 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure LAB_033 General mouse setup using a cryoprobe bed at 9.4T</p>	<p>Version #2</p>
	<p>Institutional author: Centre for Advanced Imaging AEC Reviewed & Approved: December 2024 SOP Expiry: December 2027</p>	<p>Page 1 of 4</p>

LAB_033 General mouse setup using a cryoprobe bed at 9.4T (Expiry: December 2027)

I. OBJECTIVE

MRI cryoprobe can provide up to 250% increase in signal-to-noise ratio compared to the conventional room-temperature probes. This unprecedented increase in sensitivity **allows much better measurements of functional and structural changes in mice, especially the brain**. The cryoprobe setup, however, must be performed with extra care due to limited space and complexity of the mouse bed.

II. POLICY

- This procedure must be performed by a CAI approved experienced operator.
- Two trained personnel are required for all after hours experiments.
- Each mouse setup must be tested using the Bruker mock cryoprobe before inserting to the scanner

III. COMMENTS / RECOMMENDATIONS

- Users must have:
 1. Read the Bruker manual for the cryoprobe mouse bed
 2. Completed the induction and training by Facility Manager and be deemed competent in the procedure
 3. (Users must understand the components and the steps required for the installation/uninstallation of the cryoprobe, **however only the Facility Manager and registered personnel are allowed to do the install/uninstallation**)
 4. Practiced performing the cryoprobe animal setup using cadaver, particularly the placement of the ear bars
 5. Completed animal handling and anaesthesia training given by UQBR
- Note: this SOP is not intended to cover mouse brain functional MRI setup as there are variations in ancillary equipment and anaesthesia.
- PPE should include disposable gloves, face mask, long sleeved lab gown, closed in shoes.
- **Warning! Incorrect setup of the ear bars can result in injury to the mouse.**


IV. EQUIPMENT

- Bruker 9.4T MRI with cryoprobe, a mock cryoprobe
- Bruker cryoprobe bed

Note: Bruker capacity guideline is mice which weigh 15-45g,

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CAI recommends 20-35g mouse (lighter mice may result in instability of anaesthesia, heavier mice may result in difficulty of placing ancillary equipment)

- Cryoprobe temperature regulator unit (set at 36-37 °C).
- Warm water circulation unit (set at approximately 45 °C)
- Vaporous isoflurane anaesthetic unit, including:
 - Precision isoflurane vaporiser
 - Induction chamber
 - Nose cone
 - Rodent anaesthetic circuit (one-way)
 - Isoflurane scavenging system
- Animal physiology monitoring: respiratory and body temperature sensors
- Heating pad for animal anaesthesia induction and recovery

V. PROCEDURE

One day before experiment:

1. Ensure that the cryoprobe is in operating (cold) condition 24h before the experiment.
2. Warning! If not prepared, cryoprobe will need at least 5h to cool down before it can be ready for use.
3. Ensure that the cryoprobe coil temperature unit is set at 36-37°C.
4. Ensure that the batteries for animal monitoring are charged.


During experiment:

1. Fill the isoflurane unit with isoflurane, record the use.
2. Check the temperature regulator is set at 36-37 °C

WARNING! Failure to setup the temperature regulator unit can result in death of the animal due to

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hypothermia



- Fill the warm water circulation unit with distilled water. Set the water circulation at 45°C. The water temperature is set higher than physiological temperature to compensate for heat loss when the water flows through a long tubing, the animal bed and foam surfaces.
- Important: Water temperature needs to be carefully adjusted according to the reading of the temperature probe. Temperature at the animal should be maintained between 36-37°C, dependent on the animal's body temperature.**
- The mouse cryoprobe bed should be pre-warmed using the water circulation.
 - Place a small piece of foam in the bed groove to support the animal.
Place the respiratory pad and temperature probe on the animal bed.
 - Ensure anaesthetic unit is operational and scavenging system is connected. Place the mouse in the anaesthesia chamber.
 - Start the manual and automatic recording of animal physiology
 - Induce anaesthesia with isoflurane at a dose of 4-5% at 2 L/min in a closed anaesthetizing chamber. Wait until the mouse breathing is approximately 60 beats per minute (bpm).
Reduce the isoflurane to 2-3% at 1.5L/min
 - To "flush" the anaesthetic line, connect the isoflurane line to the mouse bed and allow it to run for 10 seconds. Then place the mouse's incisors on the tooth hook, and fasten into the head cone.
 - Fasten the ear bars carefully (if using). Refasten the tooth bar as necessary. The mouse's head position should be straight and level.
- Caution! Ears bars should only be used for the ear. The feet of the ear bars should not protrude past the guide lines. Protruding ear bars will move during the insertion of mouse bed into the cryoprobe and will cause injury.**
- Place the animal respiratory pad (under the diaphragm) and the temperature probe. Fasten using tapes as necessary.

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12. Important! Check the animal positioning using the cryoprobe mock to ensure that the mouse can fit safely into the cryoprobe.

13. Insert the mouse and the bed into the cryoprobe.

Pull the lever $\frac{3}{4}$ down and insert a small piece of foam to maintain the tilt of the mouse bed towards the cryoprobe surface.

14. Start the MRI scans. Mouse must not be under anaesthesia longer than 3 hours, unless specifically approved by the AEC.

15. Maintain the animal respiratory rate at approximately 50-60 bpm using isoflurane to 1-1.5% at 1L/min, body temperature (36-37°C) using warm water circulation. Keep the manual recording of the body physiology.

16. At the end of the scan, remove the animal and stop isoflurane. Return animal to the cage placed on heating pad. Monitor continuously until fully recovered and for at least one hour.

17. Consider providing analgesia if any injury or bruising may have occurred. Animals should be monitored for at least 2 days following the procedure.

18. Sign off the manual animal monitoring sheet.

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