RETROVIRAL PRODUCTION PROTOCOL

Producer Cells Used: PhoenixAmpho PhoenixEco Bosc 23 Bing Cak 8

Day/Time/Notes	Activity
1.	Plate 7 x 10^6 Cells/100 mm plate in DMEM+10%FBS + pen/strep
	Incubate 24 h at 37C/ 5%CO_2
2.	Transfect with plasmid DNA a. Re-feed cells with 8 ml growth medium and add 10 ul of 1000X chloroquine (25 mM stock) b. Dilute DNA:_____ug (36-48 ug/plate) into a 875 ul volume of sterile H2O c. <u>Clone Name</u> '_____': ____ul stock + _____ul H20 '_____': ____ul stock + _____ul H20 '_____': ____ul stock + _____ul H20 '_____': ____ul stock + _____ul H20 '_____': ____ul stock+_____ul H20 c. Add 125 ul 2M CaCl2 to each tube d. Add 1 ml of 2X BES with bubbling or vortex e. Add to cells drop-wise
Actual time of transfection	Incubate 6-12 hr at 37C/5% CO2
	Re-feed with 27 ml of fresh medium 24 h after the start of transfection, switch to 9 ml of medium and transfer to 32C/5%CO2 incubator
	a. Harvest 24-48 h supernatant 24 h after re-feeding. Re-feed plates with 6 ml of medium and return to 32C. Filter harvest through 0.45 um syringe tip. Freeze supernatant tubes in liquid nitrogen and store @ -80C
	b: Harvest 48-60 h supernatant 12 h after (a), filter and freeze. Re-feed with 6 ml medium and return to 32C.
	c. Harvest 60-72 h supernatant 12 h after (b), filter and freeze. You can assay producer cells at this point for your gene expression, if possible.