**Sample Prep for Sequencing and Shipping Lab Protocol**

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| Purpose |

This SOP outlines the required steps for preparing and sending samples for sequencing to the CHOP Sequencing Core and the Seq Center in Pittsburgh; methods used across various studies. For the purpose of this document, the MSPACMAN study is used as an example for CHOP sequencing.

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| Materials & Equipment |

**CHOP Sequencing Core**

* Styrofoam box
* Dry ice
* Completed Microbiome Center Sequence Core Sample Receipt form

**SeqCenter**

* Styrofoam box with outer shipping packaging
* Dry ice label (UN1845) **with dry ice weight listed**
* Category B label: UN3373
* Shipping label
* Dry ice
* Printed quote information sheet

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| Procedures |

## **CHOP Sequencing Core**

## Generating and Sending Box Maps

* + - * 1. Find and record Box IDs of interest by selecting Storage 🡪 Boxes and sorting by Study ID

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* + - * 1. Go to Sample Management 🡪 Lab Operations and sort by Box ID-Sorted. Paste the box ID into the field

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* + - * 1. Select all samples listed in the box and download an excel file

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* + - * 1. Remove columns “Visit”, “Label”, “Quantity”, “Tissue”, “Study”, “CD”, “Custodian”, “Status”, “Colony Morphology”, “ID Microbial Species”, “Quarantined?”, “Expired?”, “Expiration Date”, “Testing Complete”, “Archive Sample”, “SPRISOL ID” .
        2. Highlight the row of headers and select Sort and Filter icon 🡪 Filter

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* + - * 1. Select the A to Z (Ascending Order) filer on the Ext Participant ID Column using the drop arrow on column header

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* + - * 1. Save document as Box Name mm-dd-yyyy using the date the file was made:

For example, using MSPACMAN Seq Cryovials Box 1, file name = Seq Cryovials Box 1 01-01-2024

* + - * 1. Send the file to the PI and team.

## Shifting Samples in Boxes for Sequencing

For MSPACMAN: This step is used to shift samples in cases where the endotracheal sample from Wednesday was not collected. Therefore, Katie will indicate that samples from either Monday or Friday collections will need to be added into the sequencing box. Other cases that will require checking in and out samples include inadmissible samples meaning there were less than 4 collections of specimens due to participant being discharged early or unenrolling in the study. In this case samples will be transferred to inadmissible boxes that have been made.

* + - * 1. Collate a list of sample barcodes (S#s) in a document. Go to the Lab Operations page🡪Select the “…”🡪Select Other Tasks🡪Select Alias Search
        2. Paste list of barcodes into window 🡪 select Search
        3. Check all the samples of interest 🡪 select the “Check Out” button 🡪 Add “need to move” to text box 🡪 select OK
        4. Go to Storage🡪File Samples. Scan barcodes of samples that need to be added to sequencing or inadmissible and file sample into respective box.

## Confirming Samples

* + - * 1. Generate a box map for the box of interest
        2. Print a copy of the box map
        3. Check each diagonal of the box as well as three samples in every row to spot check that samples are in the correct position as listed on the map
        4. If there are differences about the location of the sample, reconcile by shifting samples into correct position and generating new box maps. Send final copy to MSPACMAN group email with the most updated version of the box maps

## Sample Drop-Off

* + - * 1. PI will reach out to Ahmed Moustafa ((moustafaam@chop.edu) to coordinate sample drop off time (open from 10am-4pm). Penn-CHOP Microbiome drop off is located Abramson 9th floor room 903G.
        2. Add dry ice to Styrofoam box to transport samples
        3. Print out completed Microbiome Center Sequence Core Sample Receipt Form and bring with samples for delivery

For MSPACMAN, Project ID is CHOPMC-208\_Chiotos\_16s

## **SeqCenter**

## Shipping Preparation

* + - * 1. Go to the SeqCenter website and have the PI generate a quote
        2. Create file of samples of interest for sequencing
        3. Have PI set up a PO for payment
        4. Using Eship, generate a shipping label

Go to Eship 🡪 select Address Book 🡪 select New 🡪 Add SeqCenter address

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Go to Ship 🡪 select Yes 🡪 select Domestic 🡪 select Biological Materials

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select Dry Ice

If sending Category B biological specimens, select the following:

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Enter name of samples in material name field. List an approximate quantity in package (need to complete this field even if samples are solid or non-liquid). Type “1” for Net Value in USD field. Select “Continue”

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Select Load from Address Book to add SeqCenter. For package info, select “Dry Shipper” for type, approximate the weight, and enter and measure the dimensions. For special instructions, check “Dry Ice” and enter weight of dry ice in the respective field. For email notifications, check “Notify Shipper”, “Notify Shipper on Delivery”, and “Notify Others on Delivery”. Enter the PI(s) email addresses into the respective field.

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Select “Show Quote” 🡪 Select cheapest overnight shipping option

Save and print shipping label

## Packing Isolates for Shipment

* + - * 1. Weigh the empty Styrofoam box; tare
        2. Add the dry ice to the box and weigh, providing the weight of the dry ice alone
        3. Put samples in box
        4. Print out a copy of the SeqCenter quote information and enclose it in the shipment
        5. Affix the proper labels and shipping label to the cardboard box

Category B label

Dry Ice Label

Address Label

* + - * 1. Deliver package to pick up location (located in lobby by loading deck in BRB or in Blockley Hall). Times for pickup change depending on the time of year so be sure to check these prior to drop off.
        2. Email SeqCenter (CC the PI or any other relevant personnel) to notify about shipment (include tracking information) and that they received the sample intake form

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| Change Control |

Please note any changes made to the laboratory protocol following study launch by completing the table below. Changes must be approved by the study principal investigator and laboratory director. The SOP should be saved with the date of change saved as part of it’s file name.

|  |  |  |  |
| --- | --- | --- | --- |
| **Date of Change Implementation (MM/DD/YYYY)** | **Brief Description of Change** | **Change Completed By** | **Approved By** |
| 06/26/2024 | Document put into use in correct format, added shipping label instructions for cat B and dry ice | Laura Cowden | Laura Cowden |