

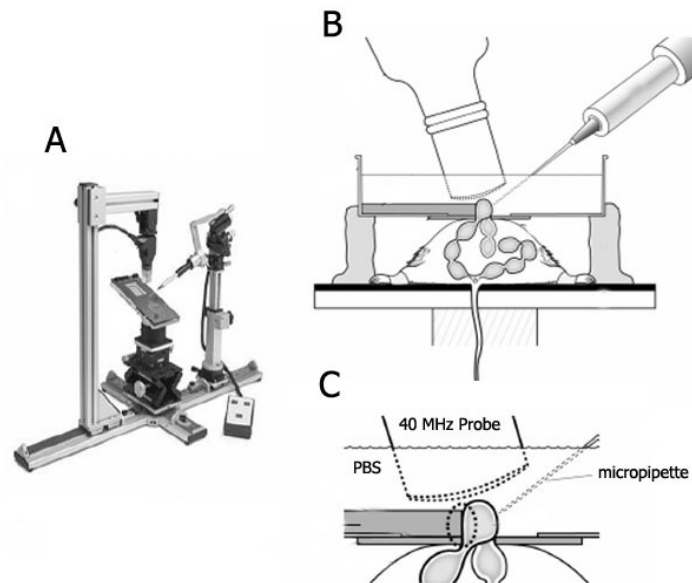
- SOP No:** AHP 79
- SUBJECT:** Embryonic microinjection, *in utero* electroporation, and embryo marking under Ultrasound Biomicroscopy guidance.
- POLICY:** This procedure must be performed by an experienced operator.
- PRECAUTIONS:** Gloves, eye protection, long-sleeved gown, closed in shoes, face mask, hair net.
- EQUIPMENT:**
- Ultrasound Biomicroscope (Vevo 770, Visualsonics Inc)
  - 40 MHz transducer
  - Rail system (Visualsonics Inc. Fig A)
  - Nanoject II (Drummond Scientific Company)
  - Microinjection pipette
  - ElectroSquarePorator™ (BTX Harvard Apparatus)
  - Tweezerrodes (Nepagene)
  - Anaesthetizing chamber
  - Rectal probe
  - Heart rate monitor
  - Animal clipper
  - Sterilised surgical instruments
  - Hair removal cream (eg Nair)
  - Isoflurane
  - 70% ethanol
  - Sterilised Phosphate-buffered saline (PBS)
  - Silk suture

**PROCEDURE:**

*Laparotomy to expose embryos*

1. Restrain pregnant mouse
2. Induce anaesthesia with isoflurane at a dose of 5% in a closed anaesthetizing chamber then maintain at 1.5% by nose cone for the duration of the ultrasound scan.
3. Shave the abdominal fur region of the isoflurane-anaesthetized pregnant mouse with an animal clipper first.
4. Completely remove the remaining fur with a standard chemical hair removal cream (Nair) then cleanse the skin with 70% ethanol.
5. Mount the mouse on the stage of a rail system (Visualsonics, Fig.A). This permits the operator to

- readily adjust the position of the mouse while maintaining the microinjection pipette within the transducer-imaging plane. Monitor the body temperature via a rectal thermometer and maintain at 35°C-38°C using a heating pad and lamp. Monitor the heart rate with electrodes attached to the paws (Indus Instruments).
6. Perform a laparotomy along the vertical midline (~1.5-2 cm incision length).
  7. Gently expose/exteriorise the uterine horn, one side at a time, and count the total number of embryos in each horn before carefully placing them back into the abdominal cavity.
  8. Position a modified Petri dish (100 mm diameter, 25 mm deep) with a 25 mm hole above the vertical incision site. The Petri dish is sealed from below by a thin transparent rubber membrane (40 mm square). The membrane has a 10x1 mm slit cut in the centre, which is positioned above the skin incision. Fine forceps are passed through the slit in the rubber membrane, the skin edges are gently apposed, and pulled up while the dish and membrane are placed against the maternal skin. Pulling up on the skin in this manner helps create a watertight seal as well as keeping the skin accessible so it could later be held in place while retrieving and replacing the uterus. Using fine forceps, the slit edge and its apposing skin edge are lifted to reveal the underlying uterine segment.
  9. Use a second pair of forceps to gently exteriorise the uterine segment by grasping between the embryonic yolk sac/implantation sites to expose 1-3 embryos at the ovarian end of the uterine horn and positioned just below the skin at the incision site.
  10. Support the Petri dish with plasticine blocks and fill with PBS (Fig.B). The PBS serves as an ultrasound-coupling medium, maintains the hydration of the uterus, and is used at room temperature to reduce normal resting myometrial activity.
  11. Support the exposed uterine segment with a semi-circular sheet of silicone rubber that is submerged next to it inside the Petri dish on the side opposite that of the microinjector (Fig.C, inset of Fig.B).
  12. Use the ultrasound biomicroscope (UBM) with a 40 MHz probe to microvisualize the embryo and microinjection pipette (Fig.C).



### *Microinjection and Electroporation*

1. Carefully monitor body temperature, heart rate, respiratory vigour, and anaesthetic depth throughout the ultrasound procedure.
2. Image the microinjection pipette in PBS prior to the experiment.
3. Align the microinjection pipette in the scan plane using the XYZ controls, so that the tip is centred within the focal zone of the transducer (area of image with the greatest resolution). This position, 'the guide point', is recorded by the UBM and remains displayed on the monitor. Retract the pipette leaving the tip in PBS to prevent dehydration-induced obstruction of the lumen.
4. During experiments, move embryonic sites into the scan plane using the XYZ controls on the mouse stage. Position the target region of the embryo at the guide point displayed on the UBM's monitor and then advance the microinjection pipette until the tip is visible within the target region.
5. Accuracy is maintained/improved by ensuring the uterine segment is directly adjacent to the 'uterine

- stabilizer' at the time of microinjection and by the sharpness of the microinjection pipette, both of which minimize the movement of the uterus as the microinjection pipette is advanced.
6. Use a lateral approach to microinject the three-targeted regions to improve the accuracy. This approach avoids passage of the needle through non-targeted regions of the embryo and the associated risk of mechanical injury or deposition of substances/beads in these regions.
  7. Administer the injectate at a specified rate (nL/sec) using the Nanoject II. The injectate comprises:
    - a. plasmid DNA, RNAi vectors, cells or viruses AND
    - b. fluorescent microspheres or inorganic glass microbeads (for marking which embryos have been injected)
  8. Injected substances/beads are echogenic/visible when injected into cavities thereby permitting a visual impression of targeting success.
  9. Coordinate the time of microinjection with the spontaneous motion of the uterine segment (caused by uterine muscle activity) to optimise alignment of the uterine segment against the 'uterine stabilizer' and the path of the microinjection pipette to the target.
  10. In some experiments the injection of DNA or RNA will be followed by electroporation of the construct into the brain.
    - a. Gently place the head of the embryo between tweezerrodes (disc electrodes with a 7 mm diameter (or smaller) tip).
    - b. Deliver electric pulses (30-35V for 50 msec) 5 times with the intervals of 1second using an ElectroSquarePorator.
  11. After injecting (and electroporating) the 1-3 exposed embryos, replace them in the abdominal cavity and carefully remove the uterine segment containing the next 1-3 embryonic sites, pull gently through the water seal membrane into the PBS-filled Petri dish and lay next to the uterine stabilizer.
  12. Patency of the microinjection pipette is confirmed before continuing.
  13. After microinjecting all sites as described in detail above, close the maternal abdomen using silk suture for the peritoneum, abdominal muscles and the skin respectively.

### *Recovery*

1. **Total time for anaesthesia from induction to conclusion of microinjection experiment is one hour.**
2. **Discontinue anaesthesia and place the mouse in a heated recovery chamber. Usually the mouse is awake and active within 5-10 minutes.**
3. **Administer analgesic (buprenorphine, 0.05-0.15mg/kg) sc; lasts for 12 hours.**
4. **Return the animal to a preheated cage (cage sitting on a warming pad) with ad libitum access to food and water.**
5. **Monitor the animal's recovery over the next hour for any signs of discomfort, bleeding, or preterm abortion.**

### **RECOMMENDATION**

**DATE ISSUED:** 22.10.2008

**REVISED:**

### **REFERENCES**

1. Slevin JC, Byers L, Gertsenstein M, Qu D, Mu J, Sunn N, Kingdom JC, Rossant J, Adamson SL. (2006) *High resolution ultrasound-guided microinjection for interventional studies of early embryonic and placental development in vivo in mice.* BMC Dev Biol. 6:10.
2. Kulandavelu S, Qu D, Sunn N, Mu J, Rennie MY, Whiteley KJ, Walls JR, Bock NA, Sun JC, Covelli A, Sled JG, Adamson SL. (2006). *Embryonic and neonatal phenotyping of genetically engineered mice.* ILAR J. 47(2):103-17.