

## IUE Surgery Prep Protocol

### PULL THE PIPETTES

1. To make the micropipettes for surgery, start with Drummond glass capillary tubes (cat# 3-000-210-G).
2. Pull pipettes using the Narishige device:
  - a. Slide the capillary tube into the groove at the top, through the heating coil and down into the groove in the weight block. Center the thick orange band inside the heating coil.
  - b. Tighten the top knob to hold the tube in place.
  - c. Move the black weight at the bottom up to its highest position and tighten the bottom knob.
  - d. Close the cover. Set a heater to 80.6°C, switch the knob to this heater. The coil will heat up; wait for the weight block to drop.
  - e. Turn the heater off, and open the cover once the coil cools.
  - f. With one hand, hold the bottom of the capillary tube as though you were pulling it down. At the same time, loosen the bottom knob. The pulled, thin portion in the middle of the tube should break.
  - g. The bottom part you are now holding is what will become the micropipette. Discard the top portion. Most likely, the tip will be very long - you will cut this down to the desired length at surgery time.

### STERILIZE THE SURGERY TOOLS

1. Place a piece of autoclave tape on the inside of the stainless steel sterilization container. Add to tools:
  - a. hemostat
  - b. bent spatula
  - c. Dumont forceps
  - d. forceps with teeth
  - e. flat forceps
  - f. scissors

You will also use the wound clip stapler, but it doesn't have to be sterilized

2. Lock the lid in place.
3. Autoclave on dry cycle #2 (15 or 20 minutes).

## MAKE THE PLASMIDS

Each experimental and control preparation should include the desired shRNA, scrambled shRNA and/or overexpression construct and plasmids encoding eGFP, mRFP, or both. Prepare plasmids in microcentrifuge tubes on ice before starting surgery.

1. Final concentration in the plasmid solution should equal:
  - a. shRNA, scram, OX:  $1.5 \mu\text{g}/\mu\text{L}$ - eGFP, mRFP:  $0.75 \mu\text{g}/\mu\text{L}$
  - b. WGA Tg:  $3.0 \mu\text{g}/\mu\text{L}$
2. To calculate the amount of each plasmid needed, use the following formula:

$[\text{stock plasmid}](x) = [\text{final}](x)(\text{desired total volume}) = y$ , where "x" is the dilution factor and "y" is the amount to add to the mix

For example, to determine the amount of  $5.57 \mu\text{g}/\mu\text{L}$  DYX shRNA stock solution needed in  $30 \mu\text{L}$  of plasmid mix:

$$(5.57 \mu\text{g}/\mu\text{L}) * (x) = 1.5 \mu\text{g}/\mu\text{L} \quad x = 0.27 \quad (0.27) * (30 \mu\text{L}) = 8.1 \mu\text{L}$$

3. Once these calculations have been performed for each experimental and fluorescent protein plasmid required, add the necessary amounts to a microcentrifuge tube and bring the solution up to the desired total volume with diluted FastGreen dye.

For example,  $8.1 \mu\text{L} + 4.8 \mu\text{L eGFP} + z \mu\text{L FG} = 30 \mu\text{L} \rightarrow z = 17.1 \mu\text{L FG}$

4. The concentration of FG can be adjusted based on need. The purpose of the dye is to make the plasmid mix visible upon injection - use the lowest concentration at which this is still possible (for the above example,  $0.75$  or  $1.5 \mu\text{g}/\mu\text{L}$  would be adequate). Too much FG in the plasmid solution may block cerebrospinal fluid drainage from the ventricle, causing hydrocephaly.
5. To load the plasmid into a glass micropipette (capillary tube), use an extra long pipet tip. This tip should fit into the open end of the capillary tube, so that the plasmid can flow directly down the glass tip. After the plasmid solution has been loaded into the body of the capillary tube, it may not fully reach the tip. To fix this, you can remove and reload the pipet tip with air and pump it into the micropipette until the fine tip is fully colored.

### **PREPARE THE SALINE (0.9%)**

0.9% saline solution is meant to be isotonic to the blood.

1. In a two-liter beaker, combine one liter 0.1M Sodium Phosphate Buffer and 9 grams of sodium chloride (NaCl). Do not fill the beaker, or saline will spill out during the autoclave cycle.
2. Cover the opening of the beaker with aluminum foil and place autoclave tape over the foil.
3. Autoclave the saline for 15-20 minutes on the liquid cycle.
4. Saline should be allowed to cool and is kept in a 37°C water bath during surgery.
5. Before use, add Penicillin/Streptomycin in a 1:200 ratio: for each 100mL saline, use 500µL Pen/Strep (Pen/Strep should be frozen in 500µL aliquots to prevent spoiling).

### **ANESTHETIZE THE RAT**

Use a **10:1 ratio of Ketamine to Xylazine**. It is crucial not to reverse this ratio! Ketamine is an anesthetic and causes the rat to lose consciousness, while Xylazine is a muscle relaxant. If you reverse the ratio, the rat will be conscious and paralyzed = not good.

Ketamine is a controlled substance, and must be kept in a locked drawer. Log all use, as members of the IACUC will come to the lab for inspection.

1. Using a hypodermic needle, draw up more liquid than you are planning to use for the anesthetic mix. With your finger, tap on the barrel of the syringe so that any bubbles rise to the top. Push the excess fluid out - air bubbles should come out at this time.
2. It is also very important that the tip of the hypodermic is cleared of Xylazine before it is loaded for the initial injection. Even the small amount of Xylazine that remains in the tip after measuring the 10:1 solution can overdose and kill an adult rat. It does this through respiratory depression, so if breathing appears more shallow than usual and seems to slow, immediately inject either Yobine (a Xylazine antidote) or warm saline. To expel the majority of liquid from the hypodermic, draw air up into the chamber, tap the side with the needle facing down, and depress the plunger over a waste basket. Repeat as necessary to ensure no liquid remains.
3. Dosage for the rats is by weight. For example, a dam weighing 250 g should be given 0.25 cc of 10:1 K/X mixture.
4. Pre-anesthetize the rat using an isofluorane vaporizer. Once the rat is out, remove her from the chamber and place on a lab bench or table.

5. Lift the skin and underlying muscle on the belly. The idea is to create a "tent" and insert the needle horizontally through the skin and muscle into the cavity. Do not angle the needle down into the body cavity; this increases the risk of piercing the uterus and damaging the embryos.
6. As the rat begins to wake up (usually the nose starts wriggling first), pump the anesthetic into the body. Return the rat to its cage - it should wake up briefly and then begin to go out once again. Avoid injecting while the rat is still sleeping from the isoflurane, otherwise you risk overdosing the animal.
7. If breathing appears more shallow than usual and seems to slow, immediately inject either Yobine (a Xylazine antidote) or warm saline.