

**Production of Hematopoietic Chimeras**  
**IACUC Standard Procedure**  
**Effective Date: August 2025**

**Description of procedure:**

Bone marrow or fetal liver cells (typically  $1-5 \times 10^6$ ) 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 X-ray irradiator within a barrier facility. 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 350R (sublethal) - 900R (lethal). The optimal dose may vary per strain<sup>(1-7)</sup>. To accomplish complete myeloablation, use the lethal dose (e.g., 900R for adult C57BL/6 mice), which may be fractionated into two doses (e.g., separated by 3 hours). In Section I of the protocol, list the dose to be administered and the interval between doses if splitting. Split dose irradiation limits non-hematopoietic toxicity, usually intestinal damage. After 2-3 days, mice with lethal irradiation may experience acute illness (e.g., if there has been intestinal damage) and should be monitored for potential adverse effects, though when following the SOP (900 rad given as a split dose), the majority of mice remain healthy during the reconstitution process, including during the first 10 days. Failure of hematopoietic reconstitution leads to illness during the 2<sup>nd</sup> week after irradiation, and mice should be monitored for this potential adverse effect.

Irradiated mice are then injected within 0-24 hours after the second irradiation via the tail vein or retro-orbitally as described in your protocol.

Each irradiated mouse will receive a single injection of donor bone marrow or fetal liver cells. Review the standard procedures for [Tail Vein Injection](#) and [Retro Orbital Injection](#). When using irradiated genetically immune-compromised (e.g., RAG-deficient) mice as recipients, it is recommended to maintain them on antibiotic-containing water or feed for approximately 4 weeks. This is advised even for a sublethal dose of irradiation. The use of antibiotic-containing water or feed is not typically required when using irradiated wild-type mice as recipients. Duration of the entire procedure is typically 6-16 weeks (*deviations must 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

**References:**

1. Kallman RF, Kohn HI. The influence of strain on acute x-ray lethality in the mouse. I. LD50 and death rate studies. **Radiat Res. 1956 Oct;5(4):309-17.**
2. Grahn D, Hamilton KF. Genetic Variation in the Acute Lethal Response of Four Inbred Mouse Strains to Whole Body X-Irradiation. **Genetics. 1957 May;42(3):189-98.**
3. Grahn D. Acute Radiation Response of Mice from a Cross between Radiosensitive and Radioresistant Strains. **Genetics. 1958 Sep;43(5):835-43.**
4. Mori N, Okumoto M, Morimoto J, Imai S, Matsuyama T, Takamori Y, Yagasaki O. Genetic analysis of susceptibility to radiation-induced apoptosis of thymocytes in mice. **Int J Radiat Biol. 1992 Aug;62(2):153-9.**
5. Iwakawa M, Noda S, Ohta T, Ohira C, Lee R, Goto M, Wakabayashi M, Matsui Y, Harada Y, Imai T. Different radiation susceptibility among five strains of mice detected by a skin reaction. **J Radiat Res. 2003 Mar;44(1):7-13.**
6. Ohta T, Iwakawa M, Ohira C, Noda S, Minfu Y, Goto M, Tanaka H, Harada Y, Imai T. Fractionated irradiation augments inter-strain variation of skin reactions among three strains of mice. **J Radiat Res. 2004 Dec;45(4):515-9.**
7. Biedermann KA, Sun JR, Giaccia AJ, Tosto LM, Brown JM. scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. **Proc Natl Acad Sci U S A. 1991 Feb 15;88(4):1394-7.**
8. Eng J, Orf J, Perez K, Sawant D, DeVoss J. Generation of bone marrow chimeras using X-ray irradiation: comparison to cesium irradiation and use in immunotherapy. *J Biol Methods* 2020;7(1):e125. DOI: 10.14440/jbm.2020.314
9. Gibson BW, et al. (2015) Comparison of Cesium-137 and X-ray Irradiators by Using Bone Marrow Transplant Reconstitution in C57BL/6J Mice, *Comparative Medicine*, Vol 65(3), 2015.
10. Wittenborn TR, Fahlquist Hagert C, Ferapontov A, Fonager S, Jensen L, Winther G, et al. (2021) Comparison of gamma and x-ray irradiation for myeloablation and establishment of normal and autoimmune syngeneic bone marrow chimeras. *PLoS ONE* 16(3): e0247501. <https://doi.org/10.1371/journal.pone.0247501>