GST-Protein Binding Assay

1) Use 15 ug GST-YFP (test) and 15 ug of GST (neg. control)
2) Wash 10 uL of Glutathione-Sepharose beads in 400 uL TBST, repeat (in 0.5mL tubes)
3) Add 50 uL of TBST (supplemented with 10 mM B-Me) + GST-tagged proteins of interest
4) Mix for 1 hr on rotater at 4 ºC
5) Remove supernatant
6) Add 50 uL of TBST + 5% milk + 10 mM B-Me
7) Mix for 1 hr on rotater at 4 ºC
8) Remove supernatant (beads are hard to see, so be careful)
9) Add 50 uL of TBST + 5% milk + 10 mM B-Me
10) Add 15 ug of test protein. Keep 1.5 ug as input.
11) Mix for 1 hr on rotater at 4 ºC
12) Wash beads 4X with 400 uL TBST + 10 mM B-Me
13) Remove all extra sup with pipette and add 20 ul of 2X SDS-SB and boil 5-10 min
14) Also add 20 ul 1X SDS-SB to input
15) Load 10 uL of input (10%), and 18 uL of bound
16) Run through stacker at 80 V and then 100 V until dye front reaches bottom of gel
17) Carefully remove gel and cut off stacking gel
18) Stain gel in coomassie blue stain