Introduction
Mesenchymal Progenitor Cells (MPC) have been implicated as potential targets in a range of cellular therapies for the treatment of defects of both the hematopoietic and skeletal systems and as vehicles for gene therapy. In order to utilize the potential of these cells in various therapies, a pre-clinical animal model in which both the biology and potential therapeutic applications of these cells can be assessed is of fundamental importance. The goal of the current study was thus to develop a simple, robust and reproducible method for the isolation of Mesenchymal Progenitors from mouse compact bone (CB).

Methods
CB cells were harvested from tibiae and femurs of C57BL/6j mice.

Cell Culture
Colonies forming unit-fibroblast (CFU-F) assay: Pre-enriched CB cells were plated in a 6-well plate at densities ranging from 10,000 - 50,000 cells/well. Enriched hematopoietic mouse cells from bone marrow (SCA-1+ cells; 92% purity) isolated with EasySep® Mouse SCA-1 Positive Selection Kit were plated under maintenance of primitive hematopoietic cells. Enriched hematopoietic mouse cells from bone marrow were differentiated into adipocyte, chondrocyte or osteogenic cells and stained with Oil red O stain, Alcian Blue stain and Alkaline Phosphatase stain.

Figures
Figure 1. Isolation of MPC from mouse CB (Technical Manual available, StemCell catalog # 28374)

Figure 2. EasySep® procedure for column-free enrichment of MPC (StemCell catalog # 19771)

Figure 3. Culture in low (5%) oxygen leads to a 35-fold increase in CFU-F derived colony numbers from CD45 / Ter-119 depleted CB fraction; n=3.

Figure 4. Enrichment of MPC by immunomagnetic depletion of CD45+ and Ter-119+ cells

Figure 5. Sca-1 expression on CB cells pre- and post-enrichment

Figure 6. Differentiation of CB-enriched cells into adipocytes, chondrocytes, and osteogenic cells

Figure 7. Phenotype of enriched mouse MPC from CB culture at Passage 2 (P2)

Table 1. CFU-F Enrichment and Recovery

Table 2. LTC-IC frequency (preliminary results)

Conclusions
- Culture of MPC in low oxygen is crucial for optimal CFU-F formation
- MPC can be highly enriched from CB using a simple and rapid (<1 hr) EasySep® magnetic separation procedure
- CB enriched MPC are capable of supporting primitive hematopoietic cells in long-term culture