Sample Custody, Labels, and Worksheet Instructions for Molecular Biology Samples

1. INTRODUCTION

1.1 This method provides instruction 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: PCR-HF183, PCR-HF183B, PCR-HF183S, PCR-GULL2, PCR-GULL2B, and PCR-GULL2S.

1.2 This method also 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 for qPCR methods.

2. SAMPLE COLLECTION AND PRESERVATION

2.1 The Receiving Room staff is responsible for receiving samples shipped from the 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 in order 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.

3.1.1 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.

3.2 Sample Check Out

3.2.1 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. Log into LIMS. Select **Laboratory/ Receiving/Custody** from the main menu.

3.2.2 In the window that appears (**DEP Central Laboratory Custody**), select the first button to **Check Out** samples.

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3.2.3 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. PCR samples are collected in duplicate, so there will be 2 bottles for each sample ID.

3.2.4 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 Microbiology Lab for filtration.

3.3 Prep Worksheets

3.3.1 Each prep worksheet can contain up to 13 samples for each analysis (HF183 and/or Gull2). It should also include one lab duplicate, one method blank, one extraction blank, and one extraction control for each analysis (HF183 and/or Gull2). Each worksheet batch is extracted and analyzed together. Multiple filtration batches can be added to the same worksheet. Continue adding samples to the worksheet until it contains 13 samples. Create a new worksheet after every 13 samples (Row number reaches 17) or if current worksheet has been extracted.

3.3.2 Log in to LIMS on the Pre-PCR Lab computer. Select **Laboratory /Technical/ Prep Worksheet Manager** from the main menu. Select the first shortcut button to **Create a prep worksheet**.

3.3.3 In the "Preparation ID…" section, select **MICRO-PCR** from the list. 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 less (see Section 3.3.1).

> **Note:** As a shortcut, use the **Samples Checked Out** button to automatically highlight samples and analyst name, but this must be done before checking samples back in.

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

3.4 Print Labels

3.4.1 Go back to the LIMS main menu and select Laboratory /Receiving/Print Preparation Labels or click on the label button in 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).

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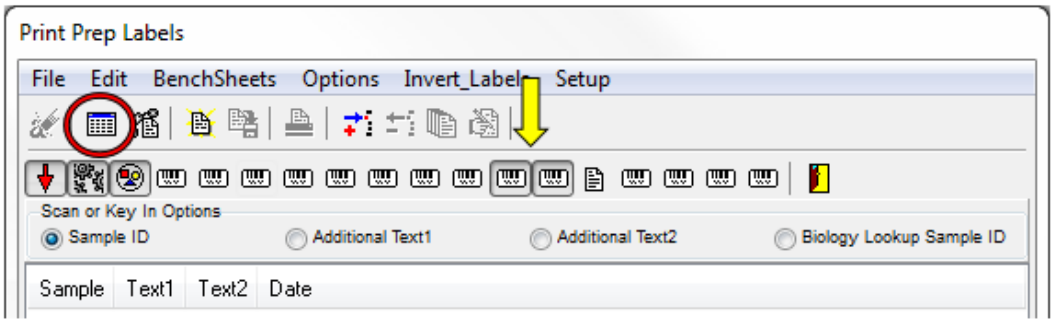


Figure 1

3.4.2 From the Options menu, select PCR Cryo Sample (small).

3.4.3 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 for the samples) and click OK. Verify that the samples shown correspond to the samples that you checked out. There will be one line for each analysis that has been scheduled for each sample. Multiple worksheets can be added.

3.4.4 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," respectively.

3.4.5 Create an additional row for the method blank with the Duplicate selected item icon. Rename the Sample and FieldID fields to "MBLK_Worksheet#" and "METHOD BLK" respectively, by highlighting the row and selecting the Sample and FieldID headers consecutively.

3.4.6 Create a space to write in the filter volume on the label by highlighting all the samples, selecting the FilterVol header, and pressing OK in the FilterVol pop up window. Leave the Enter Filter Volume (mL) field blank. This will populate the FilterVol field as "F-." Highlight the method blank row and enter 50 for the FilterVol using the same process (the method blank will always have a filter volume of 50 mL).

3.4.7 Select the Print List icon ( ) to open the print menu. Select SATO CG408 (Small Label) as the printer and click OK to print. Repeat this process an additional time to create 2 sets of labels. Alternatively, select 2 copies in the Print window. Click on the Exit label printing icon ( ) to exit the module. Place one set of labels on bead beat tubes and the 2nd set of labels on 2 mL centrifuge tubes. Each sample and blank will need one bead beat tube and one 2 mL tube.

3.5 Sample Check In

3.5.1 Open the custody module by selecting **Laboratory/Receiving/Custody** from the LIMS main menu.

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3.5.2 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.

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

4. SAMPLE FILTRATION PREP WORKSHEET DATA

4.1 After filtering samples, 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 ().

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

4.3 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.

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

4.5 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 (Aliquot 2) and add the suffix "_DUP_B".

4.6 Add the Worksheet ID number to the method blank **Sample ID** as a suffix (e.g. MBLK_55).

4.7 Also add the Worksheet ID number to the extraction blank (EB_) and the extraction control (EC_).

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

4.9 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.

4.10 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.

4.11 Freeze the samples and enter the correct date/time samples were frozen in the **Date/Time Frozen** header field.

4.12 Save and close the worksheet.

5. SAMPLE EXTRACTION

5.1 Prep Worksheet Data

5.1.1 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.

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5.1.2 Enter the analyst that is extracting the batch in the **Extracted by** header field. If more than one analyst is extracting, add the second analyst to the **Prep Comment** header field.

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

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

5.1.5 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.

5.1.6 Add the serial number of the *B. dorei* extraction control spike to the **Spike 2** header field. Enter the amount added to the appropriate line (Extraction control HF183) in the **Spike 2 Amount** sample grid field. If a Gull2 analysis is required, add the serial number of the Gull2 extraction control spike to the **Spike 3** header field. Enter the amount added to the appropriate line (Extraction control Gull2) in the **Spike 3 Amount** sample grid field.

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

5.1.8 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. If so, adjust accordingly, where the dilution factor is 1/percent dilution in decimal (i.e. 50% dilution of extract into a well is a dilution factor of 2 (1/0.5)).

5.1.9 Save and complete the worksheet by clicking the **Complete** button. Print the completed worksheet.

5.2 Print Labels

5.2.1 Follow Steps in Section 3.4.1-3.4.5.

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

5.2.2.1 Rename the **Sample** and **FieldID** fields to "EB_Worksheet#" and "EXTRACTN BLK" respectively by highlighting the row and selecting the **Sample** and **FieldID** headers consecutively for the extraction blank.

5.2.2.2 Rename the **Sample** and **FieldID** fields to "EC_Worksheet#" and "EXTRACTN CNTRL" respectively by highlighting the row and selecting the **Sample** and **FieldID** headers consecutively for the extraction control. Indicate the appropriate analysis (HF183 or Gull2) in the name.

5.2.3 Use the prep worksheet to fill in the appropriate volumes for each of the samples.

5.2.4 Enter a dilution of 1 for each line. If sample is further diluted at the end of extraction, reprint a label for the new tube with the corrected dilution factor.

5.2.5 Print one copy as shown in Section 3.4.7.

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5.2.6 Place the labels on the final extraction 1.5 mL tubes. Compare the printed filter volumes to the written volumes on the bead beat tubes to verify data entry.

5.2.6.1 Make corrections on the label and prep worksheet as necessary.

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

6. SAMPLE PURIFICATION

6.1 Prep Worksheet Data

6.1.1 For samples that need to be purified, change their status from "V" back to "W". In LIMS **Prep Worksheet Manager** select **Worksheet**/**Change LIMS Status** from the menu or click the  icon. Select **From: C (or P)** and **To: W** in the drop down lists in the "Select status to change…" section. Choose **MICRO-PCR** from the list in the "Select Preparation" section. Select the correct samples from the list in the "Select Samples" section. Click the **All** button in the "Select Components". Click the checkmark button to change the status. Click the **All** button in the "Confirm" pop-up window.

6.1.2 Once the status is changed, the sample will be added back on the **Create a prep worksheet** list in **Prep Worksheet Manager**. Create a new worksheet for all the samples in the purification batch per Section 3.3. Once the worksheet is created, a new **Prep Batch ID** will be generated and the **Date/Time of Filtration** and **Filtration Volume (mL)** fields should be automatically entered from the previous worksheet.

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

6.1.4 The method blank should read "MBLK_Worksheet#_PUR". The extraction blank should read "EB_Worksheet#_PUR". The extraction control should read "EC_Worksheet#_PUR".

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

6.1.6 Add the Mo Bio kit name and lot number in the **Cleanup Procedures** field in the header.

6.1.7 Save and complete the worksheet by clicking the **Complete** button. Print the completed worksheet.

6.2 Print Labels

6.2.1 Refer to Section 5.2 to print labels and verify filtration volumes.

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

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7. SAMPLE RE-RUN PREP WORKSHEET DATA

7.1 If a sample is to be rerun change the status to "W" per Section 6.1.1.

7.2 Add the sample to the prep worksheet that corresponds to the batch in which it will be run. If the sample will be re-run at a different dilution, make sure to adjust the **Sample Dilution Factor** appropriately and reprint the label for the diluted extract tube. If the re-run samples are analyzed in a batch that does not include new samples (no prep worksheet) a new worksheet does not need to be created. Make a comment in the existing worksheets that includes the re-run ID.

8. REACTION PLATE WORKSHEET

8.1 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

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. The initial 2 digits refer to the analysis (HF for HF183, G2 for Gull2, and GD for GFD). The second part denotes purified samples or rerun samples (P for purified and R for rerun). The number signs indicate worksheet number and the final part is the date of the run.

8.4 Primer/Probe Mix Preparation Worksheet Section

8.4.1 Select the assays being analyzed from the drop down lists. The SKETA assay should be selected second (on the right side of the worksheet).

8.4.2 Enter the date/time of the primer/probe (P/P) mix preparation and analyst. If using a previously made P/P mix, add the Run ID of the original mix in the comment and leave the Serial#s and Amount Added lines blank for that P/P mix.

8.4.3 Enter the total volume for each of the assay P/P mixes. The typical volume is 600 µL. The correct amount added should be automatically filled in by the form, when the total volume is typed in.

8.4.4 Enter the serial number for each primer/probe reagent.

8.5 Master Mix Preparation Worksheet Section

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

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

8.5.3 Enter the Serial# for each reagent.

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

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

8.6.2 Enter the Serial# and concentration of all the standards added to the plate.

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8.7 When the worksheet is complete, use "Save As" to save the file in the \\Fldep1\dear\Labs\Lab3\Biology\qPCR\RxnPlatePrepWS folder with the Run ID as the filename.

8.8 Convert the file to .pdf format and print.

9. REFERENCES

9.1 DEP SOP FS 1000, *General Sampling Procedures*

9.2 DEP SOP LB-016, *Standard Operating Procedure for Sample Receipt and Entry into the LIMS*

Appendix of Changes

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

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