Greg's RetroPrep in 293T cells by Fugene-6 Transfection

Values/10cm plate
Plate the 293T ¼ from a confluent plate the day before. They should be just sub-confluent before transfection. See the pictures.

Make up DNA mix with 1µg gag-pol expresser (p8.91)
1µg pMDG (VSV-G expresser)
1.5µg vector (Hairpin vector or CSGW)
make up to 15ul in TE (10mM TRIS pH 8, 1mM EDTA)

Take 200µl optimem and add 10µl Fugene-6 (Roche) without allowing Fugene-6 to touch the sides of the tube. Mix by flicking.
Add DNA.
Mix by flicking.
Spin for 2 seconds to remove the mix from the sides of the tube.
Leave at room temperature for 15 minutes

Change media on cells for 8ml complete media (DMEM, 10%FCS, Pen/Strep).

Add DNA mix to cells drop wise and swirl plates to mix.

Return to incubator.

Change media next day for 8ml fresh.

Collect sup at 48, 72 and 96 hours post transfection, filter (0.45µm) and store at -80. On a good day the titre does not go down between days 1 and 2 and 3 is only a little lower.

To reduce the scale 1 million 293T/well in 6 well plates works well. Divide the protocol by 3 ie 3ul fugene, 300ng 8.91 etc.

For HIV plasmids see
Zufferey, R., D. Nagy, R. J. Mandel, L. Naldini, and D. Trono. 1997. Nature Biotech 15:871-875.
Bainbridge, J. W., Stephens, C., Parsley, K., Demaison, C., Halfyard, A., Thrasher, A. J. & Ali, R. R. (2001) *Gene Ther* **8,** 1665-8.

Thank Didier Trono University of Geneva, Switzerland for p8.91 and pMDG and Adrian Thrasher Institute of Child Health, University College London, UK for pCSGW and reference the protocol Besnier, C., Takeuchi, Y. & Towers, G. (2002) *Proc Natl Acad Sci U S A* **99,** 11920-5.

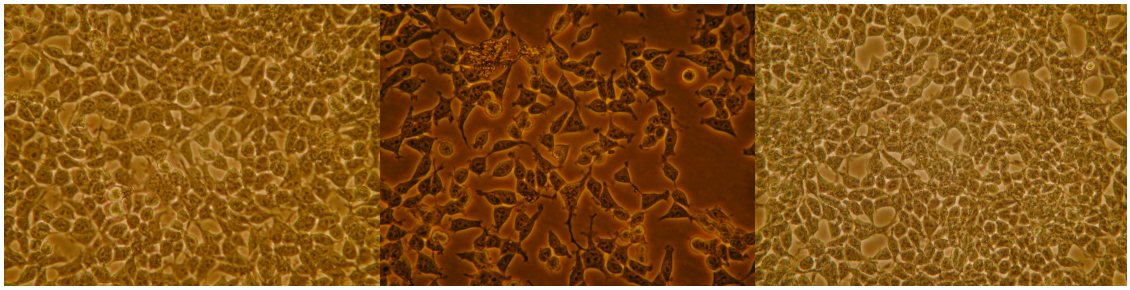
Any questions call Greg on the 293T helpline 020 3108 2112 or g.towers@ucl.ac.uk
We can show visitors how we do all our retrovirus experiments by appointment.

Good luck

Happy 293T cells

The titre of the virus absolutely depends on how you keep the 293T cells. Titres can vary between 10^5 and 10^8 i.u./ml but will be high if you keep the cells appropriately. The key is to keep them pretty dense most of the time. 293T come off the plate easily with trypsin but make sure you trypsinise them properly so that they are individual cells when you plate them. A few times up and down in a 10cm pipette helps. Remove bubbles and make sure the shelf in the incubator is level. We often let the cells stick down before we stack the plates. We split ¼ Mondays and Wednesdays and 1/6 on Friday. Use DMEM with Glutamax and high Glucose throughout. They must also be almost confluent when you transfect them. The aim is to transfect them at or just before the point they become confluent and then they don't divide and virus release stays high for several days. If cells look ill during normal passage try 15% serum. Happy 293T=very high virus titre.

When you transfect them they should look like the first picture. You might be surprised how fast they grow. If they don't look good in the morning leave them until last thing to transfect.



The second is not confluent enough. The third picture shows what the cells look like when they're not very well. They make a kind of reticular pattern and don't plate evenly, note the gaps. They do this when they don't like the serum, or when you've split them too hard. We batch test our serum using 293T.

Infections

We infect cells plated at 10^5/well in 6 well plates in 1ml in the presence of 8μg/ml polybrene. Make polybrene at 1000X in water and filter (0.45μm).

You can increase the titre by spinning the virus onto the cells. We use 500G (1.6K in a Sorvall Legend) for 1 hour at room temp in a bench top centrifuge. Use 1.5ml/well in a 6 well in this case. After spinning leave the sup on the cells overnight and then change it. If you have toxicity reduce the time the virus is on the cells but don't go below about 4 hours if you can help it.