Standard Operating Procedure for:

Preparation of *Bacteroides dorei* (*B. dorei*) Assay Reagents and Standards for qPCR analysis

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1. INTRODUCTION

1.1. A purified and quantified *Bacteroides dorei* DSM 17855 genomic DNA is used as a standard in the *Bacteroidales* quantitative Polymerase Chain Reaction (qPCR) assay. DNA standards are used to generate standard curves for the determination of performance characteristics of qPCR assays.

1.2. Extraction control and third well spike: The extraction control contains an added known concentration of *B. dorei* DNA that is extracted and analyzed in the same manner as environmental samples. The third well spike also contains an added known concentration of *B. dorei* DNA and is added as a third replicate well to each environmental sample during reaction plate setup. Both extraction control and third well spikes are used as positive controls for the *Bacteroidales* target sequence and qPCR assay.

2. EQUIPMENT AND SUPPLIES

2.1. Gloves, latex or nitriles

2.2. Tube racks

2.3. Centrifuge tubes, 0.5ml, 1.5 ml, and 2.0 ml

2.4. Cryodot labels

2.5. 50 Nalgene bottles, 50 mL

2.6. Micropipettes with sterile PCR grade filter tips

2.6.1. Calibrate and use per SOP PCR-2.4.

2.7. Forceps

2.8. Syringe, 1 ml

2.9. Hypodermic needle, 23 gauge

2.10. Gassing cannula -Nitrogen or Nitrogen/Argon mix gas cylinder

2.11. Bunsen burner

2.12. Mettler Precision Balance Model XA105DU (S/N B314195659)

2.12.1. Calibrate and use per SOP PCR-2.2.

2.13. CBS Optimizer PCR Workstation #1 (S/N 007631)

2.14. CBS Optimizer PCR Workstation #2 (S/N 007632)

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2.15. Fisher Mini Vortexer

2.16. Fisher Minicentrifuge

2.17. Eppendorf Centrifuge Model 5418 (S/N 5418AG715992)

2.18. Qubit 2.0 Fluorometer (S/N 1301008411)

2.18.1. Calibrate and use per SOP PCR-2.0

2.19. Analog dry bath incubator with thermometer (S/N C1648131158860)

2.20. PCR#3, Thermo Refrigerator (S/N 0121179201121219), maintained at 4ºC ± 2ºC, Room B246B

2.21. PCR#2, Thermo Freezer (S/N 0121174101121218), maintained at -20ºC ± 10ºC, Room B246A

2.22. PCR#4, Thermo Freezer (S/N 0121174201121218), maintained at -20ºC ± 10ºC, Room B246B

2.23. PCR#6, Thermo UXF Freezer (S/N 0132376801130826), maintained at -80ºC ± 10ºC, Room B246

2.24. Incubating mini shaker (S/N 150747)

3. REAGENTS AND CHEMICALS

3.1. Deionized (DI) water

3.2. PCR grade water – Autoclave ultrapure water for 15 minutes. Record in LIMS Autoclave Log.

3.3. Bleach solution, 10% (v/v)

3.4. TE Buffer ()

3.5. PRAS (Pre-reduced anaerobic system) PY Glucose w/Tween™ 80 broth tubes

3.6. *Bacteroides dorei* (*B. dorei*) DSM 17855 freeze-dried culture

3.6.1. May also be purchased as genomic DNA(gDNA)

4. HF183 PRIMER/ PROBE STOCK

4.1. Always purchase primer/ probes together as a set. Primers should be dry, salt free, and at a 1 µmol scale. The probe should be dry, HPLC purified and at a 1 µmol scale. The probe needs to be ordered with the 5’ 6-FAM dye and 3’ TAMRA

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quencher. When ordered, a confirmation email is sent. Verify that sequences are correct on the confirmation email. Keep stored at -20°C.

4.1.1. Forward Primer HF183: 5'-ATCATGAGTTCACATGTCCG- 3'

4.1.2. Reverse Primer BthetR1: 5'-CGTAGGAGTTTGGACCGTGT- 3'

4.1.3. Taqman® Probe BthetP1: 5'-6FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA

4.2. Each order includes an Oligonucleotide Data Sheet. Record received date on this sheet and review the sequences on the sheet for correctness. Scan the data sheet into the following folder: \\Fldep1\dear\Labs\Lab5\Biology\AppliedBiology\Molecular Biology\Certificates of Analysis\Oligo Data Sheet

4.3. Record each preparation in Laboratory Information Management System (LIMS) Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker**).

4.3.1. In LIMS select **Laboratory/Technical/Standard Preparation Tracker** from the menu. Choose **BIO-PCR** from the "Group" drop down list.

4.3.2. Select **Purchased/New Reagent/Material** from the menu. If a similar reagent exists then select **Purchased/Duplicate Reagent/Material** from the menu and choose the reagent to duplicate from the list.

4.3.3. In the "Purchased Material" pop-up window, enter the following information:

4.3.3.1. For Forward Primer HF183: Identity: = HF183F; Description = HF183 Assay Forward Primer; Entered by: = Analyst Name; Type: = Powder; Grade: = blank; Purity: = desalted; Received: = Date of receipt; Expires: = 1 year from receive date; Manufacturer: = Eurofins Operon; Lot # = Barcode ID from data sheet; Storage Location: = B246A; Storage Temperature: = Freezer at <-10 degC; Container Size: = weight in mg (shown on data sheet in µg); Units: = mg;

4.3.3.2. For Reverse Primer BthetR1: (NOTE: Reverse Primer BthetR1 will be entered as HF183R for consistency with the HF183 qPCR assay). Identity = HF183R; Description = HF183 Assay Reverse Primer BthetR1; Entered by = Analyst Name; Type = Powder; Grade = blank; Purity = desalted; Received: = Date of receipt; Expires: = 1 year from receive date; Manufacturer = Eurofins Operon; Lot # = Barcode ID from data sheet; Storage Location = B246A; Storage Temperature = Freezer at <-10 degC; Container Size = weight in mg (shown on data sheet in µg); Units = mg;

4.3.3.3. For BthetP1Probe: (NOTE: Probe BthetP1 will be entered as HF183P for consistency with the HF183 qPCR assay). Identity = HF183P;

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Description = HF183 Assay Probe BthetP1; Entered by = Analyst Name; Type = Powder; Grade = HPLC Grade; Purity = HPLC; Received: = Date of receipt; Expires: = 1 year from receive date; Manufacturer = Eurofins Operon; Lot # = Barcode ID from data sheet; Storage Location = B246A; Storage Temperature = Freezer at <-10 degC; Container Size = weight in mg (shown on data sheet in µg); Units = mg;

4.3.3.4. Select "Yes" for the "Has Certificate of Analysis" drop down list.

4.3.4. If a component is not already in the components list, click **Add**, and "Identity" name will self-populate in the "Components" table.

4.3.5. Double click the component and add the concentration of the reagent. Use the concentration in nmol from the data sheet. The **Add** or **Remove** buttons can be used to add or remove components.

4.3.6. Click **OK** to save the entry and receive a serial number. Click **Yes** on the "Print label?" pop up window. Select the **3/4" x 1-1/4"** label size in the "Select Label Size & Copies" pop up window. Uncheck the **Use Increment Range** and click **OK.** Choose the Large label printer in the "Print" pop-up window and click **OK**. Place the label on the Oligonucleotide Data Sheet, before scanning it (from Step 6.2).

4.3.7. The concentration of primer/probes will vary with each probe. This is listed in the "nmol" column of the data sheet. The data sheet will list how much TE buffer is needed to reconstitute the primer/probe to a 100 µM concentration in the "Add X µL for 100 µM" column. Use this amount for a 100 µM concentration. For a 500 µM concentration, divide this number by 5.

4.4. All primer/ probe mixing and pipetting must be completed in PCR Workstation #1 (DNA free) using sterile, DNA free supplies. Use only aerosol barrier tips for micropipettes. Before opening the dry oligonucleotide tube, pulse centrifuge to make sure all the powder is at the bottom of the tube and help prevent a spill. Do not over mix the oligonucleotide. The primer/probes are made up of very small DNA fragments that are fragile.

4.5. For each primer, pipette appropriate amount of TE buffer into the tube containing the dried primer to make a 500 µM concentration. Dissolve by gentle vortexing (or tapping the tube). Allow the solution to equilibrate for 5 minutes. Mix again and pulse centrifuge to coalesce droplets. Aliquot 12 µL volumes into blue 0.5 ml centrifuge tubes. Label clearly, and affix cryodots (green dot – forward primer; pink dot – reverse primer) to the top of each tube.

4.6. For the probe, pipette appropriate amount of TE buffer into the tube containing the dried probe to make a 100 µM concentration. Dissolve by gentle vortexing (or tapping the tube). Allow the solution to equilibrate for 5 minutes. Mix again and pulse centrifuge to coalesce droplets. Aliquot 12 µL volumes into blue 0.5 ml

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centrifuge tubes. Label clearly, and affix cryodots (yellow dot) to the top of each tube.

4.7. Group in sets of forward primer, reverse primer, and probe in a tube rack and store at -20°C.

4.8. Record each primer/probe preparation in Laboratory Information Management System (LIMS) Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker**).

4.8.1. In LIMS select **Laboratory/Technical/Standard Preparation Tracker** from the menu. Choose **BIO-PCR** from the "Group" drop down list.

4.8.2. Select **Prepared/New Reagent** from the menu. If a similar reagent exists then select **Prepared/Duplicate Reagent** from the menu and choose the reagent to duplicate from the list.

4.8.3. In the "Prepared Reagent" pop-up window, enter the following information:

4.8.3.1. For HF183 Forward Primer: Identity: = HF183F; Type: = Working Reagent; Entered by: = Analyst Name; Holding Time: = 180 Days; Prepared = Date/Time of preparation and Analyst; SOP: = PCR-3.2; See Also: = leave blank; Location: = B246A; Description = HF183 Assay Forward Primer; Storage Temperature = Freezer at <-10 degC; Additional Information: = TE Buffer S#; Solvent Used: = AE Buffer; Final Volume: = final total volume of preparation in µL; Units: = uL;

4.8.3.2. For HF183 Reverse Primer: Identity = HF183R; Type: = Working Reagent; Entered by: = Analyst Name; Holding Time: = 180 Days; Prepared = Date/Time of preparation and Analyst; SOP: = PCR-3.2; See Also: = leave blank; Location: = B246A; Description = HF183Assay Reverse Primer; Storage Temperature = Freezer at <-10 degC; Additional Information: = TE Buffer S#; Solvent Used: = AE Buffer; Final Volume: = final total volume of preparation in µL; Units: = uL;

4.8.3.3. For HF183 Probe: Identity = HF183P; Type: = Working Reagent; Entered by: = Analyst Name; Holding Time: = 180 Days; Prepared = Date/Time of preparation and Analyst; SOP: = PCR-3.2; See Also: = leave blank; Location: = B246A; Description = HF183 Probe; Storage Temperature = Freezer at <-10 degC; Additional Information: = TE Buffer S#; Solvent Used: = AE Buffer; Final Volume: = final total volume of preparation in µL; Units: = uL;

4.8.4. Click **Add**. Choose the **Purchased Materials** radio button in the "Select Source:" section. In the "Enter Serial #" pop up window, choose the primer/probe that was used to make the preparation being entered from the list, or enter its serial number in the text box. In the "Enter Amount & Units for.."

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pop up window, enter weight of the primer/probe added in µg and choose “ug” from the “Added Units:” drop down list. Click **OK**.

4.8.5. Click **OK** to save the entry and receive a serial number. Click **Yes** in the “Confirm” pop up window to print labels. In the “Select Label Size & Copies” window, select the 1/2” x 9/10” label size in the “Label Size” section. Make sure that **Use Increment Range** is checked and the “Increment Range:” reflects the number of aliquots made for the primer/probe. Click **OK**. Choose the small label printer in the “Print” pop-up window and click **OK**. Place labels on all aliquotted tubes before storage in the freezer.

5. *B. dorei* CULTURE

5.1. Start with the purchased lyophilized (freeze-dried) culture of *B.dorei* in a double-vial ampule. Before opening the ampule, identify the culture by the label on the inner vial which indicates the strain number and date of preservation. Confirm that the ampoule is under vacuum by checking the color of the desiccant at the bottom of the outer vial.

Note: The freeze-dried pellet of most anaerobic strains available from the DSMZ is protected against short exposure to oxygen by amorphous ferrous sulfide (FeS) which confers a black color to the pellet.

5.2. Open ampule and rehydrate the freeze-dried culture pellet:

5.2.1. Remove the glass ampoule from the secondary packaging.

5.2.2. While wearing proper protective glasses, heat the tip of the ampoule on a Bunsen burner. Place two or three drops of water onto the hot tip to crack the glass.

5.2.3. Carefully strike off the glass tip with forceps. Remove the insulation material with forceps and gently take out the inner vial containing the freeze-dried pellet.

5.2.4. Lift and remove the cotton plug using forceps. Flame the top of the inner vial and immediately insert a gassing cannula to keep the pellet under oxygen-free conditions. Replace the cotton plug half-way so that it keeps the gassing cannula snugly inside the vial.

5.2.5. Aseptically pull approximately 0.5 ml of the PRAS medium from a PRAS PY Glucose w/Tween™ 80 broth tube using a 1 ml syringe with a hypodermic needle and add to the vial. Resuspend the cell pellet completely. This may take up to 5 minutes. Transfer the cell suspension by using a 1 ml syringe with the hypodermic needle to the PRAS tube.

5.2.6. Invert tube 2-3 times to mix well and incubate in incubating mini shaker at 37.0°C ± 0.5°C for 24-48 hours.

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5.2.7. The culture can be transferred to new broth for up to 5 passages. Bring the stored culture to room temperature and mix with gentle vortexing at speed 7 for 1 minute before transferring to a new broth tube. After 5 passages, lab strains may develop and new culture should be purchased. Always use freshly grown (24-48 hours) culture to purify for gDNA.

5.2.8. After removing culture for standards, the remaining culture broth can be kept at 4°C for up to one week. For long term (up to 1 year) storage keep frozen at -20°C.

5.3. Record the *B. dorei* culture in LIMS Standard Preparation Tracker module (Laboratory/Technical/Standard Preparation Tracker).

5.3.1. In LIMS select **Laboratory/Technical/Standard Preparation Tracker** from the menu. Choose **BIO-PCR** from the "Group" drop down list.

5.3.2. Select **Purchased/New Standard** from the menu. If a similar standard exists then select **Prepared/Duplicate Standard** from the menu and choose the standard to duplicate from the list.

5.3.3. In the "Purchased Standard" pop up window enter the following information:

5.3.3.1. For *B. dorei* culture: Identity: = B. DOREI CULTURE; Description: = Bacteroides dorei DSM 17855 pure culture; Entered by: = Analyst Name; Prepared = Date of reviving the culture; Received: = Date received; Expires: = 365 days; Type: = pellet; Has certificate of Analysis: = YES; Manufacturer: = DSMZ Germany; Lot #: = (enter from the vial); Storage Location: = B246B; Storage Temperature: = Freezer <10 degC; Additional information: = Use with SOP = PCR-3.2.

6. STOCK *B. dorei* gDNA, AND WORKING STANDARDS

6.1. Using a sterile syringe and needle aspirate 2 mL of culture from the PRAS tube, and add to a 2 mL micro centrifuge tube. Centrifuge at 14,000 rpm for 10 minutes to pellet the *B.dorei* culture. Remove supernatant without disturbing the pellet.

6.2. Add 2 mL of TE buffer and resuspend the pellet by vortexing. Centrifuge at 14,000 rpm for 5 minutes to pellet the *B.dorei* culture. Remove supernatant without disturbing the pellet.

6.3. Repeat step 6.2. two more times to finish washing the media from the culture. Resuspend the pellet in 50 µL TE buffer.

6.4. Extract total gDNA from the culture per SOP PCR-4.2, using the MO BIO PowerLyzer PowerSoil DNA extraction kit.

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6.5. Make a serial dilution (1:10, 1:100, 1:1000, 1:10000) of the *B. dorei* gDNA using TE buffer as the diluent. Gently mix each dilution and pulse centrifuge to coalesce droplets. Overmixing can affect DNA amplification.

6.5.1. Mix 50 µL of *B. dorei* gDNA with 450 µL of TE buffer to make a 1:10 dilution of *B. dorei* gDNA.

6.5.2. Mix 100 µL of the 1:10 *B. dorei* gDNA with 900 µL of TE buffer to make a 1:100 dilution of the *B. dorei* gDNA.

6.5.3. Mix 100 µL of the 1:100 *B. dorei* gDNA with 900 µL of TE buffer to make a 1:1000 dilution of the *B. dorei* gDNA.

6.5.4. Mix 100 µL of the 1:1000 *B. dorei* gDNA with 900 µL of TE buffer to make a 1:10000 dilution of the *B. dorei* gDNA.

6.6. Analyze the 1:100 and 1:1000 dilutions (and 1:10000 if needed) in triplicate on the Qubit fluorometer, per SOP PCR-2.0, to determine the actual concentration of each dilution. Use 10 µL as the sample volume. Each dilution should be 10X greater than the next higher dilution. If the 1:100 and 1:1000 dilutions are not 10X from each other, but the 1:1000 and 1:10000 are 10X from each other, then the 1:100 may be too concentrated and can be ignored in the calculations below. If none of the dilutions are 10X from each other. Remake dilutions and re-read on the Qubit fluorometer.

6.7. Determine the concentration of stock *B. dorei* gDNA in ng/mL.

6.7.1. For each rep for each dilution, multiply the stock concentration (from Qubit data file) measured on the Qubit by the dilution factor (100X for 1:100, 1000X for 1:1000, etc.).

6.7.2. Use the average of these as the final measured concentration of the stock *B. dorei* gDNA. The stock should have at least a 10^5 concentration. If not, not enough DNA was recovered from the culture and the stock should be remade.

6.8. Determine the concentration of the stock *B.dorei* gDNA in GEU/5 µL using the following equation with the conversion factor of 1 ng of *B.dorei* double-stranded gDNA = 1.69 X 10^5 copies of *B.dorei* genome.

$$\frac{GEU\ B.dorei}{5\ uL} = (Stock\ Conc.\ in\ ng/mL)\left(\frac{1\ mL}{1000\ uL}\right)\left(\frac{1.69 \times 10^5\ GEU}{1\ ng}\right) \times 5$$

6.9. Determine the concentrations of the dilutions in GEU/5 µL units and continue the serial dilution down to a concentration of 10^5 in 1000 µL volumes.

6.9.1. For example, if the concentration of the stock *B. dorei* gDNA is 7.03 X 10^6, the dilution 1:10 is 7.03 X 10^5.

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6.9.2. Using blue 0.5 mL tubes, dispense the stock *B. dorei* gDNA and any dilutions that are 10^6 or greater in 120 µL aliquots. Store the stock *B. dorei* gDNA and dilutions that are 10^6 or greater in a cryobox in the -20°C freezer in room B246B. For 10^5 dilution, aliquot in 30 µL volume. Place red cryodots on the tops of all tubes. Label the dots with the concentration (e.g. E5) and the volume. Store 10^5 tubes in the -20°C freezer in room B246B. This will make it easier to retrieve when performing standard curves.

6.9.3. When setting up the reaction plate, perform a serial dilution of the 10^5 standards to create a standard curve set and 3rd well spike for the HF183 assay. See Section 7.

6.10. Record the stock *B. dorei* gDNA and dilutions in LIMS Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker**).

6.10.1. In LIMS, select **Laboratory/Technical/Standard Preparation Tracker** from the menu. Choose **BIO-PCR** from the "Group" drop down list.

6.10.2. Select **Prepared/New Standard** from the menu. If a similar standard exists then select **Prepared/Duplicate Standard** from the menu and choose the standard to duplicate from the list.

6.10.3. In the "Standard Solution" pop up window enter the following information:

6.10.3.1. <u>For Stock *B. dorei* gDNA</u>: Identity: = B. DOREI DNA; Type: = Stock Standard; Holding Time: = 365 days; Entered by: = Analyst Name; Location: = B246B; Prepared = Date/time of preparation and analyst; SOP: = PCR-3.2; See Also: = leave blank; Description: = *B. dorei* gDNA standard at x.xxEx GEU per 5 uL; Additional Information = TE Buffer S#; Storage Temperature: = Freezer at <-10 degC; Solvent Used: = AE Buffer; Final Volume: = 120.00; Units: = µL;

<u>Note</u>: The stock gDNA will essentially be used up to make working standards. It still needs to be added to LIMS.

6.10.3.2. <u>For working gDNA Standard</u>: Identity: = x.xxEx B. DOREI DNA (use the measured concentration of the standard in GEU/5 µl), e.g. 7.03E5); Type: = Working Standard; Holding Time = 365 days; Entered by: = Analyst Name; Location: = B246B; Prepared = Date/time of preparation and analyst; SOP: = PCR-3.2; See Also: = leave blank; Description: = *B. dorei* gDNA standard at x.xxEx GEU per 5 uL; Additional Information = TE Buffer S#; Storage Temperature: = Freezer at <-10 degC; Solvent Used: = AE Buffer; Final Volume: = 1000.00 ; Units: = µL;

6.10.3.3. Click **Add**. In the "Enter Serial #" pop up window, choose the standard that was used to make the standard being entered from the list, or enter its serial number in the text box. For *B. dorei* stock gDNA, the standard is in the **Purchased Materials** "Select Source:" list. For the other

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working standards, choose **Existing Standards** for the source list. In the "Enter Amount & Units from.." pop up window, enter the amount of standard added in µL and "Final Conc. Units:" should be entered as "ng/mL". Click **OK**.

6.10.3.4. Click **OK** in the "Standard Solution" pop up window. Click **Yes** in the "Confirm" pop up window to print labels. In the "Select Label Size & Copies" window, select the 1/2" x 9/10" label size in the "Label Size" section. Make sure that **Use Increment Range** is checked and the "Increment Range:" reflects the number of aliquots made for the standard. Click **OK**. Choose the small label printer in the "Print" pop-up window and click **OK**. Place labels on all aliquotted tubes before storage in the freezer.

7. HF183 ASSAY STANDARD CURVES AND 3RD WELL SPIKE

7.1. Make a serial dilution of the working gDNA standard (10^5 concentration) and TE buffer to create a dilution series of 10^5, 10^4, 10^3, 10^2, and 10^1 GEU/5 uL, and the 3rd well spike has a concentration of 10^4 GEU/5 uL.

7.1.1. Mix 10 µL of the working gDNA standard with 90 µL of TE buffer to make a 10^4 standard.

7.1.2. Mix 10 µL of the 10^4 standard with 90 µL of the TE buffer to make a 10^3 standard.

7.1.3. Mix 10 µL of the 10^3 standard with 90 µL of the TE buffer to make a 10^3 standard.

7.1.4. Mix 10 µL of the 10^2 standard with 90 µL of the TE buffer to make a 10^1 standard.

7.2. Record the 3rd well spike standard in LIMS Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker**) as a working standard per Step 6.9.7.2, except add SPK to the end of the Identity name.

7.3. When new standards are needed, use the highest dilution available in the freezer. Dilute the standard to 10^5 as shown in section 6. If no higher dilutions are available, make stock HF183 as described in section 5 and make dilutions for the standard curve.

8. *B. dorei* EXTRACTION CONTROL

8.1. The extraction control (EC) has a concentration of 10^5 GEU/5 uL of *B. dorei* gDNA.

8.2. Use the 10^6 *B. dorei* gDNA working standard to make the 10^5 EC dilution by mixing 40 µL of the 10^6 dilution with 360 µL of TE buffer.

8.3. Aliquot the EC into 0.5 mL tubes in 6 µL volumes and keep frozen at -20°C in B246B. Place white cryodots on the tops of all tubes. Label the dots with the concentration E5 and the volume.

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8.4. Record the standards in LIMS Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker**) as a working standard per Step 7.12.3, except add SPK to the end of the Identity name.

9. QUALITY CONTROL

9.1. Maintain control charts of HF183 standard curves and extraction controls. If recoveries show a decreasing trend, DNA standards may be deteriorating and need to be remade.

9.2. When new standard curve sets are made, analyze alongside an older set for comparison. New standards should perform similar to older standards. If older standards are not available, compare to the control chart information.

10. REFERENCES

10.1. FDEP Bureau of Labs Quality Manual http://www.dep.state.fl.us/labs/library/progplan.htm

10.2. Health and Safety Plan http://depnet/burlabs/safety.htm

10.3. EPA Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay (October 2012)

10.4. DEP SOP PCR-2.0 *Calibration, Use and Maintenance of Qubit 2.0 Fluorometer*

10.5. DEP SOP PCR-2.2, *Calibration, Use and Maintenance of Mettler Toledo XA105DU Balance*

10.6. DEP SOP PCR-2.4 *Calibration and Use of Mechanical Pipettes using PCS® Pipette Calibration System and Artel® Pipette Tracker™*

10.7. DEP SOP PCR-4.2 *Purification of Filtered Samples Using the MO BIO PowerLyzer Powersoil DNA Isolation Kit*

Appendix of Changes

06/14/2017 – Added table of contents and cover page, made major revision to working stock standards procedure and added new culturing procedure.