

## Bone Cells Core

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# Osteoblasts From DMP1-GFP Mice for Osteocytes isolation (Sorting)

### Osteoblasts from DMP1-GFP mice for osteocytes isolation (Sorting)

Plate primary osteoblasts isolated from DMP1-GFP mice in 10 cm dish.

Change medium (a-MEM + 10%FBS+1%PS) on Monday-Wednesday and Friday

For sorting cells (everything should be sterile):

- 1) aspirate medium
- 2) wash once with PBS
- 3) add 2 ml trypsin and wait-3-5 min or until all cells are lifted from the plate
- 4) add 3-4 ml of **PBS-2%FBS** and resuspend the cells well
- 5) transfer all cell suspension in 15 ml conical Falcon Tube
- 6) centrifuge at 4C at 850-900 RPM for 10 min (new table top centrifuge)
- 7) aspirate the supernatant (careful not to aspirate cells. Leave some volume behind)
- 8) resuspend the cells in 300-400ul (depending on the size of cell pellet) of **PBS-2%FBS**
- 9) transfer the cells into a 5 ml round-bottom tube with blue cell-strainer cap (BD Falcon 352235).
- 10) Keep cells on ice. Cells are ready for sorting (GFP-positive only)
- 11) Bring some medium (30 ml) along with the cells for sorting
- 12) Sorted cells need to be spun (850-900 RPM for 5-10 min) and plated in 1 well of a collagen coated 6-well plate
- 13) Change medium Monday-Wednesday and Friday