Phosphorimage Protocol for Radioactive Gels **PLC 12/03/01**

General Precautions:
- wear gloves
- dump silver waste in proper container, not down sink
- empty gel dryer cold trap before turning on; do not forget to pre-chill
- do not leave gel dryer heater on when not in lab

1. Run gels and stain.

2. Transfer gels to filter paper. The easiest way to do this is to arrange the wet gels on Saran Wrap in the REVERSE of what one would like to be their final orientation. Make sure edges of multiple gels are squared up (this will make image processing easier). Trim a piece of filter paper (Whatman 3MM CHR) to the proper size, leaving about two inches on all sides for slop. Bow the paper into a “U” shape and contact with the center of the wet gels. Flatten out the “U” onto the gels and pat down the paper to insure good contact, taking care not to slide hands. Flip the whole thing (paper, gels and plastic wrap) over and trim excess paper and wrap.

3. Put gel sandwich on dryer, between plastic fiber mat and clear plastic sheet. Cover with flexible plastic membrane and turn on vacuum. Insure there is a good seal, then turn on heat for 7 hr at 75°C.

4. Check gels for dryness. If they are not dry at this point, a few more hours at 75°C may be necessary. Another option is drying for several hours without additional heating (I sometimes do this if I want to leave something drying O/N). Do NOT leave the lab for the day with the heater running!

5. When dry, turn off the vacuum and remove gel sandwich. Remove plastic wrap. Trim filter paper edges to close to gels.

6. Blank phosphor screen on light source for about 20 minutes.

7. Tape gels to grid of phosphor cassette; make sure gels are labeled. When screen is blanked, place on gels and close. Store in drawer for O/N.

8. When ready to read (after 16-24 hr), take the screen to the phosphorimager (Galvin Biology); open lid of imager. Make sure adapter is in place if using small screen. Open screen, place on imager and close lid.

9. Take note of what part of the screen grid is covered by the gels. Log onto the computer (username: mduser) and select this area on the grid shown in the scanning software window (be careful; you do not want to re-scan as it will result in a less-intense image). When prompted for a file name, first make sure that the directory is set correctly and then type in a name (ending in “.gel”). Type in some identifying notes in the scanning window, and press “Scan” (scanning will take a couple of minutes).

10. When scanning is complete, the result should be dumped automatically into ImageQuant (it is not necessary to start the program beforehand). Typically, the intensity range is set too wide by default, meaning that one sees light or non-existant bands. To correct this, go to Edit and select Color/Greyscale (or press F8). The default range is 0-100000 (or 99999); try 0-1000 and keep adjusting until the bands look nice. There are fancier corrections that can be made; see the ImageQuant manual for help.

11. Move files off of the phosphorimager computer and onto your AFS space. Tweak in lab using ImageQuant or NIH Image 1.62.