Generating RAds

1. After transfecting your pAdZ construct into 293TREx cells (if tet-repression is important to your experiment) or other cells (293, 911 etc) you should see plaques after 7-10 days.
2. Change media when it goes yellow, and eventually all the cells will round up and detach from the monolayer. Highest titers are obtained by taking the cells when they are all rounded up, but not yet all detached. Normally there's ~1 day between the cells all rounding up, and detaching.

   Note. 293TREx cells do not adhere very strongly to the flask. Be gentle, or the entire monolayer can detach prematurely. You only want the cells to detach when they are all infected!! Various companies do coated flasks that can help – we use cellbind from corning – and they make a big difference to ease of use.
3. Hit the flask to detach the infected cells into the supernatant.

These cells contain your initial virus stock. To make a useable amount of virus you must infect more cells though, so:

1. Seed approx 6×10^6 293 TREx cells (or 293/911 cells if tet-repression is not important to your experiment) into a T150 (again, coated flasks such as cellbind can help). Cells should be almost confluent.

   Note. The number of flasks you infect depends on how much virus you want. You should get at least 2×10^9 pfu from a single T150, so scale up the number of flasks by how much you expect to need.

2. Add infected cells from first step to these T150's containing fresh cells.
3. Change media when it goes yellow until all cells are rounded up and about to detach from the flask. May take anywhere from 3-10 days depending on how many infected cells you added.
4. Hit flask to detach all infected cells.
5. Pellet cells (~1500rpm for 3 mins).
6. Resuspend cells in PBS to wash off media, and combine all cells from all flasks (assuming more than 1 flask was infected).
7. Pellet cells again.
8. Resuspend cells in 1ml PBS for every T150 that was infected. I.e. if 5 T150's were infected, resuspend in 5mls PBS.
9. Add an equal volume of tetrachloroethylene.
10. Shake vigorously until a single phase is formed. This lyses the cells, releasing the virus.
11. Centrifuge to separate layers. Lower layer is tetrachloroethylene, then a layer of cell debris, and an upper layer of PBS containing the virus prep. Upper layer sometimes remains slightly turbid. This is fine. Usually 2000rpm for 20mins is sufficient, this can be increased if upper layer is still very turbid.
12. Pipette off upper layer and store at -80°C in 300-500µl aliquots.

This prep can be used directly, or if higher purity is desired a Cesium-chloride gradient can be performed.

Cesium Chloride gradients

1. To prepare the CsCl gradient. Pipette 1.6ml of 1.45g/ml CsCl (3.6M solution in 5mM Tris-HCl, pH7.8) into 14x 89mm ultra clear Beckman centrifuge tubes and then carefully overlay 3ml of a less dense CsCl solution (1.33g/ml 2.6M in 1mM EDTA, 5mM Tris HCl, pH7.8). Add the extracted virus carefully on top of the gradient (approximately 7mls) until the tubes are filled to within 2.5mm of the top. Load the tubes into a SW41 Ti rotor and spin at 90,000g (23,000 rpm) for 2h at room temperature.
2. After 2 hours the virus should appear as an opalescent layer resting between the higher and lower density CsCl solutions. A second band higher up & resting on the top of the low density CsCl solution is derived from cellular components.
3. The virus is harvested from the gradient by puncturing the tube just beneath the virus band with a 21 guage needle and gently pulling the virus into a 2ml syringe.
4. Make up the CsCl extracted virus solution to 2ml with dialysis buffer and then dialyze against a solution of 1mM MgCl2, 135mM NaCl, 10mM Tris HCl pH7.8, 10% glycerol with two changes of buffer. I make up 2 L of buffer per virus purification and change the dialysis buffer twice with an overnight incubation at 4°C. Put on a magnetic stirrer to keep the buffer moving while dialysing.
5. After dialysis, extract the virus from the dialysis tubing/cassette and make up to the appropriate volume using dialysis buffer (1ml per T150 that was infected) before aliquoting and storing at -80°C.