

PROTOCOL:  
**MOUSE**  
**PERFUSION**  
LU LABORATORY

Gage, G. J., Kipke, D. R., Shain, W. Whole Animal Perfusion Fixation for Rodents. *J. Vis. Exp.* (65), e3564, doi:10.3791/3564 (2012).

## BACKGROUND

# WHAT IS PERFUSION?

(GAGE, 2012)

The goal of perfusion-fixation is to rapidly and uniformly preserve tissue in a life-like state for further research analysis.

Perfusion is a low-cost, rapid, and uniform fixation procedure using 4% paraformaldehyde or PFA. PFA is suffused through the heart of a rodent via the vascular system in order to obtain the best possible preservation of the brain.

The advantage of directly perfusing fixative through the circulatory system is that the chemical can quickly reach every corner of the organism using the natural vascular network.

(Gage, 2012)

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## PROTOCOL STAGES

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**1. PREPARATION**

**3. PERFUSION**

**2. SURGERY**

**4. HARVEST**

## STAGE 1

# PREPARATION

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## CHECKLIST

- **4% PFA**

Remove from -20°C Fridge and leave at room temperature.

- **SURGICAL TOOLS**

Obtain forceps, tweezers, scissors, and IV Set (syringe + needle).

- **SURGICAL PLATES**

Obtain a plastic tub and a foam plate with at least 5 push-pin needles.

- **EUTHANASIA JAR**

Prepare Euthanasia Jar with Isoflurane droplets.

- **4% PFA WITH IV SET**

Fill the syringe with 4% PFA. Then purge any air bubbles from the tube.

## IMPORTANT NOTES

- Wear the appropriate PPE **at all times!**
  - Perform **all** of the following procedures in the Fume Hood!
  - Leave Euthanasia Jar and Isoflurane in the Fume Hood **at all times!**
  - Be careful using the Isoflurane: it is **very heavy and will spill easily** through the pipette!
  - Be careful using sharps and needles: these are sharp!
  - Hold the syringe upright to effectively purge the air bubbles out through the IV needle.
  - Have fun being a surgeon!
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## STAGE 2

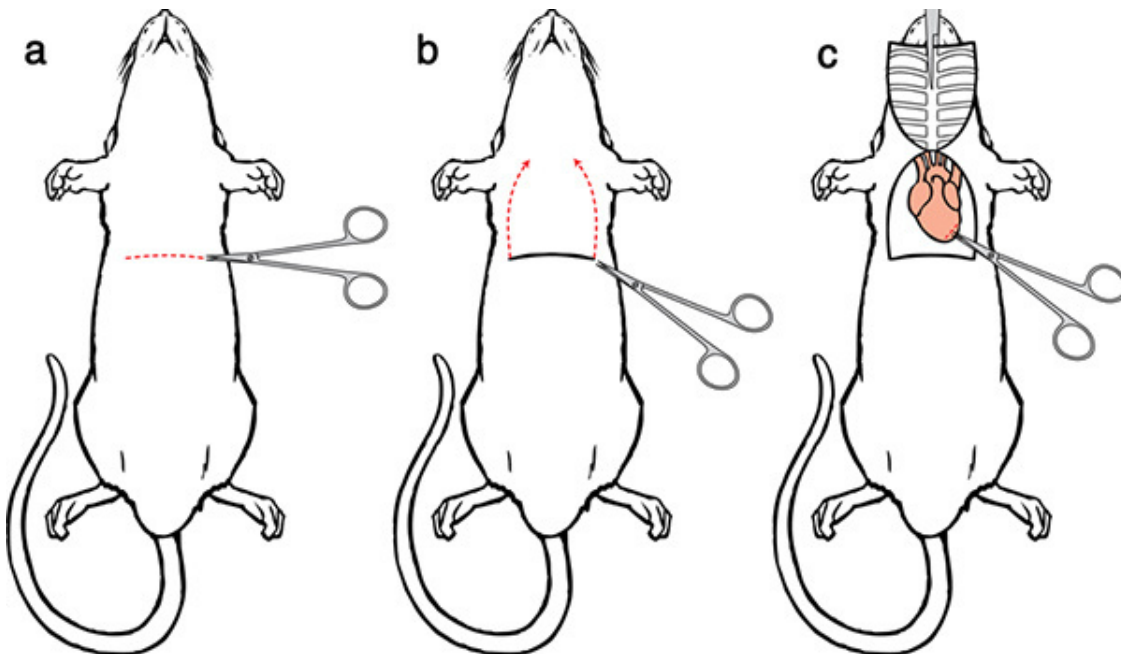
# SURGERY

### CHECKLIST

- **ANESTHETIZE MOUSE**  
Place mouse into Euthanasia jar.
- **FIX MOUSE TO PLATE**  
Use push-pins to fix the mouse to the foam plate (1 for each paw).
- **OPEN CHEST SURGERY**  
Use scissors and forceps to open the chest of the mouse.
  1. Remove the skin (from the liver to the heart).
  2. The 'white' thymus tissue will be on top of the heart.

### IMPORTANT NOTES

- Grab the mouse **by the tail** for a swifter transition into the jar.
- Wait until the mouse **stops coughing/ wheezing/ moving** before removing it from the jar.
- Spray the mouse with alcohol to **minimize fur clots & shedding!**
- Use a **long push-pin** to keep the severed skin flap out of the way.
- **Try not to damage the heart, lungs, or liver in the process!**



## STAGE 3

# PERFUSION

### CHECKLIST

- **CUT THE HEART**

Carefully use the scissors to cut a small hole in the **Right Atrium**.

- **PIERCE WITH NEEDLE**

Use the IV needle to pierce the **Left Ventricle**.

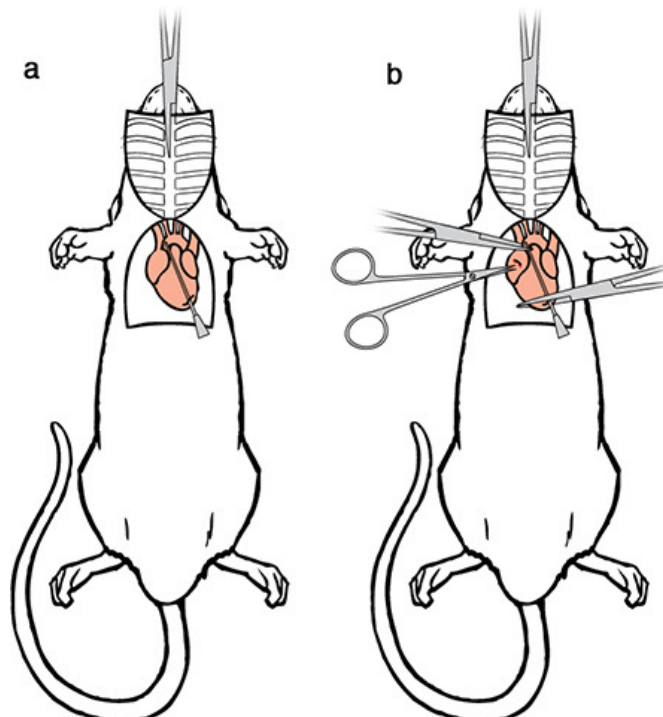
- **ADMINISTER 4% PFA**

Slowly perfuse PFA throughout the mouse's circulatory system. Perfusion is successful if:

1. The color of the liver changes to white/yellow.
2. The tail & body become stiff.

### IMPORTANT NOTES

- The Right Atrium will be on the **UPPER LEFT SIDE** (looking down at the mouse).
- The Left Ventricle will be on the **LOWER RIGHT SIDE** (looking down at the mouse).
- Be careful not to poke the heart **TOO DEEP** with the needle!
- Try **holding the heart** with the **tweezers in your left hand** before making the puncture.



## STAGE 4

# HARVEST

### CHECKLIST

- **SLICE THE NAPE**  
Sever the head at the spinal cord.
- **CARVE THE SKULL**  
Carefully slice the skull open down the midline.
- **PEEL THE SKULL**  
Use tweezers to carefully peel the skull from the mouse cortex.
- **SCOOP THE BRAIN**  
Use curved forceps to lift the brain out of the open skull.

### IMPORTANT NOTES

- Spray the mouse with alcohol to minimize fur clots & shedding!
- Detach the head to allow more space for skull carving.
- Place scissors **at the base** of the skull and **lift up on the tip** as you cut to prevent damage.
- Twist the tweezers with your wrist to easily peel the skull.
- Scrape along the bottom of the skull with the curved forceps to prevent damage to the brain.

