# Competitive Transplant

## Adapted from Carmen Da Silva (Miller Lab) and previous PCY experiments

## Materials

* Donor mice (age and sex matched)
* Recipient mice (age and strain matched)
* 70% ethanol
* Dissection tools
* FACS buffer
* 1ml insulin syringes
* 50ml tubes
* Ice
* RBS lysis buffer (ACK)
* Cellometer slides
* AO
* Irradiator pie
* Alcohol pads

## Preparation

1. Sterilize all supplies a few days before they are needed (dissection tools, mortar and pestle)
2. Label all tubes for tissue and cell isolation the night before
3. Make/ aliquot sterile FACS buffer
4. Reserve procedure room for 3 time points (book a week in advance)

## Protocol

Irradiation: Animal Facility

1. Place up to 10 animals at a time in the irradiator pie
	1. Be careful to label the slots that each mouse was placed in
2. Administer first dose between 7-8am
	1. 475 cGy
	2. Give hydrogel, check lixit
3. Administer second dose 3-4 hours later (around 11am)
	1. 475 cGy
	2. Check lixit

Tissue and Cell Isolation: On the Bench

1. Euthanize mice with CO2
2. Spray liberally with 70% ethanol
3. Remove femurs and tibias, cleaning away any muscle/ tissue
	1. Remove top of the bone to expose the BM
4. Spin-flush the bones and pool BM from each mouse
	1. 200ul FACS in 1.5ml tube, 0.5ml tube with hole, spin down max. speed for 30 seconds
5. Resuspend with P200
6. Add 200ul FACS
7. Pool samples from same mice and transfer to a filtertop FACS tube, final volume ~800ul
8. Count with AO
9. Resuspend cells in FACS at 40 million/ml

Flow Check

1. Take 200ul of each cell sample for flow staining
2. Add 5ml ACK lysis buffer and transfer to a 15ml conical
3. Incubate on the rocker for 5’
4. Add 5ml FACS to neutralize ACK
5. Spin down at 500xg for 5’ at RT
6. Stain with CD45.1, CD45.2, and 7AAD

Transplant Injections: Mouse Facility Procedure Room

1. After the second dose of radiation, bring cells up to the animal facility procedure room on ice
	1. Keep cells on ice for duration of procedure
	2. Resuspend cells before loading syringes
2. Load syringes with desired volume (between 100 and 200ul)
3. If doing RO injections
	1. Fully anesthetize mice
	2. Inject into eye socket behind the eyeball
4. If doing TV injections
	1. Put cage under heat lamp
	2. Restrain mice
	3. Clean tail with alcohol pad
	4. Bend tail and inject
5. Check on mice everyday for the first week after transplant
	1. Replace hydrogel everyday
6. Check on mice every other day for the second week
	1. Replace hydrogel every other day
7. Check mice periodically for remainder of experiment (at least 1-2 times per week)
	1. Check at 4, 8, 12, 16 weeks with peripheral blood flow/ CBC checks
	2. Takedown at 20 weeks with full flow panel