Protocol 1 / 2 / 4 production of Lenti and Pseudo Retro viral particles.

Virus producing cell lines (with large T antigen): 293FT, HEK293T with combinations of plasmids:

| | Protocol 1 | Examples of vectors in comination with | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| psPAX2 | 2nd generation packaging (gag-pol-rev-tat) | pRSI12 | pLKO.1 | pLV | pLM | etc | | | |
| pCMV-VSVG | Envelope | | | | | | | | |
| pCMV-VSVG | Envelope | | | | | | | | |
| pCMVd8.74 | 2nd generation packaging (gag-pol-rev-tat) | pTRIPZ | pHIV | pLenti | pLKO.1 | pGreenFire lenti-reporter (pGF1) | | | etc |
| | Protocol 2 | | | | | | | | |
| pMDLg/pRRE | 3rd generation packaging (gag-pol) | FUGW | FUW | pWPT | pLKO.1 | pLenti | etc | | |
| pRSV-Rev | 3rd generation packaging (rev) | | | | | | | | |
| pMD2.G | Envelope has VSVG | | | | | | | | |
| | Protocol 3 | | | | | | | | |
| LentiX systeem | 4e generation packaging | pLVX | | | | | | | |
| pLentiX Gag-Pro | | | | | | | | | |
| pLentiX Pol | | | | | | | | | |
| pLentiX TR | | | | | | | | | |
| pTet-off | | | | | | | | | |
| | Protocol 4 | | | | | | | | |
| pv-pack-gp | Retro Pseudo packaging | pBabe | pQCXIH | etc | | | | | |
| VSV-G | | | | | | | | | |

Virus production and transduction.

We use the virus production cell line 293FT:

http://tools.invitrogen.com/content/sfs/manuals/293ft_cells_man.pdf

take in a T75 in culture, thaw in hand, pipet into 15ml tube , add 10ml drop by drop of cold while swirling 10% FCS/DMEM High glucose/PenStrep

Spin down 5 min 1200rpm, discard supernatant, resuspend pellet in 10ml

10% FCS/DMEM High glucose/PenStrep pipet in T75

in incubator, expand and freeze down

Once in a while put them under G418 to retain Large T antigen, according to the manual for the cell line from invitrogen.

Our lab packages lenti ant retroviral vectors.

Protocol:

Your lenti HIV promotor based vector of interest and packaging plasmids: VSV-G/ envelope (addgene 8454) and pCMV(delta)R8.74/packaging (addgene 22036) viral polemerase/etc. You will be able to tranduce any cell type.

For retro viral transduction you use your retroviral contstruct in combination with VSV-G/ envelope (addgene 8454) and pv-packgp/ packaging (Stratagene). You will be able to tranduce any cell type.

Use the plasmids plasmid combination in a ratio of 1:1:1

Lenti and retro viral transduction:

Pipet with care, 293FT's can come loose easy; virus is very infectious, put all used items in 70% etOH with 1% SDS for at least a day (then tape up in a plastic bag and discard in green bin).

Day 0 Transfection:

- 10 cm dish 293FT cells 95-100% confluency (no G418) (having 10 ml DMEM 10% FCS) (you can also use a 15cm dish, just double your DNA and PEI, and medium without serum)

- add DNA 24ug DNA (15 cm : 48ug 16 16 16, 140 PEI)
  - o 8 ug viral vector DNA + 8 ug packaging + 8 ug envelope in 2 ml serum-free DMEM
  - o Optional add a teaspoon of GFP (if your viral vector has no GFP)
  - o vortex
  - o add 72 ul PEI
  - o swirl 5x
  - o incubate 20 min RT
  - o put mixture on medium 293FT using droplets over the whole plate, swirl plate finally and put in incubator

Day 1 First tap and infection (or collection).

Check GFP expression in your 293FT, did transfection work?

Caution: live virus! tape your dish for transport in box with biohazard sticker to fluorescent microscope to check fluorescence. Disinfect surfaces first.

- Make sure you have your acceptor cell line ready at 10 – 15% confluency in 6 well plate (take off medium) if you trypsynize in the morning and infect when they are just attaching, infection works even better.

- Take medium 293FT cells and push it through a 0.45 filter

o pipet on the acceptor cell line ( one control well, not for infection)

o left over virus medium: store at 4°C

o put 8 ml new DMEM (10% serum) on 293FT cells

Day 2 Second tap and infection (or collection).

293FT should be very bubble-ly and show polynuclei, you know they are producing good

- Take medium 293FT cells and push it through a 0.45 filter

o add 2 ml virus medium in tube and add 2 ul polybrene, swirl leave for 5 min, pipet on the acceptor cell line left over virus medium: store at 4°C

o put 8 ml new DMEM (10% serum) on 293FT cells

Note: As a visual control, cells should be fused and multinucleated thirty-six to forty-eight hours after transfection,

due to the presence of the VSV-G protein. This morphological change is expected and does not

effect the production of lentiviral vectors.

Day 3 Third tap and infection (or collection).

- Take medium 293FT cells and push it through a 0.45 filter

o add 2 ml virus medium in tube and add 2 ul polybrene, swirl leave for 5 min, pipet on the acceptor cell line (if cells donnot look good leave polybrene out, we normally use it only at Day 2)

o left over virus medium: store at 4°C

Day 4

- trypsinize 6 well plate (acceptor cell line) and put in 10 cm dish (T75 flask), select if you have a selection marker and expand, (you can use your cells in an experiment after you at least passaged your cells three times or two weeks after your last infection, change the medium regularly). Split some cells to culture without PenStrep for a mycoplasma test, so they are allowed into the clean culture room.

For the stored virus: centrifuge at least 2h at 18000 g pipet medium off except for 1 ml and resuspend the pellet  aliquot in 100 ul samples and flash freeze dry ice with ethanol or liquid N2 Store in screw cap vials and then in 15 ml tubes at -80.

You can also skip all separate infection steps, and infect your acceptor line, with this concentrated virus once before freezing down. Future infections, just thaw an aliquot and add it to your cells.

Goodluck