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

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Plate primary osteoblasts isolated from DMP1-GFP mice in 10 cm dish.
Change medium (α-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 cell 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