MAKING TAQ POLYMERASE FOR GENOTYPING
WITH DAVID DANKORT

NOTE: This protocol is to produce Taq polymerase for the purposes of genotyping. The method exploits the thermostable properties of Taq polymerase where host E.coli (strain BL21 used here) proteins are precipitated after their denaturation by freezing and high temperature thawing. As a rule of thumb this procedure will produce enough Taq for 2000 reactions per 100ml of initial bacterial culture. The total time required is 3 days. I tend to do larger preps so QC takes only a small amount. There are several places in the protocol (**) where you can stop and store the lysates at -80°C to enable larger scale processing.

Reagents Required

- BL21 bacteria containing His-tagged Taq polymerase (Amp^R)
- LB-Amp

1000 ml	SOC media
0.5M	IPTG (GoldBio cat # ____)
buffer A	50 mM Tris-HCl pH 7.9, 50 mM glucose, 1 mM EDTA
15g	Ammonium Sulphate
8L	Dialysis buffer** (50 mM Tris-HCl pH 7.9, 50 mM KC1, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50% glycerol)
~25cm	Dialysis Tubing* (Spectra/Por* Dialysis Tubing, Regenerated Cellulose, MWCO 12000 to 14000, Spectrum* Laboratories, 32mm wide, cat # S732703) [Fisher VWR 25225-248]

* Dialysis buffer can be made as a 20x stock, diluted with ddH20 and glycerol and where DTT and PMSF are added just before use.

1. Innoculate a colony of transformed BL21 cells into 3ml LB containing ampicillin (100 ug/ml) and, if BL21 derived chloramphenicol (at 35 ug/ml for pLysS selection) and incubate overnight at 37°C.

2. The next morning transfer 200ul of the overnight culture into each 2L flask containing 500ml SOC-Amp at 37°C. It is best to do at least 1L.

3. Culture at 35°C until the broth had reached an OD_{600} of 0.4-0.6. I have found that this takes ~6hrs.

4. Add IPTG to 0.75mM and transfer culture to 32°C while shaking overnight (220rpm)

5. The next morning bacteria are harvested by centrifugation (3500 rpm, 15 minutes), washed once in buffer A, and resuspended in buffer A to a twentieth of the culture volume (i.e. 5ml/100ml original culture). I often do this in 50ml falcon tubes.**

6. The suspension was subjected to three cycles of freezing and thawing at temperatures of -70°C and 75 °C (5-10 minutes). This step serves two functions: to lyse the bacteria and to denature the E. coli proteins.**

7. The lysate is cleared by centrifugation. I do this in two steps. Falcon tubes are centrifuged for 7 minutes at 4000 rpm. The supernatant is transferred to 1.7 ml eppendorf tubes and centrifuged at top speed (15,000 rpm) for 15 minutes. If you think that the lysates are not yet cleared transfer to new eppendorf tubes and spin again at top speed.

8. Pool the lysates into 10ml aliquots in 15ml tubes. (Steps 9-11 result in cleaner Taq and minimizes shadows at the bottom of DNA gels and additionally can be used to increase concentration of Taq).**

9. Add 3.0 g of powdered $(NH_4)_2SO_4$ [ammonium sulphate] per 10.0 ml of cleared lysate and mix at room temperature to get salt into solution. (the last time I did this I incubated the mixture at 4°C for 10 minutes before spinning)

10. Spin at 15000 rpm for 10 minutes to recover precipitated protein. To do this you will have to transfer the solution to eppendorf tubes. You can remove the supernatant and freeze at -80°C until needed (it is likely best to proceed to dialysis though).**

11. Resuspend precipitate in buffer A such that to $1/20^{th}$ of the original culture volume, i.e. the equivalent of each 200ml original culture should be resuspended in 10ml of buffer A. A rule of thumb here is that each eppendorf should receive about 1ml of buffer A.

12. Transfer to dialysis bags (12,000-14,000 cutoff)

13. The resuspended protein was dialyzed against multiple changes of Dialysis/storage buffer (50 mM Tris-HCl pH 7.9, 50 mM KC1, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50% glycerol).

    Dialysis: Dialysis functions as follow:
    Volume within tubing / volume dialysis buffer. So if you have 10ml of Taq lysate and dialyze against 1L of buffer this is a 1:100 dialysis. It is better to change dialysis buffer frequently. I tend to do three successive 1000ml dialyses, 2x1000ml at room temp (2 hours each) with the last one overnight at 4°C

14. Test Taq in a standardized reaction. Here I make dilutions of the Taq such that 1ul containing either 1ul, 0.75ul, 0.5ul, and 0.25ul is used to set up reactions tested against a previous batch of Taq similarly diluted.

15. Pool Taq preps into a single 15 or 50 ml tube diluting with fresh dialysis buffer if necessary, mix, spin briefly and aliquot to screwtop microfuges.

This procedure tends to produce ~ 10ml of Taq/ 1L of starting material after using ammonium sulphate precipitation. This Taq functions at 0.5ul/PCR (i.e. ~ 20,000 PCRs)