Lentivirus Packaging Protocol

The following protocol allows for the production of recombinant lentiviral particles up to a 10^6 IU/mL titer. We recommend including a negative control (without DNA or transfection reagents) in your experiments to help evaluate your results.

Before starting the lentiviral packaging protocol, please ensure that you have an adequate volume of expression DNA (10 µg plasmid /10 cm dish). A DNA amplification step will usually be required using standard bacterial transformation protocols. We recommend using *E.coli* DH5-alpha strains for amplification of all DNA plasmids from **abm**, this strain has been tested to produce high yields of plasmid for lentiviral packaging with minimal risk of recombination occurring.

Protocol

DAY 1: A day before transfection, seed cells roughly at 40-50% confluency.The following day (dayof transfection) the confluency should be ~70-80% .

DAY 2: (Carry out steps 2 - 6 in the morning on the day of transfection)
2. Check to make sure the cells are 70-80% confluent.

3. a) **For each 10 cm dish** prepare the transfection complex as follows:
Solution A: Dilute 20 µg DNA plasmids (10 µg expression vector and 10 µg of **abm**'s Second Generation (LV003) or Third Generation (LV053) Packaging Mix) in 1 mL serum-free, antibiotic-free medium. Solution B: Dilute 80 µL of DNAfectin™ Plus Transfection reagent (G2500) in 1 mL serum-free, antibiotic-free medium.

b) Incubate both solutions at room temperature for 5 minutes.
c) Mix Solutions A and B together well and incubate at room temperature for 20 minutes. This will create the transfection complex.

4. Add 4.5 mL serum-free medium to the transfection complex.

5. Remove medium from the cells in the 10 cm dish.

6. Add the complete transfection complex from step 4 to the cells and incubate at 37°C for 5-8 hours. Avoid dislodging the cells by gently adding the mixture against the side wall of the dish.

7. Add 0.65 mL FBS to the 10 cm dish and incubate at 37°C overnight.

DAY 3:
8. Remove the transfection medium from the cells.

9. Add 10 mL complete culture medium to the cells.

10. Incubate at 37°C for 24 hours.

DAY 4 (Harvest):

11. Collect the supernatant medium from the culture dish.

12. Centrifuge the supernatant at 3000 rpm for 15 minutes at 4°C to pellet cell debris.

13. Transfer the cleared supernatant to a fresh tube. Filter the cleared supernatant with a low-protein binding 0.45 μM sterile filter.

14. The viral titer of the first harvest is approximately 10^6 IU/mL. The filtered supernatant will be ready for *In vitro* infections or further concentration and/or purification. Alternatively, it can be stored at -80°C as viral stock for future applications. Aliquotted volumes are preferred for long term storage to reduce the loss of viral titer through multiple freeze-thaw cycles.

15. A second harvest can be carried out by adding 10 mL of complete medium to the cells after the first harvest and incubating at 37°C for a further 24 hours. The first harvest can be stored at 4°C overnight to allow the second harvest to be added to it the following day (freezing the supernatant would result in a greater loss of titer).

16. Collect the second supernatant on Day 5 (as in steps 11-13) and combine this with the first harvest.

Note: Expression of the VSVG glycoprotein causes 293T cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect the production of the lentivirus.

17. For viral titers that are 10^6 IU/mL and higher, you can quickly and easily titer your virus preparation using the qPCR Lentivirus Titer Kit (LV900) available from **abm**.

In addition, our Ultra-Pure Lentiviral Purification Kit (LV998) will allow you to concentrate the virus to a higher titer if desired.