**Homemade Taq purification**

protocol from Brady lab (UCD) and modified by Kazu (Maloof lab, Feb 26, 2013)

**Prepare**

LB/Carb (100 µg/ml) plate, LB/Carb (10 ml, 200 ml in flask), 100 mM IPTG (filter sterilized), 100% sterile glycerol (autoclaved)

buffer A (50 ml)

50 mM TrisHCl (pH 7.9) 2.5 ml of 1M TrisHCl (pH 7.9) found in lab stock cabinet (room 2011?)

50 mM dextrose (glucose) 1.25 ml of 2M stock or add 0.45g

1 mM EDTA 100 µl of 0.5M EDTA (found in lab stock cabinet)

buffer B (10 ml) make fresh (during step 7 in Day4 because half-life of PMSF is 30 min).

10 mM TrisHCl (pH7.9) 100 µl of 1M stock

50 mM KCl 500 µl of 1M stock

1mM EDTA 20 µl of 0.5 M stock

1 mM PMSF Phenyl Methyl Sulfonyl Fluoride

100 µl of 100 mM stock in EtOH (voltex well, prepare fresh)=1.74 mg

0.5% Tween20 50 µl

0.5% Nonidet P40 50 µl (=Igepal, CA-630 Sigma)

(option) Taq storage buffer for dilution (for 100ml)

10 mM TrisHCl (pH 7.9) 1ml 1M stock

50 mM KCl 5ml 1M stock

1 mM EDTA 200µL 0.5M EDTA

0.5% Tween 20 500µl

0.5% Nonide P40 500µL(=Igepal, CA-630 Sigma)

1 mM PMSF 1 mL 100mM (make fresh)

50% Glycerol 50mL

**Day1**

1. Streak pTaq E. Coli (Maloof lab glycerol stock box I-J3) on LB/Carb (carbenicillin) (100 µg/ml, found in [lab antibiotics stock](http://openwetware.org/wiki/Maloof_Lab:antibiotics).

**Day2**

1. Grow 10 ml starter O/N (overnight) in LB/Carb (100 µg/ml) at 37˚C.

**Day3**

1. In the next day, transfer 200 µl into 200 ml LB/Carb. Grow until OD (optical density) =0.2 (~3.5 hr) at 37˚C.
2. Add IPTG to a final conc of 0.5 mM (eg. 1 ml from 100 mM stock or 2.5 ml of 40 mM stock).
3. Grow O/N at 37˚C (after you can keep at 4˚C).

**Day4**

Prepare Cold Sorvall centrifuge (in front of Sinha lab) and Water bath (75˚C)

1. Centrifuge the culture 10 min in 6000 x g (in 250 ml bottle) (4˚C)
2. Resuspend the pellet in 40ml buffer A (= ⅕ of original culture volume, on ice)
3. Transfer the resuspension to 30 ml tubes). (on ice)
4. Centrifuge the resuspension 10 min in 6000 xg (4˚C)
5. Resuspend the pellet in 2 ml buffer A (1/20 of volume added in previous stage, on ice).
6. Add 8 mg Lysozyme (final is 4 mg/ml). (RT)
7. Incubate at room temperature for 15 min.
8. Add equal volume of buffer B. (RT)
9. Incubate at 75˚C for 1 hr.
10. Centrifuge in 4˚C at 8000 rpm for 15 min. (Cold room)
11. Collect the supernatant and transfer to a new tube.
12. Add equal volume (2-4 ml) of 100% sterile glycerol. (on ice)
13. Divide it to aliquots and keep in -80˚C for long-term storage or -20˚C for daily use.
14. Try different dilution (0.5, 0.25, 0.1 µl) for PCR on plasmid and genomic DNA.