

## Protocol

# Mammary Fat Pad Tumor Preparation in Mice

Rakesh K. Jain, Lance L. Munn, and Dai Fukumura

The host microenvironment influences tumor biology, affecting parameters such as gene expression, angiogenesis, growth, invasion, metastasis, and responses to therapy. Consequently, the use of tumor models growing in appropriate orthotopic locations is necessary to obtain a rigorous understanding of tumor pathophysiology and to correctly study antitumor treatments. The mouse mammary fat pad serves as an orthotopic site for breast cancer and can be used to study various aspects of this disease, including the effect of host–tumor interactions on tumor biology and therapeutic response. This protocol describes mammary fat pad tumor preparation in mice.

## MATERIALS

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It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

### Reagents

Anesthetic, isoflurane 1%–3% inhalant (up to 5% for induction) or ketamine (80–100 mg/kg body weight [BW])/xylazine (5–10 mg/kg BW)

Breast carcinoma cells or tumor slurry

Phosphate-buffered saline (PBS)

### Equipment

Coverslips, glass

Cyanoacrylate adhesive, biocompatible

Equipment for injection of tumor cells or slurry

Heating pad or similar device

Metal ring support, lollipop-shaped

Microscope

Surgical tools

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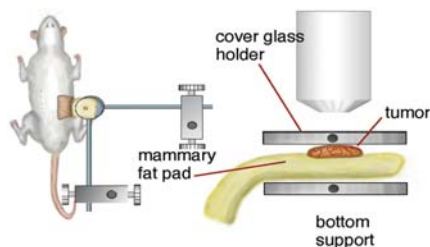


FIGURE 1. Mammary fat pad model setup.

## METHOD

Perform all surgical procedures with the animal under appropriate anesthesia and with full approval by the Institutional Animal Care and Use Committee. During the surgical procedure, maintain the animal's core body temperature at 36°C–37°C using a heating pad or similar device.

1. Inject breast carcinoma cells ( $3 \times 10^6$ ; final volume of 30  $\mu$ L in PBS) or tumor slurry into the mammary fat pad just inferior to the nipple of a female mouse.  
*Four to six weeks later, tumors grow to ~5–8 mm in diameter.*
2. When tumors are of the proper size, make a midline incision through the skin and fascia. Gently elevate a flap by blunt dissection being careful to avoid disrupting the vasculature and irritating the tumor vessels.
3. Gently glue the flap to a specially designed stage, such as the one shown in Figure 1. Place a glass coverslip over the tumor using a lollipop-shaped metal ring support.

## RELATED INFORMATION

See Monsky et al. (2002) for more information on the mouse mammary fat pad as an orthotopic site for breast cancer.

## ACKNOWLEDGMENTS

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## REFERENCE

- Monsky WL, Carreira CM, Tsuzuki Y, Gohongi T, Fukumura D, Jain RK  
2002. Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: Mammary fat pad vs. cranial tumors. *Clin Cancer Res* 8: 1008–1013.



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