Purification of Taq DNA Polymerase - for 1 Liter culture

Modified from the protocol presented in F.G. Pluthero (1993) Rapid purification of high-activity *Taq* DNA polymerase. Nucleic Acids Research 21 no.20: 4850-4851.

Day 1

Start 3ml overnight culture of taq from glycerol stock in LB/amp (75ng/ul)

Day 2

Add 1ml of overnight culture to 1L of LB/amp. Incubate at 37C and 220rpm for 11 hours, or until A600 of 0.800.

Add 125mg/L pf IPTG and incubate an additional 12 hours

Day 3

Pre-heat a water bath to 75C

Transfer culture to 2-500ml centrifuge bottles and spin at 4800rpm, 5 minutes at 4C in Beckman JA10 rotor.

Discard supernatant and resuspend pellet in 100ml Buffer A (50ml/bottle)Resuspend very well with a pipet, but do not vortex.

Spin at 4800rpm, 5 minutes at 4C. Resuspend pellet in 50ml pre-lysis buffer (25ml/bottle). Combine in a 250ml flask and incubate at room temp for 15 minutes.

Add 50ml of lysis buffer,mix well and incubate at 75C for one hour.

Transfer to 50ml centrifuge tubes and spin at 15,000rpm for 10minutes at 4C in Beckman JA-20 rotor.

Pour supernatant into sterile 250ml flask with a stirring bar and add ammonium sulfate at a rate of 30g/100ml lysate. Add slowly – over about 15 minutes- Stir well to precipitate protein (at least another 10-15 minutes). ***It is very important to do this slowly and will make a difference in your yield.***

Transfer to 50ml centrifuge tubes and spin at 15,000rpm for 10minutes at 4C. Protein pellet will form on surface and walls of tube. Remove it from all tubes with sterile pasteur pipet and resuspend in a total of 20ml of Buffer A *Note: it is not easy to get the protein out – it kind of floats around and/or sticks to the walls. You can try to pick it up or suck it up. Sometimes I find it easiest to try to remove all of the liquid and leave the pellet and resuspend it in that tube.*

Transfer suspension to dialysis tubing and dialyze at 4C for 12 hours with gentle stirring in 450ml of storage buffer.

Day 4

Drain storage buffer off and replace with 450ml of fresh storage buffer. Dialyze for 12 hours longer under same conditions

Add equal amount of storage buffer and aliquot into 1.5ml tubes and store at -80C.

Always thaw aliquots of taq and dilution buffer on ice.

BUFFERS

	Needs for 1 L of culture Stock	 Add
Buffer A		**(200ml)**
50mM Tris-HCl pH8.0	1M	10ml
50mM Dextrose (D-glucose)		9g
1mM EDTA pH8.0	0.25M	800ul
ddH2O	up to 200ml	

Pre-Lysis Buffer

50ml bufferA
200mg lysozyme

Lysis Buffer		**(50ml)**
10mM Tris-HCl pH7.9	1M	500ul
50mM KCl	1M	2.5ml
1mM EDTA pH 8.0	0.25M	200ul
1mM Pefabloc SC (do not autoclave)	100mM	500ul
0.5% Tween 20		250ul
0.5% Igepal CA650		250ul
ddH2O	up to 50ml	

Storage/Dilution Buffer			**(1000ml)**
50mM Tris-HCl pH7.9	1M	50ml	
50mM KCl	1M	50ml	
0.1mM EDTA	0.25M	400ul	
*1mM DTT(not autoclavable)	1M		1ml
*0.5mM Pefabloc SC (do not autoclave)	100mM	5ml	
50% glycerol	100%	500ml	
ddH2O	up to 1L		

*add right before use.
Store this buffer in freezer while not in use. Save out a little of this buffer to use as dilution buffer which should be stored in small aliquots at –80C.

Supplies (sterile) per 1L of starting culture:

4 centrifuge bottles (500ml and 50ml)

4 oakridge centrifuge tubes (50ml)
2-200ml flasks with stir bar, covered with aluminum foil
dialysis tubing [from Gibco/BRL (now Invitrogen)#15961-022] and clamps
500ml beaker w/ stir bar
Pasteur pipettes
STERILIZE ALL GLASS AND PLASTICWARE

Check concentration by making a dilution series and using the dilutions in PCR. Results usually vary between 1:5 dilution and 1:30 dilution.