

SOP No:	AHT 52
SUBJECT:	Skin grafting of rodents
REASON FOR USE:	This technique is used to help analyse components of the immune system
POLICY:	Operator must be experienced and competent at this technique
PRECAUTIONS:	Gloves, long-sleeved gown, closed-in-shoes, hair cover, face mask. Read and sign off on relevant UQ animal handling Risk Assessments. Read MSDS before use of hazardous chemicals. Care must be taken when handling sharp objects (scalpel blade, needles and surgical instruments) and hazardous chemicals.
EQUIPMENT:	Sterile glass bottles Sterilised surgical instruments (“grafting” quality - scalpel handle, large scissors, small scissors and two pairs of fine curved forceps) Small petri dishes Sterile gauze Sterile Whatman filter paper (cut into strips) Bactigras (Smith and Nephew 10 x 10 cm antiseptic dressing with chlorhexidine acetate) Vet flex self adhesive stretch bandage (Lyppard, Global flex 10cm roll) Micropore tape, 25mmx9M Animal Clippers Ear Clips Heating Pad Cotton Buds 2,2,2 tribromoethanol (Sigma cat# T48402) tert-Amyl alcohol ReagentPlus 99% (Sigma cat# 152463) anaesthetic of choice 70% Ethanol Sterilised Phosphate-buffered saline (PBS) or Baxter saline Lacrilube Temgesic (buprenorphine hydrochloride, Reckitt Benckiser, Sydney, Aust.)

PROCEDURE: Donor ear tissue preparation

1. Constantly clean the surgical instruments with 70% ethanol throughout the grafting procedure to ensure contamination does not occur
2. Sacrifice donor mice by CO₂ asphyxiation using [SOP AHT 36 Euthanasia of Mice \(Carbon Dioxide\)](#).
3. Spray mouse head with 70% ethanol.
4. Cut the ears off the mouse at the ear base with large scissors. Float ears in a small petri dish containing PBS or 0.9% saline.
5. Separate the dorsal and ventral layers of each ear using curved forceps.
6. Place ear pieces hair-side down on Bactigras backing paper.
7. Using the flat end of curved forceps remove dermis and cartilage from the ear by firm scraping until the surface is uniformly flat and no further large pieces of cartilage can be removed.
8. Trim to the desired size and shape (sufficient to cut around the outline of the ear skin)
9. Float prepared graft with dermis down (paper-side up) in another small petri dish containing sterile PBS or 0.9% saline.

Grafting

(The animal technician will assist in steps 2-8 and steps 20-28)

1. After completing donor skin preparation, change latex gloves and spray gloves with 70% ethanol regularly during grafting procedure.
2. Anaesthetise each graft recipient. The amount administered is dependent on weight, sex and strain of the mouse.
3. Check mouse muscle reflexes by gently pinching the foot. If the mouse starts to flinch or wake up during surgery, immediately apply a “nose cone” containing a gaseous inhaled anaesthetic. Monitor mouse respiration throughout surgery and if the breathing rate drops, remove nose cone.

- 4. Working with one mouse at a time, remove hair from site of graft (usually right flank) with fine clippers and number each mouse by ear clip.**
- 5. Apply lacrilube to prevent eyes from drying out.**
- 6. Wipe grafting site with 70% ethanol and wipe dry to remove loose hair.**
- 7. Place mouse on heatpad covered by tissues.**
- 8. Grafts are placed on the right flank, on top of the ribs in the middle between the front and back limbs leaving sufficient space towards the head to bandage below the arms. Cut 3 sides of the graft site using a scalpel (top edge and 2 sides) approx 1cm square.**
- 9. By applying pressure along the length of the mouse, pull the skin of the right flank taut. With an even stroke, incise the epidermis and dermis down to the panniculus carnosus (avoid cutting so deep that blood is drawn). The panniculus carnosus (PC) appears frosty white, not as shiny as the body wall and contains many small blood vessels which can be moved above the body wall. Conserve the PC and blood vessels at the graft site. Male skin is thicker than female skin and may require extra downwards pressure to cut through the epidermis and dermis.**
- 10. Release hand pressure regularly to check size of the cut (make sure it isn't too large or too small) and allow the mouse to breathe freely. If cutting 2 graft sites (double graft), leave at least 2mm of recipient skin in-between adjacent graft beds.**
- 11. If any bleeding occurs, blot with a piece of sterile gauze.**
- 12. Once 3 edges of 1cm x1cm graft site have been cut, pick up top corner of the recipient skin graft area with forceps and tease apart the PC and the upper layers of the skin. Move along the graft bed and continue separating the skin until the graft beds are approximately the same size as the donor grafts (should have a bed containing a layer of white tissue (panniculus carnosus) and small blood vessels).**
- 13. Cut off the recipient skin from the graft site along the bottom edge using small scissors.**
- 14. Remove an appropriately sized donor graft from the petri dish containing PBS/saline and blot ear skin gently on sterile filter paper to remove excess liquid.**
- 15. Place donor ear graft on the graft bed of recipient mouse with the straight edge of the ear on the side closest to the head.**

- 16. Using forceps, gently centre the graft on the graft bed and align the edges of the donor and recipient skins. Trim off excess donor tissue if necessary. There should be no donor skin overlapping the edge of the graft bed. Stretch the recipient flank skin with the back of the forceps to fit donor skin inside graft bed border and to remove air bubbles. The donor skin should cover most (90-100%) of the graft bed. It is better to have a small graft site, completely covered by donor skin than a large graft site with exposed tissue.**
- 17. Cut a piece of vaseline gauze (Bactigras) to cover graft leaving approximately 5mm of extra gauze around the edges. The gauze prevents the graft from adhering to the dressings and minimises graft dehydration. Remove gauze from backing sheets using forceps and place directly on top of graft and pat down edges firmly (avoid touching the graft with fingers or patting it down with the sharp end of forceps).**
- 18. Cut 2 pieces of micropore tape approximately 15cm long.**
- 19. Lift the mouse by the front and hind legs while the operator holds the micropore tape taut (holding each end) on the bench.**
- 20. Align the tape with the graft and lower the mouse at 90°C to the tape (spine down).**
- 21. Roll the mouse to cover the new graft by the tape (graft down).**
- 22. The mouse is gently stretched by the animal technician to firmly apply micropore tape around the body but allowing the mouse to breathe and walk (care should be taken to not move the graft while applying the dressing).**
- 23. Trim off excess micropore and discard.**
- 24. Cut a piece of vetflex bandage wide enough to cover the graft and micropore (approx 4 cm wide). Wrap it around the mouse securely overlapping the vetflex ends firmly (this is like trying to roll a sleeping bag).**
- 25. Apply another layer of micropore tape (as above). The dressings should be firmly secure around the body to prevent the mouse from removing the dressings and dislodging the graft.**
- 26. Place the mouse in a fresh box on the heat pad to keep mouse warm and monitor mouse until full recovery.**
- 27. Administer a dose of Temgesic analgesic (a schedule 4 drug and should be administered by a trained operator) and monitor mouse until full recovery**

28. Return mouse to recovery cage.

Recovery

- 1. When mice have fully recovered from the analgesic, transfer the mice to a clean cage with food and water bottle (some food pellets placed onto the bedding or moistened mashed up pellets placed into a small dish can also be provided).**
- 2. The mice will be monitored post operatively daily for 3 days. It is important to check the bandages are firm, no limbs or teeth are trapped in bandages, mice are moving freely and there are no signs of distress. Wipe away any ano-genital discharge with saline-moistened facial tissue.**

Graft assessment

- 1. The mice should be regularly monitored post-grafting.**
- 2. Bandages are removed 7 days post-grafting. The mice are temporarily anaesthetised. The blunt/curved edge of the large surgical scissors is passed up under all the dressings from the abdomen towards the head (avoid poking the mouse). Carefully cut the dressings without cutting the mouse skin and peel the Bactigras from the graft using forceps (it is common for some hair to come away when removing the Bactigras).**
- 3. If there are excessive wounds present or failed grafts, mice must be culled immediately (the graft should appear pink, not excessively bloody and have a dull lustre. Graft may be missing entirely if dislodged or may be red and scabby).**
- 4. Use a grafting assessment sheet to frequently record visual assessment of grafts (start experimental graft assessment at day 8 as this allows time for the grafts to dry and be groomed by the mice after bandage removal).**
- 5. Grafts should be checked two or three times per week up to a maximum of 100 days, with frequent checking in the first week after bandage removal to ensure the graft does not get dislodged or infected.**
- 6. At each timepoint, score grafts for accepted/ rejected status. Accepted grafts will have clear graft margins, and light downy ear hair may be visible from approx 2/3 weeks after grafting. Rejected grafts may initially be red and inflamed or scabby. Rejected grafts produce scar tissue which tends to shrink in from all 4 edges and**

is shiny, there may be loss of graft margins, and overall 80% of the original graft area will be rejected/ “gone.”

7. At the end of the experiment, euthanase all mice.

REFERENCES

1. **Mayumi H, Nomoto K, Good RA et al. A surgical technique for experimental free skin grafting in mice. Japan Journal of Surgery 1988; 18:548-557.**
2. **Dunn LA, Evander M, Tindle RW, Bulloch AL, De Kluyver RL, Fernando GJ, et al. Presentation of the HPV16E7 protein by skin grafts is insufficient to allow graft rejection in an E7-primed animal. Virology 1997; 235:94– 103.**
3. **Frazer IH, De Kluyver R, Leggatt GR, Guo HY, Dunn L, White O, et al. Tolerance or immunity to a tumor antigen expressed in somatic cells can be determined by systemic proinflammatory signals at the time of first antigen exposure. J Immunol 2001;167: 6180 –7.**