Submitting Samples to the KOMP Analysis Pipeline
Date: 8/12/2017

We want to encourage investigators who have developed or studied mice that have abnormalities in their skeletal health to submit these animals the KOMP-bone characterization pipeline. It is our goal to develop a publically shared database of mice that have been characterized in an objective and consistent manner so that inter-laboratory variations in technique and interpretation can be eliminated. We also want to provide an attractive resource for bone histomorphometry that is comprehensive, relatively low cost and rapidly produced.

The workflow (see left panel of the website, www.bonebase.org) is designed to populate our web-database with the results of investigator-initiated studies that eventually can be searched by the skeletal biology community. However, data that is posted to the web-database will be restricted to the investigator who initiated the study until we receive his/her permission to make the information searchable by the public. It is from that site that the investigator can download results in a excel format to be utilized for their publications. Images of the histology can also be obtained from the site.

From a cost/efficiency perspective, the workflow is based on a sample size of 8. The µCT and the histology routine are all geared to process the work in 8 sample blocks. Thus the investigator is requested to provide the 8 samples from each site per sex since the cost and effort is the same if fewer samples are provided. In addition our statistics become less meaningful when fewer than 6 samples are available for the analysis, so an experiment with fewer than 6 acceptable samples will not be available for distribution to the public on the web site (will still be available to the investigator). The analysis includes both sexes and two skeletal sites (axial and appendicular) because our KOMP studies clearly show dimorphic differences at both sites and between each sex at each site. We want to provide a comprehensive view of the impact of your genetic model for each skeletal system and each sex. The web portal describes how we envision (see vision link) this phenotyping capability can expand to other research platforms.

Table 1 illustrates the three models for our µCT/histology workflow that is designed to intersect with an external research groups that want to submit samples into our workflow (Models 1A and 1B, mice generated external to the IMPC program). The one exception is Model 1C in which the breeders are sent to UCONN for generation and harvesting of the KO/KI and control lines. The model also can be applied to KO

Modes of Interaction Between an External PI and the KOMP Pipeline

	Breeding, Genotyping. Sample Collection		µCT		Histology				Data Generation	
					Embed Section		Mount Image		Image Analysis	Place result on web
	Ext PI	KOMP	Ext PI	KOMP	Ext PI	KOMP	Ext PI	KOMP	KOMP	KOMP
Model 1A	x			x		x		x	x	x
Model 1B	x		xx			x		x	x	x
Model 1C		x		x		x		x	x	x
Model 2A	x			x	x			x	x	x
Model 2B	x		x		x			x	x	x
Model 3	x		x		x		x		x	x

Table 1: Different modes of sample submission, work up and analysis between an external user and the KOMP pipeline. Model 1A is the full analysis being performed by the KOMP staff of externally submitted samples, while 1B the µCT is performed by the PI and histology performed by KOMP. Models 2 is designed for an institutional histology core to perform some of the embedding and sectioning prior to having the sections scanned and imaged by the KOMP staff. In Model 3, the extenal staff performs all the µCT and histological steps, but the image analysis and data presentation is performed by the KOMP staff. They are not discussed in this document.

lines that were acquired from the IMPC program. The reanimated homozygous or heterozygous breeders can be expanded at UCONN and do not need a control for the analysis since they already are being used for the KOMP studies. In addition to saving the external PI group the need to breed/harvest/send, model C has the advantage that the microbiome and breeding/harvesting techniques are similar to those used in KOMP. Models 2 and 3 is designed for the external group to perform an increasing number of the workflow steps to reduce cost while maintaining consistency of the analysis and data presentation.

Preliminary planning:

A statistically meaningful study will require 8 male or female animals from the test and control group. The minimum age is 12 weeks of age to ensure that the mice are beyond their rapid somatic growth phase. Because it may not be feasible to enlarge the mouse colony to obtain the desired number of animals, our protocol allows you to collect samples as they achieve of desired age and send them to us as they are harvested. It is important to plan to harvest all animals within 2-4 days of the same birth age. We will accumulate the samples under conditions that will preserve enzymatic activity until the desired number is met. Our workflow is optimized to utilize 8 males and 8 females for the test group and the same numbers for the littermate control group in which both long bone and lumbar vertebral column are acquired for analysis. Once the full sample number is accumulated, they will be processed together. The analysis will progress in 3 stages.

Stage 1: Steps required of the requesting laboratory (figure 1):

a) A password protected web portal to our data management will be established and we will instruct you directly in how to utilize the site for data entry.

b) You will provide background information into the web portal on the mice to be studied. It will include the formal genetic information with maps if possible and whether the animals underwent any treatment. Other non-skeletal features should also be included.

c) With the exception of Model 1C, we will send you labels, sample vials for the samples as they are harvested. The web site will provide the naming convention that is consistent with our workflow. More than 8 labels for each mouse group will be provided in case some of the submitted samples need to be replaced.

d) Breed the test and control animals: Identify each animal with a unique ear tag or other identifier and associate it with our nomenclature. Record the birth date, weight of each animal, the dates of injection of mineralization dyes prior to sacrifice and the sacrifice date.

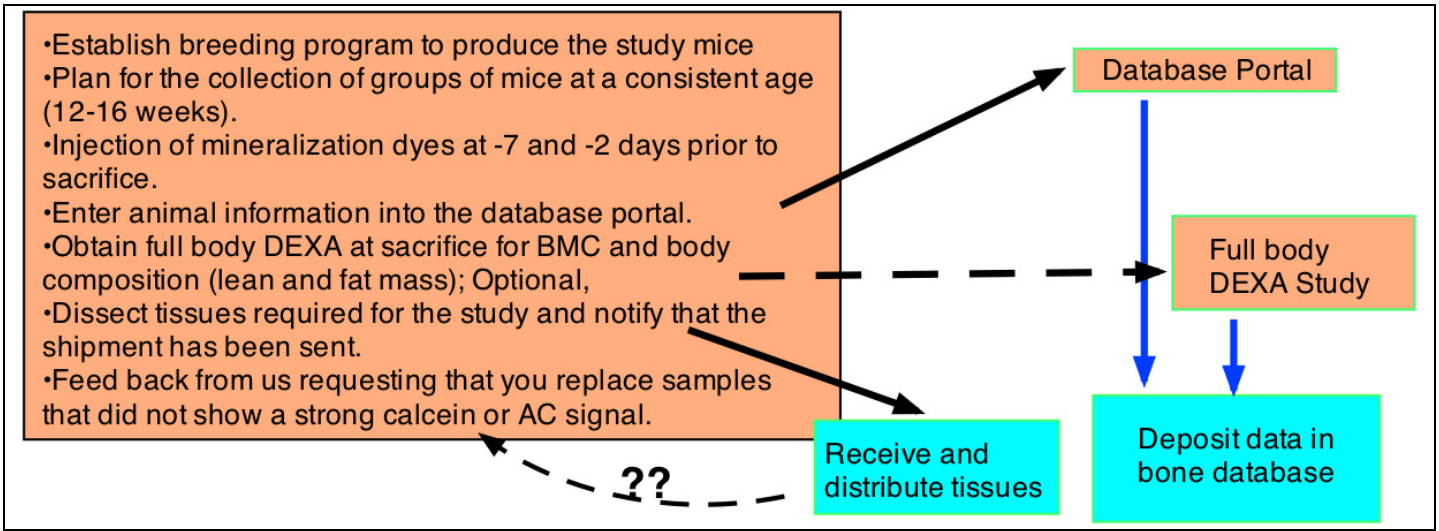

Figure 1: The steps by external user (brown) that interface with the KOMP pipeline (blue). The required information is entered into a password protected webportal. We will report back to you **(?)** if an animal is missing a mineralization label, and we will request a replacement animal. Plan to sacrifice the animals with 2-4 days of the same birthage.

e) Perform whole body DEXA study (optional): Enter the results performed at your site for the corresponding animals into the web portal. This study provides somatic data (fat and muscle content which may provide additional information relevant to the bone study).

f) Dissect tissues for study and fix in ice-cold 10% formalin. The details of the sample dissection, sample labeling, fixation volumes and containers are described in the protocol. Exposing the bone marrow space to the fixation medium is important for optimal morphology.
g) You need to check the tail snips for brightness of the red and green mineralization labels. Replace animals if either label is missing. We will check also, but if you can pick up the problem, it will save time.
h) Ideally a schedule of the calcein injection on Wednesday, AC injection on the following Monday.
i) Send the freshly harvested samples by overnight mail in wet ice with an email notification. Please have the sample information entered into the webportal prior to sending.

Stage 2: Steps required for tissue analysis (figure 2):

Once all the samples have been collected, they will be entered into the workflow in units of 8. All the test and control samples from either sex will be processed simultaneously.

a) µCT Study: The right femur and vertebra L4-L6 will be used for the µCT study. When the study is completed, you can decide if you want to progress to histomorphometry. Our webportal or a direct interaction with our program staff can help you make the decision as to whether further analysis at the histological level is likely to be worth the additional expense.

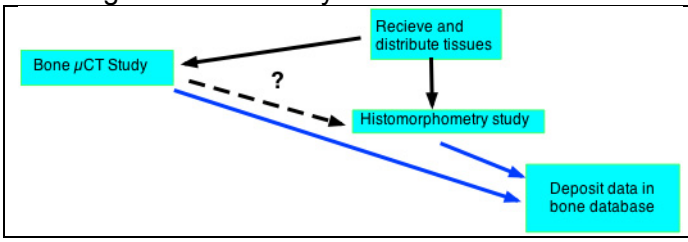

Figure 2: Internal processing of the completed sample set. Bones are first imaged by µCT and the results reported in the webportal. The user decides **(?)** if the analysis will continue after the µCT results are examined. Once permission to proceed it received, the histomorphometry is performed and the results deposted in the webportal.

b) Histomorphometry study: Once you decide to progress to histomorphometry, we will enter the samples into the histomorphometry pipeline. See the protocol for sample embedding and sectioning. Once completed, the plastic slides containing the sections will be shipped to UCHC where our staff will mount on glass slide for the imaging and image analysis steps.

3) Analysis, interpretation and dissemination of results (figure 3):

The visual images (original-microscope and computer-interpreted) and the calculated results from both studies will be placed in our LIMS database and transferred to the web portal where they can be viewed. The data is password protected so you can control who sees the information. The webportal will contain our interpretation of the data. You will be able to download the data files and the visual images that populated the webportal from our LIMS system for your further analysis and publication purposes.

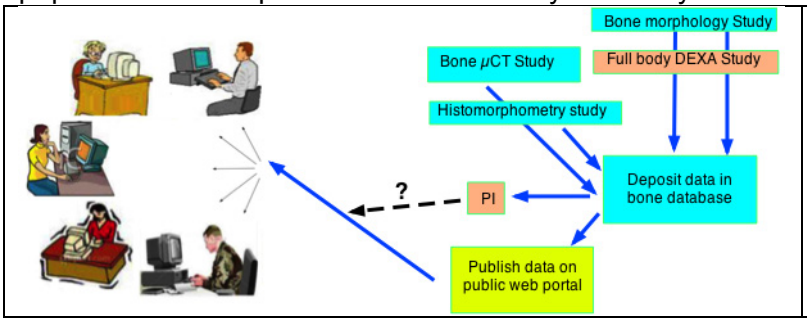

Figure 3: Accumulation of the datapoints from the bone studies within the laboratory database (LIMS) and its subsequent uploading to the webportal for the private use of the submitting investigator. Once approval is obtained from the PI **(?)**, the data will be made available to the general public.

Upon gaining your permission, we will post the results of your study on the web data site with full acknowledgement of your contribution. It will be included in the searchable studies performed through the KOMP project.

4) Initiate the study:

Once an agreed upon study is established (see study costs), an experimental protocol will be developed on our web portal that will list all the samples that will be collected. We will send you the labels that will identify the samples prior to initiating the collection. The website will ask for the details of the animals as they are collected that include your identification number, birthdate, sex, date of calcein and AC injections, weight of the animals taken for the calcein injection and date of sacrifice. More detailed information such as size of litter, breed pair id, sex of parent who contributed the mutation, temperature of

the animal room, chow feed to the animals will be requested but is not needed for us to proceed with the study.

It is important that you follow the detailed steps for injecting the animals for the mineralization dyes and for collecting the samples. We anticipate that a number of mice in each group will be collected and grouped as a batch as they reach the desired age. Each collection will receive a batch number that will be assigned in the web portal to tell us when a group has been collected. This batch collection will be repeated until the full number of animals in each group is collected.

5) Progress of the pipeline:

You will have access to the progress of your samples through our analysis pipeline. You will be able to view the images and values of all the individual samples from the database as they are deposited. Upon completion of the study you will be able to download excel tables of the results and the full resolution images from our web server. The dual video conference can be arranged by direct email communication and the results of the meeting will be included in the database. The information gathered in the database and its interpretation will be submitted to the public website.

6) Study Costs:

We have established a set price for a complete analysis that is significantly less than the traditional per sample pricing because our workflow is optimized to perform a complete study consisting of 8 samples (3 sections per sample) from males and females that include the femur and vertebra. We want to encourage users to submit a complete set of samples that are used for the screening µCT study first (by us, Model 1A or by you, Model 1B), followed by the morphology and histological study at the discretion of the

Table 2: Cost of study by Model 1B
(You do the µCT study and we do the histomorphometry)

Bundled Prices	NIH funded Non-profit	Commercial/ For profit.
Histomorphometry for the 32 samples (3 replicates per femur and vertebra) from the test group that was characterized by µCT.	$2,750	$3,500
The same study will be required of the control because each inbred line varies, particularly after genetic manipulation Control group for 1 test line	$1,500	$2,000
If the KO line is recessive and a heterozygote will be included in the study	$1,500	$2,000

- If you choose not to have the information published, add 50% for both the test and control samples.
- If the study was performed under the agreement conditions that permission to open the results to the general scientific community through the web portal would be granted but the permission is never received, we reserve the right to open the portal 2 years after the entire study process has been completed.
- The study will be performed prior to submission of an invoice for payment. The results will be available in a read-only format until the payment is received. Then the entire dataset will be available by direct downloading of the excel data and jpg image files.

Table 3: Cost of study by Model 1A and 1C:
(We do the µCT and the histomorphometry study)

Bundled Prices	NIH funded Non-profit	Commercial/ For profit.
µCT for the 32 samples from the test group.		
From UCONN breeding	$1,500	$2,250
Shipped from PI to UCONN	$2,000	$3,000
Add Histomorphometry for the 32 test samples.	$2,250	$3,000
Each control or heterozygous group for µCT	$1,000	$1,750
Each control or heterozygous group for histomorphometry	$1,500	$2,000

See table 2 for requirements for this cost structure.

submitting customer. The PI will be responsible for the breeding, harvesting and shipping of the samples to UCONN for the analysis (histomorphometry and µCT) as well as entry of the sample details into the web portal that is established for this study. In Model 1C, the samples are produced locally (see table 4) which reduces the expenses because all of the sample identification is performed locally and avoids the expense of interactions with the PI's laboratory staff.

Model 1C is based on the PI submitting breeders to UCONN and having our KOMP staff perform all the breeding, weaning, genotyping and sample collection (table 4) prior to performing the µCT and histological analysis. It is primarily designed to encourage PI to utilize KOMP-derived lines and all of the

Table 4: Cost for Breeding, Genotyping and Sample Harvesting at 12 weeks

Bundled Prices	NIH funded Non-profit	Commercial/ For profit.
IMPC Derived line – homo x homo (no control or genotype needed).	1,800	2,400
IMPC Derived line – het x wt (genotyping required, but no control needed)	3,750	5,000
Non-IMPC line – homo x homo and wt x wt (quarantine, genotyping)	4,500	6,000
Non-IMPC line – het x wt -> het, wt (quarantine, genotyping)	4,500	6,000
Non-IMPC line – het x het -> homo, het, wt (quarantine, genotyping)	7,500	10,000
Non-IMPC line – het1 x het2 > het1,2, het1, het2, wt ((quarantine, genotyping)	8,250	11,000

steps used in the standardized protocol for bone phenotyping. The PI needs to arrange the purchase of the breeder mice from IMPC and have the mice shipped to UCONN for expansion and analysis. Table 5 estimates the cost for the complete µCT and histology analysis once the breeder animal are introduced into our KOMP colony.

Table 5: Estimated Cost for a µCT and Histological analysis of an IMPC-Homozygous KO Line

Homozygous KO breeders obtained by PI from IMPC	NIH funded Non-profit	Commercial/ For profit.
Breed and harvest tissues from an IMPC Derived line homo x homo (no control or genotype needed).	$1,800	$2,400
µCT for the 32 samples from the test group.	$1,500	$2,250
Histomorphometry for the 32 samples from the test group.	$2,250	$3,000
Total	$5,500	$7,650

Histomorphometry performed in part or in totality by an
Institutional Core Facility

Models 2 and 3 will require training of the histology staff from the participating site to produce sections and images that fit into the workflow. Workshops are being scheduled to introduce users to the methods used from which a decision by an institutional core facility would make the decision on which model they would prefer to use. The estimated user cost for either model is shown below.

Model 2B
(Institutional Core performs some of the sample collection and processing steps, but the imaging and staining and image analysis steps are performed at UCONN.)

Bundled Prices	NIH funded Non-profit	Commercial/ For profit.
Histomorphometry for the 32 samples (3 replicates per femur and vertebra) from the test group that was characterized by µCT. This cost assumes that we will obtain your permission to display the results in the web portal.	$1,500	$2,500
The same study will be required of the control because each inbred line varies, particularly after genetic manipulation. We will reduce the cost of the control in proportion to the number of test lines that are studied and have the same genetic background. Control group for 1 test line	$750	$1,250
Total cost of test and controls: 1 test and 1 control	$2,250	$3,750

Model 3B
(Institutional Core performs all of the sample collection and processing steps including the imaging and staining step. Only the image analysis and web portal steps are performed at UCONN.)

Bundled Prices	NIH funded Non-profit	Commercial/ For profit.
Histomorphometry for the 32 samples (3 replicates per femur and vertebra) from the test group that was characterized by µCT. This cost assumes that we will obtain your permission to display the results in the web portal.	$350	$500
The same study will be required of the control because each inbred line varies, particularly after genetic manipulation. We will reduce the cost of the control in proportion to the number of test lines that are studied and have the same genetic background. Control group for 1 test line	$175	$250
Total cost of test and controls: 1 test and 1 control	$525	$750

If either of these models might work for your institution and you would like to explore the concept further, please contact David Rowe at drowe@uchc.edu.