Protocol : Purification of *Taq* DNA polymerase

Reference: Pluthero, F.G. Nucleic Acids Research (1993) **21**, 4850-4851

Buffers:

		per 1 L	
Buffer A	50 mM Tris-HCl pH 7.9	50 ml 1 M pH 7.9	
	50 mM dextrose	50 ml 1 M	
	1 mM EDTA	2 ml 0.5 M	0.25M

		per 75 ml	per 25ml
Lysis Buffer	10 mM Tris-HCl pH 7.9	0.75 ml 1 M pH 7.9 →	0.25 ml
	50 mM KCl	3.75 ml 1 M →	1.25 ml
	1 mM EDTA	0.15 ml 0.5 M →	0.05 ml
	1 mM PMSF	0.75 ml 100 mM →	0.25ml
	0.5% Tween 20	1.875 ml 20% →	0.625ml
	0.5% Nonidet P40	1.875 ml 20% →	0.625ml

		per 1 L
Storage Buffer	50 mM Tris-HCl pH 7.9	50 ml 1 M
	50 mM KCl	50 ml 1 M
	0.1 mM EDTA	0.2 ml 0.5 M
	1 mM DTT	1 ml 1 M
	0.5 mM PMSF	5 ml 100 mM (omit from final)
	50% glycerol	500 ml

Grow overnight culture of *E. coli* INV1αF'/p*Taq* in L-broth + ampicillin
Inoculate 0.25 ml overnight culture into **500** ml L-broth supplemented with 80 mg/L ampicillin in 2 L baffle flask at 37°C
Grow ~ 11 hr to an OD_{600} ~0.8 = 0.5mM (= 0.25 ml of 1M IPTG)
Add IPTG to 125 mg/L. Continue growing for 12 hr.
Harvest cells by centrifugation (8K, 15 min). (2 purple capped 500ml bottles ~250ml in each.)
Wash in 25 ml **Buffer A**, centrifuge as above.
Resuspend pellets in 25 ml **Buffer A** + 4mg/ml lysozyme ~12.5ml/pellet
Incubate 15 min at RT
Add 25 ml **Lysis Buffer**. Transfer to a Pyrex flask. Incubate 75°C 1 hr.
Transfer to centrifuge bottles. Fuge 15,000 rpm, 10 min, 4°C.
Transfer clarified lysate to a clean Pyrex flask, get stirring with stir bar at RT.
Add 15 gm $(NH_4)_2SO_4$ (powdered) to stirring lysate at RT.
Transfer to centrifuge bottles. Fuge 15,000 rpm 10 min.
Harvest precipitate from pellet and surface.
Resuspend in 10 ml **Buffer A**.
Dialyze against storage buffer for 12 hr at 4°C.
Change buffer and repeat dialysis step Dont' add PMSF to dialysis buffer.
Dilute 1:1 with sterilized **Storage Buffer (No PMSF)** and store in small (25 µl) aliquots at -80°C.