Standard Operating Procedure for:

Microbial Source Tracking (DNA.doc)

Missouri State University

and

Ozarks Environmental and Water Resources Institute (OEWRI)

Prepared by: _____ Date: _____
OEWRI Quality Assurance Coordinator

Approved by: _____ Date: _____
Microbiology Professor

Approved by: _____ Date: _____
OEWRI Director

Table of Contents

1 Identification of the test method

Identifying fecal source contamination using host specific *Bacteroides* PCR assay.

2 Applicable matrix or matrices

This method is suitable for use with surface water samples.

3 Detection Limit

Primers and thermal cycling conditions have been selected that are highly complementary to desired DNA sources that when amplified provide specific host contamination information. Agarose gel electrophoreses is used to size the DNA fragments and the fragments are visually compared to a DNA ladder which contains fragments of known size.

4 Scope of the test method

This standard operating procedure describes the test methods for the collection and analysis of water samples for the presence of nonspecific and host specific cow and human *Bacteroides.*

5 Summary of test method

Surface water samples are collected in EPA-accepted 500ml Whirl-Pak® bags. Samples are filtered and DNA is extracted from the samples using a Qiagen fecal DNA kit. A PCR assay, using host specific primers, is used to analyze the extracted DNA. PCR products, or DNA fragments, undergo agarose gel electrophoreses and are visually sized and compared to a DNA ladder. Results are recorded using a Kodak UV analyzer and are reported as positive or not positive for host specific *Bacteroides*. Specific base values are reported to indicate fragment confidence and accuracy. Laboratory duplicate results are reported to indicate precision.

6 Definitions

6.1 Analytical batch: The set of samples processed at the same time

6.2 Chain of Custody (COC): Is a term that refers to the maintenance of an unbroken record of possession of a sample from the time of its collection through analysis. Chain of custody forms will be completed as described in the Chain of Custody SOP # 1030R01. Chain of custody (COC) forms are located on a board in Temple Hall 125. COCs will be given to the laboratory director upon returning from the field.

6.3 Check standards: These are measures that are used to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. Fragments are visually compared to a DNA ladder which contains fragments of known size and specific base values. Each host specific *Bacteroides* primer is analyzed with the samples in the batch.

6.4 Control: Laboratory factors or checks that are used to eliminate alternate explanations of experimental results.

6.5 Deoxyribonucleic acid (DNA): A nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms.

6.6 Electrophoresis: A method of separating substances, especially proteins, and analyzing molecular structure based on the rate of movement of each component in a colloidal suspension while under the influence of an electric field.

6.7 Field duplicate (FD): Two samples taken at the same time and place under identical circumstances and that are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage as well as laboratory procedures.

6.8 Field blank (FB): An aliquot of deionized water treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

6.9 Laboratory blank (LB): A negative control prepared in the laboratory and treated as a sample in all aspects. The purpose is to determine if sources of contamination are present in the laboratory environment, the reagents, or the apparatus. Water is generally used as the template during PCR analyses.

6.10 Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation or storage procedures.

6.11 Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

a. To calculate the MDL:
b. Prepare a solution with the concentration of TN near the estimated MDL
c. Analyze seven portions of this solution over a period of at least three days
d. Include all sample processing steps in the determination
e. Calculate the standard deviation (*s*).
f. From a table of the one-sided *t* distribution select the value of *t* for 7 – 1 = 6 degrees of freedom at the 99% level. This value is 3.14
g. The product 3.14 times *s* is the desired MDL.

6.12 Negative control: A laboratory blank that is used to determine if sources of contamination are present in the laboratory environment, reagents, or apparatus. Water is generally used as the template during PCR analyses.

6.13 Nucleotides: Chemical compounds that consist of a heterocyclic base, a sugar, and one or more phosphate groups and are the structural units of RNA and DNA.

6.14 Polymerase chain reaction (PCR): A technique for exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism (such as *E. coli* or yeast).

6.15 Polymerase: Is an enzyme that polymerizes DNA and RNA against an existing DNA or RNA template in the processes of replication and transcription. In association with a cluster of other enzymes and proteins, they take nucleotides from solution, and catalyze the synthesis of a polynucleotide sequence against a nucleotide template strand using base-pairing interactions.

6.16 Positive control: Primers used to amplify a target sequence that is used to evaluate amplification of samples and assure correct amplification. One positive control will contain *Bacteroides* DNA (B-DNA), another will contain a plasmid with a partial cow-specific 16S rRNA gene (C-RNA), and another will contain a plasmid with a partial human-specific 16S rRNA gene (H-RNA).

6.17 Primer: Deoxyoligonucleotides, or a short sequence of DNA that binds to its complementary target sequence. Primers generate a place for a polymerase (enzyme) to bind and initiate DNA synthesis during PCR procedures.

6.18 Relative Percent Difference (RPD): Calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

6.19 Thermocycler: Laboratory apparatus used for polymerase chain reactions (PCR). The device has a thermal block with holes where reaction tubes are inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps.

7 Interferences

To optimize PCR conditions, various laboratory protocols are used to separate pre-PCR reactions from potential DNA contaminants. Aseptic techniques are followed in setup, analysis, and purification areas associated with PCR analyses. Primers and thermal cycling conditions have been selected that are highly complementary to desired DNA sources that when amplified provide specific host contamination information. Agarose gel electrophoreses is used to size the DNA fragments and the fragments are visually compared to a DNA ladder which contains fragments of known size.

8 Health and safety

The analysis involves handling of freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently. Aseptic techniques will be followed and eye protection will be used during analytical processes.

The reagents used in this method pose no unusual hazard to an analyst employing standard safety measures. Analysts should review the MSDSs for all chemicals used in this analysis.

This procedure requires use of a centrifuge, thermocycler, water bath, and electrophoresis apparatus. All safety directions for using these devices should be followed carefully.

9 Personnel qualifications

Laboratory and field personnel shall have a working knowledge of this analytical procedure and will have received training from an MSU employee knowledgeable of the proper sample analysis procedures and instrumentation in the laboratory.

10 Equipment and supplies

Whirl-Pak® bags: 500 mL and sterilized. www.enasco.com Nasco Product number: B00736WA

Sampling pole (for Whirl-Pak bags): 12 feet long, with retainer rings to hold the bags.

Heating units: A thermocycler (Eppendorf Mastercycler EP gradient No. 5341-016929), autoclave, and water bath (Polyscience No. 5L-M) are used to modify sample temperature or to sterilize supplies.

Filtration apparatus:

a. Vacuum pump
b. Collection flask
c. Glass 47mm filter funnel with base, stopper, and clamp
d. Vacuum tubing

Computer imaging unit: Kodak Image Station 1000

Separation units: A micro-centrifuge and vortex are used to separate substances.

Gel apparatus:

a. Horizontal gel base
b. Cover
c. Gel tray
d. Tray combs
e. Power supply

Laboratory glassware as needed: sterilized beaker; place in a 170°C oven for 2 hours, then allow to cool.

Micropipettes and tips: www.midsci.com Midwest Scientific product numbers: 5111YSK and FR-10R.

Plates: EU one-piece, non-skirted, thin-wall, and 96 well x 0.2ml. Midwest Scientific product number: B70501.

Tubes: Eppendorf (1.5ml) and sterile conical plastic tubes (15ml). Midwest Scientific product number: C15B.

Tabs for 96 well plates: EU Q-PCR optical flat cap- Thin wall 8-cap strip low background. Number: B79701.

Gloves: Latex.

Filters: Mixed cellulose ester membrane (0.45µm).

11 Reagents and standards

DNA extraction kit: QIAamp DNA stool mini kit (50). www.qiagen.com Qiagen product number: 51504.

Master Mix: GoTaq® Green Master Mix 100 reactions. www.promega.com Promega product number: M7122.

DNA Ladder: 1kb 500µl (100 lanes). Promega product number: G5711.

Primers: 20µM. www.operon.com Custom-made via Primer sequence of bases listed in Table 1- Primer sequence and corresponding optimal annealing temperature.

Primer	Sequence (5'-3')	Target	Annealing Temperature °C
Bac32F	AACGCTAGCTACAGGCTT	*Bacteroides*-	53
Bac708R	CAATCGGAGTTCTTCGTG	*Prevotella*	53
CF128F	CCAACYTTCCCGWTACTC	CF123 cluster	58
CF193F	TATGAAAGCTCCGGCC	CF 151 cluster	55
HF183F	ATCATGAGTTCACATGTCCG	HF8 cluster	59

Table 1 - Primer sequence and corresponding optimal annealing temperature (Bernhard, Field et.Al. 2000).

1xTAE buffer: Tris-acetate buffer solution that is prepared by diluting the 50x Tris-acetate buffer solution. The 50x Tris-acetate buffer solution is prepared by combining 240g tris base, 100ml of 0.5 M EDTA, and 57.1ml glacial acetic acid and diluting to 1000ml with deionized water.

Ethidium bromide: 10mg/ml concentrate.

Ethanol

Agarose: Genetic analysis grade, low melt.

HPLC Water

12 Sample collection, preservation, shipment and storage

Pre-label sample bags with a sampling site number or name.

Arrive at site and record site name or number, date, time, stream flow or gauge reading, appearance of water as either cloudy or clear, and precipitation within the last 24 hours in the field log book. Additional water parameter collection will be dictated by the project manager.

Rinse the sampling pole by dipping it into the water twice at the current site.

Collect a 500 mL surface water sample from the shoreline by placing a sterile Whirl-Pak bag into the retainer attached to a twelve foot pole. Lower the bag into the water facing upstream approximately 6 - 10 feet from the shoreline. Lower the bag into the water far enough so that the water flows freely into the bag. Avoid capturing large particulate matter in the bag by moving the bag (maintaining the upstream direction).

The samples will be kept in the possession of Missouri State University personnel who collected the samples until transferring the samples to the laboratory with the appropriate chain of custody forms where they become the laboratory's responsibility.

Samples will be transported to the laboratory in coolers containing ice. Transport should not take longer than three hours.

13 Quality control

10.1 Quality control program: The minimum requirements of the quality control program for this analysis consist of an initial demonstration of laboratory capability and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory must maintain performance records that define the quality of the data that are generated.

a. Analyses of check standards or positive controls are required to demonstrate method accuracy. Three positive controls will be prepared and analyzed with each batch. One will contain *Bacteroides* DNA (B-DNA), one will contain a plasmid with a partial cow-specific 16S rRNA gene (C-RNA), and one will contain a plasmid with a partial human-specific 16S rRNA gene (H-RNA).

b. Analyses of laboratory duplicates (LD) are required to demonstrate correct laboratory practice as well as precision. One LD will be prepared and analyzed with each batch. The LD will be collected at a different site during each sample collection event.

c. Analyses of laboratory blanks or negative controls are required to demonstrate freedom from contamination. One negative control without DNA will be prepared and analyzed with each batch.

d. Agarose gel electrophoreses is used to size the DNA fragments and the fragments are visually compared to a DNA ladder which contains fragments of known size.

e. The laboratory will maintain records to define the quality of data that is generated.

10.2 Initial demonstration of performance. The following must be satisfied before a new analyst may analyze samples.

a. Initial Precision and Recovery – To establish the ability to generate acceptably precise and accurate results, the PCR laboratory supervisor will train the technician, review laboratory techniques with the technician, and will oversee data produced by the technician.
b. The PCR laboratory supervisor will routinely verify performance.
c. All data generated from the PCR laboratory will be reviewed for quality assurance and quality control by the QA/QC manager before being transferred to project managers.

10.3 Agarose gel electrophoreses will be used to size the DNA fragments produced from each batch of samples. A DNA ladder containing fragments of known size will be used to compare fragments from each batch of samples.

10.4 A specific base value will be reported for each sample and all quality control checks.

14 Calibration and standardization

Calibration procedures for this method consist of using primers and thermal cycling conditions that are highly complementary to desired DNA sources that when amplified provide specific host contamination information. Standardization procedures for this method consist of using positive and negative controls, agarose gel electrophoreses, and a DNA ladder.

15 Laboratory Procedure

DNA Extraction

a. Set up filtration apparatus with vacuum pump, collection flask, funnel, and tubing.

b. Filter each water sample through a 0.45µm mixed cellulose ester membrane filter. Prepare a laboratory duplicate (LD) by filtering 500ml of the sample in the 1000ml sample bag through one filter and then the remaining 500ml from the sample in the1000ml bag through a separate filter. Discard filtered water.

c. Transfer each sample filter from the filter apparatus using flame sterilized tweezers and place into a sterile 15ml plastic conical tube.

d. Add 1.4ml of “ASL Buffer”, provided with the kit, to each tube. Place tubes in the vortex and process for 10 minutes to remove cells from filter.

e. Place the tubes in a 70°C water bath for 10 minutes to incubate.

f. Use a pipette to by-pass the filter, pull analyte into the pipette tip from each conical tube, and transfer the analyte to separate 1.5ml Eppendorf tubes. Discard each filter and conical tube into the red biohazard bag.

g. Place the Eppendorf tubes in the centrifuge and process for 1 minute to pellet cell debris.

h. Pipette each supernatant from its tube and transfer it into a different Eppendorf tube.

i. Add an InhibitEX tablet, from the kit, to each Eppendorf tube.

j. Place tubes on the vortex for 1 minute to dissolve tablets and separate residual matter from DNA product.

k. Place tubes in the centrifuge and process for 6 minutes.

l. Pipette supernatant and transfer into a separate tube.

m. Centerfuge to pellet residual inhibitEX matter for 3 minutes.

n. Pipette 200 µl of supernatant into another Eppendorf tube, add 15 µl Proteinase K, add 200µl of buffer solution Al and vortex to mix.

o. Incubate at 70 C for 10 min.

p. Add 200 µl of ethanol and vortex to mix.

q. Add all of the lysate from step 15.1p to the spin column.

r. Centrifuge 1 min. place column into new collection tube and discard the collection tube containing filtrate.

s. Add 500µl of buffer AW1. Centrifuge 1 min. place column into new collection tube and discard the collection tube containing filtrate.

t. Add 500µl of buffer AW2. Centrifuge for 3 min. discard collection tube containing filtrate.

u. Transfer the spin column to a new eppendorf tube and add 200µl of buffer AE. Incubate for 1 minute at room temperature. Centrifuge for 1 min.

v. The final result of the extraction yields 200µl of product that contains approximately 25-70ng/µl DNA.

PCR Assay

a. The PCR assay will amplify the partial 16S rRNA gene sequence.

b. Twelve microliters of DNA product produced from each sample extraction from Section 15.1 will be used to produce the PCR assay. Four aliquots of 3 µl (hence a total volume of 12 µl) will be combined with each Forward

Primer plus the Reverse Primer, Master Mix, and HPLC Water to produce a final volume of 25 µl. Each sample will be primed with a Bac32F, CF128F, CF193F, and HF183F. Use Table 2 - Preparation of PCR Assay, to prepare **each sample** for the PCR assay using a well plate. Prime the laboratory duplicate and process identically with the other samples.

Identification	DNA product from each extraction from section 15.1o (µl)	20µM Forward Primer (see identification) (µl)	Bac708R Reverse Primer (µl)	Master Mix (µl)	HPLC Water (µl)	Final (µl)
Bac32F	3	1.25	1.25	12.5	7	25
CF128F	3	1.25	1.25	12.5	7	25
CF193F	3	1.25	1.25	12.5	7	25
HF183F	3	1.25	1.25	12.5	7	25

Table 2 - Preparation of PCR Assay.

c. Take the positive control DNA out of the freezer and use Table 3 - Preparation of Positive and Negative Controls to prepare each control.

Identification	DNA product from each extraction from section 15.1h (µl)	20µM Forward Primer (µl)	Bac708R Reverse Primer (µl)	Master Mix (µl)	HPLC Water (µl)	Final (µl)
B-DNA	3	1.25	1.25	12.5	7	25
C-RNA	3	1.25	1.25	12.5	7	25
H-RNA	3	1.25	1.25	12.5	7	25
LB (negative control)	---	1.25 Bac32F	1.25	12.5	10	25

Table 3 - Preparation of Positive and Negative Controls.

d. Load assay into thermocycler by placing a 96 well-tray into the cradle and sliding the cover over the tray until it is not visible.

e. Set thermocycler to mirror the program illustrated in Table 3 – PCR Assay Thermocycling Program.

Temperature (°C)	Time (seconds)	Process	Cycles
94	240	First Denaturization	1
94	60	Denaturization	35
55	40	Annealing	35
72	60	Extension	35
72	300	Final Extension	1

Table 3 – PCR Assay Thermocycling Program (Bernhard, Field et.Al. 2000).

Electrophoresis

a. Place the gel tray into the horizontal gel base.
b. Weigh out 1.2g of agarose and transfer to a flask.
c. Add 120ml of 1xTAE buffer to the flask.
d. Microwave the solution until it boils slightly and becomes clear indicating that the agarose has completely dissolved.
e. Let it cool to the touch and add 2 drops of 1µg Ethidium bromide to the flask and swirl to combine.
f. Pour the solution into gel tray and place a comb into the tray and gel. Allow the solution to gel.
g. Position the gel tray into the horizontal base so that the wells are at the opposite end of the positive electrode. Remove the comb and add 1xTAE buffer as a layer over the gel, this should fill up the horizontal gel base.
h. Prepare the 1kb ladder by combining 1µl dye and 5µl 1kb ladder and add it to the first well.
i. Pipette each PCR product into the individual wells.
j. When wells are full of PCR products, place cover over the base which connects the electrodes.
k. Connect the electrodes to a 120 volt power supply.
l. Let gel process for approximately 40 minutes.
m. Disconnect the power supply, place the gel under the Kodak UV analyzer, and close the lid.
n. Turn on UV analyzer light source, select ETHBR on computer, and click capture.

DNA Ladder Comparison

a. Compare bases to the DNA ladder to determine correct size. The 1Kb ladder is set as follows: the largest fragment is 10,000 bases long; the next fragment is 8000 bases long; the next fragment is 3000 bases long; and the final fragment is1000 bases long. There will be additional faint lines between these brighter bands.

b. The *bacteroides* fragment should occur at approximately 676 bases, the cow 128 fragment should occur at approximately 580 bases, the cow 193 fragment should occur at approximately 515 bases, and the human 183 fragment should occur at approximately 525 bases.

15.5 Report results on the bench sheet.

15.6 The completed bench sheet should be reviewed by the analyst, the microbiology faculty supervisor, and the OEWRI QA manager.

16 Data acquisition, calculations, and reporting

This method does not quantify fecal source contamination therefore no concentration calculations are necessary.

Method detection limits, recoveries, and precision values cannot be quantified with this method.

Samples are not diluted therefore no correction calculations for dilution are necessary.

Laboratory duplicates are collected and processed but are not quantified. Positive and not positive results will be included in the laboratory report to indicate precision.

Results will be reported as positive or not positive for each host specific *Bacteroides*.

DNA fragment confidence will be quantified by comparison with a DNA ladder. The base value, which indicates where the fragment is located on the ladder, will be reported on the bench sheet along with the qualitative “positive” or “negative” results. The base value, or location of the DNA fragment as compared to the ladder, indicates accuracy and should be ±10% of the “true” base specific value (*bacteroides* = 676, cow 128 = 580, cow 193 = 515, and human 183 = 525).

17 Computer hardware and software

Word: This document and attached bench sheet are prepared using Microsoft Word. The Word document file name for this SOP is: DNA.doc

Excel: Quality control charts and laboratory reports are created using Excel.

18 Method performance

18.1 The desired performance criteria for this measurement are:

a. The laboratory duplicate must mirror all results of the source sample.
b. The negative control must not produce positive results.
c. The positive controls must produce positive results for the respective host specific *bacteroides* DNA used to prepare the positive control.

18.2 There are no published method performance data for this method. The positive control mixtures have been tested and have been found to work optimally for the parameters of interest. These methods are based on previous successful microbial tracking research, see References Section 23.

19 Pollution prevention

All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU Biology Department. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

20 Data assessment and acceptable criteria for quality control measures

The analyst should review all data for correctness (e.g., use of MPN table).

The laboratory duplicate must mirror all results of the source sample. The qualitative results will indicate precision.

The negative control must not produce any positive results

The positive controls must produce positive results for the respective host specific *bacteroides* used to prepare the positive control and the base value must be within ±10% of the anticipated DNA ladder range. The *bacteroides* fragment should occur at approximately 676 bases, the cow 128 fragment should occur at approximately 580 bases, the cow 193 fragment should occur at approximately 515 bases, and the human 183 fragment should occur at approximately 525 bases.

The completed bench sheet is reviewed by the analyst's supervisor or the OEWRI QA coordinator.

21 Corrective actions for out-of-control or unacceptable data

The results for precision and blank data are compared to the acceptable values for this analysis.

If a blank value produces positive results for any source the analyst will note the results in the bench sheet and inform the QA/QC coordinator.

The samples cannot be reanalyzed because the sample volume will be depleted after the initial analysis.

If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

22 Waste management

The wastes generated in this method are not hazardous. They can be discarded in the following manner: the initial sample water can be discarded in the laboratory sink; all other wastes associated with this method are placed in a red biohazard bag, are autoclaved, and then are discarded with other waste.

23 References

Anne E. Bernhard and Katharine G. Field. 2000. A PCR Assay to Discriminate Human and Ruminant Feces on the Basis of Host Differences in *Bacteroides-Prevotella* Genes Encoding 16S rRNA. Appl. Env. Micro. 66:10:4571-4572.

Orin C. Shanks, Christopher Nietch, Michael Simonich, Melissa Younger, Don Reynolds, and Katharine G. Field. 2006. Basin-Wide Analysis of the Dynamics of Fecal Contamination and Fecal Source Identification in Tallamook Bay, Oregon. Appl. Env. Micro. 72:8: 5537-5546.

24 Tables, diagrams, flowcharts and validation data

Appendix A contains the bench sheet associated with this method. The analyst should make a copy of this form for each batch of samples analyzed.

These methods vary from the stool kit instructions due to the fact that the kit was made to use with solid fecal matter versus water samples. Bacteria are filtered from the water. Alterations in the original kit instructions include:

a. Set up filtration apparatus with vacuum pump, collection flask, funnel, and tubing.
b. Filter each water sample through a 0.45µm mixed cellulose ester membrane filter. Prepare a laboratory duplicate (LD) by filtering 500ml of the sample in the 1000ml sample bag through one filter and then the remaining 500ml from the sample in the1000ml bag through a separate filter. Discard filtered water.
c. Transfer each sample filter from the filter apparatus using flame sterilized tweezers and place into a sterile 15ml plastic conical tube.
d. Add 1.4ml of “ASL Buffer”, provided with the kit, to each tube. Place tubes in the vortex and process for 10 minutes to remove cells from filter.
e. Place the tubes in a 70°C water bath for 10 minutes to incubate.
f. Use a pipette to by-pass the filter, pull analyte into the pipette tip from each conical tube, and transfer the analyte to separate 1.5ml Eppendorf tubes. Discard each filter and conical tube into red biohazard bag.
g. The rest of the procedure will follow the manufacturers instructions in the kit.

Appendix A

Ozarks Environmental and Water Resources Institute

Microbial Source Tracking

Analyst: _____ Project: _____

Date collected: _____ Date analyzed: _____

Were samples processed before the hold time of 3 hours expired? Yes No Explain: _____

Sample Data	Results											
Sample Identification	Bac32			CF128			CF193			HF183		
	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value

Sample Data	Results											
Sample Identification	Bac32			CF128			CF193			HF183		
	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value	Pos.	Neg.	Base Value
LB Negative Control												
B-DNA Positive Control												
C-RNA Positive Control												
H-RNA Positive Control												

Comments: _____

_____

_____

_____

_____

_____