Standard Operating Procedure for
Leica EM AFS2

AFS2 Set up

- Plug in and turn on the AFS

- Choose User name and input temperature ramping program or Choose a program that is already set up

- Fill AFS with LN_2:
  Attach screw-on funnel to nitrogen port. Pour LN2 into the funnel until the fill gauge reads:
  - 50% for 3 day program
  - 75% for 4 day program
  - 100% for longer

- Start the program, pause it at the starting temperature for the chamber to cool down. It can take up to 1 hour for the sample chamber to reach the desired starting temperature.

- Place your freeze subsitution solution in a 20 ml scintillation vial or 2ml cryo tube in the AFS chamber to equilibrate.

Sample transfer into AFS

- After HPF, place your frozen samples in cryo tubes and store under LN2 until you can do your FS run. After the AFS has reached the desired starting temperature (-90C or lower), take out the sample tubes from the LN2 storage, quickly dump out any remaining LN2 in the sample cryo tubes and place them into AFS chamber. Use a rack or one of the small metal containers to keep the tubes upright.

- Allow the sample to equilibrate, add the pre-chilled freeze subsitution solution from the scintillation vial to the cryo-tube. If you use a pipet make sure it is pre-chilled, better to pour directly.

- Resume the program (or restart if it was running).

NOTES

- This whole process is extremely dependent on the samples staying frozen at all times!
- The best results will come from slow and smooth temperature ramping, but a single moment of thaw/refreeze before fixation is complete will ruin the sample.
- For every step, consider the temperature of all tools, solutions, jars and surroundings. Make sure everything is always at the correct temperature, and always minimize the time transitioning between areas (ex: LN2 to AFS).