

**Production of Hematopoietic
Chimeras**
IACUC Standard Procedure
Effective Date: May 2016



Description of procedure:

Bone marrow or fetal liver cells of a desired mouse strain are isolated and collected from euthanized donor mice. These will be delivered alone or mixed at a 1:1, 1:10, or other desired ratio, with bone marrow or fetal liver cells from another mouse strain of interest and are then transferred into lethally irradiated recipient mice.

Irradiation of recipient mice is performed by using an irradiator within one of the barrier facilities. Mice are placed into a rotating pie-shaped holder (to limit mobility and ensure equal irradiation), which is then secured in the irradiator to deliver a dose of 350-600R. The exact dose that is optimal may vary with strain. For B6 mice irradiated with an irradiator the optimal dose for hematopoietic ablation is 550R. The mice will be in the irradiator 2-5 minutes. Irradiation is repeated 3 hrs later for a total dose of 700R - 1200R (split dose irradiation is used to limit the non-hematopoietic toxicity -- usually intestinal damage). Mice will be monitored to assure there is no acute illness.

Doses or irradiation protocols other than these will be noted as an exception to the standard procedure in the PI's approved IACUC protocol.

Irradiated mice are injected within 18h after the second irradiation via the tail vein with donor bone marrow ($1 - 5 \times 10^6$) or fetal liver ($0.5 - 5 \times 10^6$) cells in 200-400 ul of sterile phosphate buffered saline. Alternatively, the cells may be injected retro-orbitally into anesthetized mice.

Cell sources or numbers outside this range will be noted as an exception to the standard procedure in the PI's approved IACUC protocol.

Each irradiated mouse will receive a single intravenous (tail vein) or retro-orbital injection of donor bone marrow cells. Anesthesia is required for the retro-orbital injection, and is recommended for the tail vein injection (as these mice will be debilitated). Review the standard procedures for Tail Vein Injection and Retro Orbital Injection. Recipient mice are maintained on antibiotic-containing water or feed for approximately 4 weeks. Duration of the entire procedure is typically 6-12 weeks (*deviations to be noted in approved IACUC protocol*).

Agents:

All agents administered to animals should be listed in the "Agents" section of the RIO IACUC protocol.

Adverse Effects:

Adverse effects should be listed in the "Adverse Effects" section of the RIO IACUC protocol.

Examples of potential adverse effects include: Transplant failure, anemia, infection, intestinal bleeding