

## Photographing westerns

1. After final PBS washes, seal membrane in plastic bag (seal three sides only). Do not have large gap between bag and filter

2. Set up camera

- switch on camera FIRST otherwise computer will crash
- start FluorChem program
- set filter to position 1
- insert black board on lower level and position filter. Focus on membrane (should fill most of screen). Note position of membrane so that you can put in back into exactly the same position later (eg leave tape strategically positioned)
- open aperture to maximum ( $f=1.2$ )
- sensitivity to High/Low
- check 'chemi display'
- can check 'noise reduction'

set initial exposure for say 2 min (but will depend on antibody)

3. Mix 1 ml of chemiluminescence reagents (ie 0.5 ml from brown bottle, 0.5 ml from white bottle) Make sure you use clean tip for each bottle! DON'T make up until just before use!

4. Mix and pour or pipette mix into bag. Seal 4th side. Place on flat surface and use falcon 'rolling pin' or your fingers to make sure that the liquid is well distributed around bag. After 1 min cut corner off bag and expel excess liquid from bag.

5. Place membrane back in camera box (probably best if side that proteins are attached to are upper most - ie the side that was in contact with the gel). if necessary tape down so that it is flat.

6. close door, close camera door, check lights are off, expose  
save image

7. reexpose adjusting exposure as appropriate - save images

8. On complete of satisfactory exposure take a picture of the membrane in white light (chemi display off)- do not alter position or any parameters except you will need a very short exposure (stop down aperture if necessary). This allows you to record the location of the mw markers.

9. remember to switch off camera and lights