ATPase Sampling Protocol

Materials/Equipment:

- Sucrose (Sigma cat. no. S9378)
- Ethylenediaminetetraacetic acid, disodium dihydrate [EDTA] (Sigma cat. no. E4884)
- Imidazole (Sigma cat. no. I0250)
- 500 ml glass storage bottles (Fisher cat. no. 06-414-1C)
- Magnetic hot plate stirrer (VWR cat. no. 12620-970)
- Magnetic stir bars (VWR cat. no. 58948-150)
- pH meter (Fisher cat no. 13-645-521)
- Metal blocks (2x USA Scientific cat. no. 9124-2000)
- 0.5 ml tubes (VWR cat. no. 87003-290)
- Disposable transfer pipettes (VWR cat. no. 16001-180)
- Forceps (Fisher cat. no. 12-000-127)
- Curved dissection scissors (Fisher cat. no. 08-951-10)

Sample Collection

1. Make SEI buffer:
    - 42.79 g Sucrose
    - 1.86 g EDTA
    - 1.7 g Imidazole
    - Dissolve all into approximately 400 ml diH_2O
    - Adjust pH to 7.3, adjust volume to 500 ml with diH_2O, store at 4° C
2. Place metal block and SEI buffer on ice.
3. Using transfer pipette, fill 0.5 ml tube about halfway full of SEI buffer.
4. Cut a small section of gill, including filaments and arch, and place tissue into SEI buffer, about a 1:20 ratio of tissue to buffer is ideal. The tissue tends to become gluey if there is too much in the tube.
5. Using a transfer pipette, top off tube with SEI buffer. Make sure that the tissue is completely submerged in buffer.
6. Storage Options:
    a. Leave samples in the metal block on ice until they are transported to a freezer at the end of the day. If the available freezer is not a -80° C, transfer to a -80° C as soon as possible.
    b. Freeze samples periodically during sampling with dry ice or a -20° C freezer. Transfer to -80° C as soon as possible.
    c. Freeze at -80° C periodically during sampling.

    (The general theme here is keep samples as cold as possible given available resources and transfer samples to -80° C as soon as possible)