

**Fernald Community Cohort
Protocol**

Cyropreserved Biospecimens Sample Selection, Processing, Inventory and Storage

Identify participants who meet study criteria

1. Participants for the research study will be determined by Dr, Pinney and/or the research associate according to study requirements. A project code will be assigned to the researcher and the study by Dr. Pinney.
2. A search of the FCC database will be done to identify UCID's of participants who meet the research study criteria.
3. A spreadsheet with the identified UCID's will be used to determine sample availability for participants who meet study criteria.

Preparing for the sample pull:

1. The research associate, using the spreadsheet of UCID's of potential study participants, will identify the sample availability for the UCIDs of the persons from whom a sample (your study subjects) is needed.
2. Go to S:\OCCH\Fernald Residents\FMMP Freezer Inventory\ and using the Reports/Queries function in the Biospecimen Inventory Database, choose report "info for Specific UCID and Sample Type" and click continue, add UCID and Sample type into boxes (Whole Blood, Serum, Plasma, Non-buffered Urine and Buffered Urine), click on "Query" and click preview. Copy and paste the rows of data into your spreadsheet all of the samples of that type in the inventory for that FMMP participant. The information on the query should include the sample ID in addition to the UCID.
3. Dr. Pinney or the research associate will review the spreadsheet and determine which samples may be used according to the Priority Level of the FMMP participant (Priority 1, 1A or 2) or other criteria. During this review, the samples to be pulled will be noted by placing an X in the "pull" column.
4. If using whole blood for DNA for genotyping, you may be directed to use samples collected at a later date (2006-2008) so that earlier collected samples may be used for identification of predictive markers.
5. Select the samples (one per study subject for a certain Sample Year) for your pull.
6. Create two subset worksheets from the main worksheet on the spreadsheet, one of "Pull" samples and one of "Reserve" samples. (Copy and paste, so that you retain the main worksheet.) Sort the list of Pull samples by Freezer, Shelf and Tray and Box. Sort the Reserve samples by UCID.

Scheduling the Sample Pull

1. A sample pull team usually consists of four persons, so that you can get in and out of the freezers quickly. For a small number of samples, a team of only two or three persons may suffice.
2. There must be at least one experienced, regular UC staff member on the team for the sample pull. This is an absolute requirement. A team may not consist entirely of students.
3. A member of the study research team will be encouraged to participate in the sample pull or inventory if a person is available. Our policy for Access to Biospecimens includes this expectation for all in-town researchers. We will try to give them as much notice as possible of a future sample pull/inventory.
4. Dr. Pinney or the Research Coordinator, Jeanette Buckholz, must approve the composition of the sample pull team, and any changes to the composition, by email. If any last-minute changes to the composition of the sample pull team need to be made on the scheduled day of the pull, Dr. Pinney or Ms. Buckholz must approve that change, either in-person, by phone or by email.
5. The UC staff member on the sample pull team has the responsibility to see that every member of the team has read the sample pull protocol.
6. The UC staff member has the responsibility to see that the freezer at Kettering will be accessible when you return to Kettering with the samples.
7. Each member of the sample pull team must be appropriately dressed for this activity – shoes with closed toes, long sleeves, long pants, long hair tied back.
8. Each member of the sample pull team must have their UC or CCCHMC ID badge with them when they go to the freezer area.
9. The FCC program coordinator will notify the lab at CCHMC and ask them to unlock the door to the freezer room at the designated arrival time of the sample pull team.
10. The UC staff member will record the names of the persons on the sample pull team on the Sample Removal Report..
11. After the sample pull is completed, the UC staff member who is on the sample pull team must call the CCHMC lab (636-8362) and let them know that the sample pull team has left the freezer room.

Pulling the samples:

1. Before leaving Kettering, check again to be sure that the freezer at Kettering will be accessible when you return. Gather all supplies that you will need to pull samples. Freezer gloves, latex gloves, forceps, towels, extra labels for trays if needed, sample box (use a cardboard box), cooler with dry ice, pens, magnifying glass, spread sheet, extra

blank box maps. Make copy of the box map of each box you will be entering so that you have a copy for the box if none is found in the box.

2. As soon as the team arrives in the freezer room, the UC staff member will direct a member of the team to record the arrival time and the temperature on each of the FMMP freezers on the sample pull report sheet.
3. Work area is set up with moving cart, towels, and supplies needed to pull and record sample removal.
4. Person #4 identifies freezer, shelf and tray number of sample to be pulled
5. Person #1 (best to be a tall person, with strong arms): Wears freezer gloves, finds tray containing the box; after tray has been removed, closes freezer (both inner and outer doors). The tray may remain out for no longer than 10 minutes, hopefully less time. If you are going to use two or more boxes in the tray, the boxes should be removed and the tray returned to the freezer immediately. (Small temperature fluctuations cause degradation of DNA.)
6. Person #4 identifies Box number, UCID and sample location in box to be pulled.
7. Person #2 Wears latex gloves and removes box from tray and opens box. Records location of all missing samples in the box on an empty "missing samples" box map. Writes the box number and date at the top of the box map.
8. Person #3: Unfolds box map. Locates sample on box map. Using forceps, removes vials one at a time. Reads off sample type, UCID and date. Gives sample vial to Person #4.
9. Person #4 (usually the UC staff member, but not necessarily): records UCID and then reads back UCID. Records the sample that is removed from the box. If the Priority A sample cannot be found, locates a priority B sample for that person, and writes in that information on the priority A spreadsheet. Sample is then placed in transport box.
10. Person #3 – crosses out the removed sample on the box map in the box. Writes date in the slot in the box map.
11. After Person #4 reads next UCID & location, Person #3 pulls out the next vial in the same box (if another vial is needed from that box), and reads off sample type, UCID and date. Person #4 records that information on the spreadsheet and Person #2 crosses out the sample on the box map and records empty and missing vials.
12. After all needed samples are removed from a box, Person #3 folds box map and places it back in box, closes box.
13. Person #2 replaces box in the tray.
14. Person #1 returns tray in freezer, and then removes the next tray on the spreadsheet.
15. If there are only 3 members of the team, the tasks of Person #2 can be shared by Person #1 and Person #3.

16. If possible, rotate the jobs of Persons #1, #2 and #3. Person #4 should consistently record information throughout the entire sample pull.
17. Continue with locating and removing each tray and samples in that freezer.
18. If at any time the alarm on the freezer should go off, record the freezer number and the time the alarm went off. Immediately cease pulling samples from that freezer, and go on to the next freezer. If there is a third time that an alarm goes off, finish with the box that you have open, but immediately after that finish the sample pull session. (When a freezer alarm goes off it also is heard up in the lab office.)
19. After you finish pulling samples in one freezer, go to the next freezer, and pull samples from that freezer.
20. After all samples are pulled, or there has been a third alarm, or the UC staff member has decided that enough time has been spent for that day, please close the sample pull. The UC staff member will need to record the stop time, and the temperature on each freezer at that time.
21. Please remember to gather the gloves, forceps and any paper copies of the spreadsheets before you leave the freezer room.
22. Transport the samples to Kettering Laboratory, using sample boxes and an insulated cooler. It is usually best to have some ice in the cooler – dry ice is best if available.
23. The original copy of the sample removal report and the sample pull spreadsheets should stay in the offices at Kettering.

Preparing samples for transfer to the researcher

1. Upon return to Kettering, the research associate will make a box map of samples pulled from the FCC freezers. The box and map will be labeled with the researchers name and stored in the Kettering freezer until transfer. A copy of the map will be placed in the box and the original will be kept with the freezer pull list.
2. A material transfer form will be created to document the transfer of samples to the researcher. Material transfer form can be found at S:\OCCH\Fernald Residents\Archived Specimens\Fernald\Material Transfer Form. The samples will be listed by UCID number order with columns for sample type, year of sample, amount (1 or 2 ml), and date transferred. At the top of the form will be the name of study and protocol number, and lines for signature and date for those transferring the samples (UC personnel and researchers representative).

Transferring samples to the researcher

1. Contact the researcher to arrange a time and place for transfer. The research associate will prepare the samples for transport by placing them in a cooler and have the material transfer form available for checking sample ID's and signatures.
2. The research associate and the researcher (or designee) will check each sample ID in the box with the sample ID on the box map. To minimize the time that the samples are out of the freezer, the sample box will be placed in the freezer after the box and map are checked. The research associate and researcher will then check off the sample ID on the box map with the material transfer form by ID order with the date of transfer recorded for each sample. After the review is complete, the research associate will sign and date the log as transferring the samples to the researcher and the researcher will sign and date receiving the samples. A copy of the material transfer form will be given to the researcher and the original will be filed in the FCC Kettering Building offices.

Tracking and documenting samples given to researchers

1. Email Dr. Pinney any problems (other than just an empty vial or a missing sample) that were identified during the sample pull by the next day. Enter missing box maps on the list to replace in box when pulling another sample from box.
2. Record the date samples were pulled from the FCC freezers on the pull or reserve spreadsheet in the database (S:\OCCH\Fernald Residents\Archived specimens\researchers file)
3. In the Sample Inventory database (S:\OCCH\Fernald Residents\Freezer Inventory\), go to view/edit specimens. Using the pull list for the samples and transfer form enter the UCID, sample type and find the date, exam year, freezer, shelf tray, box and slot number for the specimen that was pulled. Enter date distributed, project code and year transferred.
4. Using the list to pull samples, note the samples that have been pulled on the box maps in the notebooks (A,B,C, D, E, F, or G). Identify the box number (new and old) from the sample pull list and locate the binder where the box map is located. After locating the binder that corresponds to the box map where the sample is located, place a line through the sample that was removed with the date and code for the researcher.
5. Make notes in both the binder and Freezer Inventory database information on specimens (ex. missing, tube empty).
6. Split Samples: If a sample is to be split for a project; split from" Mother" becomes the "Daughter":
 - A) Create a new record for the UCID for the split (Daughter). This record will have a new sample ID (auto assigned).
 - 1) Fill in Sample Type, Sample Date (original date at which blood or urine was obtained); Exam Year (Exam at which blood or urine was obtained); Nothing entered in Freezer, Shelf, Tray etc; Date Distributed – (mmddyy); Project Code for sample distribution; Split or Return- enter SD (daughter sample); Split date- (mmddyy); Split for-(enter project code); Split from

(enter Mother sample ID #). Do not enter anything into the Return Date or Return From fields.

2) NOTE: In the Split For (project) field, you will put the same project code as the one you entered in the Project Code field under date distributed. In the Split From field, you will enter the Sample ID of the sample from which the split was taken.

B) Since the original samples will need to be removed from their boxes for the process removing the aliquots, they will be returned to the freezer in one box. Therefore, in the original record of the Mother sample from which the split sample was taken: Enter the new location of the sample; Freezer, Shelf, Tray, Box, Slot.

1) To indicate that an aliquot was removed from the "Mother" sample, on the "Mother" record enter Split or Return (SM); Split date-(mmddyy); Split for (Project Code): Do not enter anything else into other fields .

2) NOTE: If the last portion of the "Mother" (SM) sample is distributed, then fill in Date Distributed and Project Code on the "Mother" record.

3) If a second split is taken from the sample and there is still some of the mother remaining, use the Comments field on the Mother record to enter the second split information: Split or Return (SM); Split date-(mmddyy); Split for (Project Code). You will need to enter the split into a new record, as described about in 6.A.1.

7. Returned Samples: If a sample sent out has been returned:

A) Entire sample sent out and returned to us unused- Do not create a new record.

To the original record that contains the information that the sample was distributed, change the code in the Split or Return field to RX. Fill in the Return date and Return from Project code. Edit the record so that it now has the new sample location: Freezer, Shelf, Tray, Slot.

B) If entire 1 ml sample is distributed, and then remaining amount is returned after use in a lab, record the return of the sample in the "mother record". Fill in Return (R), Return Date (mmddyy), Return from (project). Edit the record to indicate the new location of the sample: Freezer, Shelf, Tray, Box, Slot.

NOTE: In the field for Return from (project) you will enter the same project code as the one you entered in the Project Code field under date distributed. Do not fill in any split sample information. In the comment field note that only approximately xx of the sample remains.

C) For a split sample distributed to lab (split or Return = SD) and then returned after having been used by the lab-Go to Daughter record and fill in (R) for Return, Return Date- (mmddyy) and Return from- (Project). In Daughter record, fill in sample location (Freezer, Shelf, Tray Box Slot). Do not add any information to the "Mother" record.

D) Split sample sent out, but not used and then returned- Go into the daughter record and fill in (RX) for Return, Return date-(mmddyy), Return from (Project). In Daughter record, fill in sample location (Freezer, Shelf, Tray Box Slot).

E) When Mother samples are pulled from freezer but never distributed-. Change the location of the samples to the current location (Freezer, Shelf, Tray, Box, Slot). In the Comments field, note that sample was pulled for XXX project but never distributed, and then returned to a new location in the freezer.

DNA extracted from FCC whole blood

1. DNA aliquots returned to FCC after extraction from whole blood
 - A. Place the DNA aliquot tubes in a box and create a box map for the DNA aliquots
 - 1) Assign a distinct inventory number to each box (DNAXXX); add additional box numbers as needed. Add these box numbers to the file Inventory-Freezer - Shelf -Trays_box_May2009.xls in S:\OCCH\Fernald Residents\FreezerInventory.
 - 2) Create a box map for each box.
 - 3) On the box map, in the slot area, note both the UCID and aliquot sample ID (which will be different from the sample ID assigned in the FCC sample inventory data base).
 - B. Pull up UCID. Create a new record for each DNA aliquot tube from the original sample, using auto assign sample number.
 - 1) Enter sample type (DNA).
 - 2) Enter the sample date (the date when the DNA was extracted).
 - 3) Enter the Exam Year when the original blood sample was drawn.
 - 4) Enter current location of the DNA sample (Freezer, Shelf, Tray, Box, Slot, and year drawn (the year the blood was drawn).
 - 5) In the area of the form with green font, enter the sample ID of the whole blood sample from which the DNA was extracted. Enter the exact number on the tube (Tube No.) DNA in the tube may be from the first, second or possibly third extraction through the column. (Information should be obtained from the person who did the DNA extraction). For DNA Order, select First, Second or Third.
 - C. Leave date distributed and Project code blank – these fields are not used for DNA samples.
 - D. If No DNA was able to be extracted from the whole blood sample (because of clot or degradation), go back to the record for that blood sample, and in the Comments field, note that “no DNA extracted because of XXXXXX”.
2. DNA distributed (plated) for genotyping
 - A. Pull up UCID. Find the record for DNA aliquot (tube) that you are going to use for plating. In the green font area, click on “DNA Splits”.
 - 1) Enter the Sample ID on the manifest, the date that the DNA was distributed (plated), and the Project code. Enter the plate number on which this DNA was plated.
 - B. If plated DNA should be returned, go into the record for the DNA aliquot (tube), and then DNA Splits, and note the Return Date and the Return From (Project).

Maintaining the hard copies of the freezer pulls and transfers.

1. The freezer pull list, sample pull record, and original checked box map used during transfer will be filed in offices at Kettering.
2. The original signed transfer log will be filed in the offices at Kettering.