Taq Polymerase Synthesis

Procedure:

Streak out pUCR-TaqPol (pEJS25) onto an LB_{Amp100} plate.

Pick a single colony into 5ml of LB_{Amp100}

Inoculate 1L of LB_{Amp100} with 1ml of the overnight culture.

Grow to OD_{590}= 0.2 (~2.5h).

Add IPTG to 0.5mM.

Continue growth for 16-20h.

Chill cells on ice.

Spin cell down at 10,000 X g for 10 min at 4°C.

Resuspend cells in 100ml buffer A.

Spin 8K, 10 min, 4°C.

Resuspend in Buffer A plus lysozyme:*

Buffer A	25ml	
Lysozyme	100mg	(4mg/ml)

Incubate at RT 15min.

Add 25ml Buffer B

Incubate at 75°C for 60 min.

Spin cell debris down at 20,000g (13,000 rpm in SS34) for 20min at 4°C.

Collect supernatant (~50ml).

Precipitate with ammonium sulfate:

Add NH_4SO_4 to 35% (208g per L or 10.4g per 50ml).

Spin 20,000 X g (13,000 rpm in SS34) for 20 min at 4°C.

Collect the supernatant by passing it through a kimwipe (the pellet will float).

Increase the [NH_4SO_4] of the supernatant to 60% (+163g per L or 8.15 per 50ml).

Pellet the 60% NH_4SO_4 cut by spinning at 34,000 X g (15,000 rpm in SS34) for 30 min at 4°C.

Resuspend the pellet in 1-2ml Taq storage buffer.

Determine the activity of the enzyme (Units) by titering it against a known standard.

*(An alternative would be to resuspend in 10 ml buffer A and French Press the cells.)

Solutions and stuff:

1. **LB Medium** — 1000 ml

(a) Bacto Tryptone	10.0g
(b) Yeast Extract	5.0g
(c) NaCl	5.0g
(d) pH with 10N NaOH	to ~pH7 (Two pellets of NaOH per 1000 ml adjusts the pH to ~7)
(e) dH_2O	to 1000ml
(f) A/C	20-30 min

2. **LB Amp Plates** — 500 ml (~20 plates)

(a) Bacto Tryptone	5.0g
(b) Yeast Extract	2.5g
(c) NaCl	2.5g
(d) pH with 10N NaOH	to ~pH7 (one pellets of NaOH per 500ml adjusts the pH to ~7)
(e) dH_2O	to 1000ml
(f) Agar	15.0g
(g) Stir bar	
(h) A/C	20-30 min
(i) Cool media to ~50°C	
(j) Add ampicillin	1ml

3. **Ampicillin**

Prepare a 100mg/ml stock solution of Ampicillin by dissolving 1g into 10ml of ddH_2O. Filter-sterilize by passing the solution through a syringe with attached 0.2μm filter sterilization unit. Collect the supernatant into sterile microfuge tubes or a 15ml sterile plastic conical tube. Store at –20°C.

4. **IPTG** (Isopropylthio-β-D-galactoside) (2g/10ml = 0.84M)

Stock	For 10 ml
IPTG	2g
dH_2O	8ml

Bring final volume to 10 ml.
Filter-sterilize using 0.2μm filter. Aliquot into microfuge tubes & store at -20°C.

5. **PMSF** (Phenylmethylsulfonyl fluoride) and AEBSF (4-(2-amino-ethyl)- benzenesulfonyl fluoride hydrochloride), which is a non-toxic alternative to PMSF)

Stock	For 1 ml
PMSF (100mM)	17.4mg
2-propanol (iso)	1.0 ml
Store at –20°C	

or

AEBSF (100mM)	23.9mg
dH_2O	1.0 ml
Store at –20°C	

6. **Buffer A**

	100ml	10ml	Final []
1M Tris (7.9)	5.0ml	0.5 ml	50mM
50% Dextrose^	1.8ml	0.18 ml	50mM
0.5M EDTA	0.25ml	0.025	1.25mM
dH_2O	93ml	9.3ml	

^(50% Dextrose = 2.8M Dextrose)

7. **Buffer B**

	100ml	10ml	Final []
1M Tris (7.9)	1.0ml	0.1ml	10mM
1M KCl	5.0ml	0.5ml	50mM
0.5M EDTA	0.2ml	0.02ml	1mM
PMSF (100mM)	1.0ml	0.1mL	1mM
Tween 20	500µL	50µL	0.5%
Nonidet NP40	500µL	50µL	0.5%
dH_2O	91.8ml		

8. **Taq Storage buffer**

	10ml	Final []
1M Tris	0.2ml	20mM
1M KCl	1.0ml	100mM
0.5M EDTA	2µl	0.1mM
80% Glycerol	6.25ml	50%
1M DTT	10µl	1mM
Tween 20	50µl	0.5%
Nonidet NP40	50µl	0.5%
dH_2O	2.45ml	