Production of retroviruses using FuGENE-6

3/21/00

1) On the day before transfection, plate 0.5-1 x10^6 293T cells onto 6cm dishes in 4-5 ml DME/10%IFS. The cells are ready for transfection after 18-20 hours, or when they are about 25-30% confluent. Plate slightly more cells when making VSV-G pseudotyped viruses. For 10 cm dishes, double the quantities given here.
2) Pipette 6μl FuGENE into serum-free medium (at room temperature) so that the final volume after the addition of the DNA will be 100μl. DO NOT TOUCH THE TUBE WITH UNDILUTED FUGENE.
3) Add 2μg total DNA (in TE). For instance, if using pCL vectors (Ampho, 10A1, or Eco; Naviaux (1996) *J. Virol*, vol 70, p 5701), use 1μg transfer vector (pBabe, pMSCV-IRES-GFP, etc.) and 1 μg of the pCL vector. For making VSV-G retroviruses by triple transfection use 0.9μg gag/pol expression vector, 0.1 μg VSV-G expression vector, and 1 μg transfer vector.
4) Do not vortex; swirl to mix.
5) Incubate the FuGENE/medium/DNA solution at room temperature for 15 minutes.
6) Drip the mixture onto the 293T cells using a Pipetman.
7) Incubate overnight at 37 C.
8) On the day following transfection feed the cells with 3-4 ml fresh medium.
9) Harvest viral supernatants at 48 and 72 hours (or 36 and 60 hours). Ecotropic and GaLV viral titers at 72 hours will be will be approximately 20-40% less than at 48 hours. VSV-G viral titers are essentially the same at 48, 72, and 96 hours. Filter with a 45 uM syringe filter.
10) In a typical viral infection protocol for adherent cells: use 3.5 ml of 36-48 hour viral sup, with 8 ug/ml polybrene, to infect 1-2 x10^6 cells (20-30% confluent) on a 10 cm dish. After 3 hours remove the sup and add fresh medium.
11) Repeat the infection 24 hours later with the 60-72 hour sup. 24 hours after the second infection, split the cells; apply drug selection or sort the day following the split. Although two infections are optimal, one infection is often enough.
12) Please occasionally take the time to sing happy songs about viruses.

Notes about using FuGENE to make retroviruses:

-Do not let FuGENE come into contact with any plastic surface without diluting first in serum free medium (do not aliquot; store in original polypropylene tubes).

-Antibiotics do not seem to impair the transfection or viral titers.

-Whether FuGENE or DNA is added first to the medium doesn't seem to matter. It's OK to add the two or three plasmids separately.

-For collecting VSV-G viruses, use medium buffered with 10-25 mM HEPES to limit pH-dependent cell killing by the VSV-G protein.

-With this protocol we have made ECO viruses with titers of 10^7/ml and VSV-G, 10A1, and AMPHO viruses with titers of 10^6/ml.

-FuGENE is made by Roche, formerly Boehringer Mannheim. Cat # 1 814 443, 1 ml, $275, about $1.65 per transfection.

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