Inadvertent
Use of 100Ml led to
loss of cells.

28. After 72 h, carefully remove the supernatant from the top, resuspend pellet in ~~200~~ 100 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using Pasteur pipette

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29. Again add ~~200~~ 100 µl wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm tissue culture dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. ~~Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA~~
35. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 5 cc syringe with 21 gauge needle
37. Determine cell concentration by transferring 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transer $2x10^6$ cells to T75 flask for mutant expression. Didn't have enough cells for $2x10^6$
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer ~~200~~ 100 µl of cell suspension (in triplicate) to 7 ml scintillation vial containing 6 ml cocktail (Ecolume)
41. Incubate tissue culture dishes for 1 week
42. Count vials for radioactivity **Date/Time:**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.