Transduction Protocol (BM progenitors)

Use freshly isolated bone marrow cells. Use 1 x 10^5 cells per vial for spinoculation.

BM medium: αMEM, 15 % FBS, 1% PenStrepGlutamine, 50 μM βME

BMC medium: BM medium + cytokine cocktail:

a. IL-3 6 ng/ml
b. IL-6 10 ng/ml
c. SCF 10 ng/ml

(can lower concentrations of IL-6 and SCF)

Keep virus supernatant on ice

1) Isolate bone marrow cells per standard protocol (typically use mice that are 3 weeks old)
2) After adhering overnight, take nonadherent fraction and put them over a Ficoll gradient
3) Resuspend cells in BMC medium in a 6 well plate for 24 hr prior to transduction. Plate them at 5 x 10^5/ml.
4) On day of transduction, count cells and resuspend BM cells in BM media at 2 x 10^5 cells/ml. Prepare 5 vials per virus condition.
5) Aliquot 0.5ml per 2ml vial (Fisher catalog no. 05-669-29). For Phoenix-based retrovirus, add 0.5 ml of retroviral supernatant and 1 μl of polybrene. For 293T based VSVg pseudo-typed virus, use 2 μl of virus and 0.5 μl of polybrene.
6) Incubate at 37 deg for 1 hr
7) Spin at 10,000 x g in a JA20.1 rotor for 1 hr, 37 deg
8) Remove supernatant, resuspend in 0.5 ml of BMC medium and transfer to 15 ml conicals. Let recover in 37 deg incubator for 3-4 hr, with the cap loosely on. Save the 2 ml vials for the next step.
9) Transfer cells to 2 ml vials. Repeat spin as above x 1 for Phoenix-based virus and x 2 for 293T based virus.
10) Let recover in 12 well plate in 37 deg incubator overnight.
11) Optional for Phoenix based virus: The next day, transfer cells to 2 ml vials and repeat spin.
12) Expand in BMC medium for an additional 24 hr prior to differentiation.