Protocol for Ussing Chamber Recording

1. Turn on 1) the water bath (42 ^{o}C) (NESLAB EX7, Thermo Fisher Scientific, Newington, NH, USA), 2) multichannel voltage/current clamp VCC MC8 (Physiologic Instrument of Medical Research, San Diego, CA, USA), and 3) computer.
2. Preheat all test solutions to 37^{o}C in a water bath.
3. Connect electrodes (two black and two white for each chamber, filled with 4% agarose/3M KCl in the tip and backfilled with 3M KCl) to each chamber in left-to-right order white, black, black, white.
4. Add 5ml bath solution to each side of a chamber (get rid of air bubbles !!!).
5. Turn on the gas cylinder (mixed gas of 5% CO_2 and 95% O_2) and connect the air supply to each chamber half.
6. CORRECT ELECTRODE OFFSET POTENTIAL: Press the MASTER OVERIDE switch to "ON", the FUNCTION key to "OPEN" and METER switch to "V" to test all 8 channels. . The panel meter will display the difference potential between the pairs of voltage-sensing (black) electrodes. Press the OFFSET button to to the polarity opposite that displayed on the meter and use the dial to adjust the offset so that the meter reads 0.0.
7. LIQUID RESISTANCE CORRECTION: Switch the meter to I. Press the PUSH TO ADJ button to pass current across the fluid in the chamber. The meter should read ~64μA. Switch the METER to V. Press and hold the PUSH TO ADJ button and adjust the FLUID RES COMP so that the displayed voltage reads zero. The resistance of the fluid in the chamber can be read from the fluid resistance dial. This value depends on the slider used and temperature of the bathing solution. For the P2306 slider at 37^{o}C the resistance should be about 540 Ohm).

8. MOUNTING TISSUE PATCH/MONOLAYERS:
   Working with one chamber at a time, perform the following:
   a. Recheck offset and fluid resistance just prior to removing the slider to mount the tissue. Readjust if necessary.
   b. Remove the air tubes from the chambers;
   c. Suck the solutions from each side, loosen thumbscrew and spread chambers apart.
   d. Remove the slider, dry and replace layer of silicone vacuum grease. Remove any saline in the space between the chamber halves.
   e. Place the tissue over the aperture in the slider;
   f. Insert the slider containing the tissue or cell culture into the chamber so that the apical side of the cell faces to the left. Ensure that no saline reaches the blue heatblock as this can cause crosstalk between chambers.

g. Tighten the thumbscrews. These should be just tight enough that the chamber does not leak fluid, but not so tight as to cause damage by crushing the epithelium.
h. Replace the solution in each chamber half. Be careful to remove any trapped air bubbles.
i. Reconnect air tube.

9. With the FUNCTION switch on OPEN and METER on V look at the value of the open circuit voltage. Press the PUSH TO ADJ button and calculate the tissue resistance from

   $R_t = \Delta V/\Delta I$ (x1000 to get value in Ohms)

   This will provide a quick estimate of the quality of the tissue.

10. Press the FUCTION switch to CLAMP. Observe that the meter shows 0.0 for voltage. Switch the METER to I and observe the short-circuit current. The reading should be reasonably stable.

11. Open the 'Acquire and Analyze 2.3' software on the computer desktop. Verify that "DAC initialized" appears at the bottom left of the screen.

12. Click File/New experiment. Enter a file name. Select chambers from which to collect data. Choose chamber (s) individually or Shift-click to select all. Set aperture area: tissue 0.04 cm^2, snapwell 1.12 cm^2. Press Enter.

13. Open the Reference form (Acquire/Reference). Make sure the reference correction check box is checked.

14. Press the REM switch on each channel or by using the MASTER CONTROL section. To enable computer control of the clamp.

15. Press the Reference button and verify that the values reurned for each channel are reasonable (not red). Press OK.

16. Click "Running man" icon to start recording. Click "Traffic lights" to alter sampling rate.

17. For multiple windows, minimize the active window first; Graph/Create or Fire icon. To save the multiple windows as a template: Graph/View editor/Save.

18. STOP EXP:

a. Wash the chamber with dH_2O after each experiment. Suck off the water with tissue paper.
b. Immerse the tips of the electrodes in physiological saline (sans glucose) or 3M KCl. (I think the saline will provide for less electrode drift because the diffusion of KCL into the saline will be in a steady-state).

19. Turn off power to the amplifier and water bath. Close the main valve on the gas cylinder.
20. Transfer data to Excel or OriginLab for analysis