Use of "293-GPG" cells to Generate Amphotropic Retroviruses, plus infection protocol

Background Information:

- These cells were generated in Richard Mulligan's laboratory (Ory, Neugeboren and Mulligan, Proc Natl Acad Sci, **93:**11400) and allow for the production of high titer amphotropic retrovirus (using the procedure below). Many retrovirus packaging cell lines lose packaging efficiency as they are cultured due to the gradual loss of expression of the packaging genes. The packaging plasmids introduced into this cell line (293-GPG) were introduced using different selection markers. Therefore, expression of packaging proteins can be fairly well maintained by culturing the cells continuously in media containing the corresponding antibiotics. In addition, the VSV-G protein is toxic to 293 cells so expression of this gene is controlled by tetracycline. Therefore, these cells should be maintained in DME+10% FBS (+1ug/ml tetracycline, 2ug/ml puromycin, and 300ug/ml G418)].
- For safety issues, we primarily carry out amphotropic packaging of a retrovirus encoding the gene for the ecotropic retrovirus receptor (the vector we use is from Scott Lowe's laboratory, pWZL-Neo-EcoR). We then infect cells of interest (usually human) with this virus, select with G418, and use cells directly (and make freezes). These cells are now susceptible to infection by ecotropic packaged retroviruses (note: not only are the recipient cells now infectable with an ecotropic retrovirus, but the infection efficiency should be higher than in most mouse cells as a result of over-expression of the ecotropic retrovirus receptor). To carry out specific experiments, we then package retroviruses containing genes of interest in an ecotropic system. Using this virus (which cannot infect normal human cells) we can then efficiently infect the converted human cell line of interest.
- The method outlined below was derived from optimization experiments carried out by Miguel Campanero. If you perform the experiments exactly as indicated, high titer virus should be readily obtainable.

Protocol:

I. Generating Virus

- Split a ca. 90% confluent 100mm plate of 293-GPG cells 1:10 into 5 new plates containing 10 ml fresh in DME+10% FBS (+1ug/ml tetracycline, 2ug/ml puromycin, and 300ug/ml G418)] for each retroviral construct.
- The next morning, change media on each plate with 8 ml of fresh DME+10% FBS (+1ug/ml tetracycline, 2ug/ml puromycin, and 300ug/ml G418)].
- Late in the afternoon, transfect cells using the Calcium Phosphate method outlined elsewhere in the flemingtonlab web site. Use 30ug/transfection of the retroviral vector (in our case, this is usually the ecotropic retroviral receptor plasmid, pWZL-Neo-EcoR).
- The next day, the precipitate will be taken off and VSV-G expression will be induced by withdrawing tetracycline from the media. Aspirate DNA precipitate from each plate, wash 2x with 5 ml sterile 1X PBS, and add 3 ml trypsin/EDTA (Life Technologies). When cells start to come off, tap plate with fingers to release remaining cells. Neutralize with 3 ml of fresh DME (+10% FBS + Pen/Strep). Pipette up and down 10-15 times.
- Pool all cells and spin down.
- Suspend in 5ml 1X PBS and spin down again.
- Suspend cells in 10 ml of fresh DME [+10% FBS + Pen/Strep (**no** tetracycline-this is of course critical!)] and plate onto one 100 mm plate.
- The next day, replace media with 10 ml of fresh DME (+10% FBS + Pen/Strep).

- After letting cells culture for 2 days, take off media [replace with 10 ml fresh DME (+10% FBS + Pen/Strep)], spin cells out of media and divide supernatant into 1 ml aliquots (in sterile tubes) and freeze at –70°C until use.
- The next day, take off media again [replace with 10 ml fresh DME (+10% FBS + Pen/Strep)], spin cells out of media and divide supernatant into 1 ml aliquots (in sterile tubes) and freeze at –70°C until use.
- The following day, take off media again [replace with 10 ml fresh DME (+10% FBS + Pen/Strep)], spin cells out of media and divide supernatant into 1 ml aliquots (in sterile tubes) and freeze at –70°C until use.
- The following day, take off media again [replace with 10 ml fresh DME (+10% FBS + Pen/Strep)], spin cells out of media and divide supernatant into 1 ml aliquots (in sterile tubes) and freeze at –70°C until use. (Note: The viral harvest from each of these three days should yield high viral titers but subsequent days may not, so you can now discard the plate).

II. Infection with virus

- Split recipient cells either 1:10 or 1:20 (for very fast dividing cells) into a well of a 6 well plate containing fresh media.
- The next day, replace media with 1 ml of virus + 1 ml of fresh complete media + polybrene (see below for amounts of polybrene).
- Spin cells for 1 hr. at 1,000g (2,200rpm in Beckman tabletop centrifuge) at 22°C.
- Carefully (without disturbing media very much) transfer to tissue culture incubator and incubate for 4-5 hrs more.
- Aspirate off media and replace with fresh media.
- Culture for 2 days before adding selectable marker (if needed – i.e. unless you know that the titers are very high).

III. Misc. Info

- 293-GPG cells must be maintained in: DME
  +10% FBS
  +1ug/ml tetracycline
  +2ug/ml puromycin
  +300ug/ml G418

- The amount of polybrene (stock solution is 1.6ug/ul) used during infection should be the highest concentration that is tolerated without killing cells. Below lists some concentrations for cells that we have tested.

HCT15	24ug/ml
SAOS2	12ug/ml
NIH3T3	8ug/ml
IMEC	4ug/ml

Polybrene – Sigma (Cat # H-9268) Make up to 1.6 mg/ml in media (e.g. DME or RPMI) and store at –20°C.

- Listed below are the concentrations of drugs that are used to select for stable integration of the indicated selectable markers.

	G418	Puro	Hygro
HCT15	0.9mg/ml		400ug/ml
SAOS2	0.9mg/ml	1ug/ml	400ug/ml
U2OS	0.6mg/ml	1ug/ml	200ug/ml
IMEC	0.05mg/ml		25ug/ml

G418 – Media Tech (General Stores - Cat # 4361) Make up in 1x PBS. Store at –20°C.
Puro – Sigma (Cat #P7255) Store at –20°C.
Hygro – Boehringer Mannheim (Cat #843555) Store at 4°C.