The Fleischman Lab

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Title	**Bone Marrow Transduction-Transplantation**	
Introduction	Bone marrow transduction-transplantation is a valuable method used to model hematologic malignancies in mice. In this system, bone marrow cells from donor mice are infected with retrovirus (transduction) causing overexpression of an oncogene. These cells are then transplanted into recipient mice whose own bone marrow cells have been cleared by irradiation. The transduced donor cells replenish the recipient's bone marrow with oncogene-expressing cells to generate a hematologic malignancy, in our case MPN. This protocol details the steps involved in transduction-transplantation and should be supplemented with our *Isolation of Bone Marrow from the Major Leg Bones* protocol for section B. Viral supernatants used in this protocol are generated using our *Viral Production* protocol. Several changes may be made to this protocol to answer specific questions, such as transplanting sorted cells rather than whole bone marrow or performing secondary or tertiary transplantations.	
Materials	1. 5-fluororacil (5-FU) 2. Donor and recipient mice 3. 10ml syringes 4. ⅜" 25G needles 5. 70% EtOH 6. D10 (DMEM + 10% FBS + penicillin/streptomycin/L-glutamine) 7. 100µM cell strainers 8. 50ml conical tubes 9. ACK buffer 10. Pre-stim media (DMEM, penicillin/streptomycin/L-glutamine, FBS, mIL-3, mIL-6, mSCF) 11. 6-well tissue culture plates 12. Viral supernatant 13. Polybrene (10mg/ml) 14. PBS 15. MicroFACS tubes 16. Insulin syringes with ½" 27G needles 17. Serological pipettes and pipette-aid 18. Centrifuge with plate carriers 19. X-ray irradiator 20. Cytometer (BD Accuri) for counting cells 21. 37°C 5% CO_2 incubator with ≥95% humidity	
Protocol	**A. Day 0: 5-FU Treatment of Donor Mice**	*Notes*
1.	Treat donor mice with 150mg/kg 5-FU via retro-orbital injection. Donor mice should be 4-8 weeks old.	*1 donor mouse can donate enough bone marrow for 2 recipient mice.*
	B. Day 5: Isolation of Bone Marrow from Donor Mice	
1.	Sacrifice donor mice and harvest bone marrow. See our *Isolation of Bone Marrow from Major Leg Bones* protocol for detailed instructions.	
2.	After counting cells, centrifuge for 10 minutes at 1200 rpm (400g).	

3.	Resuspend cells to a concentration of 1.5-3x10^6 cells/ml in 2x prestim media: DMEM 40mL FBS 10mL Pen/Strep/L-Glutamine 1mL Filter Sterilize *Then Add: (50µg/mL) IL-3 14µL (100µg/mL) IL-6 12µl (100µg/mL) SCF 56µL	*Prestim media must be made fresh! Only make the amount you need (cut all volumes in half if you need <25mL)* *2x prestim media can be made with WEHI-CM and reduced IL-3.* *Additional prestim formulations are available in the Recipes box.*
1. 4.	Transfer cells to a 6-well plate with 4ml/well. Incubate cells for 24 hours.	
	C. Day 6: First Spinoculation	
1.	Wash bone marrow cells from plate and count on Accuri.	
2.	Resuspend cells in 2x prestim media at a concentration of 2x10^6 cells/ml. Transfer 2ml of cells to each well of a 6-well plate.	
3.	Add 2ml of viral supernatant with at least 2x10^6 pfu/ml. If the viral titer is above or below 2x10^6 pfu/ml, adjust the volume with prestim media. The final volume should be 4ml/well.	*The final solution should be at a 1:1 ratio between pfu/ml and bone marrow cells/ml.*
4.	Add 4µl Polybrene to each well for a final concentration of 10µg/ml.	
5.	Centrifuge the plates at 30°C for 90 minutes at 2500 rpm (1000-1500g) with brake off.	*Adjust the speed to reflect the centrifuge used. Note that plate carriers alter the rpm to g calculation.*
6.	After spinoculation, return the cells to the incubator overnight.	
	D. Day 7: Second Spinoculation and Irradiation	
1.	Repeat steps C1-C5 above, reusing the 6-well plate from the 1st spinoculation. After spinoculation, return the cells to the incubator overnight.	*If desired, cells may be resuspended in total viral supernatant supplemented with prestim cytokines and polybrene.*
2.	Irradiate mice on the evening before transplantation. There are different radiation doses for different mouse strains: Strain / Dose (if single) / Dose (if split) BALB/c / 1x 800 rads / 2x 400 rads C57BL/6 / 1x 900-1200 rads / 2x 450-600 rads If doing split doses, each dose should be separated by at least 3 hours.	
	E. Day 8: Transplantation	
1.	Transfer the bone marrow cells to a conical tube. Be sure to harvest all cells from the plate by pipetting. Wash the plates with PBS to remove any remaining cells and transfer to the conical tubes. Trypsinize cells to collect any remaining cells if desired.	

2.	Count cells on the Accuri. Centrifuge the cells at 4°C for 10 minutes at 1200 rpm (400g).	
3.	Resuspend cells in PBS for a final concentration of $5x10^6$ cells/ml. Keep cells on ice until injection.	*This recipe is for 500k whole bone marrow cells per recipient mouse. If other concentrations are desired, adjust the volume of PBS accordingly.*
4.	Load cells into insulin syringes with ½" 27G needles.	
5.	Inject 100µl of cells per recipient mouse via retro-orbital injection.	
6.	Monitor mice for signs of disease: • Survival beyond D14 post-transplant indicates successful engraftment of donor cells. • CBCs and %GFP should be first assessed between D14 and D30. • The time required to develop an MPN phenotype varies by model. Check CBCs and %GFP every 2 months to monitor disease progression.	