RETROVIRUS PRODUCTION

IMPORTANT NOTE

" The viral supernatants produced by these methods might, depending upon your retroviral insert, contain potentially hazardous recombinant virus. The user of these systems must exercise due caution in the production, use and storage of recombinant retroviral virions, especially those with amphotropic and polytropic host ranges. This consideration should be applied to all genes expressed as amphotropic and polytropic pseudotyped retroviral vectors. Appropriate NIH and other regional guidelines should be followed in the use of these recombinant retrovirus production systems. The user is strongly advised NOT to create retroviruses capable of expressing known oncogenes in amphotropic or polytropic host range viruses." Gary Nolan

Make sure your Phoenix cells are in good shape. For selection of gag-pol/env expression see http:/www.stanford.edu/group/nolan/phx_selection.html. These cells appear to be less than adhesive so I plate them on poly-lysine plates prior to transfection. To get them ready the day before I split Phoenix cells 1:2 to make sure they are proliferating at the time of plating. Please check out Gary Nolan's website to get information from the source himself www.stanford.edu/group/nolan/

As a rule of thumb use Phoenix Ecotropic packaging lines (Phoenix E) when infecting rodent cells and Phoenix amphotropic packaging lines (Phoenix A) to infect human and other cell types.

Reagents Required:

Phoenix A or E cells
LipofectAmine 2000 Transfection Reagent (Invitrogen)
OptiMEM (Invitrogen)
0.45uM PVDF syringe Filter Unit (Millipore cat# SLHV R25 LS)
3 or 10 ml Luer-Lok Syringe (B-D # 309585, 309604)
Polybrene (polybrene is lOOOx at 5 mg/ml)
Falcon 2058 Polysterene snap-cap tubes
Poly-D-Lysine (0.2μM filtered)

Day 1 Plating Phoenix Cells

1. (*optional step*) Treat 100mm tissue culture dishes ~2.5ml of sterile (0.2uM filtered) 50ug/ml poly-D-lysine (Sigma) at room temperature for 5 min to 2 hours. Alternatively, I also use 6 well dishes to make virus if I am to infect a single dish. Aspirate and rinse plates twice in 5-10 mls of sterile water or 1xPBS and once in 4-5ml full media. The plates are now ready to be used. If you wish to store them, simply aspirate after the poly lysine step, let them dry and store at room temperature.
2. Plate Phoenix cells such that they are at 60-80% confluent (~3-6x10^6). These cells will adhere rapidly so much so that you can transfect the cells the same day.

Day 2 Transfecting Phoenix Cells

3.* Make DNA-liposomes. I use 2-2.5ul Lipofectamine 2000 for each 1ug of plasmid DNA. For a 6 well dish use 2ug of DNA for a 100mm dish anywhere from 10-20ug works well. Set these up in a total volume of 200ul and 1000ul respectively. **NEVER use**

polypropylene tubes, **ALWAYS use polysterene** tubes (Falcon 2058). This is done at room temperature for 20-40 minutes.

* Alternatively, I have used Fugene6 (2-3ul/1ug DNA). Both work well. If using the Fugene6 one need only make the liposome-DNA conjugates, add them and leave it overnight.

4. At the point you are ready to transfect, rinse cells twice in OptiMEM (Invitrogen), add 4ml OptiMEM and place them at 37°C.
5. Add dropwise to the Phoenix cells and incubate at 37°C for 2-5 hours. Call this t=0. Agitate the plate gently every 90minutes to distribute the liquid over the monolayer.
6. Add 5ml of full media with 20% FCS and incubate overnight.

Day 3 Fresh media & ready recipient cells.

7. The next day, about 19 hours later) remove the media and replace with 6-7ml fresh media containing 20% FCS and incubate overnight at 32°C (37°C works but the retroviruses are more stable at 32°C). At this point there is little virus being produced.
8. Plate recipient cells at a density that will allow consistent cell growth over at least 3 days. These retroviruses require nuclear membrane breakdown in mitosis for the viral cDNA/ nucleoproteins to gain access to the genome. Thus your cells must go through S-phase at some point after the virus enters the cell.

Day 4-5 Virus collection/infection

9. At around 42-48 hours after t=0, remove and **save** the media.
10. Add 5ml full media with 20% FCS back to the Phoenix cells (if you want to collect more virus).
11. Filter through a 0.45uM low protein binding syringe filter. Virus can be used to directly to infect cells. Virus supernatant can be frozen at –80°C for future use (each freeze/thaw only causes a two-fold reduction in titer). A 0.22uM filter can be used but leads to a reduction in infectious units. The point is not to sterilize the virus as it is already sterile.
12. Filtered viral supernatant should be mixed with full media at least 1:1and add to recipient cells** in the presence of 2-8ug/ml polybrene.

**Note if you forget to split your recipient cells on day 3, you can add virus to cells that are still in suspension and let them settle in the presence of virus.

13. Repeat steps 9-12 at ¥t=60hr and t=72hr. (¥this one is optional but it does help)

Day 4-5 Virus collection/infection

14. Allow 48 to 72 hours post-infection (120-144hr after transfection as above) for peak expression of retroviral-encoded genes.