Chicken</b>	Chicken Bacteroidetes ID
<b>Dog</b>	Dog Bacteroidetes ID: Target 1 ( <i>Default</i> ) Dog Bacteroidetes ID: Target 2
<b>Deer/Elk</b>	Ruminant Fecal ID
<b>Ruminant</b>	Elk Bacteroidetes ID Ruminant Fecal ID: Target 1 ( <i>Default</i> ) Ruminant Fecal ID: Target 2
<b>Horse</b>	Horse Bacteroidetes ID
<b>Beaver</b>	Beaver Fecal ID
<b>General</b>	General Bacteroidetes ID General Enterococcus ID EPA Method 1611 EPA Method B

## Appendix B: Sample Locations Maps



**Rapid Creek Proposed Sampling Location 1:  
DENR Site WQM 69 - Dark Canyon**

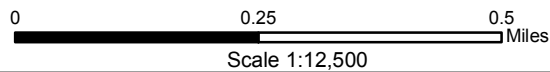
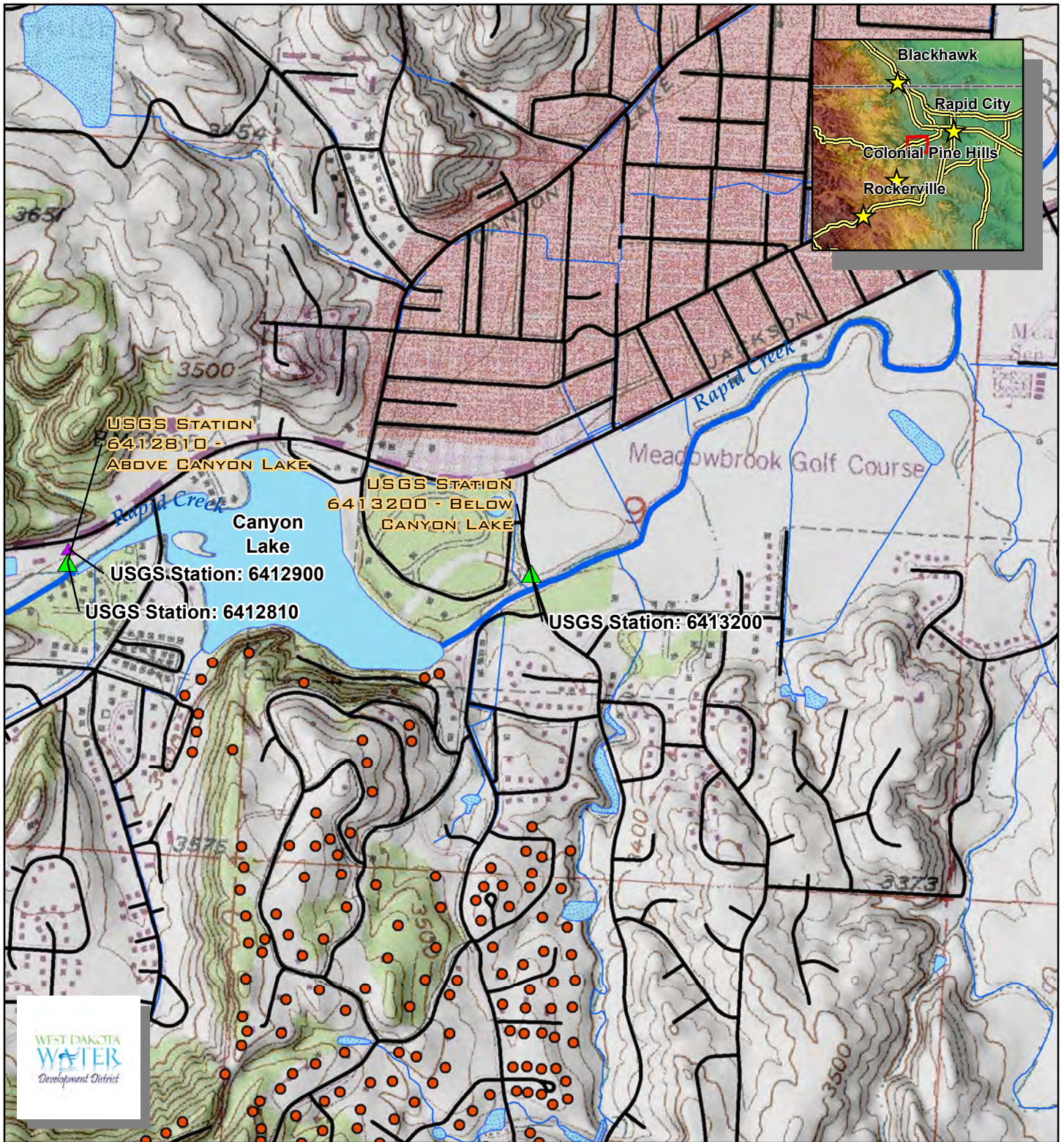
-  Local Roads
-  DENR Existing Sampling Location
-  Septic System Locations (2007)






Prepared by:



Date: 2/17/2016  
User Name: eenglund



**Rapid Creek Proposed Sampling Location 2:  
USGS Station 6413200 - Below Canyon Lake**

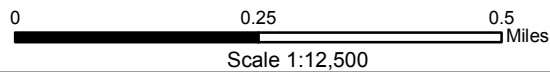
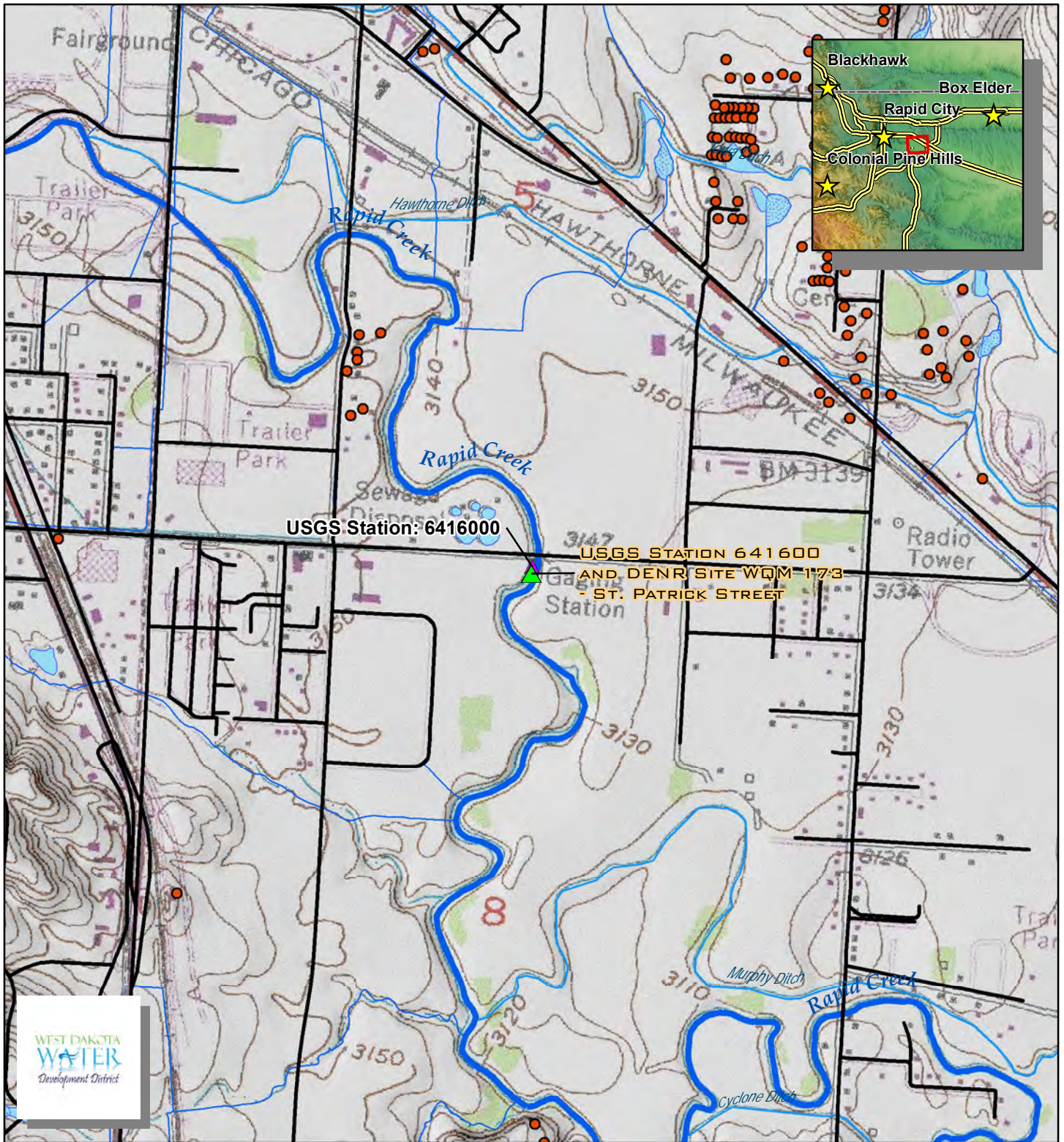
-  Local Roads
-  USGS Guaging Station Sampling Location
-  Septic System Locations (2007)






Prepared by:

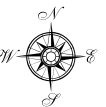


Date: 2/17/2016  
User Name: eenglund



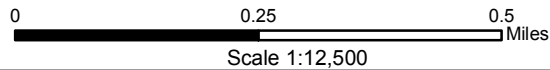
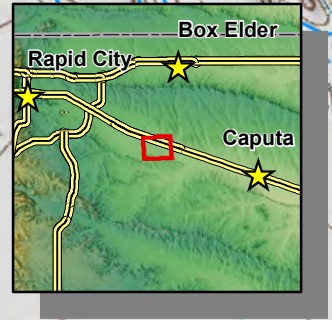
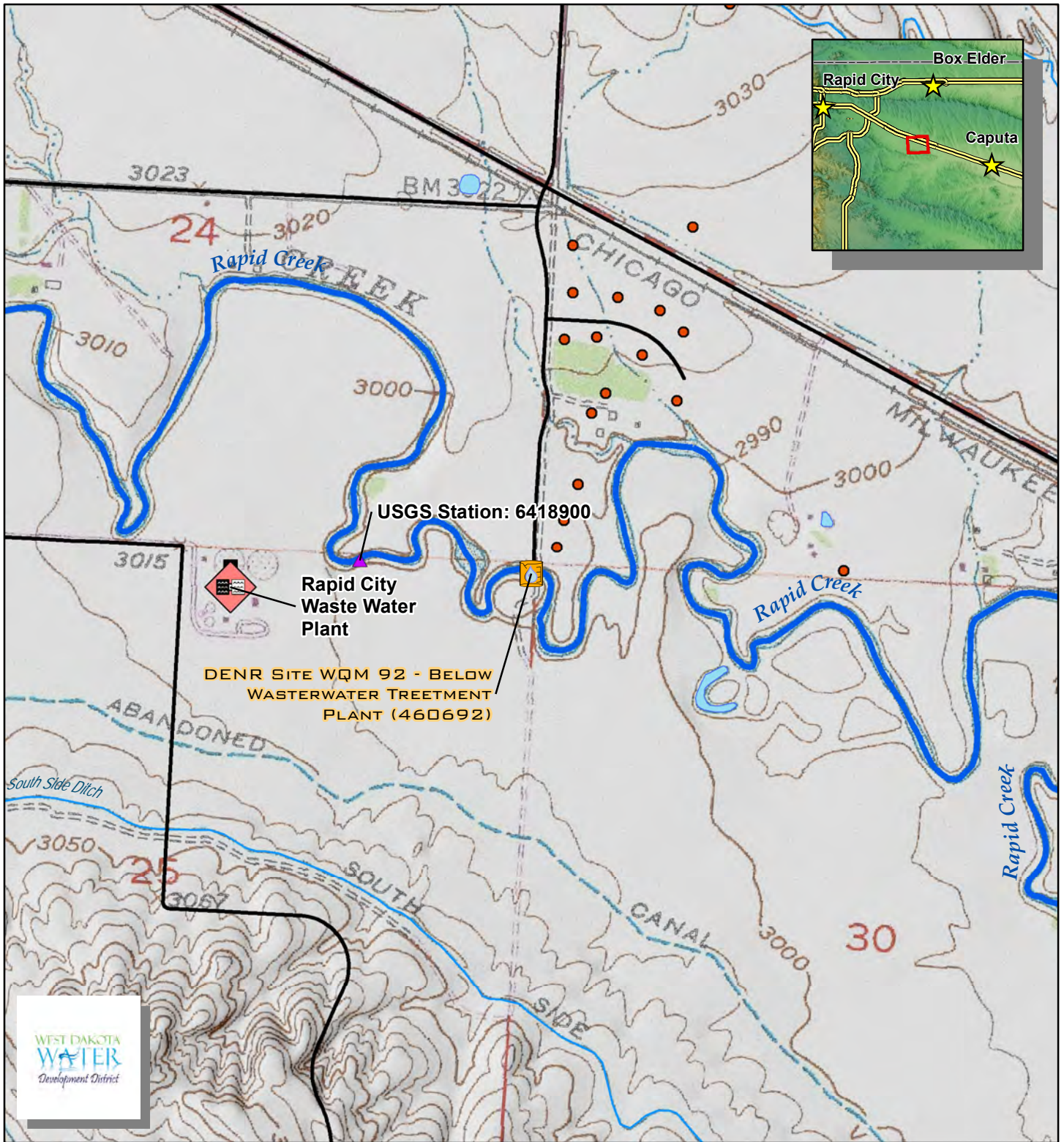
**Rapid Creek Proposed Sampling Location 3:  
USGS Station 641600 and DENR Site WQM 173 - St. Patrick Street**

-  Local Roads
-  USGS Guaging Station Sampling Location
-  Septic System Locations (2007)






Prepared by:  


Date: 2/17/2016  
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**Rapid Creek Proposed Sampling Location 4:  
DENR Site WQM 92 - Below Wasterwater Treatment Plant (460692)**

-  Local Roads
-  DENR Existing Sampling Location
-  Septic System Locations (2007)



Prepared by:



Date: 2/17/2016  
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Appendix C: Detailed Project Budget

## Detailed Budget

Project Funds	
WDWDD Committed Funds	\$ 40,000.00
Additional Requested Funds from WDWDD	\$ 13,014.00
TOTAL	\$ 53,014.00

Project Costs	
	Total Cost
<b>A. Personnel</b>	
Total Personnel	\$ -
<b>B. Fringe Benefits</b>	
Total Fringe Benefits	\$ -
<b>C. Travel</b>	
Travel for Staff: 50 mi/mo x \$0.55/mi x 12 mo	\$ 330.00
Total Travel	\$ 330.00
<b>D. Equipment</b>	
Total Equipment	\$ -
<b>E. Supplies</b>	
Disposable coolers: 12 sampling trips x 1 cooler/trip x \$16.00/cooler	\$ 192.00
Total Supplies	\$ 192.00
<b>F. Contractual</b>	
H2E, Incorporated	
(1) DNA Sampling Plan: \$74.50/hr x 60 hours	\$ -
(2) Sample Collection	
*Monthly Sampling: 1 FTE x \$74.50/hr x 6 hrs/mo x 12 mo	\$ 5,364.00
(3) Developing Outputs	
*Final Report/Action List: \$74.50/hr x 8 hrs/week x 8 weeks	\$ 4,768.00
Source Molecular: Sample Testing	
(1) 4 samples/mo x 12 mo x 5 tests/sample x \$215/test x 0.65 (30% discount)	\$ 36,120.00
(2) Sample Collection QA/QC: 2 samples/mo x 12 mo x \$175/sample	\$ 4,200.00
Total Contractual	\$ 50,452.00
<b>G. Construction</b>	
Total Construction	\$ -
<b>H. Other</b>	
Overnight sample shipments: 12 coolers x \$170/cooler	\$ 2,040.00
Total Other	\$ 2,040.00
<b>K. TOTALS</b>	<b>\$ 53,014.00</b>

## Appendix D: Source Molecular QA/QC Summary

## Source Molecular QA/QC Summary

### Special Training/Certification

Individuals appointed to MST projects hold at minimum a Bachelor's degree and have a sound knowledge in genetics and molecular biology. Individuals must have had 1 year of previous hands-on qPCR experience at another laboratory. Trainees undergo supervised hands-on training by the Laboratory Manager, which typically lasts 1-4 months depending on experience. An initial demonstration of technical capability is required before personnel are permitted to work independently on client projects. This involves:

- Successfully preparing five, 5-point standard curves that satisfy accuracy and precision criteria; and
- A side-by-side comparison test in which the trainee's results are compared to a qualified individual's results after both independently prepare and analyze the same randomly selected client samples (5 batches up to 100 samples).

Training records are documented by the Laboratory Manager and hard copies are kept on file in the company's office.

### Quality Objectives and Criteria

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. Key performance acceptance criteria and Data Quality Indicators are described below.

#### Data Quality Indicators and QC Requirements for MST Tests

<b>Data Quality Indicators</b>	<b>QC Item/Activity Used to Assess Measurement Performance</b>	<b>Purpose</b>	<b>Frequency</b>	<b>Measurement Performance Criteria</b>
Accuracy/Bias	Extraction blank	Evaluates contamination during DNA extraction/purification	Once every week samples are extracted	No detection or detection at least 3 $C_T$ units above sample $C_T$ values
Accuracy/bias	Diluted sample	Monitors for sample matrix inhibition affects	Every sample analyzed	$C_T$ value must be greater than that of unknown sample
Accuracy/bias	Positive control	Monitors for false negatives	One reaction for every sample analyzed	$C_T$ value below 35. No false negatives

Accuracy/bias	Negative control	Monitors for false positives	Three reactions for every sample analyzed	No detection or detection at least 3 C <sub>T</sub> units above sample C <sub>T</sub> values
Accuracy/bias	Standard Curve	Monitors overall reaction performance and efficiency  -Ensures confidence and comparability between sample data  -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification	R <sup>2</sup> : ≥0.98 Efficiency: 80-110% Slope: -3.0 - -4.0  Analytical Limit of Quantification (copies). Sample unknown within the linear dynamic range limits
Precision/Comparability	qPCR duplicates	Ensures precision and confidence in data	Every sample analyzed	± 1 standard deviation unless CT value ≥33

## Analytical Methods

Test methods meet the needs of the project and are appropriate for the tests undertaken. The microbial source tracking tests aim to identify potential animal host sources of fecal contamination in water samples. Currently, no standard methods exist for microbial source tracking. Genetic markers used for microbial source tracking tests are adopted by the Source Molecular laboratory from published, peer-reviewed scientific texts or journals whenever possible. Tests have been validated internally and/or externally in the microbial source tracking research community. If possible, reference methods published as international, national or regional standards are used. The laboratory ensures that the latest edition of a standard is used unless it is not appropriate or possible to do so.

## Quality Control

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reported to client.

Statistical calculations are calculated automatically by the qPCR software. These include qPCR replicate standard deviations, replicate means and standard curve efficiency, slope, y-intercept and coefficient of linear regression ( $R^2$ ).

<b>QC Item/Activity</b>	<b>Data Quality Indicator</b>	<b>Frequency</b>
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	Once every week samples are extracted
qPCR duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed
Diluted sample	Accuracy/Bias -Monitors for sample matrix inhibition affects	Every sample analyzed
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction for every sample analyzed
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions for every sample analyzed
Standard Curve	Accuracy/Bias, Comparability, Sensitivity -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification

### **Instrument/Equipment Testing, Inspection, and Maintenance**

Access to laboratory equipment is controlled to ensure that only authorized personnel use the equipment. Instructions on the use and maintenance of equipment are readily accessible by authorized personnel.

Generally, the handling, transport, storage, use and maintenance of equipment are outlined in the manufacturer's manual. Manuals are located in the laboratory at all times. Specific requirements, if any, are outlined in the test method standard operating procedures.

The manufacturer's manual is critical in describing the safe handling requirements of the equipment, to avoid any damage, alteration, contamination, deterioration, change of integrity or reliability and condition of the equipment (or samples). The manufacturer's manual also provides guidance for suitable environmental conditions for the calibrations, inspections, measurements and tests performed. These guidelines should be followed at all times unless specified otherwise in standard operating procedures.

Routine test work is completely discontinued on equipment that shows minor non-conformances. Not only do we do this for ethical reasons in support of our customer, but minor non-conformances are often indicative of major breakdowns in expensive equipment. These breakdowns need to be avoided wherever possible. Out of service equipment is clearly marked with an "out of service" label.

### ***General Equipment***

General service equipment is maintained by cleaning and performing safety checks as necessary. Calibrations or performance checks will be necessary where the setting can significantly affect the test or analytical result (e.g., the temperature of a water bath). Instructions on the use and maintenance of general equipment are located in the laboratory at all times.

### ***Volumetric Equipment***

The correct use of volumetric equipment is critical to analytical measurements. Volumetric equipment are suitably maintained and calibrated as specified in the Equipment Records and Inventory datasheet located in the web-based storage system.

Attention is paid to the possibility of contamination arising from the equipment or cross-contamination from previous use. The type used, cleaning, storage and segregation of volumetric equipment are critical. Volumetric equipment should be sterilized with 10% bleach solution and 70% ethanol, DNA Away, or autoclaved as appropriate. Instructions on the use and maintenance of volumetric equipment are located in the laboratory at all times.

### ***Measuring Equipment***

Measuring equipment, which include the real-time qPCR instrument, must be used correctly, with care and requires stringent calibration and maintenance plans to ensure adequate performance. Such equipment shall not be used for measurement of customer test items if they go out of calibration. If this occurs, items must be re-measurement once the equipment has been re-calibrated. Operating instructions for the Applied Biosystems StepOnePlus Real-Time qPCR System are located in the laboratory office and also in the StepOnePlus Software Instrument Maintenance Manager.

### **Instrument/Equipment Calibration and Frequency**

All measurement and test equipment having an effect on the accuracy or validity of tests are calibrated and/or verified before being put into service. Calibration records for these equipment, including calibration dates and due dates, are maintained in the Source Molecular web-based storage system. Equipment may be calibrated internally or externally. External calibration

services must be conducted by a calibration laboratory that demonstrates competence by being accredited and demonstrating measurement capability and traceability. The frequency of calibration depends on the accuracy requirements of the test, the stability of the instrument and manufacturer recommendations. It is crucial that calibration measurements are traceable to the International System of Units (SI) whenever possible. Calibration reports, traceability certificates and certificates of analysis are maintained in the Calibration Certificates binder that is kept in the laboratory office. Records are retained for 5 years or more at the discretion of the laboratory.

The procedures for checking newly received equipment are as determined by manufacturers' specification and/or those determined by the laboratory during procurement.

After repair, equipment must be calibrated, when appropriate, and verified to perform correctly by following procedures in the manufacturer's manual and/or by comparing pre-nonconformance and post-repair tests.

Anytime the equipment goes outside the direct control of the laboratory, the function and calibration status must be verified before the equipment can be returned to service. This is done by ensuring that calibration stickers and calibration reports are correct, calibration values are within a specified range (if applicable) and that all components of the instrument are functioning properly. This, along with other key information, is recorded and documentation is stored for 5 years or more at the discretion of the laboratory. When verification of the calibration status and functionality of the equipment is not possible, the equipment must be re-calibrated and serviced, respectively.

Generally, spare parts do not have to be kept on hand in the laboratory. Any parts that are needed as part of equipment servicing are provided and installed by the manufacturer or service contractor.

### **Inspection/Acceptance of Supplies and Consumables**

For all test methods, only services and supplies of the required quality and grade are used. If the specified reagent or material is discontinued by the manufacturer, an alternative from a different manufacturer may be purchased as long as the grade and specifications are identical to the discontinued item. The Laboratory Manager verifies and approves the alternate items and the change is made in the appropriate SOP. Supplies, materials and consumables to be purchased are determined by the Laboratory Manager and entered into an electronic "order list" that includes a description of the item, the name of the vendor, the item catalogue number, the quantity and the cost and is stored for a minimum of 5 years.

Shipments are received at the receiving area and brought to the laboratory. The Laboratory Manager or other authorized personnel is responsible for checking shipments for accuracy. Packing slips are checked against package content labels and matched with the electronic order list. Certificates of analysis (COA) are verified (when applicable) to ensure the received item



meets minimum specifications. All standards, reagents, filters, and other consumable supplies are purchased from manufacturers with performance guarantees and industry recognition, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality. Reagents are marked with the “date received”. Primers and plasmid DNA standards are quantitated and aliquoted for storage at -80°C.

Once the materials are verified, the appropriate box is checked next to the item in the order list and an electronic signature is created. If a discrepancy is found that could affect the quality of laboratory output, the supplier is contacted and the material is replaced.

All supplies will be stored as per manufacturer labeling and discarded past expiration date. Long term storage of nucleic acids is in a -80°C freezer. Whenever possible, consumables and reagents that come into contact with test samples are received pre-sterilized and disposable (e.g. filtering funnels). They are used once and not re-used. Specific information of supply and consumable vendors are specified in individual Test Method SOPs’ materials list.