

Bone Marrow Chimera Protocol

Prep 1 week before:

Make sure appropriate antibiotic is available: Diltrim (sulfadiazine/trimethoprim) or Sulfatrim (sulfamethoxazole/trimethoprim, to be added to the drinking water (5ml/200ml) of the irradiated animals for 2 weeks.

Coordinate with Animal Facility personnel for irradiator use.

Prep 1 day before:

Gather necessary materials:

- Sterile PBS
- DME media (no serum or antibiotics)
- 27 gauge needles and 1 cc syringes (a few)
- 30 gauge needles and 1 cc syringes (one for each recipient)
- sterile cell culture dishes
- surgical instruments (sharp, sturdy scissors for cutting through femur), medium forceps
- Kim wipes and bench diapers
- alcohol spray bottle
- cell counting equipment (trypan blue dye and hemocytometer)
- clean pipetmen & sterile tips
- sterile Falcon tubes and eppendorf tubes
- ice bucket

Mice: make sure recipient mice (C57BL/6J, 8-14 weeks) are in good health, and food is removed the night before (but water can stay).

Irradiation of recipients:

- check in at animal facility and get let into irradiator room
- wipe down mouse container from irradiator with antiseptic (bleach or alcohol spray in irradiator room), place mice in container (2-3 mice at a time)
- irradiate container with mice for appropriate amount of time to deliver 900-1000 Rads (90-100 Gy) = as of 03/07 should be about 14.2 minutes for their Cs-137 (but make sure at least 90 Gy)
- place mice back in cage and add food and antibiotics to water

Harvesting marrow from donor mice:

- One mouse (4 bones: femurs & tibias) should yield over 50 million cells
- Sac donor mouse, spray down with alcohol and remove skin from lower half of animal
- remove leg at hip joint (can feel with fingers where to cut)
- remove skin and muscle by cutting skin off and using Kim wipes to "rub" off muscle
- place bones in cell culture dish with PBS
- move to hood & use sterile technique from now on
- rinse bones with fresh PBS and transfer to new dish of PBS
- separate femur from tibia/fibula at the knee
- cut ends of bone off and transfer to new dish containing media
- flush bone marrow out with 27 gauge needle and syringe, and repeat with all
- pass marrow through a 22 gauge needle to break up any clumps

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- pass cell suspension through cell strainer basket to remove large fragments
- count cells and store on ice until ready to inject (only if more than 2 hours)
- when ready to inject, spin cells 10 minutes at 500 rpm and resuspend in PBS to give a final concentration of 67 million/mL

Injecting bone marrows cells into recipients (must be done within 24 hours of irradiation):

RETRO-ORBITAL PROCEDURE:

- Anesthetize with isoflurane
- Pick an eye and pull eyelids away from eye while pressing gently but firmly on skull bones around eye to make eyeball pop out slightly
- Insert 30 gauge needle below eyeball but above lower lid at an angle towards cheekbone - keep going until you just touch the cheekbone, then pull back a little bit and inject 150 ul (10 million cells) into the venous network there
- Check to make sure the cell mixture doesn't all come out the mouse's nose - if not, it was successful
- Give mice food and maintain them on anti-biotic water for 2 weeks.
- Analyze after 4-6 weeks. If donor cells don't repopulate bone marrow, mice will die in about 10 days.

TAIL VEIN PROCEDURE:

- Heat mice up in cage with heat lamp until they exhibit whisker grooming behavior, get spikey fur, or seem much more active. Then make sure they can get shade so they don't get too hot.
- Place mouse into restrainer with tail sticking out. Wipe injection site with alcohol swab and inject 150 ul (10 million cells) with 30 gauge needle. Watch for clearing of vein lumen (=success). If not in vein, move more proximal until successful. Apply pressure to injection site.
- Give mice food and maintain them on anti-biotic water for 2 weeks.
- Analyze after 4-6 weeks. If donor cells don't repopulate bone marrow, mice will die in about 10 days.