

## **Parabiosis Protocol**

The protocol is a revision based on Kamran et al. JoVE e50556, 2013.

### **Preparation of surgery area and reagents and tools:**

1. Sign up to reserve the surgical room MR4-G0.
2. Bring isoflurane and heating pad.
3. Bring sterile tools: 2 forceps, scissors, Ointment, 4-0 Chromic gut suture and 2-0 silk suture, and 1 ml-syringe.
4. Bring 0.9% NaCl, Ketamine/Xylazine 20 mg/2 mg/ml, Ketoprofen 1 mg/ml.
5. Sterile gloves, Povidone-iodine, 70% ETOH.

### **Preparation of animals:**

1. Use mice of the same genetic background and gender. Similar in body weight.
2. Anesthetize mice by IP injection of Ketamine/Xylazine at 0.1-0.15 cc/25 g body weight (80-120 mg/8-12 mg/kg body weight).
3. Remove the hair from 0.5 cm above the elbow to 0.5 cm below the knee joint.
4. Lay down the mice back to back on the heating pad. Clean up surface by using Providone-iodine 3 times, follow by 70% ETOH 3 times.
5. Cover the mice with sterile drape exposing only the operation area.
6. Put some Ointment on the mouse eyes.

### **Parabiosis**

1. Cut the skin from 0.5 cm above the elbow to 0.5 cm below the knee using sterile scissors.
2. Blunt dissect to detach the skin with the forceps.
3. Use 4.0 Chromic gut suture to suture the knees (in a bended position) between the mice.
4. Suture the elbows similarly by a double surgical knot.
5. Suture the skin of the two mice with 2-0 silk suture, ending with a double knot.
6. Verify the continuity of the suture and confirm the absence of openings.
7. Injection 0.5 ml of 0.9% NaCl subcutaneously to each mouse to prevent dehydration.

### **Postoperative recovery**

1. Keep mice on heated pad until they recover completely.
2. Place mice in clean cage with wet food pellets in it.
3. Give them Ketoprofen for 5 days 100  $\mu$ l/25 g body weight (i.p. 4 mg/kg).
4. Add Antibiotic Baytril in drinking water at XXXX/ml.
5. It takes 10-14 days before blood sharing, which can be confirmed by Western blot.