Adult Mouse Cardiac Myocyte Isolation and Culture
(Dr. Kranias laboratory protocol)

I. Clean the modified Baker apparatus (do before and after prep)

- Rinse each syringe with 70% EtOH
- Rinse with filtered ddH20 through both syringes (~ 300ml each)
- Rinse with filtered ddH20 with P/S through both syringes (~80 ml each)

II. Prepping the Baker apparatus

1. Turn on circulating heater so that the outflow at the tip of the cannula is 37C.
2. Run Perfusion Buffer through both syringes to clean out any remaining water. Be sure to get all air-bubbles out of the system.
3. Add Perfusion Buffer to the left syringe
4. Add Digestion Buffer to the right syringe

III. Dissection and Perfusion/Digestion

1. Prepare dissection area by laying out:
    a. Sterile dissection tools (forceps & scissors)
    b. paper towel
    c. 4 pieces of tape 1.5" long
    d. Two 100MM petri dishes with ~ 60ul of 1000u/ml Heparin filled with perfusion buffer.
    e. Put the 20 gauge cannula on a 1cc syringe and fill with perfusion buffer. Set it up for cannulation in second petri dish.
    f. Cut ~1.5 inch piece of 7-0 silk and rinse in 70% EtOH. Place on the cannula with 1 overhand knot leaving the loop.
    g. Prepare 0.1cc of 1000u/ml Heparin and 0.1cc of Nembutal into a 1ml insulin syringe
2. Anesthetize the mouse with the heparin/Nembutal IP injection.
3. Wipe the chest of the mouse with 70% EtOH
4. Remove the heart and place in petri dish with perfusion buffer.
5. Clear tissue from the aorta, move to the second petri dish with the cannula in it. The aorta should be 2mm long from the entry on the base of the heart.
6. Cannulate the heart and tie the pre-looped suture around the aorta to secure it to the cannula. The cannula should be distal to aortic valve.
7. Depress the syringe and be sure the coronary arteries are being perfused.

IV. Perfusion and Digestion

1. Have a slow drip of Perfusion Buffer going through the perfusion system catheter when you are attaching the cannulated heart
2. Perfuse heart for 4 minutes with Perfusion Buffer at a flow rate of 3ml/min.
3. Perfuse the heart for 10 minutes with Digestion Buffer.-(collect the digestion buffer).
4. Heart should become pale and swollen.
5. When digestion is complete, the rate of perfusion will increase.
6. Remove cannula from Baker App. and place both heart and cannula in a 50MM petri dish. 2.5ml of Digestion Buffer.
7. Remove atria
8. Cut heart in pieces and gently tease the ventricles apart using forceps…
9. Gently dissociate the tissue by triturating.
10. Transfer solution to a 15ml conical tube
11. Rinse 50MM petri dish dish with 2.5ml of Stopping Buffer #1 and add to 15ml conical tube (total volume is now 5 ml).
12. Continue to dissociate tissue by triturating
13. Centrifuge cells for ~ 8 seconds and remove supernatant.
14. Resuspend cells in 10ml Stopping Buffer #2 and place in a sterile 50MM petri dish.

V. Calcium Add-Back

1. Add 5µl of 100mM $CaCl_2$ to cells, mix, and incubate for 4 min.
2. Add 5µl of 100mM $CaCl_2$ to cells, mix, and incubate for 4 min.
3. Add 10µl of 100mM $CaCl_2$ to cells, mix, and incubate for 4 min.
4. Add 30µl of 100mM $CaCl_2$ to cells, mix, and incubate for 4 min.
5. Add 50µl of 100mM $CaCl_2$ to cells, mix, and incubate for 4 min.
6. Rock the 50MM petri dish back and forth and side to side, DO NOT swirl the dish to mix the Ca++
7. 4 minutes After the last Calcium Add-Back (50µl of 100mM $CaCl_2$) Transfer supernatant to a new 15mL conical tube.
8. Centrifuge cells for ~ 8 seconds and remove supernatant.
9. Resuspend cells in 3mL of plating medium.
10. Gently mix cells and plating media using a glass Pasteur pipette
11. Count cells.
12. Dilute cells to $1X10^5$ rod-shaped cells/ml and plate.

VI: Plating myocytes:

1. Set up 6-well plates with cover slips for functional studies under the hood. UV plates and cover slips for 10 minutes.
2. Get an aliquot of laminin and thaw on ice.
3. Once laminin has thawed, put 100µl of laminin on the coverslips in hood. UV for 10 minutes.
4. Aspirate laminin off cover slip and place 200ul of cell suspension on each cover slip for a coverslip for a concentration of 2×10^4 rods/cover slip.
5. Allow cells to adhere to cover slip for 2 hours.
6. After 2 hour incubation carefully remove plating media. Add 2mls of Culture media** or perform virus-infection.

**Culture media should be warmed and equilibrated in incubator prior to adding to cells.