Amplifying Adenovirus

1) **Thaw Vial of human kidney 293 obtained from liquid nitrogen stock in 37 C water bath and place into 100 mm dish containing l MEM w/10% FCS pen strep/ glutamine**
2) **Passage cells using trypsin when they are nearly confluent 1:3 or 1:5 depending on when they will be used.**
3) **Prepare several 150 mm plates by subsequent passage and add either 1-3 ul of CsCl purfifed virus to each dish or, titrate lysates from plaque purifications (5-100 ul). (see addendum for use of plaque isolates)**
4) **Watch for signs of cytopathicity (cells rounding and coming off the plate) which should be 48 hours after infection.**
5) **Collect the infected cells by vigorous pipetting and transfer to 50 ml falcon tube (no trypsin needed).**
6) **Centrifuge cells 3000 rpm for 20 minutes. Use about 5 ml to combine all the cells together from all the infected plates (about 1 ml/plate so it ends up in one tube)**
7) **Freeze (-70 C) and thaw (37 C) this material 5 times to release the virus from the cells**
8) **Centrifuge the material (minimum 3000 rpm, 20 min) or 7000 rpm shorter time).**
9) **Remove the supernatant which is the HTL and store in aliquots at – 80 C**
10) **Proceed with further purification using CsCl gradients, Virapure or use directly after performing a titration to determine the optimal concentration for your application.**

Addendum for amplification from Plaque isolates:

1) **Set up several plates of 293 cells**
2) **When they are confluent add a varied dose of the fluid in the vial ranging from 10-250ul (say, 10, 25, 50, 100, 125).**
3) **Watch for Cytopathic effects over the next 48 hours. Lower doses may not show effects until 60 hours, higher doses will be earlier (the cells will make more virus that can infect other cells over time).**
4) **When cytopathic effects are visible (you will see the cells lifting/rounding from the plate) vigorously pipette them from the plate and transfer them in to a sterile 50 ml falcon tube. Proceed to step 5 above. Resuspend the material in 1ml/plate of and proceed to step 9.**
5) **Use ~25 ul of this newly amplified material/150 mm of 293 for further amplification (resuspending in 1 ml/150 mm plate)**