Protocol for Preparation of Tag Sequence

1. Remove 1 colony from pMK Tag LB amp plate and inoculate 3-4 mls of LB amp (100 µg/ml) O/N at 37°C

2. Use 1 ml O/N culture to inoculate 1 liter of LB media (15 g Bacto tryptone, 7.5 g yeast, 7.5 g NaCl & 150 µl 10N NaOH). Shake inoculated culture to O.D at 600 nm of 0.4-0.6.

* 8 2 liter baffled flask @ 1 liter LB amp = 12 liters.

3. Add IPTG to a final concentration of 0.5 mM to induce and Continue shaking for 2 hours at 37°C

4. Harvest cells by centrifugation at 7,000 rpm 3 minutes 4°C Pool cells in 1 liters per 500 ml Centrifuge bottle.

5. Resuspend cells in 200 ml Buffer A / 1 liters and Repellet (Buffer A 50 mM Tris pH 7.9, 50 mM dextrose, 1 mM EDTA)

6. Resuspend each pellet in Buffer A 40 mls @ 40 mg/ml lysozyme. Incubate at RT for 20 minutes

7. Add 40 mls Buffer B to Each tube (Buffer B - 10 mM Tris pH 7.9, 50 mM KCl, 1 mM EDTA, 0.5% Triton X, 0.5% NP40, 1 mM PMSF). Incubate at 75°C for 60 minutes

8. Spin in cold GSA Rotor 15 minutes, 8000 rpm. Save Supernatant

9. Transfer Supernatant to Cold flask 250 ml bottles

10 Precipitate with Polyethyleneimine (PEI) to a final conc of 0.15%.
PEI stock made as 10% w/v pH 7.5 in HCl Stored at 4°C.
- Add 12 mls of cold 10% PEI dropwise to each 80 mls of Supernatant on ice with gently shaking to mix and, let Stand at ice for 45 minutes.

11. Collect precipitate at 8000 rpms for 20 minutes 4°C.

12. Resuspend each pellet in 20 mls Buffer C plus 0.25M KCl using a dounce and pestle loose
(Buffer C = 20 mM Hepes pH 7.9, 1 mM EDTA, 0.25% Tween 20, 0.25% NP40, 0 5mM PMSF)

13. Respin. Collect Supernatant. Add 10 mls Buffer C with 0.15M KCl in dounce & tight pestle couple of Strokes. Stand 20 minutes on ice. Respin and pool Supernatant. Add 10 mls Buffer C with 0.35M KCl and further dounce & tight pestle Repeat

14. Pool supernatants and adjust KCl conc to 50 mM

15. Equilibrate BioRex 70 Column 100-200 Size or 200-400 with buffer C. Use 120 ml packed resin per 12 liters Cell Culture

16. Wash Column with buffer C 50 mM KCl 2 times with 120 mls buffer C each time

17 Elute Tag Polymerase with buffer C 200 mM KCl Slowly. Approx. 90-95% will be Tag Pol Should be about 20 100 units/ml (Compare to Cetus)

18. Dialyze the enzyme collected in Storage buffer at 4°C.
First w/o glycerol 1 liter and w glycerol 1 liter
(Storage Buffer = 50% glycerol, 20 mM HEPES, 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 0.5 mM PMSF).

19. Add PMSF to final conc 1 mM after dialysis.

20. Aliquot in 100 µl and Store at −80°C after Snap freezing in EtOH, dry ice. Stable to 5 freeze thaws

* Use 1-2 units per PCR rxn, Too much can inhibit PCR Rxn.
you can dilute and titrate. Compare to 10 units/rxn.

Buffer A 2 liters

50 mM Tris pH 7.9 - 100 mls 1M Tris pH 7.9

50 mM Dextrose - 18 g of Dextrose

1 mM EDTA - 4 mls 0.5M EDTA

Buffer B 500 mls

10 mM Tris pH 7.9 - 5 mls 1M Tris pH 7.9

50 mM KCl - 25 mls 1M KCl

1 mM EDTA - 1 ml 0.5M EDTA

1 mM PMSF - 5 mls 100 mM PMSF

Buffer C w/o KCl - 500 mls

20 mM HEPES pH 7.9 - 10 mls 1M HEPES pH 7.9

1 mM EDTA - 1 ml 0.5M EDTA

0.25% NP40 - 1.25 mls Stock NP40

0.25% Tween 20 - 1.25 mls Stock Tween 20

0.5 mM PMSF - 2.5 mls 100 mM PMSF

(1) to 50 mM KCl
+ 25 mls 1M KCl

(2) to 150 mM KCl
+ 75 mls 1M KCl

(3) to 250 mM KCl
+ 125 mls 1M KCl

(4) to 700 mM KCl
+ 100 mls 1M KCl

Storage Buffer - 2 liters

20 mM HEPES - 40 mls 1M HEPES pH 7.9

0.1 mM EDTA - 0.4 mls 0.5M EDTA

1 mM DTT - 2 mls 1M DTT

100 mM KCl - 200 mls of 1M KCl (14.8g)

1 mM PMSF - 20 mls 100 mM PMSF

50% glycerol - 1 liter glycerol

$(2000 \text{ mls})(50) = (1500 \text{ mls})c_2$

$150 = c_2$

$c_2 = 47\%$ glycerol

Buffer A = 50 mM Tris pH 7.9
50 mM Dextrose
1 mM EDTA

* need 1.6 liters / 12 liters culture
* need 320 mls / final lysozyme

Buffer B = 10 mM Tris pH 7.9
50 mM KCl
1 mM EDTA
1 mM PMSF

* need 320 mls / 12 liters culture

Polyethyleneimine (PEI) = 10% w/v pH 7.5
w HCl
Store at 4°C

Final conc. 0.15%
to precipitate enzyme
on ice 30-45 mins.

Buffer C = 20 mM Hepes pH 7.9
1 mM EDTA
0.25% Tween 20
0.25% NP40
0.5 mM PMSF

a. Plus 50 mM KCl (5ml mls)
b. Plus 150 mM KCl (20ml mls)
c. Plus 250 mM KCl (20ml mls)
d. Plus 2M mM KCl (20ml mls)

Storage Buffer = 20 mM HEPES
0.1 mM EDTA
1 mM DTT
100 mM KCl
1 mM PMSF

a. Plus 50% glycerol
b. w/o glycerol

= need 1 liter each.