Supplemental Protocol and Methods

**1. Prepare procedure space and vector delivery materials.**

1.1. To warm the procedure area, place a heating pad covered by a disposable underpad.

1.2. Set up the pulse oximeter to measure heart rate and peripheral capillary oxygen saturation (SpO2). Prepare a rectal thermometer by coating with a lubricating jelly.

1.3. Pass the microsprayer nozzle through a 2.0 mm inner diameter endotracheal (ET) tube and mark the base of the microsprayer when the tip exits the ET tube by ~1 mm. This will serve as a guide for how far to insert the microsprayer into the ET tube once its placed in the animal’s airway.

NOTE: Not all ET tubes are the same length, so this step should be repeated for every new ET tube used in a procedure.

1.4. Remove the microsprayer from the ET tube and screw the microsprayer onto to a luer locking syringe loaded with 1-2 ml of viral vector. Set aside.

NOTE: A test spray through the microsprayer with water or saline is recommended prior to setup.

NOTE: The appropriate pressure on the syringe plunger to generate a mist should be practiced beforehand. Too little pressure will result in a stream instead of a spray.

NOTE: Prefilling the spray nozzle with viral vector is not necessary.

1.5. Insert a stylet into the ET tube to support intubation.

1.6. Pigs will be anesthetized using isoflurane, a suggested range is 2-4%. Assemble an isoflurane vaporizer. 1) Connect an O2 tank with a pressure regulator and flowmeter to the vaporizer. 2) Connect the vaporizer through tubing to deliver the isoflurane through an anesthesia mask and 3) an anesthesia gas filter canister to collect the waste anesthesia gas from the operating room environment.

1.7. Within the procedure area, arrange a laryngoscope (4” elongated blade, the microsprayer with syringe containing vector, and ET tube (lined with stylet). Pre-coat ET tube tip with lubricating jelly.

**2. Sedate pigs**

2.1. Use a pulse oximeter to measure oxygen saturation and heart rate. Place a wraparound SpO2 sensor around the pig’s hind leg and ensure the readings register on the pulse oximeter. Record pre-anesthetic reading.

NOTE: Animals must fast prior to sedation to prevent aspiration during delivery.

2.2. Turn on O2 tank (flowmeter set to 2 L/min) and isoflurane vaporizer to begin flow to the anesthesia mask.

2.3. Place anesthesia mask over the pig’s snout and hold pig until sedated. This may take approximately 4-5 minutes but will vary depending on the age and weight of the animal. Begin by holding the pig during initial stages of anesthesia. Once pig is sedated, lay it on the prepared procedure space (underpad over a heating pad). Confirm anesthesia by testing the pedal reflex.

NOTE: The animal should never be left unattended.

2.4. Record rectal temperature and respiratory rate.

2.5. Continue with procedural record keeping every 15 minutes throughout sedation and recovery (SpO2, heart rate, and respiratory rate).

**3. Intubate sedated pigs with an endotracheal tube**

3.1. Confirm that the ET tube (with stylet) has been lightly coated with lubricating jelly to facilitate intubation (Step 1.5).

3.2. Remove the anesthesia mask from the pig and turn off the flow of isoflurane.

3.3. Lay the pig supine on the procedure space and visualize the larynx using a laryngoscope.

3.4. Pass a 2.0 mm ET tube through the vocal folds of the larynx and into the trachea (Supplemental Movie 1). If properly intubated, the SpO2 levels will start to decline due to the airway obstruction by the ET tube.

NOTE: The exact tracheal region reached by the ET tube will vary depending on the size of the animal. Placement can vary from just beyond the larynx for larger animals (~3-4 kg) to near the carina for smaller animals (~0.8-1 kg). In small animals, there is risk of one side intubation and trimming 3-5 cm from the ET tube may be warranted.

3.5. Remove stylet.

**4. Aerosolize the viral vector using the microsprayer**

4.1. Pass the microsprayer (connected to viral vector-containing syringe) through the ET tube until reaching the mark at the base of the microsprayer (as prepared in 1.3).

4.2. Spray the solution intratracheally by pressing the syringe plunger with firm and consistent force to generate a mist. This will take approximately 3-4 seconds.

NOTE: For our studies, we limited the volume delivered to ~1 ml/kg.

4.3. A post-spray “air chaser” of ~500 µl will help ensure complete delivery of vector from the syringe and nozzle.

4.4. Gently remove ET tube and microsprayer from the intratracheal intubation at the same time. (Successful delivery will typically result in the sound of crackles when breathing.)

NOTE: A typical procedure time from initiated anesthesia time to extubation is 10-15 minutes.

**5. Monitor pigs as they come out of sedation**

5.1. Apnea is a common response to intubation in newborn pigs. Sporadic breathing may last 2-3 minutes. Gentle chest compressions can help facilitate normal breathing.

5.2. Monitor SpO2 levels until they return to 95-100% then remove the SpO2 sensor from the pig’s hind leg.

5.3. Continue with post-procedural monitoring every 15 minutes until pig is alert, sternal, and walking. Typically, pigs will recover within 15 minutes.