July 25, 2016

Quality Assurance Project Plan (QAPP)

2016 US EPA Workforce Development Fund

PhyloChip Microbial Source Tracking (MST) Project

RFA 16126

US EPA
Office of Environmental Measurement and Evaluation, North Chelmsford, MA
&
OECA

Project Work/QA Plan Acceptance

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Signature:_____	Date: _____
Region 7 Project Lead:	Steven Baker
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OECA Project Lead:	Esteban Herrera
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UC Berkeley Project Lead:	Dr. Gary Andersen
Signature:_____	Date: _____

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1.0 Project Background

The purpose of this document is to describe the 2016 Workforce Development Fund (WFD) PhyloChip Project (See **Addendum A** for original WDF proposal) and associated quality controls measures that will provide quality assurance for collecting, processing, and analysis of water sample concentrates from water samples for the purpose of identification and relative quantification of microbial indicators.

Despite spending millions of dollars in water quality monitoring and a variety of programs like Pollution Identification and Correction (PIC) grants, National Estuary Program (NEP) funding, Clean Water Act 104 Funds to state, tribal, and local entities, identification and mitigation of fecal pollution sources remains elusive. Fecal contamination from agriculture and other sources have been a nationwide problem over the last decade making CAFO's a National Enforcement Initiative for EPA.

In all regions high fecal bacterial levels degrade water quality resulting in waters unsuitable for their designated uses, such as fishing, boating, and swimming. Tribal nation and general public health have been adversely affected in all regions and EPA continues to invest annually hundreds of millions of dollars nationally for sampling, monitoring, and investigations, but despite these efforts bacteria levels are increasingly the cause of closures of recreational waters and shellfish beds for tribal and recreational purposes. Both closed tribal and public shellfish beds and closed recreational waters raise public health concerns and put tribal treaty rights at risk.

Current methods have limited ability to evaluate the extent to which agricultural industries and practices contribute to the water quality degradation. Regional partners collaborating together in this project will investigate and or validate the PhyloChip DNA Microarray…

1. Utility to distinguish between sources of microbial contamination (e.g., bacteria from septic breakthrough vs farm run-off).
2. Potential, in conjunction with traditional Microbial Source Tracking Methods, to identify contributing sources of bacteria and nutrients in surface waters, and
3. Suitability for various water program needs, including enforcement and compliance.

2.0 Project Goals

This project will help the US EPA demonstrate the efficacy of the PhyloChip compared to more traditional and EPA approved analysis. The Goals for sampling in VT is to…

1. Evaluate whether or not the PhyloChip can identify and distinguish between bacterial flora DNA associated with different cow/waterfowl/human fecal contamination and
2. Determine the best use of the technology to educate and transfer capability to EPA Regions.

3.0 Project Organization

Project Officers/Collaboration Partnerships are identified in Table 1. Jack Paar will coordinate/oversee activities conducted by Region 1 including field sampling, sample handling, processing, the shipment of sample containers and preservatives from OEME to UC Berkeley, OEME filter storage, and analysis according to SOPs (see **Addenda C: SOPs**). While the portion of R1 water samples (and filters) will be collected by EPA R1, the sample filters will be analyzed by PhyloChip DNA micro array by Dr. Andersen, DOE/LBNL/UC/Berkeley laboratories (see Table 2.). No Workforce funds will be used to support work conducted by Region 1. Collaboration between DOE/LBNL/UC/Berkeley and the US EPA is of mutual interest to foster further development of source tracking methods and procedures. Data will be shared among project partners once analyses are complete.

Steven Baker, US EPA R7, will coordinate the shipment of glass bead tubes, sample bottles, FILTERS, and Pall filtrations units. Storage and shipment of sample filters to Dr. Andersen will be arranged by each US EPA regional contact. Contact information for Project Research Partners is listed in Table 1.

Table 1. Project Organization I-Administration

Project Officers	Contact Info
Jack Paar	**US EPA R1 - OEME/ECA** (781) 526-6763 paar.jack@epa.gov **EPA New England Regional Laboratory** Ecosystems Assessment Microbiology & Genomics 11 Technology Drive North Chelmsford, MA 01863-2431
	US EPA R1 - OEME/EQA (617) 918-8335 conlon.nora@epa.gov
Steven Baker	US EPA R7 - STC (913) 551-5299 baker.steven@epa.gov
Esteban Herrera	US EPA OECA (303) 462-9305 herrera.esteban@epa.gov
Gary Andersen, PhD.	US DOE/LBNL (510) 495-2795 glandersen@lbl.gov Head, Ecology Department Earth Sciences Division, Lawrence Berkeley National Laboratory Berkeley, CA 94720

Table 2. Project Organization II-Research Flow Chart

Project Officers	Role	Workforce Development Funds	Regional Sampling: S Oversight: O
Steven Baker R7	1. Contract Management/ 2. Acquire/distribute expendables 3. Review data analyses 4. Project Report 5. Technology transfer to other regions	Yes: Supplies	S O
Jack Paar R1	1. QAPP 2. Collect water samples 3. Review data analyses 4. Project Report 5. Technology transfer to other regions	No	S O
Nora Conlon	1. Quality Assurance Review of QAPP	NO	O
OECA			
Esteban Herrera	1. Project Liaison	NA	O
DOE/LBNL/UC Berkeley Laboratory			
Gary Andersen	1. PhyloChip Analyses 2. Project Report	Yes	NA

3.0 Data Usage

The data collected in this project will be used to demonstrate the efficacy of PhyloChip DNA micro array to identify bacterial fingerprints and assist in the determination of microbial pollution sources. While this

project may have future regulatory implications a variety of water related assessment programs, there are currently no Standards or criteria for DNA fingerprinting to be measured as part of this study, therefore there are no Project Action Levels (PALs) for the data generated by this project. However, results from the DNA micro array analyses will be evaluated by the following:

- Comparisons of overall bacterial community structure by multivariate statistics (i.e., Bray-Curtis distance metric).
- Community "fingerprint" differences will be visualized by Nonmetric Multidimensional Scaling (NMDS).
- Analysis of Similarity (ANOSIM) will be used to test whether community structures are different between near/farfield and/or up/down stream samples.
    - Temporal differences in spatially related samples* will be evaluated by measuring the differences in taxonomic richness between different indexing periods by ANOSIM (insignificant at $p>0.05$).
    - ANOSIM R values range from 0-1, with values close to 1 indicate strong separation between groups and values close to 0 indicate no significant separation.
    - Similarity Percentage (SIMPER) analysis will be used to identify taxa that are primarily responsible for observed differences in community structure between groups in spatially related samples.

4.0 Design and Rationale

This project is not intended to determine the minimum detection limits of this method nor to comprehensively characterize the scope and variety of human and nonhuman fecal indicator sources in any specific watershed/water body, but rather to demonstrate the utility of the method to detect or identify fecal indicator signals (i.e., DNA fingerprint) of concern. See **Addendum B** for proposed regional specific sampling plans/locations. Additional sites may be selected if these sites prove unsuitable for the project.

5.1 Sampling Design

At each location samples will be collected in accordance with sample collection SOP (ECASOP-Ambient Water Sampling2, **Addendum C**). Collected samples will be vacuum filtered in the field and filters will be stored in cryo tubes in zip-lock bags and placed in a cooler on dry ice for transport to the lab the New England Regional Laboratory for storage at -80°C until subsequent shipment Attention: Gary Andersen, Lawrence Berkeley National Lab, Building 70A-Room 2253,1 Cyclotron Road, Berkeley, CA 94720.

5.2 Sampling Materials

The following materials will be provided to each participating region:

- GeneRight Pre-Loaded Bead Tubes PN S0205-50 w/ Sigma G1277 Glass Beads. Lot #: GB178-L166
- Idexx 120mL Vessels PN 98-09222-00 Lot #: CM016 Exp 3/23/21
- Pall Microfunnel Filter Unit .4um Polycarbonate membrane PN FMFNL1050 Lot #: FZ4142 Exp 2018-02

5.3 Sample Naming Convention and Chain of Custody

In order to ensure traceability of sample and analysis replicates back to the original sampling event, the following sample naming convention has been designed for this project.

Station#- State-YYMMDD-Region

For example, Region 1 samples taken from Station 01 in Vermont on August 10, 2016 would be designated as **01VT160810R01**

Chain of Custody forms will include both sample name as well as a conventional site description in the comments section, e.g. *Jewett Brook Outlet into St. Albans Bay*.

5.4 Sampling Procedures

The field crew will collect a fecal indicator sample at the sites. Using a pre-sterilized bottles, the sample will be collected at approximately 0.3 meter (12 inches) below the water surface. Samples will be chilled for at least 15 minutes on wet ice and will remain on ice until filtration. Samples will be vacuum filtered within 6 hours of collection using assembly provided and the filters frozen on dry ice for up to 48 hours then stored at -80°C for up to one year before being shipped to UC/Berkeley. During sample collection the crewmembers will look for signs of disturbance throughout the reach that would contribute to the presence of fecal contamination to the water body and record these on the data sheet. Record these disturbances on the Site Assessment Form.

1. Label sample bottle with site name and GPS location, date, time and collector's initials.
2. Don protective clothing) including "powder-free polyethylene, PVC, or nitrile gloves) and sampling gear then proceed to sampling site.
3. If water has a current or flow approach sampling location slowly from downstream or down wind. Enter water or proceed with boat to location possessing 3-ft water depth. If sample collection is performed using a boat, dipping pole, sampler at depth or depth integrated sampler refer to the EPA OEME ECA Ambient Water Sampling SOP (see Addendum C).
4. Remove sample container cap. Reaching upstream or up-current submerge the container quickly through the water surface to avoid surface scum. If a scum is present, record the observation in the field notes, and use a swirling motion to clear it before plunging the bottle to depth.
5. Avoid contacting the sample bottle with the pond or the lake sediments, bank, rocks or debris. If water depth is less than 3-ft sample at mid-depth of the water column.
6. Collect the water sample(s) at approximately 1-ft below the surface of 3ft-deep water by submerging an inverted un-capped 250-mL sterile plastic Coliform bottle. Water is collected by righting the bottle at depth and raising it up through the water column to the surface filling it.
7. An alternative method is to submerge the capped container to the 1-ft depth, to remove the cap allowing the container to fill, then re-capping at depth. The method selected for use in a study should be used consistently.
8. Upon reaching the water's surface a small amount of water is poured off from the sample bottle to allow air head-space and the sodium thiosulfate tablet is added to the sample. The sample bottle cap is threaded on the bottle and re-tightened. The bottle is shaken 25-times to aerate and mix the sample then placed on ice for 15-min to chill before initiating filtration.
9. Water samples may be collected and preserved on ice for a period of time up to 8-h (max. hold time) or may be filtered after chilling on ice a minimum of 15-min.

Sample Filtration & Storage: Utilize the disposable filtration assemblies and the 0.4 µm pore size Polycarbonate filters. Each team should filter 100-mL of water sample per filter. Record the field location of the sampling site using Global Positioning System (GPS).

10. Filtration personnel shall don a new pair of "powder-free" latex, or nitrile gloves before beginning filtration.
11. Set up sample filtration apparatus on benchtop and set out sterile 60-mm Petri dish, polycarbonate (PC) filter box and 2 filter forceps.
12. Aseptically transfer PC filters from filter box to base of opened Petri dish. Close filter box and set aside.
13. Chill Filter Extraction tubes with glass beads on dry ice for transports or in -80°C freezer for up to 6 months.

14. If so equipped, remove cellulose nitrate (CN) filter from funnel and discard.
15. DO NOT discard the underlying filter support pad.
16. Load filtration funnel with sterile Polycarbonate (PC) 0.4 µm filter onto support pad.
17. Replace the funnel on the base.
18. Shake sample bottle(s) 25 times to mix well.
19. Note if turbidity is high on COC form.
20. Pour approximately 50 mL of sample volume into filter funnel.
21. Replace cover on filter funnel.
22. Begin to vacuum until all liquid is in filtrate collection flask.
23. Care and patience should be exercised to filter a total of 100-mL of sample water per filter If filtration of first 50-mL volume is performed readily, repeat steps with second 50-mL volume. If filter is plugged and volume cannot be easily filtered, carefully dump out sample volume and aseptically remove and discard filter. Repeat steps 15-21, but only filter a lesser volume that can be filtered completely.
24. Remove filter funnel from base without disturbing filter.
25. **Umbrella Folding**: Using sterile disposable forceps or two flame-sterilized filter forceps fold the filter in half, in quarters, and then in eighths. Download video: Filter Folding video
26. Insert filter into chilled filter extraction tube (with beads). Replace and tighten the screw cap, insert tube(s) into Ziploc bag on dry ice for preservation during transport and shipping to LBL or interim holding lab.
27. Record the volume of water sample filtered through each filter on the COC.
28. Discard the disposable filtration funnel, Petri dish, and forceps in between the filtration of each individual sample collected. New sterile equipment should be procured for each sample.

Table 3.1: Field Sampling & Sample Handling

Parameter for Analysis/ Matrix	Sample/Container	Preservation	Sample Holding Time
DNA/water	1x 100 mL HDPP Wide Mouth, sterile	Wet Ice ≤-10ºC Pre-filtration	6 hrs
Post filtration filter storage		Dry Ice -20ºC	48 hrs
		-80ºC	6 months

Table 3.2: Analytical Parameters & Sample Storage

Sample Container	Preservation	Holding Time
Screw Cap cryotube Tube	-80ºC	6 months

Table 3.3: QC Samples

Number of Samples per region		
Regions	Trip/Field Blanks	Field Dups
1,4,5,7	1 each	1 each
6, 10	2 each	2 each

Region 1 Sample Station Designations and Site Locations

Sample Number	Station Designation	2016 US EPA PhyloChip Project Station Description	Lat.	Long.
1	01-VT160811-R01	Jewett Brook @ Dunmore Rd. Bridge	44° 50' 10.25" N	73° 8' 58.16" W
2	02-VT 160811-R01	Jewett Brook outlet @ St. Albans Bay	44° 48' 38.09" N	73° 9' 6.52" W
3	03-VT 160811-R01	Jewett Brook Xing @ Janes Rd. Swanton	44° 52' 38.79" N	73° 8' 22.00" W
4	04-VT 160811-R01	Near Bittersweet Farm	44° 49'19.46" N	73° 8' 15.15" W
5	05-VT 160811-R01	Jewett Brook @ Lower Newton St. Middle Rd.	44° 51' 22.39" N	73° 9' 3.92" W
6	06-VT 160811-R01	St. Albans Bay Shoreline @ Park/Boat Ramp	44° 48' 30.64" N	73° 8' 29.36" W
7	07-VT 160811-R01	St. Albans Bay South of Jewett Brook Discharge	44° 48' 32.36" N	73° 9' 13.67" W
8	08-VT 160811-R01	Stevens Brook near 2820 Lower Newton St.	44° 50' 56.51" N	73° 7' 9.17" W
9	09-VT 160811-R01	Stevens Brook Xing on Kellogg Rd.	44° 50' 55.80" N	73° 6' 15.42" W
10	10-VT 160811-R01	Tributary to Steven Brook near 2403 Kellogg Rd.	44° 50' 36.73"	73° 6' 35.10" W
11	11-VT 160811-R01	Stevens Brook @ Gas Pipeline RR Track off Jewett Rd.	44° 50' 51.18" N	73° 5' 52.93" W
12	12-VT 160811-R01	Selected Duplicate	TBD	TBD
13	13-VT160810-R01	Trip Blank	NA	NA
14		TBD	TBD	TBD
15		TBD	TBD	TBD

Field Quality Control Samples

OEME Field Blanks will be prepared using autoclave sterilized deionized water with sample containers provided by OEME to be filled in the field. Field Blanks will be packaged with, and in the same manner as, the associated field samples. Duplicate sites will be designated and selected in the field.

6.0 Analysis and QC

Methods

Project will follow Standard Operating Procedures (SOPs) or other methods appropriate to their respective analyses. The methods for the analyses in are summarized in **Table 4**.

7.0 Health and Safety

All field samplers will be approved by their immediate supervisors to perform field work. The supervisors are responsible for assuring that the field personnel are trained in safety issues regarding the work assignments.

8.0 Data Quality Requirements and Assessments

The quality of the data is to be within the ranges associated with the specific approved protocols. All PhyloChip analyses are being performed by Dr. Gary Andersen's laboratory according to the DOE/LBNL and UC/Berkeley SOPs.

8.1 Data Comparability and Completeness

Data must be comparable for all samples within each media; i.e., all analysed with the same detection limits and method for each parameter.

8.2 Corrective Action

When it is found that data is incomplete or that results are unacceptable, the Project Officer may determine that one or more of the following procedures for corrective action shall be undertaken.

Table 4. Methods Summary List and Referenced Standard Operating Procedures.

Analysis	SOP	Type	Laboratory Performing Analysis
Sample Receipt	*DRAFT* ECASOP BIOLAB SAMPLE RECEIPT	NA	Regions 1, 7, 4
Sample Collection	*DRAFT* ECASOP BIOLAB Sample Collection	Water	Regions 1, 7, 4
Sample Filtration	1. Sample Filter Processing by ECASOP-PCRSOP0 2. EPA841-B-07-004	Sample Filtration	Regions: 1,4,5,7 / 6,10 Number of Samples: 15 / 20
PhyloChip	Dr. Gary Andersen SOP	DNA Micro array	UC/Berkeley

Incomplete data: Omissions from logs, notebooks and worksheets place the entire analysis in question. Incomplete field sampling data may require re-sampling of the questionable location.

Conflicting or poor quality data: When results from duplicates, spikes, blanks, etc., do not meet the described QC goals, the available data will be reviewed by the Project Officer(s) and the designated laboratory QA officer.

Reconsideration of acceptable limits with statement explaining the results of the action/rationale may be taken. Rejection of data and exclusion from the report with written explanation may take place. Rejection of the entire sample/station location with recommendation of relocation of sample station or reconsideration of results may be recommended.

When the QC goals of the analyses are not achieved, the resultant data will be reviewed by the Project Officer(s) and the designated biology QA officer with similar remedial actions applying.

8.3 Laboratory Data Evaluation

All collected field data will be reviewed by the Regional Project Officer to determine if the data met the QC goals. A final report will be reviewed and signed off for released by Regional EPA Project Leads and the OECA Project Liaison. Analysis performed by laboratories outside the US EPA will follow their respective internal data evaluation methodologies and be referenced in their final report.

8.4 Data Usability

The usability of the data will be determined by the Regional Project Officers. The Project Officers will review laboratory and field notes, laboratory QC data, field blank results, and data evaluations (see 3.0 Data Usage, above) to determine if the results are acceptable for the project goals and meet the QC criteria.

9.0 Disclaimer

Mention of trade names or commercial products in this document of in associated SOP and/or procedures does not constitute endorsement or recommendation for use.

10.0 Addenda

Addendum A: 2016 Workforce Development Fund PhyloChip Proposal

National Enforcement Initiative Title: National Enforcement Initiative: Preventing Animal Waste from Contaminating Surface and Ground Water

Project Name: CAFO - Source Identification (PhyloChip - Microbial Source Tracking-MST)

Funds requested: EPM $40,000 (60 field sampling kits plus shipping costs) PhyloChip
Contract: Environmental Services Assistance Team (ESAT)
PhyloChip Sample Analysis – Lawrence Berkley Lab

Funds leveraged:

1) Regional staffing for sampling and analysis of MST for comparison on split samples.
2) Next Gen funds may be available.
3) ORD may provide funds and comparative analysis.

Contract vehicle: OECA - ERG Contract

Project description:

Fecal contamination from agriculture and other sources have been a nationwide problem over the last decade making CAFO's a National Enforcement Initiative for EPA. In Region 10 high fecal bacterial levels in the Puget Sound have closed tribal and recreational shellfish beds, raising public health concerns and tribal treaty rights at risk. The Lummi Nation and other tribes have been adversely affected in Washington State and EPA continues to invest over two million in a variety of programs like Pollution Identification and Correction (PIC) grants, National Estuary Program (NEP) funding, and Clean Water Act 104 dollars to state, tribal, and local entities. Annually in Whatcom County $400,000 is used for sampling, monitoring, and investigations. Despite these efforts bacteria levels still are increasing cause further closures of shellfish beds for tribal and recreational purposes.

Agricultural industries in Washington State have been unwilling to accept their part in the contribution to the problem, often claiming the fecal bacteria is coming from other sources. This problem is all too common throughout the United States as water quality continues to be impacted. Region 10 proposes to use the PhyloChip technology in conjunction with traditional Microbial Source Tracking Methods (MST) developed by EPA to identify sources of bacterial contamination in streams and shellfish beds in Puget Sound. The comparison with the current MST analysis will verify and validate the results for enforcement purposes.

Region 1 has been working with Vermont to address agricultural discharges to Lake Champlain. The PhyloChip should help distinguishing between septic breakthrough and farm run-off as contributors to bacteria and nutrients in the lake. The funding for this project would be split between regions and used Workforce Deployment funds to pay for both sampling and analysis.

PhyloChip Workforce Deployment funds will be used to;

1. Identify and Screen Sources.
2. Validate new technology compared to EPA approved analysis.
3. Follow up enforcement to address bacterial contamination.
4. Learning the best use of technology to educate and transfer use to other Regions.

Technology

The PhyloChip, developed and extensively validated at the Lawrence Berkeley Laboratory, http://www.lbl.gov/ , by Dr. Gary Andersen, is a high-precision DNA microarray used to identify bacteria from environmental samples (water, sediments, soil, or air). The 1050 x 1050 grid microarray is housed in a disposable cartridge containing over 1.1 million separate reactive DNA probes, targeting 16S rRNA genes, to enable the simultaneous detection of more than 59,000 distinct bacterial species for each sample, representing 147 phyla, 1123 classes, 1219 orders, and 1464 families. Additional probes built into the microarray provide for robust quality and processing controls.

Environmental samples submitted for PhyloChip analyses are processed without the typical culturing necessary in other source tracking technologies. Beginning with vacuum filtration according to established protocols (e.g., US EPA National Water Quality Surveys) filters retaining the bacteria may be achieved frozen for more than a year. Filters may be shipped frozen within that time-frame to the PhyloChip laboratory where they are thawed and microbial DNA is extracted and amplified by PCR. The amplicon is prepared for PhyloChip hybridization and injected onto the DNA microarray for processing, staining, and fluorescent image scanning.

PhyloChip output is measured by the presence/absence of fluorescence signal across the grid array associated with specific taxa. Statistical analyses and proprietary bioinformatics workflow reduce the fluorescent signal from each sample/PhyloChip to produce a microbial community fingerprint. Fluorescent signal intensity indicates relative abundance of target genes within each sample.

The wide dynamic range of PhyloChip is capable of identifying these community fingerprints from samples with mixtures of high and low abundance (i.e., highly diluted) taxa. "Typically, these communities include a few dominant organisms and a large number of microbes at very small overall abundance levels. These rare bugs can be important in driving vital processes and ecosystem function. The PhyloChip provides the ability to identify these minority abundance community members and determine relative differences that sequencing technologies can miss."[1]

References:

1. http://www.secondgenome.com/solutions/services/phylochip/

Dubinsky, E.A., L. Esmaili, J. R. Hulls, Y. Cao, J. F. Griffith, G. L. Andersen. (2012) Application of Phylogenetic Microarray Analysis to Discriminate Sources of Fecal Pollution. *Environmental Science & Technology* **46:** 4340-4347

Hazen, T. C., Dubinsky, E. A., Desantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh. N. *et. al.* (2010) Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria. *Science*. 330:204-208

Contacts:

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Steven Potokar, CAFO Enforcement Coordinator, Region 10
Stephanie Bailey, Lead Microbiologist, Manchester Lab, Region 10
Denny Dart, Chief, NPDES enforcement, Region 1
Katrina Kipp, Chief, Ecology Monitoring Region 1
Jack Paar, Genomics & Microbiology Laboratory Lead, Region 1

Addendum B: Regional Sampling Plans

(*including schedule, maps, and photos, etc*.) in separate attachments.

- US EPA Region 1, New England Regional Laboratory, 2016 PhyloChip Project Sampling Plan

Addendum C: SOP's

- EIASOP-NPDESWTR0 - Collection of Chemical and Biological Wastewater and Water SamplesR1/EPA New England Regional Laboratory SOP. *Available on request.*

- ECASOP - Ambient Water Sampling2, The Collection of Chemical And Biological Ambient Water Samples SOP. *Available on request.*

- Survey of the Nation's Lakes, Field Operations Manual, United States Environmental Protection Agency, Office of Water, Office of Environmental Information, Washington, DC, EPA841-B-07-004 *Available on request*

- ECASOP-PCRSOP0 - Quantitative Polymerase Chain Reaction (qPCR) Assay for Enterococci in Water *Available on request*

- Standard Operating Procedure for PhyloChip Analysis, Russian River Human Impact Study Quality Assurance Project Plan – Version 1.0, prepared by G. Andersen and E. Dubinsky, Lawrence Berkeley National Laboratory, 11/07/2012, *Available on request*