Protocol Adenoviral production in HEK293T cells

-VE-cadherin WT, Y658F and Y731F, GFP-labeled. All of them tend to accumulate in the Golgi a lot, but they do go to the junctions (obviously). Try to use lower passage cells to amplify the viruses.

Workprotocol:

-Simply grow a 10cm dish of 293A to 95% confluency, then add 100uL of virus to the media.

-The cells should round up and lift off the dish in the next 48 hours or so. Don't change the media during this time.

-When this has happened, harvest the media and cells, and freeze/thaw them using a -80 freezer and a 37 degree water bath. Repeat the freeze thaw 3 times total.

-Spin down the cell debris at 3000 rpm for 10 minutes using a desk top centrifuge.

-Aliquot the supernatant into small vials and freeze at -80. Virus can be freeze/thawed about 10 times, or kept at 4 degrees for a 2-4 weeks.

-SAFETY: The viruses cannot replicate in cells other than 293A. But you should bleach everything that comes in contact with the virus, wear gloves, work in a hood, etc. The amplification step is the most "dangerous" time when working with the virus.

-Also be aware that virus can infect clean 293A cells if they are in the same incubator...so keep them separate.

-USE: Add virus to normal cell media for 4 hours. Replace media and wait at least overnight before you do your assay (although you can usually go 2 days if you want). WT can be used 1:100, the mutants are less potent.