Finishing Western Blot for Sue 7/19/17

For WB:  
-Remove primary  
-Wash for 30 min in 1Xtbs + 0.1%tween( about 5-6 changes of solution)  
-Make secondary as 1:10000 dilution in 5%milk (tbst)  
-incubate blots with secondary for 45 min  
-wash for 30 minutes again  
-Add detection reagents (fridge)  
-image ( try to get lighter a darker images with no saturation)

Large gels:

1) marker

2) clone 1 microtubule, uninduced

3)clone 1 cytosol, uninduced

4)WT microtubule, uninduced

5) WT cytosol, uninduced

6) P301L microtubule, uninduced

7) P301L cytosol, uninduced

8) N279K microtubule, uninduced

9) N279K cytosol, unindexed

10)WT microtubule, induced

11) WT cytosol, induced

12) P301L microtubule, induced

13) P301L cytosol, induced

14) N279K microtubule, induced

15) N279K cytosol, induced

For the Tau blot you probably won’t get any signal for lanes 1-9

For GFPand K18  blot you will not get signals for 3-9

Tubulin should be stronger in microtubule fractions?

Not that the clone 1 proteins (40 kDa) are much smaller than full length (75 KdA)

small gel:

1) marker

2) clone 1 microtubule, uninduced

3)clone 1 cytosol, unindexed

4) clone 1 microtubule, unindexed, Taylor

5)clone 1 cytosol, unindexed, Taylor

I messed up the antibody I wanted to use i.e. Tau will give you no signal

GFP and tubulin blots should work