Retroviral gene transfer protocol
Barton lab
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Day 0:
Split GP2-293T/**Φx** packaging cell line for transfection the following day:

6cm plate:	10cm plate:
1.75-2.0x10^6 cells	7.5x10^6 cells

Day 1:
Transfect packaging cell line by $CaPO_4$

6cm plate:	10cm plate:
5μg DNA	15-20μg DNA
3μg vsv-g	10μg vsv-g

For $CaPO_4$ transfection:

Pre-incubate packaging cells 30' with **25μM chloroquine**

6cm plate:	10cm plate:
1.5μL 50mM stock → 3mL	5μL 50mM stock → 10mL

for ea plate combine *in numbered order* in a 15mL conical*:*

1) 8-16μg DNA	25-50μg DNA
2) H_2O to 0.438 mL	H_2O to 1.314 mL
3) 62μL $CaCl_2$	186μL $CaCl_2$

using a 10mL pipette bubble DNA and add drop-wise (as slow as you can stand):

0.5mL 2x HBS	1.5mL 2x HBS

replace media after ~8hrs:

remove 2mL, add 3mL	remove 8mL, add 10mL

incubate o/n @ 37°

Day 2:
Replace media on transfection

6cm plate:	10cm plate:
remove 3mL, add 2mL	remove 10mL, add 6mL

transfer to **32**° incubator o/n

split target cell line for transduction the next day:

	6 well plate:	10cm plate:
293T	3x10^5 cells	18.0x10^5 cells
3T3	1.0x10^5 cells	6.0x10^5 cells
RAW	3x10^5 cells	18.0x10^5 cells

Day 3:
Harvest virus supernatant:

Filter sups through a 10cc syringe fitted w/ 22-45μm filter into a 15 mL conical

Optional virus concentration step from 10cm plate:

Transfer sup to tubes for Sw40 Ti swinging bucket rotor
Spin at 50,000g (16.8rpm) for 90' in ultracentrifuge
Suck sup off of viral pellet
Re-suspend in 1mL media (1mL viral sup → 1 well of target cells in a 6 well plate in 2mL media)

Add polybrene (hexadimethrine bromide) @ 5μg/mL to viral sup (1μL/mL of 5mg/mL stock)

Suck supernatant off of target line, apply retroviral sup
Incubate o/n at **32**°C

Day 4:
Suck off viral sup and replace media on the infected cells and return to 37°C

Day 5:
Analyze cells for expression of marker