Protocol for lentivirus production
(modified from Dr. Courtney Karner)

1st day; Plate 293T cells 2,000,000 on a 6cm plate.

2nd day; Transfection – cells should be about 80% confluent if they are less you can wait a day.
Change media on 293T cells. First wash 1 time with PBS to remove antibiotic then add 2mL DMEM (10% FBS) no Pen/Strep.

Make up 2 tubes. In the first tube mix the following per infection:
100 ul DMEM (no FBS or P/s), 1 ug lentiviral plasmid, 0.5 ug pPAX2 and 0.5 ug pMD2G
In the second tube mix the following per infection:
100 ul DMEM (no FBS or P/S) and either 5 ul LipofectAmine2000 or 6 ul Xtremegene9/Fugene 6 per infection

- **I prefer Xtremegene9 or Fugene as I have had better luck with these**

Incubate tubes for 5min.

Mix the two tubes and incubate at RT for 20min.
Drop ~200 uL (whatever the volume of mix 1 and 205/6 or 9uL for mix 2) onto 293T cells (in 2mL DMEM with 10% FBS). Swirl the media around to get good coverage on cells.

3rd day; Add 2mL fresh medium of the target cells (i.e. 10%FBS-aMEM for ST2 cells).

4th day; Collect the media, filter it with a 0.45 um filter.

Add 5mL fresh media

Wait for 48hr.

6th day; Collect media, filter it with 0.45 uM filter. Combine with first harvest, aliquot into 1mL aliquots and store at -80.

Infection:
Plate target cells on a 24well plate (one extra well for a negative control).

To infect cells, dilute viral media 1:2 with target cell media (i.e. 1ml of viral sup. + 1ml of aMEM for ST2 cells medium=total 4ml). Add polybrene at 1:1000 dilution. Remove media and wash cells 1X and replace with viral media.
Infect cells for 24 hours. Wash cells 1 time and replace with normal media. Let cells rest for 24 hours then initiate your experiment.

For ISR experiments, must treat for 96 hours as the viral infection results in a delayed and blunted response to both Wnt and BMP.