Gene Transduction of 1° MKs by Retroviral Infection

A. BSA (Bovine Serum Albumin) step gradient:

Purify the megakaryocyte (MK) fraction from a day 2 liver culture (~10 livers/10 mL/10 cm tissue culture dish).

1) Pipet 1.5 mL of sterile 3% BSA (made in PBS) into a 15-mL Falcon tube.
2) Slowly pipet 1.5 mL of sterile 1.5% BSA onto the 3% BSA solution.
3) Centrifuge cultured liver cells at 1200 rpm (table-top centrifuge) for 3-5 minutes.
4) Remove supernatant, loosen cell pellet by tapping gently, and resuspend in 1 mL DMEM
5) Layer this cell suspension carefully over the BSA step gradient and hold at room temp.
6) MKs will sediment within 30-50 minutes.
7) At this stage the upper layers (consisting originally of the cell suspension and the 1.5% BSA solution may either be discarded if not needed, or washed once to remove the BSA and cultured again to harvest the "second wave" of differentiated MKs.
8) Resuspend lower 1/3 of 3%BSA and cell pellet (consisting of the denser population of mature MKs) in 8 mL DMEM..
9) Centrifuge for 5 minutes at 1200 rpm (table-top centriguge) to remove residual BSA, and discard supernatant.

B. Retroviral infections:

Empirically, we have found several parameters to promote high infection rates, such as high densities of MKs, addition of polybrene, and purity of the MK fraction.

a) Prepare 2 mL of complete DMEM with 0.5% Tpo and 2 µL polybrene (2 µL of a sterile 6 mg/mL stock solution).
b) Resuspend MK pellet in 2 mL of this complete medium.
c) Pipet 0.4 mL of the cell suspension into each well of a 12-well tissue culture plate.
d) Apply 50 µL of retrovirus stock to each desired well and resuspend.
e) Place 12-well plate into 37° C TC incubator for 24 hours.
f) After 24 hours, wash virus away as follows: Pipet 450 µL of MKs/virus into labeled sterile Eppendorf tubes and centrifuge at 4000 rpm for 1-2 minutes. Discard the supernatant, taking care to treat this waste with bleach.
g) Resuspend pellet (MKs) in 400 µL complete DMEM supplemented with 0.5% Tpo and transfer cells into new wells of a 12-well TC plate.
h) Place in 37° C incubator and monitor for production of proplatelets or until cell population achieves desired maturity.