Mice exposed to DSS – dissection – walkthrough of things to remember

Time: 2h of preparation, 6/hour when you are dissecting

**Before the dissection:**

Things to prepare:

-histology: yellows cassettes, write “SANTOS” on the side, date on the other and mouse ID at the front. You need to prepare small pieces of the special paper that you will later use. Also, you need to prepare formalin boxes where the histology cassettes will be placed. Formalin is found under the fume hood.  
-Stools: Eppendorf tubes + Dx. They will be used for qRT-PCR and 16S sequencing. Marked Fs on the side of the vial, mouse ID on the top.  
-RNA later Colon: Eppendorf tubes. pour 0.75 ml (750 ul) of the product in vials (1 vial = 1 mouse), can be done before the experiment and outside of the fume hood (la hotte). A “R” sign is used to differentiate the vial.  
-Protein (in my case, colon): Eppendorf tubes. Prepare vials with mouse ID, and a mark such as “p” to differentiate with other vials.

-Iron cc – Eppendorf tubes Liver – frozen – nitrogen. Mark with “L”

-Iron cc – Eppendorf tubes Spleen – frozen – nitrogen. Mark with “S”  
  
-Blood (no CVC): Extract serum - Eppendorf tubes. yellow capped vials with mouse ID on the side, and short ID on the top of the cap. Also, need to prepare vials for after the blood is centrifuged. Vials with mouse ID on the top, and the letter “S” to differentiate from other vials.  
-You can prepare papers with mice IDs written on them, the one on which the colon will be dissected (for the picture taken)

Print: -a paper with colon size, spleen and liver weight measurements for the one working on dissecting the mice and doing the swiss roll and everything.  
-a similar paper but with only the body weight, for the one working on extracting the blood and weighing the mice.  
-a list of the mice dissected for the day, for the people from the pathology (that receive the histology cassettes), the list is given to Gabriela after the experiment

**Day of the dissection:**

-For the RNA later, take a big bowl of ice, in which the vials will be placed once there is colon parts inside  
-Get liquid nitrogen

**Steps:**-weighing the mice  
-getting blood (through the ocular thing)  
-dissecting the mice and removing: colon, spleen and liver  
-Weighing spleen and liver.   
-Measuring colon size  
-Removing and collecting stools  
-Open the colon and stick it to the paper on which it’s dissected  
-Separate in two parts in the middle.   
-Right part is separated in one bottom one top part. Top part is used for RNA later, bottom for protein assay.   
-Left part is used for histology. Make a swiss roll and then compress the thing into the cassette’s paper.

Repeat for each mouse

-The protein and stools vials are placed into the liquid nitrogen container once they have something in it.  
-Take as much stools as possible when dissecting the mice (they are used for 16S but also qRT-PCR).

**After the dissection:**

-Put the filled blood vials into the centrifuge machine for 5 minutes at 12000 rpm. Then extract the liquid and put them into the “B” marked vials.   
-Once the experience is finished, the RNA later vials are transferred from ice to -80, and same for the centrifuged blood, the protein and stools vials are transferred from liquid nitrogen to -80 refrigerator as well.  
-The histology cassettes contained into the formaline are placed into a refrigerator and the list is given to Gabriela.  
-Don’t forget to remove the mice from labtracks! (removing a cage automatically puts them as dead).