SAMPLE CUSTODY, LABELS, AND WORKSHEET INSTRUCTIONS FOR MOLECULAR BIOLOGY SAMPLES

1. INTRODUCTION

1.1. This method provides instructions for entering samples into Molecular Biology custody using the LIMS Custody module. The analyst checks out samples from the Receiving Room, creates LIMS prep worksheets, prints labels for analyses, and checks samples into Biology custody. The Molecular Biology section analyses environmental water samples using qPCR. This includes the following LIMS Analysis IDs:

1.1.1. For SOP-PCR-4.1 Worksheets: PCR-DG3, PCR-BACR, PCR-HF183, PCR-GULL2, PCR-GFD, PCR-HUMM2, PCR-COWM2 and PCR-HOF.

1.1.2. For SOP-PCR-1.1 Worksheets: PMA-HF183

1.1.3. For SOP-PCR-4.2 Worksheets: PUR-DG3, PUR-BACR, PUR-GULL2, PUR-GFD, PUR-HF183, PUR-HUMM2, PUR-COWM2 and PCR-HOF.

1.1.4. The associated biological and soil sample test codes (PCR-DG3B, PCR-DG3S, PCR-BACRB, PCR-BACRS, PCR-GFDB, PCR-GFDS, PCR-HF183B, PCR-HF183S, PCR-GULL2B, PCR-GULL2S, PCR-HUMM2B, PCR-HUMM2S, PCR-COWM2B, PCR-COWM2S, PCR-HOFB, and PCR-HOFS) are also used on SOP-PCR-4.2 worksheets.

1.2. This method provides instructions for entering data into the LIMS prep worksheet for the filtration, extraction, and purification processes of qPCR analysis and printing labels for each.

1.3. This method also provides instructions for completing the Reaction Plate Preparation Worksheet when analyzing samples using qPCR.

2. SAMPLE COLLECTION AND PRESERVATION

2.1. The Receiving Room staff is responsible for receiving samples shipped from field personnel who collect water samples throughout the state (SOP FS-1000). Molecular Biology samples are logged into LIMS and stored at the correct temperatures (SOP LB-016). Analysts have access to storage areas to retrieve the samples for analysis.

3. SAMPLE CHECK OUT

3.1. Pick up samples in the Receiving Room (C201) that have been logged in by the Receiving Room staff. Samples will have a large label with a Sample ID number, field information, and barcode. Check out samples at a computer in the Receiving Room before bringing them back to the lab.

3.2. Log into the **Laboratory Information Management System** (LIMS). Select **Laboratory/Receiving/Custody** from the main menu. In the window that appears

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(**DEP Central Laboratory Custody**), select the first button to **Check Out** samples.

3.3. Scan the barcode of each sample. Be sure that the number of samples corresponds to the total number scanned, shown at the bottom of the screen.

3.4. Click **OK** after scanning the barcodes. When prompted, the analyst will scan their ID badge twice, then click **OK**. The analyst must be a custodian in LIMS to be able to check out/in samples.

3.5. Take the samples to the qPCR filtering station in the lab for filtration (SOP PCR-4.0).

NOTE: Samples approaching the end of holding times (48 hours) should be picked up by an analyst as soon as possible for immediate processing. These samples can be signed out of the Receiving Room even if they have not been logged into the LIMS system. To sign out samples, match field IDs on the sample bottles to the custody sheets and sign the bottom of each individual custody sheet with analyst name, date/time, sample type, and number of samples picked up.

4. PREP WORKSHEETS

4.1. Each prep worksheet can contain up to 13 samples for each analysis (6 samples for PMA analysis). It should also include one lab duplicate (DUP_A/ DUP_B), one method blank (MBLK), one extraction blank (EB), and one extraction control (EC) for each analysis. Each worksheet batch is extracted and analyzed together.

NOTE: Multiple filtration batches can be added to the same worksheet. Create a new worksheet after every 13 samples or if the current worksheet has been extracted.

NOTE: When picking the lab duplicate, choose a sample scheduled for multiple analyses.

4.2. **Add samples to a new worksheet:**

4.2.1. Log in to LIMS and select **Laboratory/Technical/Prep Worksheet Manager** from the main menu. Select the first shortcut button to Create a prep worksheet.

4.2.2. In the “Preparation ID…” section, select the appropriate worksheet from the list (**SOP-PCR-1.1** for PMA, **SOP-PCR-4.1** for PCR, or **SOP-PCR-4.2** for PUR).

4.2.3. In the “Assign To…” section, select **APPLD_BIOL** in the drop-down box and the analyst’s name from the list.

4.2.4. Highlight all the molecular biology samples that were just checked out of custody. If there are more than 13 samples, only highlight the first 13. Create worksheets in batches of 13 samples or fewer.

4.2.5. Click on the check mark button to **Create the new worksheet** or select **Create/Create Now** from the menu.

4.3. **Add samples to an existing worksheet**:

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4.3.1. Log into LIMS and select **Laboratory/Technical/Prep Worksheet Manager** from the main menu.

4.3.2. Open the relevant worksheet and click **Add Sample(s)** in the bottom right corner.

4.3.3. Highlight the samples to add from the pop-up list and then click **OK**.

4.4. **Print Labels for Filtration:**

4.4.1. Go to the LIMS main menu and select **Laboratory/Receiving/Print Preparation Labels** or click on the label button under **Technical/Prep Worksheet Manager** to open the Print Prep Labels window.

4.4.2. Deselect the two tabs that are already highlighted, as shown in Figure 1 (yellow arrow), by double-clicking the shortcut label buttons marked Micro Petri Dish and Chlorophylls (text appears when mouse hovers over selection).

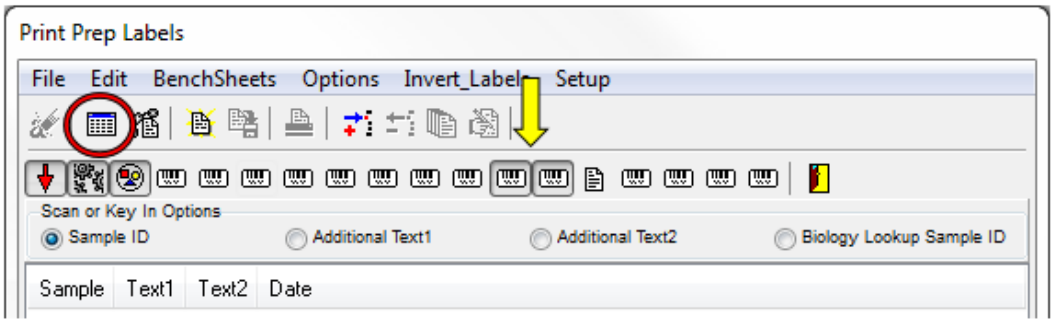


Figure 1

4.4.3. From the **Options** menu, select **PCR Sample Labels**.

4.4.4. Click on the second shortcut icon (circled in Figure 1) to Load Sample IDs from prep worksheet. Select the appropriate worksheet (that was just created or added to) and click **OK**.

4.4.4.1. Verify that the samples shown correspond to the samples that were checked out. There will be one line for each analysis that has been scheduled for each sample. Delete any excess sample IDs. Multiple worksheets can be added.

4.4.5. Duplicate sample:

4.4.5.1. Select a random sample (other than field blanks) to duplicate per sample batch. The sample selected as the duplicate should be a sample that is scheduled for all markers present on the worksheet.

4.4.5.2. Modify an existing row for the duplicate sample or create an extra row for each duplicate by highlighting the chosen sample(s) and selecting the Duplicate item icon ( ).

4.4.5.3. Rename the duplicates with suffixes "_DUP_A" and "_DUP_B".

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4.4.6. Method blank (MBLK):

4.4.6.1. A method blank must be filtered with each batch of 13 samples, and it can be added anywhere within the batch.

4.4.6.2. Modify an existing row to make the MBLK label or create an additional row for the method blank by highlighting any sample and clicking the 'Duplicate selected item' icon.

4.4.6.3. Rename the sample "MBLK_Worksheet#" (Ex: MBLK_200) by highlighting the row and selecting the Sample header.

4.4.7. When the sample list is complete, save the label list as a text file named as the date (MMDDYYYY) in the folder 'Labels for LabelMark6'. Click on the Exit label printing icon ( ) to exit the module.

4.4.8. Next, click on the BradyWorkstation icon on the desktop.

4.4.8.1. In the Print Partner, select the FilterTemplate in the Recent Files window. Click **Open**.

4.4.8.2. In the ribbon, select **Advanced Import**. A tab titled "Import from:" will appear in the bottom right corner. Click on the option **Spreadsheet**.

4.4.8.3. Select the file created in Section 4.4.6. A table will appear in the Advanced Import tab at the bottom of the screen. In the "Separate on:" drop down list select **Tab** and uncheck the box titled "First row is column header".

4.4.8.4. Click on the section labeled **Format**. Next drag and drop the columns (F1-F5) into the appropriate boxes as in the figure below.

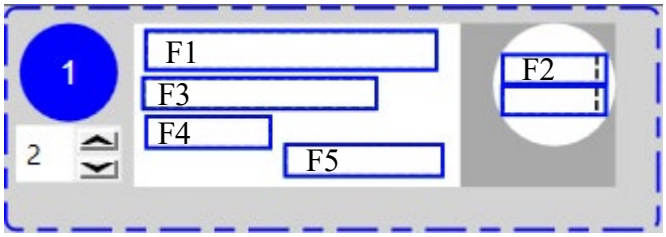


Figure 2

4.4.8.5. In the **Errors** tab at the bottom of the screen, select **Check Label Errors**. If any label errors are found, such as the sample information being too large for the printable area, select **Fix All Errors**.

4.4.8.6. Double check that the number of copies for each label is set to two. Then, print out two copies of each label.

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4.4.9. Place one set of labels on the appropriate bead beating tubes, and the 2nd set of labels on 2 mL colored centrifuge tubes or a second bead beading tube from the Qiagen kit for PMA (SOP-PCR-1.1) or purification (SOP-PCR-4.2).

4.4.10. Leave a space to write in the filter volume on the label.

4.5. **Sample Check In:**

4.5.1. In the lab, open the custody module by selecting **Laboratory/Receiving/Custody** from the LIMS main menu.

4.5.2. Select the second button in the **DEP Central Laboratory Custody** window to **Check In** samples.

4.5.3. Scan the barcode of each sample and be sure that the total number checked in corresponds to the original number of samples.

4.5.4. Click **OK** after scanning the barcodes. When prompted, the analyst will scan their ID badge twice, and then click **OK**.

4.5.4 Periodically check for samples still logged in under analyst custody. Select **Reports** in the **Custody** window and **Samples Checked Out.** Highlight analyst name and click **Add**. Then, click **Ok**. A list of sample IDs will be displayed if any are still in the analyst's custody.

5. SAMPLE FILTRATION PREP WORKSHEET DATA ENTRY

5.1. After filtering samples (SOP-PCR-4.0), log into LIMS on a workstation computer. Select **Laboratory/Technical/Prep Worksheet Manager** from the main menu. Select the shortcut button to **Open** a prep worksheet logbook ( ).

5.2. In the **Active** worksheet list (selected in the "Worksheet Status:" section), double click on the corresponding worksheet for the samples filtered.

5.3. Enter the analyst who filtered the samples in the **Prepared By** header field. If a second analyst also filtered samples, enter that name in the **Sample Comments** for the filtered sample(s).

5.4. Add the serial number of the PBS used in the **Reagent 1** header field. If a different batch of PBS was used for some samples in the worksheet, add the serial number in the **Sample Comments** for the sample(s).

5.5. Add the suffix "_DUP_A" to the sample ID of any samples that have a lab duplicate. Right click the row of the duplicate sample(s) and select **Duplicate Row**. Select each new row and add the suffix "_DUP_B".

5.6. Highlight all filtered samples and enter the **Date/Time of Filtration**.

5.7. Select each sample individually and enter the **Filtration Volume (mL)**, as written on the sample's bead tube/2 mL centrifuge tube label. Samples with the same filtration volume can be selected together and the filtration volume can be entered simultaneously.

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5.8. Freeze the filters at -80°C in a freezer box until sample extraction.

5.9. For new worksheets, enter the correct date/time samples were frozen in the **Date/Time Frozen** header field.

5.10. Save and close the worksheet. Do **NOT** click complete.

6. SAMPLE EXTRACTION PREP WORKSHEET DATA ENTRY

6.1. Print Labels for Extraction:

6.1.1. Follow steps in Section 4.4, while using section 6.1.2. to supplement previous directions.

6.1.2. Modify existing sample rows or create additional rows for the method blank, extraction blank, and extraction control with the Duplicate selected item icon.

6.1.2.1. For the method blank: rename the Sample field to "MBLK_Worksheet#" by highlighting the row and selecting the Sample header.

6.1.2.2. For the extraction blank: rename the Sample field to "EB_Worksheet#" by highlighting the row and selecting the Sample header.

6.1.2.3. For the extraction control: rename the Sample field to "EC_Assay" by highlighting the row and selecting the Sample header. Indicate the appropriate analysis (HFG2GD, BRD3, C2H2) in the name.

6.1.3. When the sample list is complete, save the label list created as the date followed by ext for extraction (MMDDYYYYext) in the folder 'Labels for LabelMark6'.

6.1.4. In the BradyWorkstation program, print **one copy** as shown in Section 4.7.

6.2. Place the labels on the final extraction 1.5 mL tubes. After extraction, place the bead beating tube label onto the printed worksheet for a record of the original label from filtering.

6.3. Prep Worksheet Data:

6.3.1. At the beginning of sample extraction, reopen the worksheet and enter the start time in the **Prep Start Date/Time** field in the header. This start/date time should also be used for the EC and EB same date/time of filtration.

NOTE: The worksheet cannot be completed until the Date/Time of Filtration is filled in for all samples. Leave the filtration volume blank for the EC and EB.

6.3.2. Select the analyst's name in the **Extracted By** header field. If more than one analyst is extracting, add the second analyst to the **Prep Comments** header field.

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6.3.3. At the end of sample extraction, fill in the **Prep Completion Date/Time** field in the header.

6.3.4. Add the extract volume to the **Final Extract Volume (mL)** sample grid field.

6.3.5. Add the serial number of the salmon extraction buffer to the **Spike 1** field. Enter the amount added to the appropriate lines (all samples/controls) in the **Spike 1 Amount** sample grid field.

6.3.6. Add the serial number of the HFG2GD extraction control spike to the **Spike 2** header field. Enter the volume added to the appropriate line (EC_HFG2GD) in the **Spike 2 Amount** sample grid field.

6.3.6.1. Repeat for all other extraction controls (BRD3, C2H2) using the remaining spike fields. Add a new line for each additional extraction control.

6.3.7. Add the units used for the spike amounts in the **Spike Units** header field (i.e., µL).

6.3.8. Add the dilution factor to all samples in the **Sample Dilution Factor** sample grid field. The dilution factor should be "1" unless the extract was diluted for the run (2-fold dilution most common). If so, adjust accordingly.

NOTE: The analyst performing the qPCR assays will change the dilution factor in the worksheet after performing the analysis. Dilution factor should be left blank until then.

6.3.9. Save the worksheet.

6.4. Completing and Reviewing the Worksheet:

6.4.1. A different analyst than the one who extracted should review the worksheet. The reviewer can make corrections to the prep worksheet as necessary.

6.4.2. Complete the worksheet by clicking the **Complete** button once you have double checked all dates, spikes, volumes, and samples on the worksheet.

6.4.3. When all is verified, complete the "**Reviewed by**" and "**Date/Time Reviewed**" fields on the prep worksheet.

6.4.4. Wait to complete the worksheet until the first plate has been run and any additional analyses added.

7. SAMPLE PURIFICATION

7.1. Print Labels for Purification:

7.1.1. Follow steps in 6.1. for printing labels.

7.2. Prep Worksheet Data:

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7.2.1. Prior to creating the purification prep worksheet, add the purified test code to the samples under LIMS **Laboratory/Administrative/Add/Remove Tests**.

7.2.1.1. Click **Sample Search** and type in the sample ID. Choose the appropriate test and component.

7.2.2. Add the sample to a worksheet, following the instructions in Section 4.2.

7.2.2.1. The **Date/Time of Filtration** and **Filtration Volume (mL)** fields should be automatically entered from the previous worksheet.

7.2.3. Open the new worksheet and add the suffix "_PUR" to all the Sample IDs in the worksheet. For duplicates, the Sample ID should read "SampleID_DUP_A_PUR."

7.2.4. At the beginning of sample purification, fill in the **Prep Start Date/Time** field in the header. Fill in the remainder of the Prep Worksheet per Section 6.2.

7.2.5. Add the comment 'Extracted using Qiagen DNeasy PowerSoil Pro kit' in the **Prep Comments** field in the header.

7.2.6. Save the worksheet.

7.3. **Completing and Reviewing the Worksheet:**

7.3.1. A different analyst than the one who extracted should review the worksheet. The reviewer can make corrections to the prep worksheet as necessary.

7.3.2. When all is verified, complete the "**Reviewed by**" and "**Date/Time Reviewed**" fields on the prep worksheet. Save and complete the worksheet by clicking the **Complete** button.

7.3.3. Wait to complete the worksheet until the plate has been run and any additional analyses added.

8. REACTION PLATE WORKSHEET

8.1. At the beginning of qPCR analysis, open the Reaction Plate Preparation Worksheet (Form PCR-PLATEPREP-version#) located in: \\floridadep\data\LABS\Lab3\Biology\qPCR\RxnPlatePrepWS.

8.2. Enter the **worksheet ID** that corresponds with the samples being analyzed.

8.3. Enter the **Run ID**. The format is XX-X-###-YYMMDD.

8.3.1. The initial 2 digits refer to the analysis:

Analysis	Digit Code
HF183	HF
Gull2	G2
GFD	GD

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BacR	BR
DG3	D3
CowM2	C2
HumM2	H2
Hof597	HOF
PMA-HF183	PMA

8.3.2. The second part denotes crude (C), purified (P), or rerun samples (R).

8.3.3. The number signs indicate worksheet number, and the final part is the date of the run.

8.4. Select the assays being analyzed from the drop-down lists. The SKETA assay should be selected second (on the right side of the worksheet). The '**Amount Added'** and '**Total Volume'** fields should be automatically filled in when the assay is selected.

8.5. **For the Primer/Probe Mix Preparation:**

8.5.1. Enter the date/time of the primer/probe (P/P) mix preparation and analyst along with the serial number for each primer/probe reagent.

8.5.2. If using a previously made P/P mix, add the date of the original mix in the comment and leave the serial number lines blank for that P/P mix.

8.6. **For the Master Mix Preparation:**

8.6.1. Enter the date/time of the master mix preparation and analyst.

8.6.2. Enter the number of reactions needed in the header of the "For # rxns Add (µL)" column. The calculated amounts needed for each reagent will be automatically filled in for the number of reactions entered.

8.6.3. Enter the serial number for each reagent.

8.7. **For the Standards Used for Standard Curve and 3rd Well Spike:**

8.7.1. Enter the date/time the final standard is diluted and analyst name.

8.7.2. Enter the serial number and concentration of the top standards and 3rd well spike.

8.8. When the worksheet is complete, use "Save As" to save the file under the correct year in \\floridadep\data\LABS\Lab3\Biology\qPCR\RxnPlatePrepWS folder with the Run ID as the filename.

8.9. After review, convert the file to .pdf format.

9. REFERENCES

9.1. DEP SOP FS 1000, *General Sampling Procedures*

9.2. DEP SOP LB-016, *Sample Receipt and Entry into the DEP Laboratory Information Management System (LIMS)*

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9.3. DEP SOP PCR-1.1, *Propidium Monoazide (PMA)-qPCR for Quantitative Differentiation of Live Human-Associated Bacteroides*

9.4. DEP SOP PCR-4.0, *Preparation of Samples for qPCR Analysis*

9.5. DEP SOP PCR-4.1, *Crude Lysate Extraction Method for qPCR Analysis*

9.6. DEP SOP PCR-4.2, *Purification of Filtered Samples Using the Qiagen DNeasy PowerSoil® Pro Kit*

Appendix of Significant Changes

10/17/2016 Update changes to naming data files and adding spike amounts to prep worksheet.

11/22/2017 Updated analysis IDs.

11/26/2018 Updated Printer and label maker information.

11/13/2019 Updated analysis IDs.

08/19/2021 Updated analysis IDs.

08/22/2022 Updated procedure for entering method blank lab location

08/20/2024 Reformatted SOP. Added instructions on using the BradyWorkstation software to print labels.

08/11/2025 Added specification about choosing duplicate sample per worksheet.

08/05/2026 Added information about Hof marker.

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