Transfection of 293 GAG/POL to produce retroviruses*

***This protocol is for 10 cm or 15 cm dishes.**

- The day before the transfection, seed $5x10^6$, ($11x10^6$) of freshly thawed[1] 293 GAG/POL cells.
- Mix 12 µg (26µg) of the transfer vector, 1.2 µg (2.6 µg) of pGAG/POL, 1.2 µg (2.6 µg) of pVSV-G in 2.3 ml (5 ml) of OPTI-MEM in one tube.
- In another tube, mix 50 µl (110 µl) of Lipofectamine 2000 in 2.3 ml (5 ml) of OPTI-MEM.
- Mix the DNA and the Lipofectamine 2000.
- Incubate at room temperature for at least 25 minutes (up to 6 hours in theory).
- Add to the DNA:Liposome complex (4.6 ml) (10 ml) to the dish already containing 7 ml (15 ml) of OPTI-MEM.
- Incubate at 37°C overnight.
- The next day, replace media with 10 ml (20 ml) of OPTI-MEM[2,3].
- Collect viral supernatant at T=48h and T=72h post-transfection. Centrifuge supernatants and filter through a 0.2µM filter[4]. Store at -80°C. To obtain higher viral titers, viruses can be concentrated by ultracentrifugation[5].
- Determine viral titers by infecting HT1080 or HeLa cells.

NOTES:

[1]I usually thaw a fresh vial in a T-150 and wait 3-4 days until the cells are fairly confluent. Then I split them into 10cm dishes for the transfection. 293 cells should not be cultured for several passages as this will result in lower viral titers.

[2]D-MEM or other media can be used at this step. I prefer to use OPTI-MEM in case I need to concentrate the virus at a later stage. Ultracentrifugation of media containing serum has been shown to also precipitate more unwanted material than serum-free media. Serum-free D-MEM might be used, although I never tested that option.

[3]For most applications, I just leave the original OPTI-MEM/Lipofectamine on the plate and I do not change the media. I just collect the viral supernatant at 48h post-transfection and replace the media at that time. I did not notice any toxicity, and I assume that most of the lipofectamine was taken up by the 293T cells so that very little would be left in the media. It also reduces the chance of losing cells from the dish as 293 cells are notorious to detach easily.

[4]Some syringe filters have been shown to bind viruses and therefore reduce considerably viral titers. Use a low protein binding syringe filter. Also, several laboratories are using a 0.45µM filter instead of a 0.2µM. I did not notice any change in viral titers with the 0.2µM filters but because the transfection is done in the absence of antibiotics, I feel more safe to use a filter that can remove bacteria and fungi at the same time (of course, if there are noticeable bacteria/yeast in the dish, discard the dish, do not filter and keep the virus).

[5]To obtain higher viral titers, viruses can be concentrated by ultracentrifugation (21 000 rpm in a SW28 rotor for 2 hours) and resuspended in 50-200 ul of Hanck's buffered saline (HBS).

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