Retrovirus Packaging Protocol

The following protocol allows for the production of recombinant retroviral 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 retroviral packaging protocol, please ensure that you have an adequate volume of expression DNA (15 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 retroviral packaging with minimal risk of recombination occurring.

Protocol

DAY 1:

1. Seed ~4.5 x 10^6 of HEK 293T cells in a 10 cm dish 16-24 hours prior to transfection.

DAY 2: (Carry out steps 2 - 3 in the morning on the day of transfection)

2. Check to make sure the cells are 40-60% confluent.

3. **For each 10 cm dish** prepare the transfection complex in an eppendorf tube as follows using ABM Calciumfectin™ Mammalian Transfection Reagent Kit (G099):

   a) Add 15 µg DNA plasmid with 7.5 µg of Retro-Combo Mix (E-510). Mix well.

   b) Fill up to 450 µl with ddH_2O (G099-3).

   c) Add 50 µl of 2M $CaCl_2$(G099-1).

   d) Incubate solution at room remperature for 5 minutes.

   e) Add 500 µl of HBS solution (G099-2) dropwise. Mix well.

4. Add the complete transfection complex from step 3 to the cells. Avoid dislodging the cells by gently adding the mixture against the side wall of the dish. Shake the dish to ensure even distribution of the transfection complex. Incubate at 37°C overnight.

DAY 3:

5. Remove the transfection medium from the cells.

6. Add 12 mL complete culture medium to the cells. *Continued*

7. Incubate at 37°C for 24 hours.

DAY 4 (Harvest):

8. Collect the supernatant medium from the culture dish.

9. Centrifuge the supernatant at 1500g for 15 minutes at 4°C to pellet cell debris.

10. Transfer the cleared supernatant to a fresh tube by filtering with a low-protein binding 0.45 µM sterile filter.

11. 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.

12. A second harvest can be carried out by adding 12 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).

13. Repeat steps 8-10 for a second collection of viral harvesting.

Note: Expression of the VSVG glycoprotein causes HEK293T 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 retrovirus.

14. For viral titers that are 10^6 IU/mL and higher, you can quickly and easily titer your virus preparation using the qPCR Retrovirus Titration(Titer) Kit (G949) available from **abm**.