# PREPARATION OF SKETA ASSAY REAGENTS AND STANDARDS FOR qPCR ANALYSIS

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# 1. INTRODUCTION

1.1. Sperm cells from salmon testes of the species, *Oncorhynchus keta* (*O. keta*), are used for the SKETA assay. The SKETA assay is used alongside the HF183, Gull2, DG3, HumM2, CowM2, GFD, and BacR assays as a sample processing and inhibition control. The salmon DNA is added to the extraction buffer which is added to all samples and controls during extraction.

1.2. Each sample for Test IDs PCR-HF183, PMA-HF183, PUR-HF183, PCR-GULL2, PUR-GULL2, PCR-DG3, PUR-DG3, PCR-CowM2, PUR-CowM2, PCR-HumM2, PUR-HumM2, PCR-GFD, PUR-GFD, PCR-BACR, and PUR-BACR are analyzed with the SKETA assay to measure extraction efficiency and check for possible inhibition. Refer to the individual method SOPs for the use of SKETA assay controls.

# 2. EQUIPMENT AND SUPPLIES

2.1. Instrumentation –

| Instrument Name | Serial Number | Manufacturer | Notes |
|---|---|---|---|
| Qubit 2.0 Fluorometer | 1301008411 | Invitrogen | See SOP PCR-2.0 for calibration and use |

2.2. Equipment –

| Equipment | Notes |
|---|---|
| Tube Racks | N/A |
| Micropipettes | See SOP PCR-2.4 for calibration and use |
| CBS Optimizer PCR Workstation #1 | S/N 007631 |
| CBS Optimizer PCR Workstation #2 | S/N 007632 |
| Fisher Mini Vortexer | N/A |
| Fisher Minicentrifuge | N/A |
| Centrifuge | N/A |
| Analog Dry Bath Incubator with Thermometer | S/N C1648131158860 |
| PCR#2, Thermo Freezer | S/N 0121174101121218; Maintained at -20 ± 10°C, Room B246A |
| PCR#4, Thermo Freezer | S/N 0121174201121218; Maintained at -20 ± 10°C, Room B246B |
| PCR#9, Thermo Refrigerator Isotemp | S/N TR1562211012; Maintained at 4 ± 2°C, Room B246A |
| PCR#10, Thermo Revco Freezer | S/N 1127920701221013; Maintained at -80 ± 10°C, Room B246 |
| Incubating Mini Shaker | S/N 150747 |
| **Or Equivalent** | |

2.3. Supplies/Consumables –

| Supplies/Consumables | Notes |
|---|---|
| Sterile Gloves, Latex or Nitriles | N/A |
| Centrifuge Tubes, 0.5 mL, 1.5 mL, and 2.0 mL | N/A |
| Brady FreezerBondz Laboratory labels | Catalog Number 19-140-586 |
| 50 mL Centrifuge Tubes | N/A |
| Pipette Tips | N/A |
| **Or Equivalent** | |


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## 3. REAGENTS AND STANDARDS

3.1. Reagents and Standards –

| Reagent/Standard | Notes |
|---|---|
| Deionized (DI) Water | N/A |
| Molecular Biology Grade Water | Corning Molecular Biology Grade Water tested to USP sterile water. Catalog Number: MT46000CV |
| Bleach Solution, 10% (v/v) | N/A |
| Tris-EDTA, 1X Solution, pH 8.0, Molecular Biology | Catalog Number: BP2473500 |
| Salmon Sperm DNA (10mg/ml) | Invitrogen; Catalog Number: 15632011 or equivalent; Store at -20°C |
| **Or Equivalent** | |

## 4. SALMON DNA 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 should be ordered with 5' 6-FAM dye and 3' Zen-Iowa Black FQ quencher from IDT.

4.1.1. **Forward Primer SketaF2**: 5'-GGTTTCCGCAGCTGGG- 3'

4.1.2. **Reverse Primer SketaR2**: 5'-CCGAGCCGTCCTGGTC- 3'

4.1.3. **Taqman® Probe SketaP2**: 5'-6 FAM/AGTCGCAGG/ZEN/CGGCCA CCGT/3IABkFQ/ -3'

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

4.3. Enter each purchased primer/probe set as soon as received in Laboratory Information Management System (LIMS) Standard Preparation Tracker module.

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, 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 SKETA Forward Primer –

| | |
|---|---|
| **Identity** | **SKETAF2** |
| **Description** | Salmon SKETA Assay Forward Primer |
| **Entered By** | Analyst Name |
| **Type** | Powder |
| **Grade** | Leave Blank |
| **Purity** | Salt-Free |


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| | |
|---|---|
| **Received** | Date of Receipt |
| **Expires** | 365 days from received date |
| **Has certificate of analysis** | Yes |
| **Manufacturer** | IDT |
| **Additional information** | Concentration in nmoles |
| **Lot Number** | Barcode ID from Data Sheet |
| **Storage Location** | B246A |
| **Storage Temperature** | Freezer at <-20 deg C |
| **Container Size** | Weight in mg |
| **Units** | mg |

4.3.3.2. For SKETA Reverse Primer –

| | |
|---|---|
| **Identity** | **SKETAR2** |
| **Description** | Salmon SKETA Assay Reverse Primer |
| **Entered By** | Analyst Name |
| **Type** | Powder |
| **Grade** | Leave Blank |
| **Purity** | Salt-Free |
| **Received Date** | Date of Receipt |
| **Expires** | 365 days from received date |
| **Has certificate of analysis** | Yes |
| **Manufacturer** | IDT |
| **Additional information** | Concentration in nmoles |
| **Lot Number** | Barcode ID from Data Sheet |
| **Storage Location** | B246A |
| **Storage Temperature** | Freezer at <-20 deg C |
| **Container Size** | Weight in mg |
| **Units** | mg |

4.3.3.3. For SKETA Probe –

| | |
|---|---|
| **Identity** | **SKETAP2** |
| **Description** | Salmon SKETA Assay Probe |
| **Entered By** | Analyst Name |
| **Type** | Powder |
| **Grade** | Leave Blank |
| **Purity** | HPLC |
| **Received Date** | Date of Receipt |
| **Expires** | 365 days from received date |
| **Has certificate of analysis** | Yes |
| **Manufacturer** | IDT |
| **Additional information** | Concentration in nmoles |
| **Lot Number** | Barcode ID from Data Sheet |
| **Storage Location** | B246A |
| **Storage Temperature** | Freezer at <-10 deg C |
| **Container Size** | Weight in mg |
| **Units** | mg |

4.3.4. Click **Add**, and "Identity" name will self-populate in the "Components" table. Double click the component and add the concentration of the


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reagent. Use the concentration in nmol from the data sheet. The **Add** or **Remove** buttons can be used to add or remove components.

4.3.5. Click **OK** to save the entry and receive a serial number. Open the Brady Workstation software and create labels (see SOP PCR-5.0 for details). Print labels with the reagent name, serial number, and expiration date and affix labels to the side of each tube. Place the label on the Oligonucleotide Data Sheet, before scanning (from Section 4.2.).

## 5. PRIMERS AND PROBE RESUSPENTION

5.1. The concentration of primers and probes will vary with each order. 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.

5.2. Primer/Probe resuspension and aliquoting 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 oligos are at the bottom of the tube and help prevent a spill. Oligos are shipped by the manufacturer as dried content and appear as either a translucent film or a white powder in a centrifuge tube.

5.3. **For the primers:** Add the appropriate amount of TE buffer into the tube containing the dried primer to make a 500 µM concentration. Dissolve by tapping the tube. Allow the solution to equilibrate for 5 minutes. Mix again and pulse centrifuge to coalesce droplets. Aliquot 12 µL volumes into pink 0.5 mL centrifuge tubes.

5.4. **For the probe**: Add the appropriate amount of TE buffer into the tube containing the dried probe to make a 100 µM concentration. Dissolve by tapping the tube. Allow the solution to equilibrate for 5 minutes. Mix again and pulse centrifuge to coalesce droplets. Aliquot 12 µL volumes into pink 0.5 mL centrifuge tubes.

5.5. When a new primers/probe set is resuspended, prepare a comparison qPCR plate and analyze alongside an older primers/probe set. The new set should perform similarly to older sets.

5.6. Group in sets of forward primer, reverse primer, and probe in a freezer box and store at -20°C.

5.7. Record each primer/probe preparation in the LIMS Standard Preparation Tracker module.

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

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


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

5.7.3.1. For SKETA Forward Primer –

| | |
|---|---|
| **Identity** | **SKETAF2** |
| **Type** | Working Reagent |
| **Holding time** | 365 days from received date |
| **Entered By** | Analyst Name |
| **Prepared** | Enter Date, Time and Analyst name |
| **SOP** | PCR-3.1 |
| **See Also** | Leave Blank |
| **Location** | B246A |
| **Description** | Salmon SKETA Assay Forward Primer 500 µM concentration |
| **Storage Temperature** | Freezer at -20 deg C |
| **Additional Information** | TE Buffer S/N |
| **Solvent Used** | AE Buffer |
| **Components Table** | Final Volume: Enter volume of TE added, Units: µL<br>Click Add: Choose Purchase Materials:<br>Enter TE S/N and volume in µL<br>Click Add: Choose Purchase Materials:<br>Click Add: Enter SKETA Forward S/N and weight in µg |

5.7.3.2. For SKETA Reverse Primer –

| | |
|---|---|
| **Identity** | **SKETAR2** |
| **Type** | Working Reagent |
| **Holding Time** | 365 Days from received date |
| **Entered By** | Analyst Name |
| **Prepared** | Enter Date, Time and Analyst name |
| **SOP** | PCR-3.1 |
| **See Also** | Leave Blank |
| **Location** | B246A |
| **Description** | Salmon SKETA Assay Reverse Primer 500 µM concentration |
| **Storage Temperature** | Freezer at <-10 deg C |
| **Additional Information** | TE Buffer S/N |
| **Solvent Used** | AE Buffer |
| **Components Table** | Final Volume: Enter volume of TE added, Units: µL<br>Click Add: Choose Purchase Materials:<br>Enter TE S/N and volume in µL<br>Click Add: Choose Purchase Materials:<br>Click Add: Enter SKETA Reverse S/N and weight in µg |

5.7.3.3. For SKETA Probe –

| | |
|---|---|
| **Identity** | **SKETAP2** |
| **Type** | Working Reagent |
| **Holding Time** | 365 Days from received date |
| **Entered By** | Analyst Name |
| **Prepared** | Enter Date, Time and Analyst name |
| **SOP** | PCR-3.1 |
| **See Also** | Leave Blank |


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| | |
|---|---|
| **Location** | B246A |
| **Description** | Salmon SKETA Probe 100 µM concentration |
| **Storage Temperature** | Freezer at <-20 deg C |
| **Additional Information** | TE Buffer S/N |
| **Solvent Used** | AE Buffer |
| **Components Table** | Final Volume: Enter volume of TE added, Units: µL<br>Click Add: Choose Purchase Materials:<br>Enter TE S/N and volume in µL<br>Click Add: Choose Purchase Materials:<br>Click Add: Enter SKETA Forward S/N and weight in µg |

5.7.4. Click **OK** to save the entry and receive a serial number. Open the **Brady Workstation** software and create labels (see SOP PCR-5.0 for details). Print labels with the reagent name (**SKETAFor**, **SKETARev**, or **SKETAProbe**), serial number, and expiration date and affix labels to the side of each tube. The top label should include the reagent name and serial number.

5.7.5. Place labels on all aliquoted tubes before storage at -20°C.

## 6. STOCK SALMON DNA AND WORKING STANDARDS

6.1. Enter the Salmon Sperm DNA into the LIMS Standard Preparation Tracker module.

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

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

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

| | |
|---|---|
| **Identity** | **Salmon DNA** |
| **Description** | 10 mg/mL Salmon Sperm DNA solution |
| **Type** | Purchased Solution |
| **Received** | Enter date received |
| **Expires** | Use expiration date in the bottle (*If specified*) |
| **Grade** | If available in datasheet |
| **Purity** | If available in datasheet |
| **Has Certificate of Analysis** | Yes |
| **Manufacturer** | Thermo Scientific |
| **Lot #** | Enter number on tube |
| **Storage Location** | B246A |
| **Storage Temperature** | Freezer at < -10 degC |
| **Additional information** | Invitrogen Brand. Use as SOP PCR 3.1. |
| **Container size** | 1 |
| **Units** | mL |
| **Component** | Salmon DNA: 10 mg/mL |


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6.1.4. Click **OK** to save the entry and print the serial number. Place serial number on the bottle and store in the fridge.

6.2. Make a set of serial dilutions (1:10, 1:100, 1:1,000, 1:10,000, 1:100,000) of the 10 mg/mL stock salmon DNA using TE buffer as the diluent (see table below). Gently mix each dilution and pulse centrifuge to coalesce droplets. Overmixing can affect DNA amplification.

| Concentration | 1:10 | 1:100 | 1:1,000 | 1:10,000 | 1:100,000 |
|---|---|---|---|---|---|
| Stock Name | 10 mg/mL Stock Salmon DNA | 1:10 | 1:100 | 1:1,000 | 1:10,000 |
| Amount of Stock | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL |
| Amount of TE | 900 µL | 900 µL | 900 µL | 900 µL | 900 µL |

6.3. Determine the concentration of each dilution

Measure the 1:1,000, 1:10,000, and 1:100,000 dilutions in triplicate on the Qubit fluorometer as per SOP PCR-2.0. Use 5 µL as the sample volume for the 1:1,000 dilution and 20 µL for the 1:10,000 and 1:100,000.

6.3.1. Each dilution should be 10X greater than the next higher dilution. The 1:1,000 dilution may be too viscous and not read accurately. If this occurs and the 1:10,000 and 1:100,000 dilutions are 10X from each other, the 1:1,000 readings can be ignored in the determination of the concentration of the stock salmon DNA. If none of the dilutions are 10X from each other, remake dilutions and re-read on the Qubit fluorometer. A more concentrated or dilute sample may be needed.

6.3.2. Insert the Qubit 2.0 USB drive into the fluorometer USB port, select Data, then the USB icon. The Qubit data are saved in a .CSV file and named with a timestamp. The USB icon should display a green dot if it recognizes the USB drive. A red dot is displayed when there is no USB drive inserted or if it is not recognized by the fluorometer.

6.3.3. Save the data on the lab computer's hard drive. Open the Excel file on the computer and copy and paste the data into a new SKETA_Quibit_Template file. Rename the file as: SKETA_Qubit_YYMMDD. Save the file in \\Floridadep\data\DEAR\Labs\Lab3\Biology\qPCR\Qubit Data.

6.4. Determine the concentration of the stock salmon DNA in ng/mL.

6.4.1. In the .xlsx, add the dilution name to the appropriate data rows.

6.4.2. Copy and paste the stock concentration column (in ng/mL) in the designated location in the excel file. The worksheet will multiply the results of the 1:10,000 dilution by ten and the 1:100,000 dilution by 100 to compare the values to the 1:1,000 dilution.

6.4.3. The worksheet will calculate the average of all the readings and use this average to calculate the concentration of the stock salmon DNA and all dilutions made.


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6.4.3.1. **The stock salmon DNA should have at least a $10^6$ concentration. If not, use a new tube of the DNA stock.**

6.5. Create 3,000 µL of the $10^3$ standard by adding 300 µL of the $10^4$ standard to 2700 µL of TE Buffer. Aliquot 50 µL volumes of the $10^3$ standard into pink 0.5 mL centrifuge tubes and store at -80°C. The $10^3$ standard is the high standard of the SKETA assay standard curve. The $10^2$ and $10^1$ standards used in the standard curve are diluted fresh from the $10^3$ standard with each plate.

6.6. Record the dilutions in LIMS Standard Preparation Tracker module.

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

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

6.6.3. In the "Standard Solution" pop up window add the following information:

6.6.3.1. <u>For intermediate DNA Standards</u>:

| | |
|---|---|
| **Identity** | x.xxEx SKETA (use the measured concentration of the standard) |
| **Type** | Intermediate Standard |
| **Holding Time** | 365 days |
| **Entered By** | Analyst Name |
| **Location** | B246 |
| **Prepared** | Date/Time of preparation and analyst |
| **SOP** | PCR-3.1 |
| **See Also** | Leave blank |
| **Description** | Salmon DNA diluted from EX (with X changing for each dilution); For the dilution from the purchased salmon stock, replace "EX" with "Salmon stock" |
| **Additional Information** | TE Buffer S/N |
| **Storage Temperature** | Freezer at <-10 deg C |
| **Solvent Used** | TE Buffer |
| **Final Volume** | Final total volume of preparation in µL |
| **Units** | µL |
| **Click Add on the Components Table** | <u>Add the TE Buffer</u>: Enter S/N for TE Buffer used from the Purchased Material list, Amount: amount in µL, Final Concentration: ng/mL<br><br><u>Add the Salmon DNA:</u> Enter S/N for the Salmon DNA used from the Purchased or Prepared Material list, Amount: Amount in µL, Final Concentration: ng/mL |

6.6.3.2. <u>For the working DNA Standards ($10^3$ standard):</u>

| | |
|---|---|
| **Identity** | x.xxEx SKETA (use the measured concentration of the standard) |
| **Type** | Working Standard |
| **Holding Time** | 365 days |
| **Entered By** | Analyst Name |
| **Location** | B246 |
| **Prepared** | Date/Time of preparation and analyst |


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| | |
|---|---|
| **SOP** | PCR-3.1 |
| **See Also** | Leave blank |
| **Description** | Salmon DNA diluted from EX (with X changing for each dilution) |
| **Additional Information** | TE Buffer S/N |
| **Storage Temperature** | Freezer at <-10 deg C |
| **Solvent Used** | TE Buffer |
| **Final Volume** | Final total volume of preparation in µL |
| **Units** | µL |
| **Click Add on the Components Table** | Add the TE Buffer: Enter S/N for TE Buffer used from the Purchased Material list, Amount: amount in µL, Final Concentration: ng/mL<br><br>Add the Salmon DNA: Enter S/N for the Salmon DNA used from the Prepared Material list, Amount: Amount in µL, Final Concentration: ng/mL |

6.6.4. Click OK to save the entry and receive a serial number. Open the Brady Workstation software and create labels (see SOP PCR-5.0 for details). Print labels with the standard name and concentration (SKETA STD, xxEx) serial number, and expiration date and affix labels to the side of each tube. The top label should include the standard name and concentration. Place labels on all aliquoted tubes before storage at -80°C.

## 7. SKETA ASSAY STANDARD CURVES

7.1. A set of $10^3$, $10^2$, and $10^1$ dilutions should be run on every plate that uses the SKETA assay.

7.2. When new standards are needed, use the stock SKETA DNA and recalculate the concentration using a dilution series as described in Section 6.

## 8. SALMON DNA WORKING STANDARD FOR EXTRACTION BUFFER (1E4 SKETA)

8.1. Use the $E^5$ SKETA intermediate standard to make the 1 X $10^4$ ng/mL standard.

8.2. To determine the volume of $E^5$ SKETA working standard needed, use the following $C_1V_1 = C_2V_2$ equation.

$$V_1 = \frac{(1 \times 10^4\ ng/mL)(V_2)\left(\frac{1000\ \mu L}{1\ mL}\right)}{[E5\ SKETA]}$$

Where: $V_1$ = Amount of E5 SKETA in µL needed
[E5 SKETA] = Concentration of the $E^5$ SKETA standard in ng/mL
$V_2$ = Final volume of stock standard in mL (i.e. 25 ml or 2.5 ml)

8.3. Working standard for extraction buffer and purification: Add the corresponding amount of E5 SKETA to a 50 mL centrifuge tube and bring up to 25 mL volume with TE buffer. Gently mix and pulse centrifuge to coalesce droplets. Aliquot 1.5 mL into pink 1.5 mL tubes for the crude lysate method and 50 µL into 0.5 mL tubes for the purification method. Store at -80°C.


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8.5. Record the standards in LIMS Standard Preparation Tracker module as a working standard per Section 6.6.3.2.

## 9. SALMON DNA EXTRACTION BUFFER

9.1. Remove 1.00E$^4$ SKETA working standard (1.5 mL per tube) and thaw at room temperature. Vortex and pulse centrifuge to coalesce droplets.

9.2. In a 50 mL centrifuge tube, add 1 mL of 1.00 E$^4$ SKETA standard and 49 mL of TE buffer to make a final concentration of 0.2 µg/mL. Gently mix and store at 4°C for a maximum of 3 months. Make additional extraction buffer as needed in increments of 50 mL.

9.3. Record the extraction buffer in LIMS Standard Preparation Tracker module.

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

9.3.2. 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.

9.3.3. In the "Standard Solution" pop up window add the following information:

| | |
|---|---|
| **Identity** | SALMON EXT BUFF |
| **Type** | Working Standard |
| **Holding Time** | 90 Days |
| **Entered By** | Analyst Name |
| **Location** | B246B |
| **Prepared** | Date/Time of preparation and analyst |
| **SOP** | PCR-3.1 |
| **See Also** | Leave blank |
| **Description** | 0.2 µg/mL Salmon DNA/TE extraction buffer |
| **Additional Information** | TE Buffer S/N |
| **Storage Temperature** | Refrigeration at 4 deg C |
| **Solvent Used** | TE Buffer |
| **Final Volume** | Enter correct final volume in mL |
| **Units** | mL |

9.3.4. Click **Add**. In the "Enter Serial #" pop up window, choose the standard that was used to make the buffer or enter its serial number in the text box. Choose **Existing Standards** for the source list. In the "Enter Amount & Units for." pop up window, enter the amount of standard added in µL and "Final Conc. Units:" as "ng/mL". Click **OK**.

9.3.5. Click **OK** to save the entry and receive a serial number. Open the Brady Workstation software and create labels (see SOP PCR-5.0 for details). Print labels with the standard name and concentration (SKETA STD, 1.00E4), serial number, and expiration date and affix labels to the side of each tube. The top label should include the standard name and concentration. Place labels on all aliquoted tubes before storage at -80°C.


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## 10. QUALITY CONTROL

10.1. Maintain control charts of SKETA extraction controls. If recoveries show a decreasing trend, DNA standards may be deteriorating and need to be remade.

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

## 11. REFERENCES

11.1. DEP Laboratory Quality Assurance Manual

11.2. Laboratory Health and Safety Plan

11.3. Sigma Catalog # D1626 Product Information Sheet and FAQ

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

11.5. DEP SOP PCR-2.4, *Calibration and Use of Mechanical Pipettes using the Artel® PCS® 3 Pipette Calibration System*

11.6. DEP SOP PCR-5.0, *Sample Custody, Labels, and Worksheet Instructions for Molecular Biology Samples.*

### Appendix of Significant Changes

| | |
|---|---|
| 09/13/2016 | Updated buffer to TE buffer, removed SOPs reference that were not needed. |
| 10/18/2017 | Updated probe quencher and manufacturer, storage of standards, and extraction buffer prep. |
| 09/23/2019 | Updated standard preparation procedures. Add instructions to print labels and the SOP-5.0 reference. Add test code HumM2 to the list of available tests. |
| 08/13/2021 | Add test code GFD TaqMan to the list of available tests. |
| 08/01/2023 | Formatted SOP and added SOP tables. Added additional guidance in Section 6 for Qubit calculations. |
| 02/14/2024 | Updated salmon DNA preparation procedure to reflect the change to the purchased 10 mg/mL SKETA solution. Removed any reference to Lyophilized SKETA and stock preparation. |


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