Date:	SJMAST	
Flank Implantation from	n Cryo	
Sample location:		
Reason for sample impl	antation:	
Procedure:		

- 1. Remove tube from -80freeze or cryo (on dry ice)
- 2. Thawed in water bath over 30sec-1min
- 3. Transfer to 15ml conical tube
- 4. Add warmed media slowly to the 15ml tube and gently swirl to mix
- 5. Aspirate and discard supernatant
- 6. Wash flank pieces again in fresh media
- 7. Place flank pieces in fresh media on ice

Implantation:

Mouse placed in the anesthesia chamber with isoflourane following the manufacturer's recommended settings to minimize distress and movement during the procedure. The mouse is removed and placed (ventral side down) with nose-cone to provide continued anesthesia. To confirm anesthetic depth, pinch the rear foot lightly. If no kicking response is present, continue with the procedure.

The area from mid-spine to the tail base is cleaned with 70% ethanol. A small horizontal 5mm x 10 mm incision in the flank area is made using sterile small surgical scissors. The tip of the sterile scissors is inserted into the incision, directly over the flank, and the scissors are opened to introduce a pocket in the subcutaneous space. One individual piece of tumor tissue is inserted into the pocket using sterile forceps. One drop of 100X penicillin/streptomycin solution is inserted into the opening over the tissue piece. The incision is closed with one drop of skin tissue glue (Vetbond) as a skin adhesive. The overlying skin is held together for 3-5 seconds with the forceps to allow adequate time for drying.