VIRUS PREPARATION

1. Remove all 10-20cc from T25 after 3-4 days incubation and spin ~1500rpm (~3.5 on control knob) x 5 min. in 50cc tubes at 4 degrees C.

2. Draw off supernatant with a 10 or 30cc syringe (18g needle) leaving 1-2cc supernatant with cell pellet and place syringe (now with the supernatant) on ice.

3. Take the tube with the cell pellet and 1-2cc of residual RPMI (see #2) and freeze/thaw cell pellet x 3 in dry ice/ethanol bath and 37 degree C. bath (tubes can be conveniently held in test tube rack for freeze/thaw). Vortex tubes after each thaw to mix.

4. Spin 2500 rpm (~5 on knob) x 5 min. at 4 degrees C. and use the syringe already holding the virus supernatant (see #2) to collect the residual 1-2cc supernatant (avoid taking cell fragments)

5. Filter virus with 0.45um filter to get rid of cell fragments.

(6. (Optional- steps 6-8, especially useful if transfection was incubated in 20cc RPMI rather than 10cc) Spin 8500rpm x 2 hrs at 4 degrees C. in sterile Beckman tubes and mark tube where virus should pellet.)

(7. Aspirate off supernatant but leave ~1.5-4cc. Let sit on ice in cold room overnight to resuspend virus and in the morning gently pipette up and down to resuspend.)

(8. In morning, infection can be done using concentrated virus (#7)).

9. Part of virus can be frozen and part can be used for infections.