# PP2A Methylation assay

BASE TREATMENT FOR DEMETHYLATION

From IP PROTOCOL:

1. Wash IP 2X with lysis buffer and 2X with PBS.
2. Wash final time with dd$H_20$.
3. Suspend bead complex and take off half into another tube. Label one as Base Treated and the other No Base Treatment (control). Spin down both.
4. Prepare solutions (all kept at $4^o$):
   A. 200 mM NaOH (in non-glass container)
   B. 200 mM HCl
   C. Tris 6.8
   D. All of the above mixed (for control) in 2:2:1 Base to Acid to Tris ratio. Test this with pH paper to ensure neutrality.
   E. 200 mM HCl (from above stock) with Tris 6.8 (from above stock) in 2:1 Acid to Tris ratio.
5. Remove sup from both sets of tubes.
6. Add 30 μL per tube of 200 mM NaOH to Base Treated tubes, shooting into beads to mix thoroughly.
7. Add 75 μL per tube of NaOH, HCl, Tris 6.8 mix (D from above) to No Base Treatment (control) tubes.
8. Mix very gently but thoroughly (base must contact all beads) by tapping bottom of tubes.
9. Incubate all tubes for 5 min. at $4^oC$
10. Neutralize Base Treated tubes by adding 45 μL of HCl and Tris mix (E from above).
11. Add 25 μL of GSD to all tubes.
12. Boil for 5 minutes.
13. Load on gel or freeze.