**Bile Acid Extraction**

**Materials:**

* Gloves
* Pipettors
* Pipette tips
* Liver sample
* Ethanol
* Methanol
* Hand held homogenizer
* Probe sonicator
* Eppendorf tubes
* Autosampler vials
* Crimper
* **Prepare sample:** 
  + Weigh out 50 mg of liver tissue or feces and place in an eppendorf tube
* **Homogenization of the samples.**
  + Add 1mL µl of ethanol to each sample.
  + Add 6-8 beads into each sample.
  + Homogenize the samples for about 30 seconds or until the liver is clearly disintegrated.
* **Incubation of the Samples** 
  + Incubate the samples at 60 degrees for 30 minutes in the shaker
  + Let the samples cool to room temperature
  + Once they cool down, incubate the samples at 100 degrees for 3 minutes
    - Under this incubation the tops of the caps should be open because the ethanol will boil
* **Extraction of the Bile Acids**
  + After the samples are cool from the incubation, spin them at 1600 g for 10 minutes.
  + After, save the supernatant and place in a separate tube.
  + Add 500 µl of **ethanol** to the dry pellets and vortex for one minute
  + Centrifuge the samples at 11200 g for one minute
  + Save the supernatant and add it to the previous samples, respectively.
  + Add 500 µl of ethanol to the dry pellets for a second time and vortex again for one minute
  + Spin the samples at 11200g for one minute.
  + Combine the supernatant and add it to the other supernatants, respectively.
* **Preparation for metabolomics:**
  + **Dry down** the pooled supernatants in a drying centrifuge
  + Add 500 µl of **methanol** to the dried samples.
  + Vortex the samples and spin them at max speed for one minute.
  + Add 300 µl of sample to autosampler vials.