Introduction:

293GP cell line is a packaging cell line derived from 293T cells, a human embryonic kidney line transformed with adenovirus E1a and carrying a temperature sensitive T antigen co-selected with neomycin. It is used for the production of high titer infectious, replication-*incompetent* retrovirus. The genes encoding the viral *gag* and *pol* proteins are stably integrated into 293-GP cells. Retrovirus is produced by co-transfection of envelope plasmid (e.g. pVSV-G or RD-114 envelope protein) and retroviral vector plasmid (e.g. gene of interest +/- marker).

The virus can infect target cell lines and transmit a target gene, it cannot replicate because the target cell lines lack the viral structural genes. By using the minimal viral sequences and separately introducing the structural genes into the packaging cell line, the chance of producing replication-competent virus due to recombination events is minimized.

The retroviral vector contains genes encoding the viral packaging signal Ψ_+, a selection marker (e.g. antibiotic and/or other marker) and a target gene.

Materials:

293GP cell line

Envelope protein plasmid (e.g. pVSV-G or **RD-114**)

Lipofectamine 2000 (Invitrogen)

Opti-MEM media (Gibco)

6-well Poly-lysine coated plates – mini production

100 mm plates Poly-lysine coated plates – for small production

150 mm plates Poly-lysine coated plates – for large production

Retroviral vector plasmid

Media:

DMEM, 10% FCS, p/s

DMEM, 10% FCS no antibiotics

Method:

Day 1:

Count 293GP cells: ________________________________

Plate 293GP cells in DMEM, 10% FCS, **ABX** media. Swirl the plate as you add the cells.

Plate	**# cells/well ($*10^6$)**	**media /well (mL)**	**# wells**	**media–total (mL)**	**# cells required ($*10^6$)**
6-well plate	0.8	2			
100 mm plates	4	10			
150 mm plates	10	22.5			

Day 2:

1. Change cells to DMEM, 10% FCS, **no ABX media** prior to transfection (30 min) by adding:

Plate	**media per well (mL)**
6-well plate	1.5
100 mm plates	10
150 mm plates	22.5

2. Make the following mix solutions in **non-polystyrene tubes**:

Mix A – required for

MIX A	6-well plate	10 cm^2 plates	15 cm^2 plates	Plates (#) __________
Retroviral Vector plasmid (µg)	**1.5**	**9**	**20.25**	
Conc: ____________________	_____ µL	_____ µL	_____ µL	
RD114 (envelope plasmid) (µg)	**0.75**	**4**	**10.13**	
Conc: 1.88 mg/ml	0.4 µL	2.1 µL	5.4 µL	
OptiMEM (mL)	**0.25**	**1.5**	**3.4**	

Mix B- required for

MIX B	6-well plate	10 cm^2 plates	15 cm^2 plates	Plates (#) __________
Lipofectamine (2000) (µL)	10	60	135	
OptiMEM (mL)	0.25	1.5	3.4	

3. Incubate A and B separately for **5 min at RT**.

4. Mix A and B together gently (do not vortex) and incubate for **20 min at RT**.

5. Add the mix to the plates dropwise, and swirl.

Plate	add per well (mL)	Total will be
6-well plate	**0.5**	2
100 mm plates	**3**	13
150 mm plates	**6.75**	29.25

6. Incubate at 37^{0}C **for 6-8 hrs (or O/N).**

7. Change media to fresh **DMEM, 10% FCS no antibiotics**

Plate	**change media per well (mL)**
6-well plate	**2**
100 mm plates	**10**
150 mm plates	**22.5**

8. Incubate at 37^{0}C for **48 hrs (from time of transfection).**

Day 4:

9. Harvest retroviral supernatant – **48 hr harvest.**
10. **Add media again as above for an additional 24 hrs (72 hrs harvest).**
11. Spin down cells, and aliquot the supernatant.
12. *Filter retroviral supernatant through a 0.22µm filter - optional*
13. Snap freeze on dry ice and store at -80°C or just store at 80°C

Day 5:

14. Harvest retroviral supernatant – **72 hr harvest.**
15. Spin down cells, and aliquot the supernatant.
16. *Filter retroviral supernatant through a 0.22µm filter - optional*
17. Snap freeze on dry ice and store at -80°C or just store at 80°C