In utero electroporation

Tools

Needles: Aluminosilicate glass OD 1.5mm ID 1 mm 10 cm length Sutter A150-100-10
Tweezertrodes – 5mm
Electroporator
DNA 1-2 ug/ul
Fast Green Dye
Sterilized PBS
Ethanol
Betadine
Silk sutures
Surgical scissors
Ring forceps
Tweezers
Sterile plastic pipette
Sterile napkins
Electroporation sheet
Pen
Isofluorane anesthesia set up

Making the needles:

* Using horizontal needle pulling machine, calibrate the heating temperature according to guidelines.
* Set the following parameters: Heat (above); Pull 0; Velocity 200; Time 0; Pressure 100
* Use a beveling machine to bevel needles at a 20 degree angle, but maintain a thin tip.

Preparation:

* Make the needles (above) and mark them every 3 mm (~1uL) with permanent marker. Sterilize
* Make DNA in proper concentration (~1uL/anticipated embryo) and add <0.2 uL of fast green dye. Mix and spin down.

Electroporation:

* Set flow of Oxygen to chamber, isofluorane level 4. Place mouse in chamber until breaths come at 1 breath every 1-2 seconds.
* Set flow of oxygen to mask, and lower isofluorane level to 2. Remove mouse from chamber, pull out tongue (to prevent choking), and place mask over nose. Monitor breathing levels. If breathing seems to be slow, intermittent gasping, lower the levels. If the breathing quickens or the mouse exhibits pain responses, increase the level slightly.
* Pinch the toes to make sure mouse is asleep. Line block abdomen using bupivacaine. Spray abdomen with ethanol, then swab with Betadine to sterilize.
* Cut through the skin using surgical scissors. Place a section of sterile napkin with a hole cut into it over the abdomen so you can observe the mouse’s breathing, and the cut is centered in the hole of the napkin. Cut through the muscle.
* CAREFULLY remove the embryos with the ring forceps, pulling only between the embryos.
* Pipette sterile PBS over embryos. Make sure to keep them wet.
* Using mouth pipette system and capillary needles, inject ~1uL (1 mark on the needle) into the ventricle closest to the placenta. Repeat for each embryo, avoiding the ones near the vaginal canal. Mark the side of electroporation and DNA construct for each embryo on the electroporation sheet
* Use the following parameters: 5 pulses, 50 ms pulses; E12.5, 13.5 – 30V; E14.5 – 40V; E15.5 and up – 50V
* Wet the embryo with PBS before electroporating. Align the tweezertrodes so that the positive connection is on the side of the cortex you want the DNA to go into and the negative connection is no lower than the shoulder area. Any lower, and you risk upsetting the heart rhythms.
* Press the foot pedal to initiate the electroporation. Wet the embryo again with PBS. Repeat for all embryos.
* CAREFULLY place embryos back inside mother. It is better to make the opening larger if necessary than squeeze the embryos. Be careful that the uterine horns do not get tangled. This can cause miscarriage.
* Suture the muscles, then the skin separately. Drop ~20 ul of bupivacaine on outside of suture, then swab with Betadine.
* Turn of oxygen and isofluorane, then put mouse on heating element at 37 deg C to wake up. The mouse should wake up in 5-10 minutes.

Post care:

* Check on mouse 3-5 hours after surgery, check for discharge, discomfort, or any signs of premature labor
* Check again next day. Discharge doesn’t necessarily mean premature labor, but is a sign to keep an eye on her. Check the incision to make sure it’s clean and free of debris, and she hasn’t torn the stitches out. Palpate to feel for embryos.