

# GST-TR purification

9/17/95

- 2 liters of LB
- inoculate with GST-TR transformed BL21
- inoculate 1 liter with 5mls Sat. o/n culture.
- grow to O.D approx. 0.6 at 600nm
- Add IPTG 400µM 1ml and induce for 4 hours at 37°C
- pellet cells freeze o/n at 20°C if needed
- Resuspend in 25mls STE buffer, pH 7.5

STE pH 7.5  
100mM NaCl  
10mM Tris  
1mM EDTA

- Add 5mls NETN pH 8.0 and resuspend pellet completely. Place on ice for 15 mins.

(Add 20µl PMSF 100mM, 50µl pepstatin, 50µl Aprotinin)

- Sonicate for 1 min. Repeat again. Keep on ice.
- Centrifuge 4°C, 10000rpm 10mins. Collect sup.
- Add 1ml glutathione beads and rotate at 4°C for 2 hours.
- pellet beads

NETN pH 8.0  
0.5% NP40  
20mM Tris  
100mM NaCl  
1mM EDTA

- Wash in NETN 5 times
- Resuspend in 1ml NETN

PMSF  
Pepstatin  
Aprotinin

## Load Gels (I)

1. GST-TR pellet 25µl set I
2. GST-TR sup 25µl
3. GST-TR beads 10µl

## (II)

1. GST-TR pellet 25µl set II
2. GST-TR sup 25µl
3. GST-TR beads 10µl

1ml  
75µl DTT  
10% Sarkosyl  
STE  
1.5mls  
10% Triton  
K100  
in STE