

**“KNOCKING DOWN” DNA w/ PHENOL**

1. Add equal volume of PCI (25:24:1, **pH 7.9**, Ambion)...door of fridge 5S-19. Sample the PCI from bottom layer (organic phase)
  - Do not use acidic phenol!!!
2. Vortex and spin ~20 sec microcentrifuge @ ~ 10,000 rpm
3. Carefully harvest aqueous (top) phase and transfer to new vial/tube
4. Add equal volume of chloroform (or CI 24:1); vortex and spin ~20 sec microcentrifuge @ ~ 10,000 rpm @ 4°.
5. Carefully harvest only aqueous (top) phase and transfer to new vial
6. Add 1/10 volume of 3M sodium acetate (to correct salt conc) and add 3x volumes of 100% ethanol. Mix well.
7. Put @ -80° for 1 hour or ON @ -20°. Spin 13,200 rpm for 20 min @ 4°.
8. Remove all ethanol; let air dry for 5-10 min
9. Add appropriate amount of water; let sit for ~20 min for DNA to go into solution
10. Proceed with second restriction digest or spec @ 260/280 for the concentration, etc