Protocol

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Skin grafting in the amphibian *Xenopus laevis* has been used to detect not only allogeneic antigens that differ by minor H antigens or by one MHC haplotype, but also to detect ontogeny-specific antigens (including both emerging adult- and disappearing larval-specific) during metamorphosis. To understand the mechanisms underlying allogeneic tolerance or immune responses against larval- and/or adult-specific antigens, a complete MHC homozygous, inbred strain is the most appropriate experi-

Steinberg's solution (10×) <R>

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Xenopus laevis (inbred J strain and/or gfp-Tg hybrid line) in rearing containers.

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Equipment

Cotton sticks (Johnson and Johnson) (optional; see Step 8)
Digital camera (AxioCam HRc; ZEISS)
Filters (0.2-µm pore size) (Millipore GSWP04700)
Fine forceps (110-mm total length) (Natsume Seisakusho [Tokyo] MA-55) (sterilized with 70% ethanol)
Glass beakers (200-mL) (Iwaki Glass; Asahi Techno Glass, Tokyo)
Kimwipes
Liquid nitrogen
Microscissors (105-mm total length, 7-mm blade length) (Natsume Seisakusho MB-50-7) (sterilized with 70% ethanol)
Microscope (Leica M60)
Petri dishes (10-cm) (Corning 353803)
Plastic scale (3×3 mm^2)
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Suture needle with thread (6-0 blue nylon polyamide) (Nescosuture ET0806NA45-KF2)
Water aspirator pump (Sigma-Aldrich)

METHOD

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1. One to two days before the operation, transfer the frogs to a rearing container of filter-sterilized (0.22 µm) or autoclaved tap water. Keep them without feeding until the day of the operation.
2. On the day of the operation, gently wash the frogs in a glass beaker 10 times using sterilized water.
3. Anesthetize the animals by immersing them in a glass beaker containing 0.05% MS222 solution.

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4. Place a donor animal on its back on a sheet of Kimwipe of suitable size in a 10-cm Petri dish. Pour ice-cold 1× Steinberg's solution with gentamicin over the animal until the top of the body is almost immersed in solution.

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5. Place a plastic scale (3 × 3 mm^2

7. Add ice-cold 1× Steinberg’s solution with gentamicin to the dish until the tops of both animals are immersed. Transfer the skin graft by gently slipping the donor skin underwater into the graft bed (the hole on the back of the host) using forceps (Fig. 1D).
8. After grafting, immediately remove the Steinberg’s solution using a water aspirator pump. Wipe off the solution around the graft >5 times, particularly at the junction between the graft and the host skin, using a Kimwipe or a cotton stick (Fig. 1E).
9. If necessary, close the back slit of the donor with sutures (Fig. 1F).
10. Post-operation, move the host frog to a cold 200-mL glass dish or beaker containing a small amount of ice-cold 1× Steinberg’s solution with gentamicin to prevent drying of the animal. Place the dish on ice for 1 h (Fig. 1G).
11. Transfer the dish and incubate for 2–4 h in the dark at 4°C.
12. Transfer the dish and incubate overnight in the dark at 16°C.
13. Rear the host animal in a narrow glass beaker for 2–3 d at normal temperature (23°C–24°C) (Fig. 1H) with a small amount of 1× Steinberg’s solution with gentamicin.

TROUBLESHOOTING

Problem (Steps 3, 11, and 12): Animals vomit during the course of grafting.

Skin Grafting in *Xenopus laevis*: A Technique for Assessing Development and Immunological Disparity

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