



Appendix E  
RNA Analysis Plan

## TECHNICAL MEMORANDUM

Date: June 9, 2004

To: Jenny Gaus, P.E.  
City of Kirkland

From: Erin Nelson, P.E.

Subject: Fecal Coliform RNA Analysis Plan  
Forbes Creek Sub-basin  
City of Kirkland

cc: Ken Ludwa, P.E.

Project Number: 558-1802-035

Project Name: City of Kirkland Comprehensive Stormwater Plan Update

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### INTRODUCTION

Fecal Coliform is a pollutant of concern in the City of Kirkland's watersheds and streams, particularly Juanita and Forbes creeks. A 1998 study of Juanita creek (Frodge 2003) identified the main sources of fecal coliforms to be ducks and geese. Secondly, seagulls and dogs also contributed to the elevated fecal coliforms. Other areas in Kirkland, including direct outfalls to Lake Washington and Yarrow Bay have not exhibited the high levels of fecal coliforms as those in Juanita and Forbes creeks. The focus of this sampling and analysis plan is Forbes Creek. Forbes Creek is rated of "high concern" by the Washington State Department of Ecology primarily because of high fecal coliform bacteria counts, low dissolved oxygen concentrations and high phosphorus concentrations (King County 2003). It is suspected that failed sewer systems may be responsible for the elevated concentrations of fecal coliforms found in the creek (Frodge 2003 and Gaus 2003). This study will help to identify sources. The results of this study will help City watershed managers target areas to be connected to the City sewer system through its Emergency Sewer Program (ESP).

### Existing Conditions

The Forbes Creek basin is approximately 1000 acres in size, extending from the eastern part of Kirkland in the highlands east of Interstate 405 to Lake Washington at Juanita Bay. Land use surrounding Forbes Creek is primarily a mix of low- and medium-density residential, with minor amounts of commercial and high-density residential land uses. In the lower reaches near Juanita Bay, the creek flows through park and open space area that is primarily wetlands.

### Historical Water Quality Data

Forbes Creek is classified as an AA-Extraordinary stream by the Washington State Department of Ecology and has certain water quality criteria for fecal coliform bacteria, dissolved oxygen, temperature, and pH. Since 1976, King County has conducted monthly water quality monitoring at a station near the mouth of Forbes creek. In the last five years (1998 to 2003), fecal coliform counts have exceeded the state criteria in 63% of the baseflow samples, and 93% of the storm event samples collected.

## PROPOSED METHODOLOGY

Fecal coliform is an indicator of sewage pollution in streams. The higher the fecal bacteria counts, the higher the probability of pathogenic bacteria pollution, which can cause illness in humans. In recent years, a number of analytical methods have been developed to identify sources of fecal coliform to distinguish between human and non-human sources. These methods include microbiological, chemical and fluorescent dye tracing (Ecology 1999). The method proposed here is microbial source tracking using DNA ribotyping/genetic fingerprinting, developed by Dr. Mansour Samadpour of the Institute for Environmental Health. The method involves isolating pure cultures of *E. coli* (or other enteric pathogens) from both receiving water and suspected sources. DNA is then isolated from the pure bacterial strains and cut into fragments using a restrictive enzyme. The DNA fragments are further separated and hybridized with a DNA probe that creates a pattern containing ribosomal RNA (rRNA) genetic information. The resulting pattern is compared to rRNA patterns from known sources (from a national library and from fecal samples collected in the region of interest) to determine if the patterns are derived from the same strain of *E. coli*. Dr. Samadpour currently has over 40,000 different strains of *E. coli* in his source library.

The microbial source tracking laboratory analysis is relatively expensive (~ \$7,500/sample station). In order to keep costs down and get the best results possible from the study, we recommend a phased approach to sampling. The first phase would involve sampling water from 10 proposed locations for analysis of fecal coliform only (~\$25/sample). This phase of sampling would help identify those locations where fecal coliform counts are highest, and where microbial source tracking sample stations should be located. Additionally, a reference sample station (with low fecal coliform counts) could be identified from this initial phase of sampling. Up to five sampling locations (at the most, four stations with high fecal coliform counts) will be identified for microbial source tracking analysis. One of the locations should be a reference station with low fecal counts to compare results.

## SAMPLE COLLECTION

### Phase 1- Fecal Coliform Sampling and Analysis

Water samples should be collected for analysis of fecal coliform only at the ten proposed locations shown in Figure 1 during two baseflow and two stormflow events. These locations are intended to be representative of different parts of the watershed (upper, middle and lower). Additionally, sampling locations were chosen to target (1) neighborhoods with high concentrations of septic systems, and (2) park areas where there may be larger populations of birds or other animals that could be contributing fecal coliforms to Forbes Creek. A list of the proposed sampling locations is in Table 1.

The results of the Phase 1 fecal coliform analyses will help identify those areas that have the highest concentrations of fecal coliform and where microbial source tracking would yield beneficial results. Up to four sampling locations with high concentrations of fecal coliform should be identified for further sampling based on the Phase 1 results. Additionally, a reference site with low fecal coliform counts should be identified for microbial source tracking analysis for comparison. If a site with low concentrations cannot be identified in the Forbes Creek watershed, a nearby stream with similar watershed and physical stream characteristics, but low fecal coliform concentrations, could serve as the reference site.

**Table 1. Proposed Phase 1 Surface Water Sampling Locations**

<b>Sample Location</b>	<b>Description</b>	<b>Target Source</b>
1	Outfall for eastern tributary to Forbes Creek west of the intersection between NE 91st Ct. and 128th Avenue NE	Downstream of numerous residential septic systems
2	Mainstem Forbes Creek upstream of Forbes Lake on north side of NE 90th St. at intersection with 120th Avenue NE	Upstream of Forbes Lake. Possible reference station.
3	Mainstem Forbes Creek downstream of Forbes Lake on south side of NE 97th St., east of Slater Avenue NE	Downstream of Forbes Lake.
4	Outfall for eastern tributary to Forbes Creek at 124th Avenue NE, south of NE 100th St.	Downstream of North Rose Hill Woodlands Park.
5	West side of Slater Avenue NE at Forbes Creek tributary located west of wetland, at approximately NE 104th St	Downstream of wetland.
6	Downstream of I-405 on mainstem Forbes creek on north edge of steep ravine off of NE 108th Street.	Downstream of horse property located immediately adjacent to Forbes creek (east side of I-405)
7	Mainstem Forbes Creek on north side of Forbes Creek Drive at 113th Ct. NE	Sample representative of mid-reach of Forbes Creek
8	South tributary to Forbes Creek on north side of Forbes Creek Drive at 400 block of NE 106th St.	Sample representative of different part of watershed
9	South tributary to Forbes Creek on north side of Forbes Creek Drive at 200 block of NE 106th St.	Sample representative of different part of watershed
10	Mainstem Forbes Creek King County long-term monitoring station at NE 108th St. and 100th Ave. NE.	Comparison with King County data.

**Phase 2- Microbial Source Tracking Sampling and Analysis**

***Water Samples***

A minimum of 20 water samples is needed at each microbial source tracking sampling location to get a good representation of E. coli strains for meaningful results. In previous King County sampling efforts near the mouth of Forbes Creek, fecal coliform counts have varied between baseflow and storm flow events. We recommend sampling two baseflow and three storm events to evaluate differences at varying flows. Four manual grab water samples per event should be collected, at a minimum of 10-minute intervals during each event; the four samples for each event should be combined to form a composite sample.

***Fecal Samples***

Fecal samples will need to be obtained from the Forbes Creek watershed or nearby locations to add local strains to the existing library of E. coli strains and to have a better likelihood of matching E. coli strains detected in the water samples.

Ten to twenty fresh fecal samples should be obtained from the each of the sources listed in Table 2. Fecal samples should be collected soon after deposition, if possible.

**Table 2. Types of Fecal Samples to be Collected**

Type of Fecal Sample	Potential Source
Human Sewage	City of Kirkland sewer system
Ducks, geese and other avian species	City parks
Horses	Bridle Trails Park horse trails
Dogs and Cats	Local animal shelter, veterinarian clinics, dog owners walking their dogs in the watershed (ask for the bag)
Squirrels and/or rodents	Local pest control contractors

**Sample Collection Procedures**

All water and fecal samples shall be collected as grab samples. Field personnel shall use aseptic techniques in the collection of water, and animal waste products. All of the water samples will be collected from Forbes Creek or tributaries to Forbes Creek using 8-ounce sterile bottles, submerged below the water surface and oriented so that the bottle opening is pointed upstream while filling. A small amount of headspace should be left at the top of the bottle to allow for homogenization at the laboratory. For stormflow events, one 8-ounce bottle will be collected per interval. For baseflow events, two 8-ounce bottles will be collected per interval to provide enough sample volume to develop fecal coliform bacteria colonies from samples that may have less than 10 CFU/100 mL. Water samples shall be placed in a cooler with ice and delivered to the laboratory within 24 hours of sample collection.

Fecal material shall be collected by placing at least one ounce of fresh material in a sterile Whirl-Pak® bag with a sterile tongue depressor. Fecal samples shall be placed in a cooler with ice and delivered to the laboratory within 24 hours of sample collection.

**Field Documentation**

The following information shall be recorded in a waterproof notebook during sample collection:

- Sampling date
- Name of person collecting sample
- Time of sample
- Weather and flow conditions
- Stream conditions (trash, sheen, unusual color, etc.)
- Type of fecal material collected (if known)
- Color size and consistency of fecal material
- Number, type, volume and location of samples collected

Photographs shall be taken at all of the sampling locations and of the undisturbed fecal material prior to collection.

## **Sample Identification**

Samples shall be uniquely identified with sample number, date, and time of sample collection, personnel collecting the sample, and project name.

## **Decontamination**

All reusable sampling equipment, such as a downhole sampling apparatus with plastic tubing, shall be decontaminated between sampling events with a 1% chlorine bleach solution followed by a distilled water rinse. Sterile sample bottles, tongue depressors, and fecal sample bags shall be supplied by the laboratory and stored in a clean location. Sample bottles shall be stored with lids on to prevent contamination prior to sampling.

## **Field Blanks**

One field blank per sampling event (baseflow or stormflow) shall be collected (10% of the total samples collected). The field blanks will consist of a sterile sample bottle filled with distilled water, leaving headspace at the top. If any sampling equipment is used, the field blank shall be collected through the same sampling equipment used to obtain the stream samples to document the effectiveness of field decontamination procedures.

## **SAMPLE DELIVERY**

### **Phase 1**

All Phase 1 water samples to be analyzed for fecal coliform only should be delivered to Aquatic Research, Inc. (ARI) or another reputable laboratory for analysis of fecal coliform using the membrane filter method. When possible, sample delivery should occur early in the week (Monday or Tuesday), so that the samples can be analyzed within the timeframe required by the method, and for normal laboratory rates. If samples are delivered on Friday, the samples will be analyzed over the weekend, resulting in higher laboratory fees.

### **Phase 2**

Phase 2 water samples and fecal samples to be analyzed using the microbial source tracking methods described above shall be delivered to Dr. Mansour Samadpour at the Institute for Environmental Health.

### **Laboratory Addresses:**

Institute for Environmental Health  
8279 Lake City Way NE  
Seattle, WA 98115

Phone: 206-240-8104

Fax: 206-522-0362

Aquatic Research, Inc.  
3927 Aurora Ave. N  
Seattle, WA 98103

Phone: (206) 632-2715

Fax: (206) 632-2417

**COST ESTIMATE**

This cost estimate only includes laboratory fees for analyzing the water and fecal samples for Phases 1 and 2 of this study. Staff field time to collect the samples, equipment, interpretation of laboratory results and report writing are not included in the estimate.

Description	Number of Samples or Stations	Price per Sample	Cost
Phase 1 Fecal Coliform Samples	40 samples (10 each for four events)	\$25	\$1,000
Phase 2 Microbial Source Tracking Samples	5 stations (4 samples each for five events)	\$7,500	\$37,500
		<b>Total</b>	<b>\$38,500</b>

**REFERENCES**

Frodge, Jonathan. 2003. Personal Communication.

Gaus, Jenny. 2003. Personal Communication.

King County Department of Natural Resources and Parks. 2003. Streams Monitoring Program website ([dnr.metrokc.gov/wlr/waterres/streams/statefedcrit.htm](http://dnr.metrokc.gov/wlr/waterres/streams/statefedcrit.htm))

Washington State Department of Ecology. 1999. Fecal Contamination Source Identification Methods in Surface Water. Ecology Report #99-345.