Seahorse checklist

Day prior to assay:

1. Make mitochondria media buffer stock solutions if needed (glucose, pyruvate, glutamine).
2. Hydrate plate.
3. Put 50 mL of XF calibrant in incubator.

Day of assay:

1. Bring incubator to JCSMR and set to 30°C.
2. Set up template at JCSMR.
3. Prepare mitochondria media. Put aliquot of 8 mL in incubator at 30°C.
4. Prepare mito stress chemical stock solutions and dilutions.
5. Collect livers.
6. Process homogenates.
7. Dilute homogenates.
8. Plate homogenates in sample plate.
9. Keep 100 ul and 10 ul aliquots of homogenates (store in -20). Bring rest of homogenates to JCSMR for flow cytometry (these should be kept in media and treated the same as samples (e.g., on ice or in incubator).
10. Centrifuge, off-gas (in incubator), and inspect.
11. Go to JCSMR (check list of things to bring).