Lab Protocol # 025

ISOLATE TAQ POLYMERASE

Ref: Pluthero, F.G. (1993) Nucleic Acids Research, Vol. 21, No. 20, pp.4850-1

Basically, follow the detailed protocol defined in the reference above. Here is an abbreviated version with notes from my own experience. For any missing details, RTFP.

SOLUTIONS:

1. Buffer A=50mM TrisCl (pH 7.9), 50mM dextrose, 1mM EDTA.

2. Pre-lysis buffer=Buffer A+4mg/ml lysozyme.

3. Lysis buffer=50mM TrisCl (pH 7.9), 50mM KCl, 1mM EDTA, 1mM PMSF, 0.5% Tween 20, 0.5% Nonidet P40

4. Dialysis/Storage buffer=50mM TrisCl (pH 7.9), 50mM KCl, 1mM EDTA, 1mM DTT, 0.5mM PMSF, 50% glycerol

PROTOCOL (for a 1L culture):

1. Start culture with 500 microliters of an overnight culture.

2. 12 hr after innoculation, induce with 125mg/L of IPTG.

3. Harvest 12hrs after induction.

4. Spin out cells, wash in Buffer A, Spin out cells again.

5. Resuspend in 50ml pre-lysis buffer, incubate for 15 minutes.

6. Add 50 ml lysis buffer, and incubate for 1 hr at 75C, spin the debris at 15K for 10 minutes at 4

7. To the recovered supe, add 30 gm of Ammonium Sulfate/ per 100 ml of supe. harvest both ppt and surface ppt. The film will appear (lots of taq in it)

8. Resuspend in 20 ml Buffer A / original 100ml of cleared lysate.

9. Dialyze at 4 degrees C with two changes over 12 hr period..

10. After dialysis, spin out the insoluble and dilute the protein 1:1 in the sterilized storage buffer.

NOTES:

1. The timing of growth, induction and harvest is critical. Don't dawdle, proceed directly with the isolation. It should take only one day from harvest to dialysis.

2. Expect a precipitate during dialysis. No precipitate=low yield. TAQ will go back into solution, some proteins won't. Volume will be reduced slightly during dialysis.

3. After dialysis, I spun out the insols and saved the clarified supernatant and proceeded to the analysis of activity

ANALYSIS:

1. To look at the protein content I ran a PAGE gel. Many extra bands were visible compared with the gels depicted in the reference other than the TAQ at ~90kd. These seemed to have no effect on activity, etc.

2. DNAse activity was checked with a sample incubated at 37 degrees for various times up to 24hrs and compared with an unincubated mixture with the composition of a standard PCR rxn mix using 250ng of Lambda DNA (cut with Hind III) as target. No DNAse activity was detected.

3. The diluted enzyme appears to be stable at -20 and undiluted enzyme is stored at -80. Previous isolations were stable at -80 for 5+ years.

4. The enzyme amplifies fragments ranging from several 100 to greater than 5000 bp equally well.