

## Mouse Mammary Intraductal (MIND) Method for Transplantation of Patient Derived Primary DCIS Cells and Cell Lines

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**[Abstract]** The MIND method involves intraductal injection of patient derived ductal carcinoma in situ (DCIS) cells and DCIS cell lines (MCF10DCIS.COM and SUM225) inside the mouse mammary ducts [Video 1 and Figure 1 in Behbod *et al.* (2009)]. This method mimics the normal environment of DCIS and facilitates study of the natural progression of human DCIS, *i.e.*, their initial growth as carcinoma *in situ* within the ducts, followed by invasion into the stroma through the myoepithelial cell layer and basement membrane (Behbod *et al.*, 2009; Valdez *et al.*, 2011). In order to demonstrate that transplantation procedure is successful, the transplanted mammary glands may be excised as early as two weeks following intraductal injection of cells followed by Hematoxylin and Eosin (H&E) staining and/or immunofluorescence staining using human specific cytokeratin 5 and/or 19 [please see Figures 2-4 in Behbod *et al.* (2009)]. Additionally, the presence of trypan blue inside the mouse mammary ducts immediately following intraductal injection is the best indicator that the injection was successful (Video 1 starting at 4:33 sec).

### Materials and Reagents

1. Hamilton syringe, 50 µl capacity, with a 30 gauge blunt-ended fixed 1/2-inch needle (Hamilton, catalog number: 80608)
2. 1 ml Tuberculin (TB) syringe (Benton Dickenson, catalog number: 309625)
3. Wound clips (Becton Dickinson, catalog number: 427631)
4. Animals: 8-12 week old female immunocompromised mice  
*Note: NSG, NOD scid gamma [NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (HE JACKSON LABORATORY, catalog number: 005557)]. Alternatively, SCID/beige mice (Fox Chase SCID<sup>®</sup> Beige CB17.Cg-Prkdc<sup>scid</sup>Lyst<sup>bg-J</sup>/CrI) may be used for the injection of DCIS cell lines only. While a formal comparison has not been made, patient derived DCIS epithelial cells seem to grow more efficiently in the NSG mice.*
5. Cells of interest [*i.e.*, cell lines MCF10DCIS.COM and SUM225 (Behbod Lab, 2009 #329) or patient derived DCIS epithelial cells] in single cells
6. PBS (pH 7.4, 1x), sterile (Life Technologies, catalog number: 10010023)  
*Note: Currently, it is "Thermo Fisher Scientific, catalog number: 10010023".*

7. Trypan blue 0.4%, sterile (Sigma-Aldrich, catalog number: T8154)
8. 70% ethanol
9. Sterile water
10. Nembutal Sodium Solution (Pentobarbital sodium injection, USP) (Akorn, Oak Pharmaceuticals, catalog number: NDC 7647850120)
11. Ketofen™ (Ketoprofen) (Patterson Veterinary Supply, catalog number: 10004029)

### **Equipment**

1. Scissors Small scale suitable for mice  
 Sharp-Blunt (Fine Science Tool, catalog number: 14028-10)  
 Castroviejo Spring Scissors (Fine Science Tool, catalog number: 15017-10)  
 Balled Scissors (Fine Science Tool, catalog number: 14086-09)
2. Clippers (Fine Science Tool, catalog number: 1501710)
3. Rounded end flat handle tweezers (ROBOZ, model: RS5095)
4. Spring scissor (Fine Science Tool, catalog number: 1501710)
5. Balled scissor (Fine Science Tool, catalog number: 1408609)
6. Rounded end tweezers (Fine Science Tool, catalog number: 1105210)
7. Rat tooth tweezers (Fine Science Tool, catalog number: 1105310)
8. Clip applier (Becton Dickinson, catalog number: 427630)
9. Heat pad (Sunbeam Products, model: PN126985900)
10. Clip remover (Becton Dickinson, catalog number: 427638)

### **Procedure**

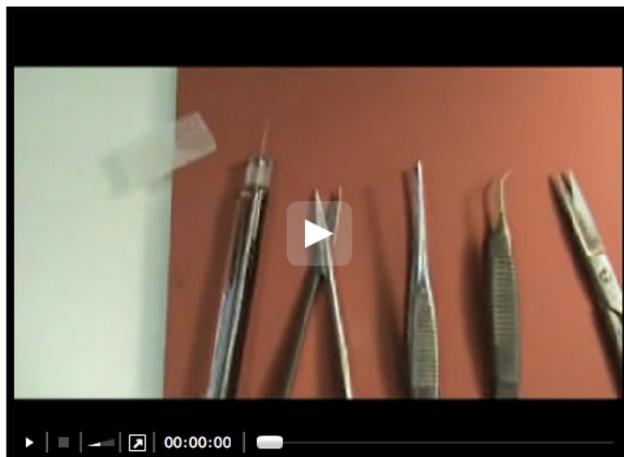
- A. Prepare the cells for injection
  1. Resuspend cells in PBS with 0.04% trypan blue. For cell lines, the final concentration should be 5,000-10,000 cells/μl. For primary cells the concentration should be 12,500-17,500 cells/μl. Trypan blue will indicate the presence of cells inside the duct after the injection procedure.
  2. Be sure all instruments have been sterilized and the Hamilton syringe washed by alternating pulling up sterile water and 70% alcohol, ending with sterile water.
- B. Prepare the mice for surgery
  1. Weigh the mouse.
  2. Using 1 ml TB syringe, administer Nembutal 40 μg/g body intraperitoneal (this will provide 1 h of sedation).
  3. Wait for mouse to fall asleep; confirm by checking hind limb reflex by pinching foot.
  4. Use clippers to shave the abdomen exposing the inguinal nipples and taking care not to cut them.

5. Using 1 ml TB syringe, administer Ketofen™ (ketoprofen) 5 mg/kg intraperitoneal for pain relief.
6. Place the mouse on surgical board and secure limbs with surgical tape.
7. Sterilize the abdomen with 70% alcohol.

### C. Surgery

1. Cut off the tip of the nipples of the inguinal glands so that the syringe can be directly inserted through the nipple. Use the rounded end flat handle tweezers to hold the skin under the nipple and force it to protrude. Use the spring scissor to cut off about the top 0.5 mm of the nipple. Take care not to remove too much or the syringe will slide under the skin. A properly cut nipple will have a volcano cone shape.
2. Using the balled scissors, make an inverted Y-incision on the abdomen beginning on the midline slightly below the inguinal nipple and extending up about 1 inch. The other two cuts of the incision should extend between and fourth and fifth nipples on either side.
3. Using the rounded end tweezers and rat tooth tweezers, separate the skin covering the inguinal mammary fat pads to expose the inguinal gland.
4. Using the Hamilton syringe, draw up two microliters of PBS (with 0.04% trypan blue) containing cells at the proper concentration; holding the skin flap up, insert the tip of the syringe into the nipple at a 90 degree angle to the skin flap and carefully inject the cells. Trypan blue will make the injected liquid visible in the duct.
5. Reposition the skin flaps and suture with wound clips.

### Video 1. Intraductal injection



*Note: The video contains material that can be disturbing to some viewers.*

### D. Post-surgery

1. Allow mice to recover on heat pad set on the lowest setting.
2. Administer Ketofen™ 5 mg/kg intraperitoneal 24 h later.

3. Remove clips after 7-10 days.

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### **References**

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