Freezing down pieces of mammary gland tissue for later transplantation

TO FREEZE:

1. Excise tissue to be frozen taking care to remove any lymph nodes or excess fat pad not containing outgrowth or tumor.

2. Mince tissue into approx. 1-mm-sized pieces (the size that you would eventually transplant).

3. Place tissue in medium containing 7% DMSO and 2% FBS (we normally use RMPI-1640 media since that is what HC11 cells grow in).

4. Slowly freeze aliquots, lowering temperature at the rate of 1°C/min. Generally, the minced tissue from one inguinal fat pad will yields two aliquots.

5. For short-term storage, freeze to -80°C and for longer-term storage, store in liquid nitrogen.

TO THAW:

1. Quickly melt frozen aliquots by submersing in a 37°C water bath.

2. Add extra medium to dilute concentration of DMSO. Note that fragments of mammary tissue will not usually pellet by centrifugation because of the high fat content; therefore, fragments of tissue outgrowths and tumors may be collected with forceps.

3. Transplant pieces into the cleared fat pads of 3 week old recipient mice.