CONCENTRATING VIRUS

1. Cool down Beckman Ultrafuge to 4ºC.

2. Wescodyne and EtOH wash buckets for tubes (for SW28 rotor) and UV sterilize for 20'. UV sterilize tubes for 20' as well.

3. Quick thaw viral sups at 37ºC and place on ice. Don't let sups warm up.

4. Filter sups through a 0.45um filter (for DF1's, use a prefilter as well). Place on ice.

5. Pour sups into sterilized tubes (each tube will hold about 35 mls). Place in buckets and spin for 3 hours at 4ºC at 21K rpms.

6. After spin, pour off sups in one motion and hold the tube upside down. You will see a big drop of media form which you should aspirate off (I usually have a pasteur pipet ready before pouring). Aspirate 2/3 up the tube to remove most of the residual media. Return tube to bucket, place on ice and cover (this is all done in the TC hood). You should have a small volume of media remaining (60-70ul if starting volume is 35ml).

7. Shake the tubes anywhere from 1 to 2 hours at 4ºC at 120-150 rpms. This step minimizes pipetting which saves your hand and helps to prevent the creation of bubbles.

8. After shaking, resuspend your virus by dialing down your pipetter to half the volume in your tube, and pipet up and down gently about 100 times. Wash down the sides as well. DO NOT CREATE BUBBLES SINCE THIS KILLS THE VIRUS.

9. Let stand for 5 minutes, pipet 20 times more and aliquot into prechilled tubes in small volumes (10-20ul). Store virus at –80ºC.