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. Prior to analysis, 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 handles qPCR samples. 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, and PCR-COWM2

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, and PUR-COWM2.

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, and PCR-COWM2S 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 that 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 the storage areas to obtain the samples for analysis.

3. SAMPLE CUSTODY

3.1. Samples approaching the end of holding times should be picked up by an analyst as soon as possible for immediate processing. These samples can be signed out of the Receiving Room 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. SAMPLE CHECK OUT

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4.1. Pick up samples in the Receiving Room 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.

4.2. Log into LIMS. Select **Laboratory/Receiving/Custody** from the main menu. In the window that appears (**DEP Central Laboratory Custody**), select the first button to **Check Out** samples.

4.3. 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.

4.4. Click **OK** after scanning the barcodes. When prompted, the analyst will scan his/her ID badge twice, then click **OK**. The analyst must be a custodian in LIMS to be able to check out/in samples. Take the samples to the qPCR filtering station in the lab for filtration (SOP PCR-4.0).

5. PREP WORKSHEETS

5.1. 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.

5.2. To add samples to an existing worksheet, log into LIMS and select **Laboratory/Technical/Prep Worksheet Manager** from the main menu. Open the relevant worksheet and click **Add Sample(s)** in the bottom right corner. Highlight samples to add from the pop-up list and click OK. Each worksheet batch is extracted and analyzed together. Multiple filtration batches can be added to the same worksheet. When picking the lab duplicate, choose a sample scheduled for multiple analyses. Create a new worksheet after every 13 samples or if the current worksheet has been extracted.

5.3. To add samples to a new worksheet, 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**.

5.4. 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). In the "Assign To…" section, select **APPLD_BIOL** in the drop-down box and the analyst's name from the list. 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.

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

5.5.1. Print Labels for Filtration

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5.6. 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. 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).

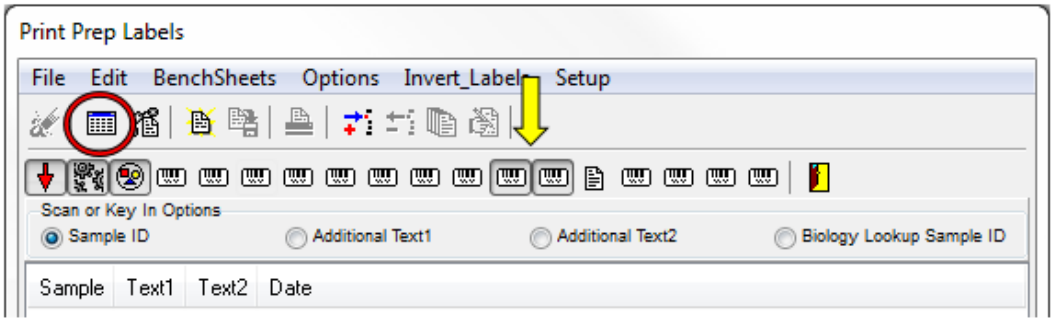


Figure 1

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

5.8. 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. 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.

5.9. Create an extra row for each duplicate by highlighting the chosen sample(s) and selecting the Duplicate selected item icon ( ). Rename the duplicates with suffixes "_DUP_A" and "_DUP_B".

5.10. Create an additional row for the method blank by highlighting any sample and clicking the 'Duplicate selected item' icon. Rename the sample "MBLK_Worksheet#" (Ex: MBLK_4.1_200) by highlighting the row and selecting the Sample header. Enter the same lab location as the samples for MB samples. Correct any other sample locations as needed.

5.11. When the sample list is complete, save the label list as a text file named as the date followed by filt for filtered (MMDDYYYYfilt) in the folder 'Labels for LabelMark6'. Click on the Exit label printing icon ( ) to exit the module. Next, click on LabelMark6 icon on desktop. Choose appropriate tab for the desired filter labels template (Ex. FilterTemplate041117-B.l6t). After choosing filter template, click Create label → External Data → Next → Data source and then select the file the labels are saved under Tab (to make labels print individually) → Next → Finish. Print out two copies of each label. 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).

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

5.13. **Sample Check In**

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

5.13.2. Select the second button in the **DEP Central Laboratory Custody** window to **Check In** samples. Scan the barcode of each sample and be sure that the total number checked in corresponds to the original number of samples.

5.13.3. Click **OK** after scanning the barcodes. When prompted, the analyst will scan his/her ID badge twice, and then click **OK**.

6. SAMPLE FILTRATION PREP WORKSHEET DATA ENTRY

6.1. 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 ().

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

6.3. 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 **Prep Comments** header field.

6.4. Add the serial number of the PBS used in the **Reagent 1** header field.

6.5. 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".

6.6. Highlight all samples that were filtered and add in the **Date/Time of Filtration**.

6.7. The EC and EB should have the same date/time of filtration as the method blank. The worksheet cannot be completed until the Date/Time of Filtration is filled in for all samples.

6.8. 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. Leave the filtration volume blank for the EC and EB.

6.9. Freeze the filters at -80°C in a freezer box based on the assigned **Lab Location** in the worksheet (PCR#8, Thermo Scientific Revco RDE Series Freezer) until sample extraction.

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

6.11. Save and close the worksheet. Do NOT click complete.

7. SAMPLE EXTRACTION PREP WORKSHEET DATA ENTRY

7.1. **Prep Worksheet Data**

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7.1.1. At the beginning of sample extraction, reopen the worksheet and enter the start time in the **Prep Start Date/Time** field in the header. Print labels per Section 5.2.

7.1.2. Select analyst name in the **Extracted by** header field. If more than one analyst is extracting, add the second analyst to the **Prep Comment** header field.

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

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

7.1.5. 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.

7.1.6. 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. Repeat for all other extraction controls (BRD3, HumM2, and CowM2) using the remaining spike fields. Add a new line for each additional extraction control.

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

7.1.8. 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.

7.1.9. Save the worksheet.

7.1.10. Complete the worksheet by clicking the **Complete** button once you have double checked all dates, spikes, volumes, and samples on the worksheet. When all is verified, complete the "Reviewed by" and "Date/Time Reviewed" fields on the prep worksheet. Wait to complete the worksheet until the first plate has been run and any additional analyses added.

7.2. **Print Labels for Extraction**

7.2.1. Follow steps in Section 3.4.1-3.4.6.

7.2.2. Create additional rows for the method blank, extraction blank, and extraction control with the **Duplicate selected item** icon.

7.2.3. For the method blank, rename the **Sample** field to "MBLK_Worksheet#" by highlighting the row and selecting the **Sample** header.

7.2.4. For the extraction blank, rename the **Sample** field to "EB_Worksheet#" by highlighting the row and selecting the **Sample** header.

7.2.5. 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, HumM2, or CowM2) in the name.

7.2.6. 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'.

7.2.7. In the LabelMark6 program, print one copy as shown in Section 3.4.6.

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7.2.8. 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.

7.2.9. The reviewer can make corrections to the prep worksheet as necessary.

7.2.10. When verified, complete the "Reviewed by" and "Date/Time Reviewed" fields on the prep worksheet.

8. SAMPLE PURIFICATION

8.1. Prep Worksheet Data

8.1.1. For samples that need to be purified, add the purified test code under **LIMS Laboratory/Administrative/Add/Remove Tests**. Click **Sample Search** and type in the sample ID. Choose the appropriate test and component and add the sample to a worksheet. The **Date/Time of Filtration** and **Filtration Volume (mL)** fields should be automatically entered from the previous worksheet.

8.1.2. 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."

8.1.3. At the beginning of sample purification, fill in the **Prep Start Date/Time** field in the header. Print labels per Section 6.2 below. Fill in the remainder of the Prep Worksheet per Section 5.1.

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

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

8.2. Print Labels

8.2.1. Refer to Section 5.2. to print labels.

8.2.2. Add "_PUR" to all the sample lines before printing.

9. REACTION PLATE WORKSHEET

9.1. At the beginning of qPCR analysis, open the Reaction Plate Preparation Worksheet (Form PCR-PLATEPREP-version#) located in: \\FLDEP1\DEAR\Labs\Lab3\Biology\qPCR\RxnPlatePrepWS

9.2. Enter the worksheet ID that corresponds with the samples being analyzed.

9.3. Enter the Run ID. The format is XX-X-###-YYMMDD. The initial 2 digits refer to the analysis (HF for HF183, G2 for Gull2, GD for GFD, BR for BacR, D3 for DG3, H2 for HumM2, C2 for CowM2, and PMA for PMA-HF183). The second part denotes crude, purified, or rerun samples (C for crude extracts, P for purified, and R for rerun). The number signs indicate worksheet number, and the final part is the date of the run.

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9.4. 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 lines should be automatically filled in when the assay is selected.

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

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

9.7. 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.

9.8. Enter the serial number for each reagent.

9.9. Standards Used for Standard Curve and 3rd Well Spike Worksheet Section

9.10. Enter the date/time the DNA is added to the reaction plate and analyst name.

9.11. Enter the serial number and concentration of all the standards added to the plate.

9.12. When the worksheet is complete, use "Save As" to save the file under the correct year in \\FLDEP1\DEAR\Labs\Lab3\Biology\qPCR\RxnPlatePrepWS folder with the Run ID as the filename.

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

10. REFERENCES

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

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

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

10.4. DEP SOP PCR-4.0, *Preparation of samples for qPCR analysis*

10.5. DEP SOP PCR-4.1, *Crude lysate extraction method for qPCR analysis*

10.6. DEP SOP PCR-4.2, *Purification of filtered samples using the Qiagen DNeasy PowerSoil Pro Kit*

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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

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