Conceptus Micro-Dissection for Brain Microarray Analysis

1. After killing the dam (cervical dislocation or CO_2), the uterus is removed and placed on an absorbent towel to blot excess blood. The uterus is transferred to a clean Petri dish (120 mm diameter) filled with buffer (PBS or saline, room temperature or warmer). Sterility is optional. This step takes about 30 seconds.
2. Conceptuses are removed under a stereomicroscope using Dumont #5 watchmaker's forceps. Removal is easiest if the uterine wall is penetrated on the anti-mesometrial face (the side opposite where the blood vessels enter). Remove all conceptuses from their yolk sacs and move to a separate petri dish of sterile buffer before proceeding with the tissue collection. For an experienced prosector, this phase takes about 5 minutes for a litter of 15.
3. EMBRYOS (E13.5 or earlier): "Brain" tissue is harvested under the stereomicroscope by grasping the torso with one Dumont forceps and then removing the head with the other. The dorsal landmark for this cut is the groove where the head joins the neck. The ventral landmark varies with the stage: at E10.5 the site is the cranial surface of branchial arch I, while for E12.5 the cut is made through the mouth. The entire head serves as the specimen. This step (including freezing) takes about 3 to 4 minutes.
4. FETUSES (E15 or later): Heads are removed from each conceptus and placed in a third dish of buffer. To remove the brain, the points of one Dumont forceps are stabbed into the eyes (to hold the head) while the other is used to remove the skin and cartilaginous skull. The brain is then peeled from the open skull using the tips of one forceps. This maneuver is easiest if the brain is manipulated from rostral (beginning at the frontal cortex) to caudal.
5. CRYOPRESERVATION: Place about 5 to 10 ml of liquid N_2 into pre-labeled, 50 ml conical centrifuge tubes. The easiest and most rapid method is to grasp the rim of the tube with a forceps and immerse it in a dewar of N_2. Three tubes can be pre-filled at one time. And placed in a beaker with their tips surrounded by dry ice. This step will be most readily accomplished with the assistance of a second person, since it should be initiated when the brain removal step is nearing completion.
6. Each head or brain specimen is preserved in its own flask. The sample is grasped by one edge using a Dumont forceps, held over a N_2-filled tube (held straight up and down), and then dropped. The tissue will settle to the bottom of the tube. The N_2 should evaporate completely before the tube is capped.
7. Store samples at -70 C until analysis.