

Pull down assay 7-8-14

Do 2 batches of tubes: one for X-linking and one for non.

- Get 100 ul of GST beads per each sample except for the GST which should be already with beads.
- Wash the beads with PBS three times.
- Do the last wash in PBS+ BSA 1%.
- Add 200 ul of PBS +BSA 1% to each 100 ul of GST beads.
- Make 300 ul aliquots of beads if you used only one tube.
- Add 20 ul of Drebrin 1mg/ml.
- Add 15 ul of old or new Zo1
- Use 15 ul of GST.
- Add 30-40 ul of CT 234 to each tube.
- Let the tubes binding over night at 4 degrees on the wheel.
- The day after, after X-linking on one batch of tubes, wash the beads at 4 degrees with PBS for 3 times.
- Aspirate the supernatant and add 20 ul of LSD+ 5% BME to each tube.
- Boil for 5 minutes and load the gel for the western blot.